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Effect of low intensity ultra sound on bone regeneration and healing: a systematic review

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ABSTRACT

Objective: Low Intensity Pulsed Ultra Sound (LIPUS) is found to have stimulatory effect on bone healing and regeneration. This review aimed to assess whether LIPUS enhances bone regeneration and healing in terms of efficiency in improving clinical, radiographic, histologic parameters or serum and tissue biomarkers. **Methods**: A comprehensive search based on PRISMA guidelines with pre-determined eligibility criteria was conducted to identify randomized controlled clinical trials evaluating effectiveness of Low intensity pulsed ultrasound in bone regeneration and healing. The title and abstract of the entries in all languages yielded from the PubMed, Google scholar and Cochrane library were screened. **Results**: 14 eligible Randomized controlled trials testing the effectiveness of LIPUS was evaluated. More heterogeneity was seen in the screened studies with respect to sample characteristics, type of bone and outcome measures. The studies that screened histological parameters state that LIPUS is significantly beneficial than control. In terms of time for radiographic union, most of the studies stated that LIPUS was more effective than control but numberof studies are very few. Whereas studies which evaluated parameters such as healing time and radiographic union were showing highly inconsistent results regarding effectiveness of LIPUS. **Conclusion**: This review cannot give a definitive conclusion that LIPUS is effective in bone healing with respect to clinical parameters but a positive influence on radiographical and histological parameters in bone healing and regeneration is promising to pursue future research.

Indexing terms: Bone healing. Bone regeneration. Low intensity pulsed ultrasound. LIPUS.

INTRODUCTION

Healing of any bone defect is a complex process requiring the recruitment of the appropriate cells and expression of the appropriate genes at the right time in the right place. Healing facilitated by bone regeneration is a complex, physiological process of bone formation, which can be seen during normal fracture healing, and is involved in continuous remodeling throughout life [1]. It is modulated in response to external stimuli, such as growth factors, hormones, and mechanical forces [2,3]. There are number of approaches that stimulate bone-regeneration process, like treatment with free vascularized graft, autogenous bone graft, allograft implantation, growth factors, osteoconductive scaffolds, osteoprogenitor cells and distraction osteogenesis [4].

Since clinical introduction in the 1950s, ultrasound at intensities ranging from 1 to 50 mW/cm^2 has been demonstrated to be osteogenic, chondrogenic, and angiogenic, thus accelerating skeletal healing in animal [5] and

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human clinical studies. Low Intensity Pulsed Ultra Sound(LIPUS) (30 - 100 mW/cm^2 of intensity) is an acoustic radiation that can be transmitted into the living tissues as pressure waves resulting in biochemical events at the cellular level. In vitro, LIPUS is very well known to stimulate bone and cartilage cells, showing that they exert direct anabolic effects such as production of growth factors and other signaling molecules, extra cellular matrix production and osteogenic differentiation [6]. In essence, ultrasound provides an optimal biological and biophysical environment promoting skeletal maintenance and repair [7,8].

Therapeutic application of LIPUS can promote bone repair and regeneration, enhance osteogenesis at the distraction site and accelerate bone fracture healing [9]. A study by Tanzer et al. showed that LIPUS influenced the extent and rate of bone growth in a positive way in a femoral bone of dog [10, 11]. Ustun et al. reported that the area, bone volume and bone-implant contact ratio values were increased by LIPUS stimulation in tibia, suggesting that LIPUS application may promote bone healing around dental implants [11]. Also, Hsu et al. demonstrated in vivo that blood flow and mature collagen fibers were more prevalent around titanium implants, and bone formation was accelerated by ultrasound stimulation.

Therefore, the aim of this review was to critically analyze the available scientific work regarding the effects of Low-Intensity Pulsed Ultra Sound (LIPUS) on stimulating bone regeneration and bone healing in humans.

METHODS

A nonregistered protocol was prepared based on PRISMA guidelines prior to the start of the literature search with a structured question; Does LIPUS facilitate bone regeneration and healing in improving clinical, radiographic, histologic parameters or serum and tissue biomarkers in humans?

The following PICO strategy was used in the literature search.

PICO (Population, Intervention, Comparison, and Outcomes)

- **P** – Patients undergoing treatment for any bone fracture or deformities
- **I** – LIPUS
- **C** – Placebo, no treatment, or only conventional treatment
- **O** – Clinical, Radiographic, Histologic parameter and serum or tissue biomarkers which reflect bone healing or regeneration

The outcomes of interest included in this systematic review were

- Clinical: Time for fracture healing, treatment period, fixator gestation period, resumption of activities, etc.;
- Radiographic: Time to radiographic union, Changes in gap area, bone mineral density, radiographic union, callus formation;
- Histological: evidence of new bone formation and characteristics of different tissue compartments;
- Serum or tissue biomarkers, example: alkaline phosphatase, osteocalcin, osteonectin, etc.

Literature search protocol

Publications of interest within the scope of this focused systematic review was searched in

- The electronic database National Library of Medicine (MEDLINE/PubMed)
- Google scholar
- Cochrane library

No restriction regarding publication type and publication date or language was set.

Article eligibility criteria

Inclusion criteria

- Articles reporting original human clinical trials on the topic of LIPUS on bone regeneration or bone healing in all languages.
- No restrictions were placed based on the age, population.
- Studies having at least one control group and one experimental group that involved the application of a LIPUS.

Exclusion criteria

- Studies with sample size less than N=6
- Studies were LIPUS was used less than 10 days.
- Studies involving patients with osteoarthritis or acute infection, pathological fracture
- Studies that analyze the effect of LIPUS with any other bioresorbable screw fixation or grafts
- Studies in which statistical analyses was not done to compare the difference.

The initial search yielded 1468 entries in PubMed database, Google scholar and Cochrane library. Excluding all animal studies, case series, case reports, systematic review and duplicate studies, 236 articles were human clinical trials. Out of this, the total number of articles selected after reviewing the titles and abstracts was 16. Two articles were excluded after full-text review. A final selection of 14 human clinical trials was made. Two independent reviewers (RV& SV) independently carried out the article selection. All the 14 articles were those in which any of the clinical, intra surgical, radiological and histological parameters were compared with LIPUS to a control or placebo device in humans with any bone defects (figure 1).

Data extraction

The data of the selected studies was extracted using standardized abstraction tables. Information extracted from each study included the following (table 1 and 2).

1) Title 2) Author and year

3) Study design 4) Duration 5) Groups 6) Sample size 7) Sample characteristics

8) Patient consent 9) Ethical committee approval 10) Sample size calculation

11) Randomization 12) Blinding 13) Outcome measures 14) LIPUS characteristics 15) Types of statistical methods used 16) Mean & SD or Mean difference or Hazard ratio 17) pvalue 18) Inference

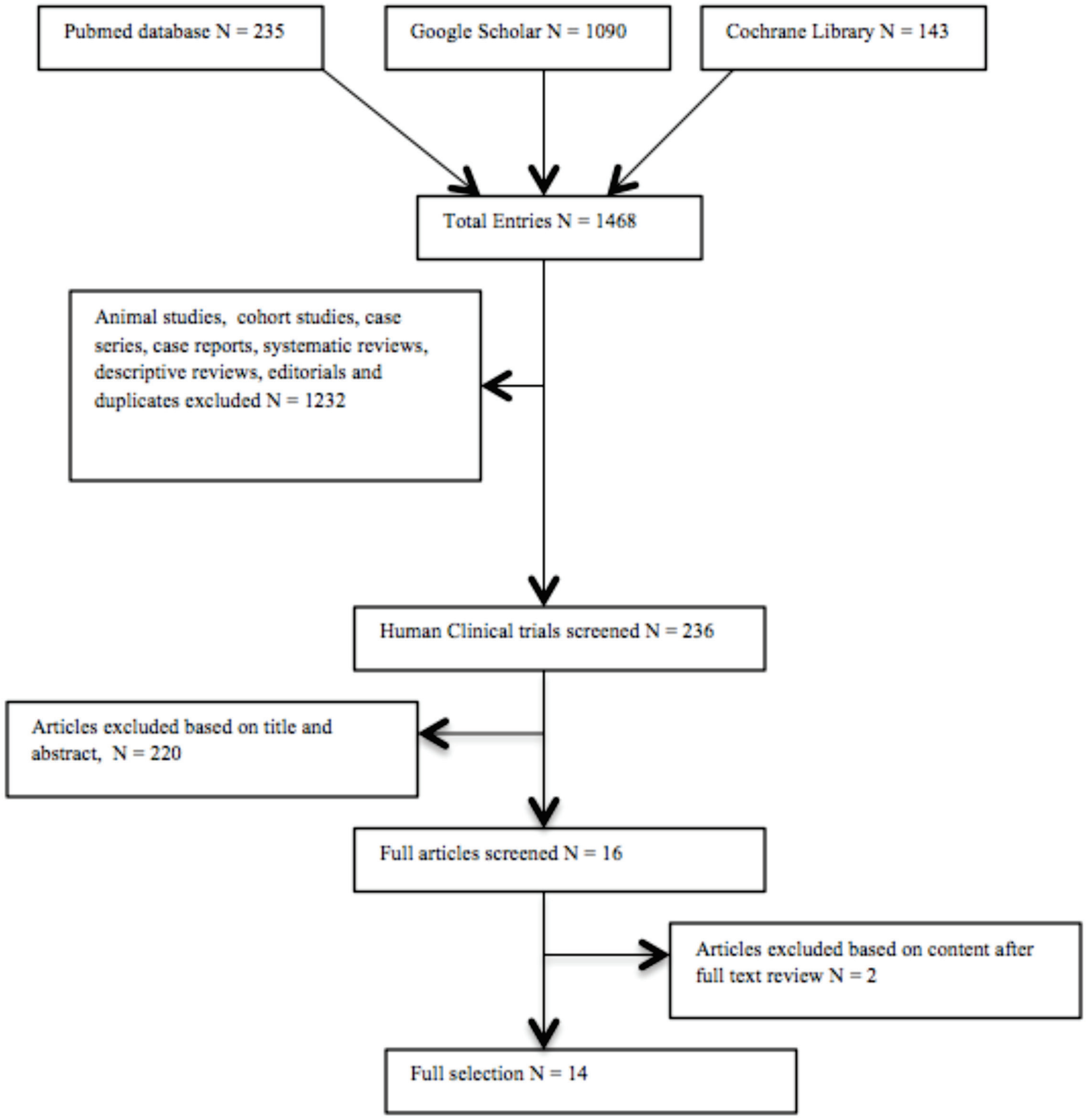

Figure 1. Search Process and article selection.

RESULTS

Fourteen eligible clinical trials testing the effectiveness of LIPUS were evaluated and study characteristics were tabulated (table1and 2). Among the 14 studies seven studies evaluated effect of LIPUS on fresh fractures [12,15,19,21,23,24,26] two each on delayed or non-unions[19,20] and distraction osteogenesis [13,16], and one each on osteotomies [14] , stress fractures [22] and sinus lift [17]. Included studies also showed variability in the type of bone they have evaluated which included lower limb [13,22], fibula [20], tibia [16,18,21,23,26], scaphoid [24], clavicle [12], ulna [14], radius [15,19]. There was also one study that used LIPUS therapy after maxillary sinus lift [17]. In all the studies except one [17] the characteristics of the LIPUS used were similar. The Spatial Average Temporal Average (SATA) intensity was 30mW/cm3, frequency was1.5MHz and pulse duration was 200 micro seconds. In the study by Kim et al. [17] SATA intensity was 240mW/cm3 and frequency was 3MHz. Due to the heterogeneity between the included studies with respect to sample characteristics and outcome measures, meta-analysis was not performed instead a qualitative synthesis of results were made (table 3).

Table 1. Characteristics of the included studies- methodology.

S. No	TITLE	Author and year	Study design	Blinding	Randomi-sation	Duration	Patient Consent	Ethical committee approval	Sample size calculation	Nature of sample population	Groups Sample size	LIPUS Chrecterestics	Types of statistical method used	Outcome measures
1	Low-intensity pulsed ultrasound (LIPUS) in fresh clavicle fractures: A multi-Centre double blind randomized controlled trial	Lubert et al. [12], 2008	Multi centre double blinded RCT	Double Blinded	Randomized (Block randomisation)	2 months	Obtained	Approved by Local medical ethical committee	Performed	Fresh clavicle fractue	Group 1-(control group) placebo transducer without LIPUS stimulation Group 2-(Test group) Active transducer with LIPUS stimulation N = 101 Group 1 = 49 Group 2 = 52	SATA intensity;30mW/cm3 Frequency-1.5MHz Puduration-200mico seconds	Differences between groups were analyzed using the Student's t-test and the Pearson Chi-square test	Clinical - Fracture consolidation (healing) according to the patient's perception, Painkiller usage, VAS score, Adverse events, Resumption of sport, professional and household activities Radiological - Nil Histological – Nil Biomarkers – Nil
2	Low-Intensity Pulsed Ultrasound as a Useful Adjuvant During Distraction Osteogenesis: A Prospective, Randomized Controlled Trial	Dudda et al. [13], 2011	A prospective, Randomised control trial	No blinding	Randomized (Method – Not mentioned)	1-28 months	Not mentioned	Not mentioned	Not mentioned	Distraction osteogenesis of lower limb	Group 1– (control group) distraction osteogenesis only Group 2 – (Test group) distraction osteogenesis + LIPUS N = 36 Group 1 = 20 Group 2 = 16	SATA intensity;30mW/cm3 Frequency-1.5MHz Pulse duration-200mico seconds	t test	Clinical – 1. Fixator gestation period (treatment period) 2. Time to bone healing (Paley index), 3. Distraction consolidation index Radiological - Nil Histological – Nil Biomarkers - Nil
3	Effect of Low-Intensity Pulsed Ultrasound on Bone Healing at Osteotomy Sites After Forearm Bone Shortening	Urita et al. [14], 2013	A prospective Multicentre, Randomized clinical trial	Investigotor was blinded	Randomised (Odd-even system)	6 months	Obtained	Approved by local institutional review board	Performed	Ulnar shortening osteotomy	Group 1 – (Control group) only Ulnar or radial shortening osteotomy Group 2 – (Test group) Ulnar or radial shortening osteotomy + LIPUS N= 27 Group 1 = 13, Group 2 = 14	SATA intensity;30mW/cm3 Frequency-1.5MHz Pulse duration-200mico seconds	unpaired t-test	Clinical – 1. Total Modified Mayo wrist score Radiological– 1.Time to Cortical union 2.Time to Endosteal union Histological – Nil Biomarkers - Nil

Table 1. Characteristics of the included studies- methodology.

S. No	TITLE	Author and year	Study design	Blinding	Randomi-sation	Duration	Patient Consent	Ethical committee approval	Sample size calculation	Nature of sample population	Groups Sample size	LIPUS Chrecterestics	Types of statistical method used	Outcome measures
4	Ultrasound treatment for accelerating fracture healing of the distal radius. A control study	Liu et al. [15], 2014	Randomized clinical trial	Investigotor was blinded	Randomised (With Random number table)	2 months	Obtained	Approved by Ethics committee of Shuguang hospital.	Not mentioned	Distal radius fracture	Group 1 – (Control) Immobilisation with cast only Group 2 – (Test Group) Immobilisation with cast + LIPUS N=81 Group1 = 40 Group 2 = 41	SATA intensity;30mW/cm3 Frequency-1.5MHz Pulse duration-200mico seconds	To compare binary variables – Chi-square test To compare means of continuous variables – student t-test To compare distribution of ordinal variables – Mann Whitney test	Clinical – 1. Healing time Radiological – 1. Ratio of the grey level , Histological – Nil Biomarkers - Nil
5	Low-intensity pulsed ultrasound does not influence bone healing by distraction osteogenesis	Simpson et al. [16], 2017	Multi centre double blinded RCT	Double blinded	Randomised (Computer generated randomisation scheme)	6 months	Obtained	Not mentioned	Performed	Distraction osteogenesis of limb	Group 1 - control – Placebo Group 2 - Test – Active LIPUS N= 62 Group 1 = 30 Group 2 = 32	SATA intensity;30mW/cm3 Frequency-1.5MHz Pulse duration-200mico seconds	Mann-Whitney U test to assess linear variables and a chi-squared to assess dichotomous variables between the groups	Clinical - 1. Distraction length 2. Time to regenerate maturation, 3. Regenerate maturation index 4. Covariates affecting the time to removal of the frame Radiological - Nil Histological – Nil Biomarkers - Nil
6	Histologic evaluation of low-intensity pulsed ultrasound effects on bone regeneration in sinus lift	Kim et al. [17], 2010	Randomized contrlled trial	Not mentioned	Randomised (Method – Not mentioned)	8 months	Not mentioned	Approved by Institutional Review Board at Dankook UniversityDental Hospital	Not mentioned	Sinus lift	Group 1 - (Control) Only sinus lifting Group 2 - (Test) Sinus lift + LIPUS N = 8 sinus lifting sites Control = 4 Test = 4	SATA intensity-240mW Frequency-3 MHz Pulse duration- Not mentioned	To compare the average new bone volume and grafted material volume for each group - The Wilcoxon signed rank test	Clinical – Nil Radiological – Nil Histomorphometric analysis – 1. Quantitative analysis of new bone, 2. Remaining grafted material Biomarkers - Nil
7	Improved healing response in delayed unions of the tibia with low-intensity pulsed ultrasound: results of a randomized sham-controlled trial	Schofer et al. [18] 2010	Multicentre, Randomized clinical trial	Blinded	Randomised (Computer generated randomisation scheme)	4 months	Obtained	Approved	Not mentioned	Delayed tibial fractures	Group 1 – control – Sham LIPUS (Inactive LIPUS) Group 2 – Test – Active LIPUS N = 101 Group 1 = 51 Group 2 = 50	SATA intensity;30mW/cm3 Frequency-1.5MHz Pulse duration-200mico seconds	To compare, proportions - Chi-square test Continuous variables – two sample t-test.	Clinical - Nil Radiological – 1. Bone Mineral Density, 2. Gap Area at the fracture site Histological – Nil Biomarkers - Nil
8	Accelerated Healing of Distal Radius Fractures with the use of specific, Low-Intensity Ultrasound	Kristiansen et al. [19], 1997	A prospective Multicentre, double blinded, Randomized clinical trial	Double Blinded	Randomised (Computer generated randomisation scheme)	4 months	Obtained	Approved by Institutional Review Board	Not mentioned	Distal radial fracture	Group 1 - control – Placebo Group 2 - Test – Active LIPUS	SATA intensity;30mW/cm3 Frequency-1.5MHz Pulse duration-200mico seconds	ANOVA	Clinical – Time to bone healing Radiological – 1. Time after fracture to early trabecular

Table 1. Characteristics of the included studies- methodology.

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S. No	TITLE	Author and year	Study design	Blinding	Randomi-sation	Duration	Patient Consent	Ethical committee approval	Sample size calculation	Nature of sample population	Groups Sample size	LIPUS Chrecterestics	Types of statistical method used	Outcome measures
8											N = 61 Group 1 = 31 Group 2 = 30			healing 2. Time after fracture to cortical bridging Histological – Nil Biomarkers - Nil
9	Low intensity pulsed ultrasound increases bone volume, osteoid thickness and mineral apposition rate in the area of fracture healing in patients with a delayed union of the osteotomized fibula	Rutten et al. [20], 2008	A randomised prospective double blind placebo controlled clinical trial	Double Blinded	Randomised (Computer generated randomisation scheme)	5 months	Obtained	Approved by Medical Ethical Review Board of the vrije universiteit medical center	Not mentioned	Delayed union of osteotomized fibula	Group 1 - control – Placebo Group 2 - Test – Active LIPUS N = 13 Group 1 = 6 Group 2= 7	SATA intensity;30mW/cm3 Frequency-1.5MHz Pulse duration-200mico seconds	Student's independant t-test	Clinical – Nil Radiological – Nil Histomorphometric analysis – 1.Bone Volume of total tissue volume 2. Mineralised volume 3. Absolute osteoid volume 4. Relative osteoid volume 5. Osteoid thickness 6. Mineral apposition rate (μm/day) 7. No. of osteoclasts per mineralised tissue area (mm^2) 8. No. of osteocytes per mineralised tissue area (0.01 $mm^{2)}$ Biomarkers - Nil
10	Complex Tibial Fracture outcomes following treatment with Low-Intensity Pulsed Ultrasound	Leung et al. [21], 2003	Adouble blinded controlled clinical trial	Double Blinded	Not mentioned	9 months	Obtained	Approved by Clinical Research ethics committee of the Chinese University of HongKong	Performed	Complex Tibial fracture	Group 1 – control – Sham LIPUS (Inactive LIPUS) Group 2 – Test – Active LIPUS N = 30 Group 1 = 14 Group 2 = 16	SATA intensity;30mW/cm3 Frequency-1.5MHz Pulse duration-200mico seconds	To compare the differences between groups on the clinical and radiological assessments – Student's t test To compare the differences between groups for bone mineral content and ALP analysis- Mann Whitney U-test	Clinical – 1. Time to the disappearance of tenderness 2. Time to start partial weight bearing 3. Time to start full weight bearing 4. Time to remove the external fixator Radiological – 1. Time to appearance of first callus 2. Time to appearance of second callus 3. Time to appearance of third callus Histological – Nil Biomarkers – 1. Percentage change in Bone mineral content 2. Percentage change in Plasma bone specific Alkaline phosphatase

Table 1. Characteristics of the included studies- methodology.

S. No	TITLE	Author and year	Study design	Blinding	Randomi-sation	Duration	Patient Consent	Ethical committee approval	Sample size calculation	Nature of sample population	Groups Sample size	LIPUS Chrecterestics	Types of statistical method used	Outcome measures
11	Low-Intensity Pulsed Ultrasound in lower limb bone stress Injuries: A Randomized Controlled trial	Gan et al. [22], 2014	A prospective, randomised, double blinded, placebo controlled trial	Double Blinded	Randomised (Method – not mentioned)	3 months	Obtained	Approved by the University of New South Wales Human Research ethics Advisory Panel	Not mentioned	Bone stress injury	Group 1 - control – Placebo Group 2 - Test – Active LIPUS N = 23 Group 1 = 13 Group 2 = 10	SATA intensity;30mW/ cm3Frequency-1.5MHz Pulse duration-200mico seconds	ANOVA	Clinical – 1. Symptomatic days of Night Pain 2. Symptomatic days of Walking Pain 3. Symptomatic days of Sitting pain 4. Symptomatic days of pain with hopping 5. Symptomatic days of Tenderness 6. Symptomatic days of running pain Radiological – 1. Change in MRI grade 2. Change in edema size Histological – Nil Biomarkers – Nil
12	Acceleration of tibial fracture-healing by non-invasive, low-intensity pulsed ultrasound	Heckmann et al. [23], 1994	A multicenter, prospective, double blinded, placebo controlled, randomised clinical trial	Double Blinded	Randomised (Computer generated code)	13 months	Obtained	Not mentioned	Not mentioned	Treatment of fracture with closed reduction and immobilisation cast	Group 1 - control – Placebo Group 2 - Test – Active LIPUS N = 67 Group 1 = 34 Group 2 = 33	SATA intensity;30mW/ cm3 Frequency-1.5MHz Pulse duration-200mico seconds	ANOVA	Clinical – 1. Time to clinical healing 2. Time to discontinuation of the cast Radiological – 1. Cortical bridging 2. Endosteal healing Histological – Nil Biomarkers – Nil
13	Does Low Intensity, pulsed ultrasound speed healing of scaphoid fractures?	Mayr et al. [24] 2000	A Randomised controlled trial	Blinded	Randomised (Random number generator)	1-3 months	Not mentioned	Not mentioned	Performed	Scaphoid fracture	Group 1 - control Group 2 - Test – Active LIPUS N = 30 Group 1 = 15 Group 2 = 15	SATA intensity;30mW/ cm3 Frequency-1.5MHz Pulse duration-200mico seconds	Mann Whitney U test	Clinical – Time to fracture healing Radiological – Trabecular bridging in % Radiological – Nil Histological - Nil
14	Re-evaluation of low intensity pulsed ultrasound in treatment of tibial fractures (TRUST) : Randomised clinical trial	Busse et al. [25], 2016	A multicenter, randomised, blinded, sham controlled, clinical trial	Blinded	Randomised (Block Randomisation)	13 months	Obtained	Approved	Performed	Tibial fracture	Group 1 - control – Placebo Group 2 - Test – Active LIPUS N = 48 Group 1 = 241 Group 2 = 240	SATA intensity;30mW/ cm3 Frequency-1.5MHz Pulse duration-200mico seconds	Cox proportional hazards	Clinical - SF – 36 PCS Health utilities index scores Radiological Nil Histological - Nil

Table 2. Characteristics of the included studies - results.

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			Outcome parameters												
S.no	TITLE	Author and year	Clinical	Mean ± SD (or) Mean difference	P value	Radiographical	Mean ± SD (or) Mean difference	P value	Histological	Mean ± SD (or) Mean difference	P value	Biomarker	Mean ± SD (or) Mean difference	P value	Inference
1.	Low-intensity pulsed ultrasound (LIPUS) in fresh clavicle fractures: A multi-center double blind randomized controlled trial	Lubert et al. [12], 2008	Fracture healing (days)	0.33	0.91	NIL	-	-	NIL	-	-	NIL	-	-	The present controlled RCT showed that the LIPUS did not influence the clinical healing time of clavicle shaft fractures.
			Number of painkiller (tablets/28 days)	4.34	0.66										
			Visual Analogue Score(VAS)	0.04	0.90										
			Adverse events (number)	0.00	0.97										
			Resumption of household activities (days)	2.86	0.16										
			Resumption of professional work (days)	1.95	0.38										
			Resumption of sport (days)	2.27	0.07										
2.	Low-Intensity Pulsed Ultrasound as a Useful Adjuvant During Distraction Osteogenesis:A Prospective, Randomized Controlled Trial	Dudda et al. [13], 2011	Average Fixator gestation period (treatment period)	Control 262.2 days Test 218.6 ± 91.9 days	-	NIL	-	-	NIL	-	-	NIL	-	-	The use of LIPUS did not show any statistically signficant influence on time to bone healing.
			Paley index (Ratio of fixator gestation period in months over the distraction gap size in centimeters)	Control 1.49 ± 0.8 9 mo /cm Test 1.1 ± 0.44 mo/cm	0.116										
			Distraction consolidation index (the ratio of fixator gestation time in days over the distraction gap size in centimeters (d/cm)	Control 44.6 ± 26.8 d/cm Test 32.8 ± 13.1 d/cm	0.116										
3.	Effect of Low-Intensity Pulsed Ultrasound on Bone Healing at Osteotomy Sites After Forearm Bone Shortening	Urita et al. [14], 2013	Total Modified Mayo Wrist score	Control 34 Test 36	. No statistically significant difference.	Radiographic union	Control 2.6 mm Test 2.8 mm	No statistical difference (P value not mentioned).	NIL	-	-	NIL	-	-	The LIPUS therapy has beneficial influence on time to bone healing.
						Time to Cortical union	Control 77 ± 26 days Test 57 ± 10 days	0.012							
						Time to Endosteal union	Control 148 ± 3 days Test 121 ± 17 days	0.019							

Table 2. Characteristics of the included studies - results.

S.no	TITLE	Author and year	Outcome parameters: Clinical	Mean ± SD (or) Mean difference	P value	Radiographical	Mean ± SD (or) Mean difference	P value	Histological	Mean ± SD (or) Mean difference	P value	Biomarker	Mean ± SD (or) Mean difference	P value	Inference
4.	Ultrasound treatment for accelerating fracture healing of the distal radius. A control study	Liu et al. [15], 2014	Healing time	Control 40.75 ± 5.12 days Test 32.04 ± 2.58 days	<0.01	Average Ratio of the grey level	Control Group 1.029±0.096 Test Group 1.109±0.147	<0.05	NIL	-	-	NIL	-	-	The bone mineral density in the test group was statistically higher than that in the control group, hence LIPUS could promote bone formation at local site. Also, The time for clinical healing in the test group was significantly shortened. This result indicated that LIPUS could increase bone formation and accelerate the fracture healing.
5.	Low-intensity pulsed ultrasound does not influence bone healing by distraction osteogenesis	Simpson et al. [16] 2017	Time to maturation of regenerate,	Control 233.8 ± 98.3 days Test 256.6± 101.2 days	0.394	NIL	-	-	NIL	-	-	NIL	-	-	LIPUS did not show statistical significant difference in relation to Distraction length, time to regenerate maturation, RMI, etc. Hence, it does not influence the rate of bone healing in patients who undergo distraction osteogenesis.
			Regenerate maturation index (Ratio of time to maturation of the regenerate over length of distraction)	Control 60.8 ± 27.3 days/cm Test 65.8 ± 24.7 days/cm	0.226										
6.	Histologic evaluation of low-intensity pulsed ultrasound effects on bone regeneration in sinus lift	Kim et al. [17], 2010	NIL	-	-	NIL	-	-	Quantitative analysis of new bone (Area of newly formed bone in proprtion to the total measured area)	Control group 15.2 ± 3.1% Test group 19.0 ± 2.8%	< 0.05	NIL	-	-	The area of the remaining grafted material did not show statistically significant difference. The percentage of newly formed bone between the groups showed statistically significant difference.

Table 2. Characteristics of the included studies - results.

S.no	TITLE	Author and year	Outcome parameters												Inference
			Clinical	Mean ± SD (or) Mean difference	P value	Radiographical	Mean ± SD (or) Mean difference	P value	Histological	Mean ± SD (or) Mean difference	P value	Biomarker	Mean ± SD (or) Mean difference	P value	
6.									Remaining grafted material (Area of remaining grafted materials that not resorbed in proportion to the total measured area)	Control group 21.8 ± 2.0% Test group 20.7 ± 3.4%					
7.	Improved healing response in delayed unions of the tibia with low-intensity pulsed ultrasound: results of a randomized sham-controlled trial	Schofer et al. [18] 2010	NIL	-	-	Changes in Bone Mineral Density,	Control 0.57 ±0.38 HU Test 0.87 ±0.67 HU	0.014	NIL	-	-	NIL	-	-	A statistically significant benefit of LIPUS was seen in terms of mean reduction in bone gap area and bone mineral density
						Changes in Gap Area at the fracture site	Control -0.097 ± 0.070 mm^2 Test -0.131 ± 0.072 mm^2	0.034							
8	Accelerated Healing of Distal Radius Fractures with the use of specific, Low-Intensity Ultrasound	Kristiansen et al. [19], 1997	Time to bone healing	Control 98 ± 6.2 days Test 61±3.4 days	<0.0001	Time after fracture to early trabecular healing	Control 43 ± 3 days Test 31 ± 2 days	<0.003	NIL	-	-	NIL	-	-	LIPUS accelerates the healing of fractures.
						Time after fracture to cortical bridging	Control 52 ± 3 days Test 37 ± 3 days	<0.001							
9	Low intensity pulsed ultrasound increases bone volume, osteoid thickness and mineral apposition rate in the area of fracture healing in patients with a delayed union of the osteotomized fibula	Rutten et al. [20], 2008	NIL	-	-	NIL	-	-	Bone Volume of total tissue volume	Control 39.8 ± 3.1 % Test 52.9 ± 3.5 %	0.02	NIL	-	-	LIPUS accelerates clinical fracture healing by increasing osteoid thickness, mineral apposition rate, and bone volume.
									Mineralised volume	Control 34.5 ± 2.9 % Test 46.2 ± 3.9 %	0.04				
									Absolute osteoid volume	Control 5.3 ± 0.4 % Test 6.7 ± 1.7 %	0.46				
									Relative osteoid volume	Control 13.7 ± 1.2 % Test 13.3 ± 3.2 %	0.91				
									Osteoid thickness	Control 11.5 ± 1.9 μm Test 16.9 ± 1.4 μm	0.04				

Table 2. Characteristics of the included studies - results.

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S.no	TITLE	Author and year	Outcome parameters: Clinical	Mean ± SD (or) Mean difference	P value	Radiographical	Mean ± SD (or) Mean difference	P value	Histological	Mean ± SD (or) Mean difference	P value	Biomarker	Mean ± SD (or) Mean difference	P value	Inference
9.									Mineral apposition rate (μm/day)	Control 1.8 ± 0.1 μm/day (n=6) Test 2.3 ± 0.2 μm/day (n=6)	0.04				
									No. of osteoclasts per mineralised tissue area (/ mm²)	Control 9.6 ± 4.8 /mm² Test 0.5 ± 0.3/mm²	0.12				
									No. of osteocytes per mineralised tissue area (/0.01 mm²)	Control 9.6 ± 4.8 /0.01 mm² Test 0.5 ± 0.3 /0.01 mm²	0.54				
10	Complex Tibial Fracture outcomes following treatment with Low-Intensity Pulsed Ultrasound	Leung et al. [21] 2003	Time to the disappearance of tenderness	Control 7.9 ± 3.5 weeks Test 6.1 ± 2.1 weeks	<0.05	Time to appearance of first callus	Control 9.5 ± 2.2 weeks Test 6.5 ± 1.8 weeks	<0.05	NIL	-	-	Percentage change in Bone mineral content	Mean and SD not mentioned	P<0.05 at weeks 6,15,18	LIPUS enhances fracture healing in open fractures.
			Time to start partial weight bearing	Control 4.1 ± 2.8 weeks Test 3.8 ± 2.2 weeks	>0.05	Time to appearance of second callus	Control 12.5 ± 2.9 weeks Test 8.5 ± 2.1 weeks	<0.05							
			Time to start full weight bearing	Control 15.5 ± 3.0 weeks Test Group 9.3 ± 2.1 weeks	<0.05	Time to appearance of third callus	Control 20 ± 4.4 weeks Test Group 11.5 ± 3.0 weeks	<0.05				Percentage change in Plasma bone specific Alkaline phosphatase	Mean and SD not mentioned	P<0.05 at weeks 12,18,27	
			Time to remove the external fixator	Control Group 17.1 ± 8.5 weeks Test Group 9.9 ± 3.8 weeks	<0.05										
11	Low-Intensity Pulsed Ultrasound in lower limb bone	Gan et al. [22] 2014	Symptomatic days of Night Pain	Control 11.4 days Test 16.2 days	0.441	Change in MRI grade	Control 2.4 Test 2.2	0.776	NIL	-	-	NIL	-	-	LIPUS is not effective as a treatment for the healing.

Table 2. Characteristics of the included studies - results.

S.no	TITLE	Author and year	Outcome parameters: Clinical	Mean ± SD (or) Mean difference	P value	Radiographical	Mean ± SD (or) Mean difference	P value	Histological	Mean ± SD (or) Mean difference	P value	Biomarker	Mean ± SD (or) Mean difference	P value	Inference
11.			Symptomatic days of Walking Pain	Control 26.1 days Test 23.4 days	0.807										
			Symptomatic days of Sitting pain	Control 29.0 days Test 26.3 days	0.937										
			Symptomatic days of pain with hopping	Control 41.5 days Test 38.0 days	0.576	Change in edema size	Control 4.1 Test 3	0.271							
			Symptomatic days of Tenderness	Control 50.3 days Test 58.2 days	0.901										
			Symptomatic days of running pain	Control 54.7 days Test 79.8 days	0.807										
12	Acceleration of tibial fracture-healing by non-invasive, low-intensity pulsed ultrasound	Heckmann et al. [23], 1994	Time to clinical healing	Control 114 ± 10.4 days Test 86 ± 5.8 days	0.01	Cortical bridging (Gradual disappearance of the interruption of the cortex at the fracture site as a result of callus formation)	Control 182 ± 15.8 Test 114 ± 7.5	0.0002	NIL	-	-	NIL	-	-	LIPUS is effective in the acceleration of the normal fracture repair process
			Time to discontinuation of the cast.	Control 120 ± 9.1 days Test 94 ± 5.5 days	0.008	Endosteal healing (Gradual disappearance or obliteration of the fracture line and its replacement by a zone of increased density formed by endosteal callus)	Control 167 ± 13.9 Test 117 ± 8.5	0.002							
13	Does Low Intensity, pulsed ultrasound speed healing of scaphoid fractures?	Mayr et al. [24] 2000	Time to fracture healing	Control 62 ± 19.2 days Test 43.2 ± 10.9 days	<0.01	Trabecular bridging in %	Control 54.6 ± 29 % Test 81.2 ± 10.4 %	<0.05	NIL	-	-	NIL	-	-	LIPUS accelerates healing of scaphoid fractures
14	Re-evaluation of low intensity pulsed ultrasound in treatment of tibial fractures (TRUST) : Randomised clinical trial	Busse et al. [25] 2016	SF – 36 PCS (Physical component score) Health utilities index scores	0.55 0.01	0.44 0.44	Time to radio grahic healing	1.07	0.55	NIL	-	-	NIL	-	-	LIPUS does not accelerate radiographic healing or functional recovery.

Table 3. Consolidated results.

Type of parameter	Parameter	Total No. of studies	Total no. of studies stating that LIPUS is significantly beneficial than the control	Total no. of studies stating that there is no difference between LIPUS and control	Total no. of studies stating that control has beneficial effects than the LIPUS
Clinical	Healing time	11	5	6	0
Radiological	Time for Radiographic union	4	3	1	0
Radiological	Radiographic union	5	4	1	0
Histological	New bone formation	3	3	0	0
Serum or tissue biomarker		1	1	0	0

Out of the 11 studies that evaluated healing time clinically, 5 showed faster clinical healing time in LIPUS group. Out of the 4 studies that have seen healing time radiographically through different radiographic parameters, all the 3 studies showed improvement in the test group and they coincided with the 5 studies that showed improvement in clinical healing time. With respect to parameters that defined bone regeneration radiographically, out of the 6 studies, 4 showed significant improvement in the test group when compared to control, whereas 2 studies showed no improvement in the test (LIPUS) group. Out of the 3 studies that included histological parameters denoting new bone formation, all the studies showed significant improvement in test group. The one study that evaluated serum biomarker as parameter also showed statistically significant result favoring LIPUS. Quality assessment of the included studies was made based on the major criteria of Consort guideline (table 4). Out of 14 studies, 5 studies had low risk of bias [12,18,20,23,26]. Moderate risk of bias was seen in 6 studies [14,15,19,22,24] and high risk of bias was seen in 3 studies [13,17,21].

Table 4. Risk of bias – major criteria

S.no	Author and Year	Randomization	Allocation concealment	Assessor blinding	Dropouts	Risk of bias
1	Lubert et al. [12] 2008	Yes	Yes	Yes	Yes	Low
2	Dudda et al. [13] 2011	No	No	No	No	High
3	Urita et al. [14] 2013	Yes	No	Yes	Yes	Moderate
4	Liu et al. [15] 2014	Yes	No	Yes	No	Moderate
5	Simpson et al. 16] 2017	Yes	No	Yes	Yes	Moderate
6	Kim et al. [17] 2010	No	No	No	No	High
7	Schofer et al. [18] 2010	Yes	Yes	Yes	No	Low
8	Kristiansen et al. [19] 1997	Yes	No	Yes	No	Moderate
9	Rutten et al. [20] 2008	Yes	Yes	Yes	No	Low
10	Leung et al. [21] 2003	No	No	Yes	No	High
11	Gan et al. [22] 2014	No	Yes	Yes	No	Moderate
12	Heckmann et al. [23] 1994	Yes	Yes	Yes	Yes	Low
13	Mayr et al. [24] 2000	Yes	No	Yes	No	Moderate
14	Busse et al. [25] 2016	Yes	Yes	Yes	Yes	Low

DISCUSSION

This systematic review was aimed to evaluate the effectiveness of LIPUS in bone regeneration and healing. On analyzing the methodology of the 14 included clinical trials it has been observed that a lot of variability exists between the studies in terms of the type of fracture or the bone involved, the method of evaluation and the outcome parameters they have assessed. While analyzing the results of all the studies, there was a lot of variability found between the studies regarding the effectiveness of LIPUS in clinical and radiographic outcome parameters. But in general none of the studies reported any deleterious effect for usage of LIPUS or that the LIPUS group was less effective than the control group. The studies that compared the histological parameters stated that LIPUS was significantly beneficial than control group but number of studies in that category was very less. On analyzing the influence of LIPUS on reducing time taken for radiographic union, most of the studies stated that LIPUS was more effective than the control. With respect to healing time and radiographic union there was variability in the results across the studies. Around 50% of studies reported that the LIPUS was more effective than control whereas the remaining studies reported that there was no difference between the LIPUS group and the control. Another observation we found from the result analysis is that LIPUS showed better results in fresh fractures [15,19,21,23,24] as compared to other type of fractures [24] or osteotomies [13,16,14] even though two studies on fresh fractures couldn’t show the same [12,26]. It has also been found that LIPUS therapy on delayed and non-unions fractures in different bones could not reveal any significant difference in healing rate and healing time as compared to control [27].

The results of the radiographic analysis were also inconsistent across the studies. Out of the 4 studies that had assessed healing time radiographically [19,21,23,26], The three studies, done in fresh fractures could show any added improvement in the LIPUS group [19,21,23]. Both these, clinical and radiographic results point out that the efficacy of LIPUS treatment on bone healing is relatively low after osteotomies and delayed or nonunion fractures when compared with acute fractures. Supporting this finding, Tajali et al. [27] in their systematic review, reported that there was weak evidence that LIPUS supported radiographic healing in delayed unions and nonunion.

Effectiveness of LIPUS was evaluated histologically in three studies and in that irrespective the fracture type all the three studies showed better results for the LIPUS group. The three studies assessed bone regeneration in various situations such as sinus lift [17], delayed union of fibula [20] and fresh tibial fracture management [21] .These results are promising even though they are not fully correlating with the other clinical and radiographical results from other studies with respect to delayed union. Histological evaluation showed that the usage of LIPUS could increase bone volume, and mineralized volume in the area of new bone formation and cancellous bone, respectively. More over a higher level of osteoid thickness and mineral apposition rate was found in the area of new bone formation, whereas they were not changed in the area of cancellous and cortical bone.

Effectiveness of LIPUS on improving the serum markers for bone regeneration was studied by Leung et al. [21] and in their study it was reported that LIPUS activated alkaline phosphatase from week 12 to week 27 after fracture. This is also a promising results and its clinical relevance need to be validated with future reseaech. Even though 14 articles were included in this systematic review the meta-analysis was not possible due to heterogeneity of the data. Quality assessment of the included articles was done and it was found that out of 14 studies, 5 studies only had low risk of bias [12,13,20,23,25]. Moderate risk of bias was seen in 6 studies [15,19,24,16,14,22] and high risk of bias was seen in 3 studies [13,15,17].

This systematic review had included articles from major databases such as PubMed, google scholar and Cochrane library. Hand searching and cross reference verification were also performed to ensure the completeness of the search. Unpublished literatures were not included in this systematic review. Even though meta-analysis was not done the qualitative synthesis of the results of the 14 included studies points out many research lacunae in establishing the effectiveness of LIPUS in bone regeneration and healing. Firstly, clinical evidences are inconclusive as there are many studies which couldn’t find any added benefit for usage of LIPUS. Secondly, even though radiographic and histological outcome evaluations were showing more consistent results favoring the usage of LIPUS, the number of studies in those categories was very less especially with histological evaluation. Most importantly the quality assessment of the included studies also need to keep in mind when interpreting the results of these studies as the majority of them were having either moderate or high risk of bias. Nevertheless the promising aspects were there was no deleterious effect reported from any of the studies with respect to usage of LIPUS and the favorable results in histological, biochemical and radiographic outcome parameters. This warrants the need for more randomized controlled clinical trials with low risk of bias to confirm the effectiveness of LIPUS in bone regeneration and healing.

CONCLUSIONS

For clinicians

This review cannot give a definitive conclusion that LIPUS is effective in bone healing with respect to clinical parameters even though positive influence on radiographical and histological parameters in bone healing and regeneration are promising.

For researcher

LIPUS has been widely used in orthopedics and physiotherapy fields. Most of the Randomized control trial performed had moderate to high risk of bias. The studies performed so far, lack homogeneity in their methodology. RCTs with high quality are required to prove the effectiveness of LIPUS. In dentistry very few studies are available to show the effect of LIPUS in alveolar bone. Hence there is abundant future scope for LIPUS in dental research to establish its various applications.

Collaborators

Radha V, Sheeja S. Varghese, conceptualization, data curation, formal analysis, investigation, methodology, resources, software, supervision, validation, visualization, writing – original draft, writing – review & editing.

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