Review

Low Intensity Pulsed Ultrasound for Accelerating Distraction Osteogenesis: A Systematic Review of Experimental Animal Studies

Jiriys George Ginini[1,2,*], Dekel Shilo[2], Omri Emodi[1,2], Adi Rachmiel[1,2]

[1] *Bruce Rappaport Faculty of Medicine, Technion, Israel Institute of Technology, Haifa 31096, Israel*
[2] *Department of Oral & Maxillofacial Surgery Rambam Health Care Campus, Haifa, 31096, P.O.B 9602, Israel*

*__Correspondence__: jiriysginini@technion.ac.il (Jiriys George Ginini)

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Abstract

The purpose of this report is to review systematically all studies performed regarding the influence of low intensity pulsed ultrasound (LIPUS) on bone regeneration in animals during distraction osteogenesis (DO). Based on the Preferred Reporting Items for Systematic Reviews and Meta- Analyses (PRISMA) checklist structure, a systematic search using PubMed and EMBASE electronic databases was undertaken utilizing the key words “distraction osteogenesis” and “low intensity ultrasound”. Human trials, review articles, case reports and non-English language publications were excluded. Data items were extracted from each eligible study, regarding the study design, risk of bias, results and whether or not LIPUS accelerated bone regeneration. The search identified 40 relevant articles, 15 of which were included for full review. Included studies were characterized by a high risk of bias and considerable variations in study design was observed. However, most studies which reported LIPUS in an intensity of 30-40 mW/cm^2 accelerated bone formation via endochondral ossification, thus shortening the consolidation period when applied during distraction and early consolidation periods. According to the current review, application of LIPUS during DO shows promise in accelerating bone formation and density during DO, that bears no adverse effects, thus shortening the consolidation period. Optimal timing of LIPUS application is during the distraction and early consolidation phases. The preferred intensity should be between 30-40 mW/cm^2. Histological analysis indicates influence via endochondral ossification. Thus, the effect of LIPUS on chondrocytes should be further investigated in order to decipher the exact molecular and cellular influence of LIPUS on enhancement of bone formation. These findings should be used in future clinical protocols and raise potential directions for future research regarding the molecular mechanisms underlying the influence of LIPUS on bone formation.

Keywords

Distraction osteogenesis; LIPUS; Review

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1. Introduction

Distraction osteogenesis (DO) is a biological process for producing new bone and overlying soft tissue by gradual controlled traction of the surgically separated bone segments. Gradual traction on living tissues creates tensile forces, which stimulate stem cells, thus promoting regeneration and active growth of the involved tissues. This process is based on the “Law of Tension Stress” [1]. The bone and its periosteum act as a guide for the newly regenerated bone. During DO, the overlying soft tissue envelope (skin, subcutaneous tissues, muscles, nerves, fascia, and periosteum) responds according to the hard tissue movements with gradual lengthening. This process is called distraction histogenesis. Simultaneous soft tissue distraction leads to decreased soft tissue resistance thus reducing bony relapse.

To achieve adequate bony elongation, preservation of blood supply, periosteum and stable fixation are required; therefore, optimal rate and rhythm of distraction is essential. Stability is an important principle, allowing for remodeling of the woven bone in the distracted gap during the consolidation period. The duration of the consolidation period may last up to several months during which the newly regenerated bone and the surrounding tissue are exposed to external forces and infections, which may interfere with the healing process and increase the complication rate [2]. In addition, due to prolonged consolidation periods, patients may experience inconvenience and psychosocial issues. Therefore, many efforts have been made to shorten the consolidation period in an attempt to overcome this major drawback of the DO procedure. This has been attempted by biophysical and biochemical strategies, including the application of growth factors, stem cells and various adjuvant modalities therapies, including extracorporeal shock wave, low level laser and low intensity pulsed ultrasound (LIPUS) [3].

Ultrasound generates acoustic energy at a high frequency (1 to 12 MHz), from a piezoelectric crystal within a transducer applied to the target tissue. Ultrasound has been widely used in medicine as a diagnostic and therapeutic tool [4]. Therapeutic ultrasound stimulates changes in cells and tissues by utilizing high energy intensities (1-3 W/cm^2). These changes may be due to the thermal effect by elevation of temperature of the local tissue [5]. In contrast, for diagnostic applications of ultrasound, low energy intensity is used and

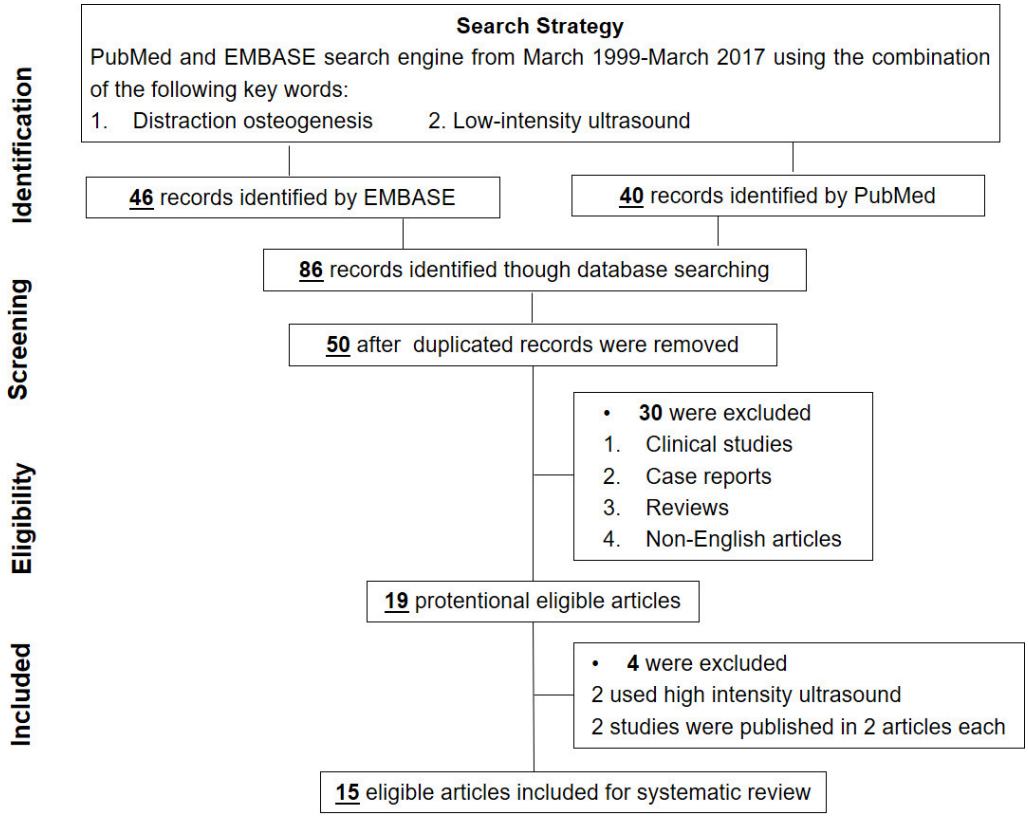

Fig. 1. Flow chart of the systematic search stratergy.

is regarded as a non-thermal mechanism producing cavitation and acoustic streaming, which stimulates biochemical events at the cellular level [6]. Duarte et al., [7] demonstrated that LIPUS enhances bone formation with minimal thermal effects [7]. This revelation generated considerable interest. The Food and Drug Administration (FDA) approved the use of LIPUS for fracture healing and for the treatment of established non-union fractures in 1994 and 2004, respectively [8, 9].

In this review, we focus on the effectiveness and safety of LIPUS as an adjuvant treatment for accelerating DO, as observed in animal models.

2. Materials and Methods

This systematic review is based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist structure. One author (JGG) searched PubMed and EMBASE electronic databases and performed a systematic search, to identify all studies from 1999 till 2017 regarding the application of LIPUS during DO in animal studies. The following keywords were used: "Distraction osteogenesis" and "low intensity ultrasound". Followed by a manual search through google scholar for additional relevant articles.

We used the PICOS format suggested by Cochrane Collaboration to describe the research question of the current review: participants – animals of any species which underwent DO on any bone type. Intervention – low intensity ultrasound during any period of DO. Comparison – control group; DO without LIPUS and DO with another intervention. Outcome – accelerated bone regeneration as evaluated by all methods reported by the authors for evaluating the effect of LIPUS during DO. Study design- animal studies. Data selection was conducted by two independent reviewers (JGG and DS). First, titles and abstracts were screened and duplicated articles were eliminated. Next, human trials, review articles, case reports and non-English publications were excluded. We then retrieved the full text of any article that was judged as potentially eligible. The reviewers independently applied eligibility criteria to the methods section of potential studies. Finally, any disagreements were resolved through discussion until consensus was reached. If there were more than 1 publication of the same experiment, the comprehensive and detailed report was selected.

Data items were extracted from each eligible study independently and included personal information, animal species and size, study design, distraction protocol, intensity and duration of the LIPUS, phase of treatment in which LIPUS was applied, assessment methods, results and whether LIPUS accelerated bone regeneration.

The Systematic Review Centre for Laboratory animal Experimentation (SYRCLE) Risk of Bias tool, which is based on the Cochrane Collaboration Risk of Bias Tool for animal intervention studies, was adopted and applied for assessing the risk of bias in this systematic review [9]. Randomization of the study, blinding of investigators and outcome assessor, attrition bias (incomplete outcome data adequately addressed), and reporting bias (selective outcome reporting) were used to assess study quality. "Yes" indicates low risk of bias; "No" indicates high risk of bias. If one of the relevant entry is answered with "No", this indicates a high risk of bias for that specific study.

Table 1. Summary of the study design of included studies.

Animal species	Bone model	Groups	Latency period (Days)	Distraction rate (mm/d)	Distraction period (Days)	Consolidation period (Days)	LIPUS application period	LIPUS intensity (mW/cm^2)	LIPUS duration (min/day)	Study reference
64 rabbits	Tibia	1. Control 2. LIPUS	7	1	10	7, 14 or 21	Consolidation	30	20	[10]
18 sheep	Metatarsal	1. Control 2. LIPUS	4	1	15	63	Consolidation	30	20	[11, 12]
26 rabbits	Tibia	1. Control 2. LIPUS	7	1	10	20	Consolidation	30	20	[13]
20 rabbits	Tibia	1. Control 2. LIPUS	7	1	10	20	Consolidation	30	20	[14]
21 rabbits	Mandible	1. Control 2. Bilateral LIPUS every other day 3. Unilateral LIPUS daily	3	2	5	28	Consolidation	30	20	[15]
34 rats	Femur	1. Control 2. LIPUS	7	0.334	21	35	Consolidation	30	20	[16]
34 rabbits	Tibia	1. Control 2. LIPUS	1	0.75	14	28	Consolidation	30	20	[17]
75 rabbits	Tibia	1. Control 2. LIPUS at latency 3. LIPUS at distraction 4. LIPUS at consolidation	7	1.5	7	14	Latency, distraction or consolidation	30	20	[18]
17 rabbits	Tibia	1. Control 2. LIPUS	7	1	18	28	Consolidation	30	20	[19]
18 rabbits	Tibia	1. Control 2. 20 min/day LIPUS 3. 40 min/day LIPUS	7	2	6	Not mentioned	Distraction	30	20 and 40	[20]
44 rabbits	Tibia	1. Control 2. LIPUS	7	1	10	10 or 20	Distraction and early consolidation	30	20	[21]
36 rabbits	Mandible	1. Control 2. LIPUS 3. Continuous ultrasound	3	3	5	7, 14 or 21	Consolidation	30	20	[22]
7 dogs	Mandible	1. Control 2. LIPUS	7	1	20	0, 7, 14, 28, 42, 56 or 84	Distraction	40	10x2	[23, 24]
24 rabbits	Mandible	1. Control 2. LIPUS	3	1	10	0, 14 or 28	Distraction and consolidation	30	20	[25]
15 rabbits	Mandible	1. Control 2. LIPUS 3. Laser	7	1	10	13 or 43	Distraction	30	20	[26]

3. Results

The search of PubMed and EMASE electronic databases provided a total of 86 records. After adjusting for duplicates, 50 records remained. Of which, 31 studies were eliminated after reviewing the abstracts which clearly showed these papers did not meet the criteria. Of the 19 potentially eligible articles retrieved in full text [10–28], 2 were excluded as they used high intensity ultrasound (2 W/cm^2) [27, 28] and 2 studies were published in 2 articles each and were thus considered as one study for the purpose of the research [11, 12, 23, 24], leaving 15 eligible studies included in the systematic review (Fig. 1). The results are summarized in Tables 1-3. Experimental designs for assessing the impact of LIPUS on DO were heterogeneous with respect to the animal species used, bone model and distraction protocol. The most common animal species used was rabbit (12/15, 80%) and the tibia was the bone model most frequently tested (8/15, 53%). Other studies utilized a mandible model (5/15, 33%), femur (1/15, 7%) and metatarsal (1/15, 7%).

The DO protocols varied widely; the latency period ranged between 1-7 days, the distraction rate was between 0.3-3.0 mm/day and the consolidation period, in accordance with time of scarification, ranged from 0 to 84 days.

All studies used the pulsed technique exclusively, except for one study which compared the pulsed technique with continuous ultrasound treatment [22]. One study compared the effect of low-level laser versus LIPUS on rabbit mandible [26].

In most experimental studies, LIPUS treatment was performed with the same therapeutic parameters; 30 mW/cm^2 intensity for 20 minutes per day (14/15, 93%) and one study used 40 mW/cm^2 intensity for 10 min twice a day [24]. Whereas another study [20] used two different durations for the LIPUS treatment, 20 min and 40 min per day.

The timing of LIPUS application varied among the reviewed studies. In one study [18], LIPUS treatment was applied during the distraction, latency and consolidation period in three different groups with the intention of investigating the optimal timing of LIPUS application during DO. Nine of the 14 other studies applied LIPUS during the consolidation period. Other researchers applied the treatment during the distraction period (3/14) or during both periods (2/14).

Although different methods were used to evaluate the effects of LIPUS on DO in the reviewed studies, bone mineral density (BMD) and mechanical tests were most prevalent. BMD was used for evaluating bone regeneration in most studies (13/15, 87%). BMD was determined using conventional X-ray, Computed Tomography (CT) and Dual Energy X-Ray Absorptiometry (DEXA) 2, 5 and 6 times, respectively, out of the 13 studies. In 10/13 studies [10, 11, 13, 15, 16, 18, 19, 22, 24, 26] a higher fraction of BMD (77%) was found in the experimental group as compared to the control group; the difference was significantly higher in 8/13 (62%) studies [10, 11, 15, 18, 19, 22, 24, 26].

In addition, mechanical testing for evaluating the stiffness, torque and strength of the distracted bone was frequently conducted (12/15 of the studies). A higher mechanical structure in the LIPUS groups was reported in 8/12 studies, compared to the control groups [10, 12, 15–18, 22, 25] of which 6 showed statistical significance.

In summary, LIPUS has a positive effect on bone regeneration in 11/15 (73%) of the studies, though in a variable manner. The results of the methodological quality and risk of bias are shown in Table 3. Six studies contained information about randomization of animals. No studies reported that investigators were blinded from knowledge, which intervention each animal received during the experiment, and only 3 studies reported blinding of outcome assessor. Regarding the attrition bias, 12 studies adequately addressed the incomplete outcome data. All of the included studies were free of selective outcome reporting. Overall, all studies were considered to be a relatively high risk of performance bias, but no reporting bias.

4. Discussion

DO is a surgical technique for bone lengthening that stimulates new bone formation and simultaneously expands the surrounding soft tissues. DO requires no donor site, thus eliminating the morbidity of a second surgical site, and is thus a safe and effective procedure that has gained widespread popularity in both orthopedic and craniofacial fields. The technique is used for treatment of congenital deformities or acquired deformities secondary to oncology, trauma, severe atrophic bone and infections. However, the prolonged consolidation period and the potential for complications during this period remain major disadvantages. Therefore, many attempts have been made to shorten the consolidation period, which would reduce costs, complications and burden on the patient. Several studies have shown the efficacy of LIPUS in stimulating bone formation during fracture repair. The current review discusses the efficacy of LIPUS during DO in animal studies for accelerating bone regeneration.

Small animals (rats and rabbits) have been extensively used, while large animal models are relatively scarce. Clearly, small animal models are easier for a large sample size and in-depth molecular and genetic analyses, but are limited in their clinical relevance to human DO patients, due to the large discrepancy in mandible size, morphology and function. Therefore, more pre-clinical studies based on large animal models are desirable.

Generally, the dosage of LIPUS treatment can be adjusted in terms of frequency, intensity and treatment duration [29]. In the reviewed studies listed in Table 1, the parameters of therapeutic LIPUS were almost identical; 20 min a day, 1-1.5 MHz sine waves repeating at 1 KHz, average intensity of 30 mW/cm^2 and a pulse width of 200 ms. In another study [24], a different intensity was applied (40 mW/cm^2 intensity for 10 min, twice a day) on dog mandibles during DO. Using this intensity, the authors also reported increased bone regeneration in comparison to the control group. These results are in accordance with other reports regarding bone fracture repair, which indicate that the appropriate intensity is generally $<$ 100 mW/cm^2 and usually in the range of 20-50 mW/cm^2 [30].

In an attempt to determine optimal duration of LIPUS treatment during rapid bone lengthening, in study [20] two different durations of treatment were selected – 20 min and 40 min per day. The results showed that LIPUS increased the bone mineral content (BMC) and the volume of mineralized tissue of the distraction callus in a dose-dependent manner. Further studies should be conducted on conventional DO in order to determine whether or not LIPUS treatment has a dose-dependent effect.

In study [22], the effects of pulsed versus continuous ultrasound treatments on rapid DO were compared. The authors reported that earlier stages of bone formation were enhanced by pulsed ultrasound to a larger extent than continuous ultrasound. This might be explained by the additional mechanical stimulation provided in the

Table 2. Summary of assessment methods, main results and accelerated regeneration of included studies

	Assessment methods	Results regeneration	Accelerated Study reference
Radiography, DEXA (BMD), Mechanical test, Histology	Radiography, BMD and mechanical tests significantly greater in LIPUS.	Yes	[10]
Radiography, qCT (BMD), Mechanical test, Histology	BMD, BMC, stiffness and callus formation were significantly higher in LIPUS.	Yes	[11, 12]
Radiography, DEXA (BMD), Mechanical test, Histology	BMD higher in LIPUS but no significant difference. Stiffness and strength not significant. Radiographically significantly larger callus in LIPUS. Histology significant less fibrous tissue in LIPUS	No	[13]
Radiography, DEXA (BMD), Mechanical test, Histology	BMD showed no significant differences. Torsional strength was significantly higher in control. Histologically, LIPUS displayed more cartilage and fibrous tissue formation	No	[14]
Radiography, Vibratory coherence, Mechanical test, Histology	BMD, vibratory, stiffness and histology were significantly higher in LIPUS groups, especially in daily unilateral LIPUS treatment.	Yes	[15]
Radiography, μCT (BMD), Mechanical test	Radiography higher density in LIPUS. Bone volume fraction significantly higher in LIPUS. BMD and BMC slightly higher in LIPUS. LIPUS group stiffer and stronger (not significant)	Yes	[16]
qCT (BMD), Mechanical test, Histology	BMD, BMC, cross-sectional area and strength showed no significant differences. Histology showed no differences in bone volume fraction	No	[17]
Radiography, DEXA (BMD), Mechanical test, μCT	Lengthening group had significantly greater BMD and mechanical strength. 3D-CT: more accelerated bone formation. Bone regeneration was enhanced more in the LIPUS applied at lengthening group.	Yes	[18]
Radiography (BMD), qCT, Mechanical test	BMD, BMC and Bone scan index were significantly greater in LIPUS at 2 weeks of consolidation, but not at 4 weeks. The maximum torque was smaller in LIPUS.	Yes	[19]
Radiography, qCT (BMD), Histology	BMC of both LIPUS groups was greater than the control group in a dose-dependent manner. BMD showed no significant differences in LIPUS groups. Histology: LIPUS enhanced endochondral formation in a dose-dependent manner.	Yes	[20]
Radiography, Mechanical test, Histological	No differences in bone mineral appositional rates or tissue composition. No difference in structural stiffness.	No	[21]
qCT, Mechanical test, Histology	BMD in the first 2 weeks was higher in Continuous US > LIPUS > Control. BMD in 3rd and 4th weeks LIPUS > Continuous US > Control. Mechanical test was higher in LIPUS > Continuous US > Control group. Histology: more bone volume and fraction in LIPUS ≥ Continuous US > Control	Yes	[22]
Radiography, mTc-MD bone imaging, DEXA (BMD), CT, Histology	Higher 99mTc-MDP uptake in LIPUS group at the early consolidation. Radiography: earlier maturation of bone, higher BMD in LIPUS group. Histology: thicker trabeculae and endochondral bone formation	Yes	[23, 24]
Radiography, μCT, Mechanical test, Histology	Mechanical tests, bone microhardness and radiopacity were significantly higher in LIPUS at early consolidation. At week 4 after the distraction - no significant differences.	Yes	[25]
DEXA (BMD)	BMD was significantly higher in LIPUS compared to control at early consolidation. BMD in laser group was significantly increased at late consolidation.	Yes	[26]

pulsed mode, which has an effect on bone cell differentiation and bone matrix production [31].

DO consists of three periods; latency, distraction and consolidation. Each period activates various molecular and cellular events including different osteogenic factors, leading to varied regeneration potential. The timing of LIPUS application during DO varied among the currently reviewed studies; however, most of the authors applied LIPUS during the consolidation period. In study [18], LIPUS most effectively accelerated maturation of newly formed bone during DO in the distraction period, as compared to the latency and the consolidation periods, suggesting that mature mesenchymal cells, osteoblasts, or chondrocytes were affected by LIPUS, rather than inflammatory cells or immature mesenchymal cells. Furthermore, study [25] reported that LIPUS stimulated more bone formation with

Table 3. Methodological quality and risk of bias of included studies

Randomization	Investigators blinding	Assessor blinding	Attrition bias	Reporting bias	Risk of bias	Study reference
No	No	No	Yes	Yes	High	[10]
No	No	Yes	No	Yes	High	[11, 12]
Yes	No	No	Yes	Yes	High	[13]
Yes	No	Yes	Yes	Yes	High	[14]
No	No	No	Yes	Yes	High	[15]
Yes	No	No	Yes	Yes	High	[16]
No	No	No	Yes	Yes	High	[17]
No	No	No	No	Yes	High	[18]
No	No	No	No	Yes	High	[19]
No	No	Yes	Yes	Yes	High	[20]
Yes	No	No	Yes	Yes	High	[21]
No	No	No	Yes	Yes	High	[22]
No	No	No	Yes	Yes	High	[23, 24]
Yes	No	No	Yes	Yes	High	[25]
Yes	No	No	Yes	Yes	High	[26]

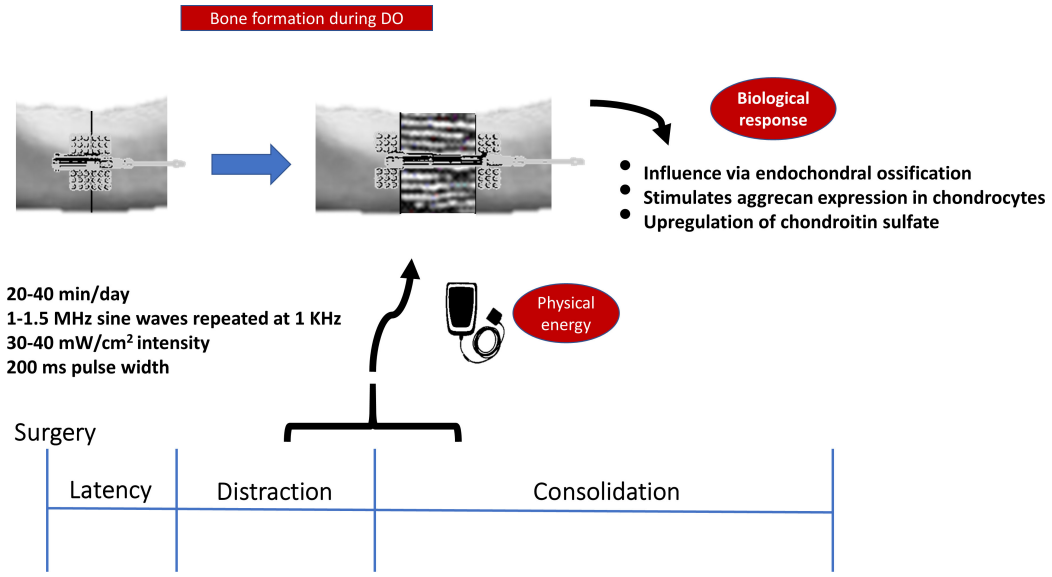

Fig. 2. Summary of the suggested effects of LIPUS on DO. The physical energy transmitted by the transducer of the LIPUS is converted into biological response, leads to up-regulation of growth factors via endochondral ossification.

higher efficacy during the distraction and early consolidation periods, with no statistical difference when applied at the late consolidation period. These results can be explained by the fact that at early stages of DO, the distraction gap is filled mostly with hyper-cellular soft tissue and less mineralized tissue, thus facilitating the transmission of LIPUS. In contrast, the results in study [21] did not support the application of LIPUS at the distraction and early stages of consolidation periods.

Similarly to LIPUS, low-level laser therapy (LLLT) produces mechanical stimulation which influences bone repair and regeneration. One rabbit model compared the effects of LIPUS and LLLT on bone maturation following DO [26]. The results suggest that both LIPUS and LLLT have positive effects in terms of accelerating bone healing during DO.

The appropriate timing of removal of the distractor device following DO is still being debated. Bone strength and stiffness are the most clinically relevant variables tested in this case, because these determine whether or not a bone will deform or fracture when the distractor device is removed [1, 10]. Most studies (75%) suggested that LIPUS improves the mechanical properties of the distracted bone and only 50% demonstrated significant differences. The lack of significant differences could be attributed to the discrepancy in test protocols, the time when the bones were tested and the small number of animals in each group. Generally, the BMD measurement is a quicker, cheaper and more available quantitative method. Several studies demonstrated a correlation between DEXA and CT for BMD determination and the biomechanical properties of bone after DO [32].

Generally, the most common method for the quantification of BMD is DEXA and particularly in the reviewed studies. BMD measurements in most studies increased in the LIPUS groups compared to the control groups. However, the differences were not significant

in the late stage of the consolidation period as indicated in some studies [10, 19, 24, 26], demonstrating that LIPUS is most effective at the early stage of the consolidation period.

In contrast to fracture healing, which is repaired in a process of endochondral ossification, it has been reported that bone formation during DO is generated mainly by an intramembranous process [33]. However, other researchers reported the presence of predominant endochondral ossification [34]. In addition, some authors postulated a third ossification mode called transchondroid, where they found predominance of endochondral bone formation in the early stages of distraction, but intramembranous ossification later [35]. Although the specific mechanism by which LIPUS affects bone formation is unclear, it appears that it stimulates aggrecan expression in cultured chondrocytes and in rat fracture repair, as well as upregulation of chondroitin sulfate [36–38]. These results are consistent with those reports showing more cartilage tissue formation during DO [12, 14, 17, 18, 20, 22] and the limited data supporting the effect of LIPUS on osteoblast and intramembranous ossification [19, 39]. Therefore, it is believed that LIPUS affects bone formation in DO via endochondral ossification. However, the exact cellular mechanism by which LIPUS accelerates bone regeneration in DO requires further investigation.

Although the reviewed studies showed considerable variations in study design, animal models, DO parameters, LIPUS parameters and the application and assessment methods, the conclusions suggest that LIPUS has a positive effect on accelerating bone regeneration during DO (11/15, 73%). In the articles that showed no advantage for using LIPUS (4/15), no negative effects were noted [13, 14, 17, 21]. In addition, LIPUS showed no adverse effects or complications in any of the articles reviewed.

A quantitative analysis (meta-analysis) of the present systematic review could not be performed when considering the lack of homogeneity related to animal species, bone model, distraction protocol, LIPUS parameters, evaluation methods and measurement unites. The quality assessment found that all studies were considered as having high risk of bias. Therefore, the conclusions in the current systematic review may be hampered by the aforementioned shortcomings.

The positive effect of LIPUS on acceleration of bone regeneration in this systematic review are consistent with the results of a previous review on five human trials (four on tibia and one on a mandible) studying the effect of LIPUS during DO [40]. This review studied the optimal time for LIPUS applications, the appropriate intensity of LIPUS and the histological aspects including the ossification pattern, which could serve as a milestone for future studies investigating the biological mechanism of LIPUS on bone regeneration.

Previous studies on human subjects were performed mainly on the tibia, yet many surgeons perform DO on the mandible. No significant results were observed in the mandible. In this review on animal models, five studies performed on the mandible were included, and in all of them, accelerated bone regeneration was reported.

In conclusion, LIPUS is a safe, non-invasive, patient-friendly technique that bears no complications or adverse effects. The current comprehensive review of the literature indicates that LIPUS can accelerate bone formation and increase BMD during DO, thus shortening the consolidation period. The optimal timing of LIPUS application is during the distraction and early consolidation phases. Sporadic human trials did not show positive results in human mandibles, yet according to the current literature review, LIPUS during DO in the mandible shows promise and should be further clinically investigated. This review strengthens the preferred timing of LIPUS application during the distraction and early consolidation phases. The preferred intensity should be between 30-40 mW/cm^2. Review of the histological effects of LIPUS in these studies indicates influence via endochondral ossification (Fig. 2). Thus, the effect of LIPUS on chondrocytes should be further investigated in order to decipher the exact cellular and molecular influence of LIPUS on enhancement of bone formation. This should be the basis for better understanding and assist in establishment of protocols for accelerating DO.

Acknowledgement

None.

Conflict of Interest

The authors declare that they have no financial or non-financial interests.

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