# Pulsed ultrasound for bone regeneration - outcomes and hurdles in the clinical application: a systematic review

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| Complete List of Authors: | Puts, Regina; Charite University Hospital Berlin, Berlin Institute of Health Center for Regenerative Therapies (BCRT)  
Vico, Laurence; University of Lyon  
Beilfuss, Nirina; Charite University Hospital Berlin, Berlin Institute of Health Center for Regenerative Therapies (BCRT)  
Shaka, Maria; Charite University Hospital Berlin, Berlin Institute of Health Center for Regenerative Therapies (BCRT)  
Padilla, Frederic; Focused Ultrasound Foundation; University of Virginia School of Medicine, Department of Radiology  
Raum, Kay; Charite University Hospital Berlin, Berlin Institute of Health Center for Regenerative Therapies (BCRT) |
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**Abstract:**

Impaired bone fracture healing is associated with long-term musculoskeletal disability, pain and psychological distress. Low-Intensity Pulsed Ultrasound (LIPUS) is a non-invasive and side-effect-free treatment option for fresh, delayed- and non-union bone fractures, which has been used in patients since the early 1990s. Several clinical reports, however, have questioned the usefulness of the LIPUS treatment for the regeneration of long bones, including those with the compromised healing. This systematic review addresses the hurdles that the clinical application of LIPUS encounters. Low patient compliance might disguise the effects of the LIPUS-therapy, as observed in several studies. Furthermore, large discrepancies in results, showing profound LIPUS-effects in regeneration of small animal bones in comparison to the clinical studies, could be caused by suboptimal parameters of the clinical set-up. This raises the question of whether the so-called “acoustic dose” requires a thorough characterization to reveal the mechanisms of the therapy. The adequate definition of the acoustic dose is especially important in elderly population and patients with underlying medical conditions, where distinct biological signatures lead to a delayed regeneration. Non-industry funded, randomized double-blind placebo-controlled clinical trials of the LIPUS application alone and as an adjuvant treatment for the bones with complicated healing, where consistent control of patient compliance is ensured, are required.
Pulsed ultrasound for bone regeneration - outcomes and hurdles in the clinical application: a systematic review

R. Puts*1, L. Vico2, N. Beilfuß1, M. Shaka1, F. Padilla34 and K. Raum1.

* Regina Puts, Berlin Institute of Health Center for Regenerative Therapies, Charité-Universitätsmedizin, Föhrer Straße 15, 13353 Berlin, Germany
Tel.: +49 30 450 539 506, e-mail: regina.puts@charite.de

1 Charité-Universitätsmedizin Berlin, corporate member of Freie Universität Berlin, Humboldt-Universität zu Berlin, and Berlin Institute of Health, BCRT - Berlin Institute of Health Center for Regenerative Therapies, 13353 Berlin, Germany

2 INSERM, U1059, University of Lyon, University of Saint-Etienne, F-42270 Saint-Etienne, France

3 Focused Ultrasound Foundation, Charlottesville, VA 22903, USA

4 Department of Radiology, University of Virginia School of Medicine, Charlottesville, VA, USA
Abstract

Impaired bone fracture healing is associated with long-term musculoskeletal disability, pain and psychological distress. Low-Intensity Pulsed Ultrasound (LIPUS) is a non-invasive and side-effect-free treatment option for fresh, delayed- and non-union bone fractures, which has been used in patients since the early 1990s. Several clinical reports, however, have questioned the usefulness of the LIPUS treatment for the regeneration of long bones, including those with the compromised healing. This systematic review addresses the hurdles that the clinical application of LIPUS encounters. Low patient compliance might disguise the effects of the LIPUS-therapy, as observed in several studies. Furthermore, large discrepancies in results, showing profound LIPUS-effects in regeneration of small animal bones in comparison to the clinical studies, could be caused by suboptimal parameters of the clinical set-up. This raises the question of whether the so-called “acoustic dose” requires a thorough characterization to reveal the mechanisms of the therapy. The adequate definition of the acoustic dose is especially important in elderly population and patients with underlying medical conditions, where distinct biological signatures lead to a delayed regeneration. Non-industry funded, randomized double-blind placebo-controlled clinical trials of the LIPUS application alone and as an adjuvant treatment for the bones with complicated healing, where consistent control of patient compliance is ensured, are required.

Key words: Low-Intensity Pulsed Ultrasound, Bone regeneration, Surgery, Acoustic dose, Non-union, Age, Osteoporosis, Compliance

Running Title: Hurdles & outcomes to LIPUS application in clinic
Introduction

According to the USA National Health Interview Survey more than a half of all chronic medical conditions reported in 2012 were associated with musculoskeletal problems (Hauser et al., 2016). Bone is an organ able to regenerate after a fracture to its full functional integrity without scar formation. However, approximately 10% of all fractures do not heal without complications (Volpin, 2014). These cases, also known as delayed- and non-union bone fractures, are accompanied by the life burdens of limited or no mobility, pain and psychological stress (Lerner et al., 1993; Mitchell et al., 2018). Moreover, the median total costs for treating a non-union in the USA were reported to comprise $25,556 (Antonova et al., 2013). With progressing age of an individual, the odds of complicated bone healing abruptly increase (Clark et al., 2017). Since the proportion of aging population continually grows, especially in the developed countries, the advances in novel technologies for efficient fracture regeneration are especially urgent.

In 1983, Duarte showed that stimulation of osteotomized rabbit fibula and femur bones with Low-Intensity Pulsed Ultrasound (LIPUS) enhances callus formation (Duarte, 1983). Currently, a device employing LIPUS is manufactured under the brand name Exogen® (Bioventus LLC, Durham, NC), which emits pulsed sine waves at an ultrasound frequency of 1.5 MHz, a pulse repetition frequency (PRF) of 1 kHz and a 20%-duty cycle (DC), generating a spatial average temporal average intensity ($I_{SATA}$) of 30 mW/cm² (Pounder and Harrison, 2008). The Exogen device is used across the globe for the treatment of fresh fractures, delayed- and non-union bones and so far, no negative side effects have been reported. The device is fully portable and does not require medically qualified staff for operation. The treatment can be applied by the patient at home and lasts 20 minutes a day for the prescribed period. However, the question of the efficiency and suitability of the LIPUS technique for fracture healing remains open for debate (Busse et al., 2014; Garner, 2017; Griffin, 2016; Griffin et al., 2014; group et al., 2016; Poolman et al., 2017; Schandelmaier et al., 2017a; Tarride et al., 2017).

Once the bone fracture occurs, the orthopedic surgeon is to decide the suitable type of treatment for the patient, with surgery being increasingly the first choice (Courtney et al., 2011; Fernandez, 2005; Schmidt et al., 2003). Should complementary methods, such as LIPUS, be used as an adjuvant to the conservative option with cast or to surgery? Can LIPUS be beneficial for bones with complicated healing? The purpose of this review is to give the readers impartial opinion to the above questions.

Materials and Methods

In this review, search and retrieval of scientific reports was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (Moher et al., 2009). Reports published between December 1950 and April 2021 were collected via PubMed and Web of Science databases using key words: (1)
Low-Intensity Pulsed Ultrasound AND (2) bone fracture. Search duplicates were first identified via EndNote software. These were then verified and further removed manually. Articles, which were not peer-reviewed, without a full-text option or written in a language other than English were excluded. Reports describing in-vitro findings and studies in animal models were not retained for the main data analysis. Additionally, articles irrelevant to ultrasound, or using ultrasound for other purposes than LIPUS stimulation, or describing reports of LIPUS application in other organs than bone were eliminated from the analysis.

Results

A PRISMA diagram describing the identification of manuscripts for the data analysis is depicted in Fig. 1. The search queries identified 449 and 357 search results via PubMed and Web of Science databases, respectively. Six publications, meeting all the inclusion criteria, were found in a google scholar free search and designated in the PRISMA chart as “other sources”. The EndNote software identified 134 duplicates and an additional 95 were excluded upon manual verification, resulting in 583 search results (Supplementary file 1). A restriction of the search results based on full-text peer-reviewed articles in English language eliminated additional 43 reports (Supplementary file 2). LIPUS application in vitro, in silico and in animal models accounted for 88, 2 and 139 entries, respectively (Supplementary file 3). These were identified via thorough screening of the full-text articles. Reports, irrelevant to ultrasound techniques (27), or irrelevant to bone fracture stimulation (10), or describing other ultrasound methods (111) were screened out manually and taken out from the analysis (Supplementary file 4). Finally, 163 articles met all of the set criteria. Out of them 77 and 24 were review articles and case reports (Supplementary files 15 and 26), respectively. Finally, 62 articles (Supplementary file 37) reporting the original findings were included in this review. Most of the clinical studies identified here employ the Exogen-like stimulation devices with the clinical acoustic parameters of 1.5 MHz, 1 kHz PRF, 20 % DC and 30 mW/cm² ISATA. These are summarized in Tables 1 (a-h), 2 (a-d) and 3. Nine reports used LIPUS parameters, which were different from the conventionally used ones or were not clearly specified (Arima et al., 2017; Bawale et al., 2020; Gan et al., 2014; Gopalan et al., 2020; Liu et al., 2014; Ozdemir et al., 2008; Patel et al., 2015; Santana-Rodríguez et al., 2019; Warden et al., 2001).

Fig. 1 is here
LIPUS and fresh fractures: surgery vs cast

There is a number of hurdles that application of LIPUS in a clinical setting encounters. First is the definition of a fresh fracture, which generally is considered way shorter than it is qualified to be (Heckman, 2017; Zura et al., 2017). This might rob some potential candidates of non-invasive treatment strategies like LIPUS. Furthermore, a big proportion of articles dedicated to LIPUS stimulation of fresh fractures are either based on case reports (Supplementary file 36), retrospective studies (Akiyama et al., 2014; Arima et al., 2017; Kinami et al., 2013; Ota et al., 2018; Ota et al., 2017; Song et al., 2019; Zura et al., 2015b) or prospective trials conducted in an unblinded manner and/or without sham controls (Arimoto et al., 2019; Brand et al., 1999; Dudda et al., 2011; El-Mowafi and Mohsen, 2005; Gan et al., 2014; Gold and Wasserman, 2005; Gopalan et al., 2020; Leung et al., 2004b; Liu et al., 2014; Patel et al., 2015; Salem and Schmelz, 2014; Santana-Rodríguez et al., 2019; Tsumaki et al., 2004; Urita et al., 2013) (Table 1 (a-h)), challenging the credibility of the LIPUS therapy. Additionally, the small size of patient cohorts of several prospective randomized double-blind placebo-controlled trials diminish the importance of their findings (Emami et al., 1999; Handolin et al., 2005a; Handolin et al., 2005b; Raza et al., 2016).

Tables 1a to 1h are here

Table 1 (a-h). LIPUS for fresh fractures and distraction osteogenesis. ‘LIPUS’ and ‘C’ stand for ultrasound-treated and control groups, respectively, and the number next to them designates a number of patients per each group. IM, PRF, DC, I\textsubscript{SATA} stand for intramedullary nail, pulse repetition frequency, duty cycle, spatial average temporal average acoustic intensity, respectively.

The discussion on whether LIPUS should be used as an alternative or an adjuvant therapy to surgical intervention has become more intense in the past several years, especially since the results of the multicenter randomized, blinded, sham-controlled clinical trial “Trial to Re-evaluate low-intensity pulsed UltraSound in treatment of Tibial fractures (TRUST)” was published in 2016 (Busse et al., 2014; group et al., 2016). The study enrolled 501 patients with tibial fractures treated surgically and fixed with an intramedullary nail. No effect of the LIPUS stimulation on the radiographically-indicated healing time and the restoration of full bone functionality was observed. The data was published soon after under Rapid Recommendations (Poolman et al., 2017), advising to remove LIPUS from clinical practice. A systematic review article (Schandelmaier et al., 2017a) further analyzed 26 randomized trials on the LIPUS therapy of all types of fracture, concluding that only 3 unbiased reports (Busse et al., 2014; Emami et al., 1999; group et al., 2016) have been published, with two of them being the results of the TRUST study. LIPUS treatment in these reports was not found
to accelerate the bone healing. The high risks of bias were defined as i) the lack of a blinded expert, ii) non-identically looking sham device, and iii) a less than 90% compliance without the appropriate sensitivity analyses. The review ruled out two well-controlled studies, in which fresh tibia fractures (closed or open grade 1) (Heckman et al., 1994) and fractures of the distal radius metaphysis (dorsally angulated, negative volar) (Kristiansen et al., 1997) were immobilized in a cast and treated by LIPUS. Both studies reported that the radiographically assessed healing time was significantly decreased by the LIPUS treatment, however. Both studies were discriminated based on low compliance of 69% (Heckman et al., 1994) and 72% (Kristiansen et al., 1997).

It should be further noted that all three unbiased studies (Busse et al., 2014; Emami et al., 1999; group et al., 2016) defined by the Schandelmaier et al. review (Schandelmaier et al., 2017a) investigated the healing of the fresh tibial fractures fixed with the reamed intramedullary nail only. The fractures treated this way are known to have a very low complication rate (Coles and Gross, 2000) and the weight-bearing with this type of the fixation can start relatively early, due to the immediately acquired stability with the preservation of subtle interfragmentary movement within the fracture gap (Perren, 2002; Schmal et al., 2020). Similarly, a lack of beneficial LIPUS effects was observed in screw-fixed lateral malleolar fractures, providing a possibility of early weight-bearing (Handolin et al., 2005a; Handolin et al., 2005b). Therefore, one of the reasons for the lack of pro-regenerative effects might be that the LIPUS application cannot override the benefits of the mechanical loading generated by natural skeletal motion (Malizos et al., 2006). This could be also true for defects with high spontaneous healing rates, where addition of the LIPUS therapy becomes redundant (Gan et al., 2014; Lubbert et al., 2008). The fractures immobilized in the cast, on the other hand, might have suboptimal mechanical environment and more significantly rely on the well-controlled mechanical component of LIPUS and thus more profound impacts were observed there (Coughlin et al., 2008; Farkash et al., 2015; Heckman et al., 1994; Kristiansen et al., 1997; Liu et al., 2014; Nolte et al., 2016). These hypotheses should be further tested in preclinical models, using ultrasound set-ups with well-controlled acoustic parameters (see section ‘Importance of LIPUS acoustic dose based on preclinical studies’), and in future clinical studies.

LIPUS and bones with compromised healing

Fractured bones with impaired healing represent a number of challenging tasks for the orthopedic surgeon. It starts with the difficulty in defining the onset of a delayed-union or non-union and propagates along the decisions on the selected treatment type and time, which must be compliant with the health status including the physiological, psychological and professional demands of the patient (Stewart, 2019). The non-union bone is defined by the FDA as a fracture with no evidence of
progressive healing improvement observed in the last 3 months of a total 9-months post-fracture period (Healy et al., 1990).

Whilst the conduction of a randomized double-blind clinical trial (RCT) involving alternative treatments like LIPUS is relatively straightforward for the patients with acute fresh fractures, the same procedure involving a large-patient cohort is more challenging to design for a non-union bone. One of the limiting factors is a lack of global standardized definition of delayed- and non-union fractures, including the absence of a universal agreement on whether radiographic or clinical or both criteria should be used to characterize those bones (Bhandari et al., 2012; Corrales et al., 2008; Özkan et al., 2019). Surgical intervention is a first-line treatment for most of bones with impaired healing (Leng et al., 2019; Özkan et al., 2019; Schmal et al., 2020), whereas ultrasound-modalities such as LIPUS, are considered inefficient (Özkan et al., 2019) and even contraindicated by some orthopedic surgeons (Busse and Bhandari, 2004; Pounder and Harrison, 2008). A prescription of the LIPUS bone-stimulators is usually advised when the surgical intervention carries high risks for the individual (Anderson et al., 2019; Leighton et al., 2017; Zura et al., 2015a). Thus, the to-date evidence of LIPUS effects on delayed- and non-unions (Table 2 (a-d)) mostly relies on either retrospective reports (Adukia et al., 2021; Carlson et al., 2015; Elvey et al., 2020; Farkash et al., 2015; Hemery et al., 2011; Lerner et al., 2004; Mayr et al., 2000; Nolte et al., 2001; Roussignol et al., 2012; Rutten et al., 2007; Teoh et al., 2018; Zura et al., 2015a) or observational studies without placebo controls (Bawale et al., 2020; Biglari et al., 2016; Gebauer and Correll, 2005; Gebauer et al., 2005; Jones et al., 2006; Majeed et al., 2020; Moghaddam et al., 2016).

Tables 2a to 2d are here

Table 2 (a-d). LIPUS for delayed- and non-union bones. ‘LIPUS’ and ‘C’ stand for ultrasound-treated and control groups, respectively, and the number next to them designates a number of patients per each group.

To our knowledge, only one multicenter randomized placebo-controlled clinical trial evaluating effects of LIPUS on delayed bone healing (minimal fracture age 4 months) and enrolling total of 101 subjects with a 91 % final compliance has been performed (Schofer et al., 2010). The study reported an increase in the bone-mineral density and a decrease of the fracture gap for the LIPUS-active group at 16-weeks follow-up, although no statistically significant difference in the number of healed fractures between the groups was found. As it was mentioned in Schandelmaier et al. (Schandelmaier et al., 2017a), this study could have been biased by the age of the fracture at the start of the trial, as the mean age in the LIPUS-treated group was higher. Although the difference in the fracture-age distribution was found to be statistically insignificant (Schofer et al., 2010), a similar study with homogenous fracture age groupings for patients with non-union bones will be of great importance.
Two more studies evaluated biopsied of fibulas with delayed healing within a randomized double-blind placebo-controlled trial, revealing that LIPUS increased osteoid thickness and bone mineralization (Rutten et al., 2008), which, most likely, occurred through the locally enhanced osteogenic differentiation of cells (Rutten et al., 2009). However, both reports are based on very small patient cohorts.

The lack of positive evidence of the LIPUS treatment in fixed fresh fractures, based on the three unbiased studies highlighted before (Schandelmaier et al., 2017a), also advised against the ultrasound technique for the patients with non-unions (Poolman et al., 2017; Schandelmaier et al., 2017b). Although one can find this conclusion logical, the biological signatures in acute fractures and chronically impaired non-unions are not alike. These are summarized in the next section.

**Biological pathogenesis of non-union bone. Can LIPUS help?**

The local biology at the fracture site, systemic conditions of the host and mechanical stability are the key factors defining the outcome of the fractured bone (Harwood, 2010). When the bone fracture is fixed and interfragmentary movement within the gap is sustained in the proper range, a process of endochondral ossification is usually observed. Through interlinked phases of inflammation, callus formation and remodeling, the fractured bone is reconstituted *ad integrum* (Loi et al., 2016; Marsell and Einhorn, 2011). If one or more phases of this well-orchestrated process is compromised, a non-union occurs. Based on radiographic and histological assessments, these non-unions can be further categorized into hypertrophic and atrophic types. For the former, biological aspects are in place, but no adequate stability of the fractured bone exists, resulting in callus formation but hindering callus union, maturation, and remodeling. For the latter, the biological components are compromised and, at times, combined with mechanical instability (Volpin, 2014). The hypertrophic non-unions can usually be managed by additional stabilization of the fractured bone (Nauth et al., 2018), whereas atrophic non-unions are more challenging to treat and complex approaches are often required.

The initial acute inflammation in the bone regeneration process is critical for the resultant organ functionality, as it was previously shown in animal studies (Grundnes and Reikeras, 1993a; Grundnes and Reikeras, 1993b; Park et al., 2002). It is usually strongest within several days to a week and declines with time in a normal healing scenario (Loi et al., 2016). The persistence of an immune reaction can result in chronic inflammation, impaired healing and bone non-union (Bastian et al., 2011; Claes et al., 2012; Hardy and Cooper, 2009; Zura et al., 2016). It has been shown that dendritic cells isolated from bone marrow and stimulated with LIPUS secreted exosomes with enhanced anti-inflammatory potential, which alleviated TNF-α-induced inflammation of endothelial cells (Li et al., 2019). The LIPUS treatment also supported the transition of inflammatory to resident macrophages, enhanced gene expression of anti-inflammatory factors and improved spinal fusion in a rat animal model (Zhang et al., 2020).
The anti-inflammatory potential of ultrasound stimulation has been as well described in a number of other studies (da Silva Junior et al., 2017; Li et al., 2003; Nakao et al., 2014; Yang et al., 2017).

When mesenchymal stromal cells (MSCs) were isolated from hypertrophic non-union fractures, they showed strong differentiation potential into all three lineages in vitro, i.e., chondrogenic, adipogenic and osteogenic (Iwakura et al., 2009). The same cells type isolated from atrophic non-unions not only underwent senescence and growth arrest, but also had a significantly lower osteogenic differentiation potential (Bajada et al., 2009). The co-stimulation of the mesenchymal cells isolated from patients with different non-union types with Bone Morphogenetic Factor-7 (BMP-7) and LIPUS significantly enhanced the osteogenic potential of these cells (Koga et al., 2013). Unfortunately, the effect of LIPUS alone was not described in this study. The expression and activation of BMPs and their antagonists were found to be out of balance in both hypertrophic and atrophic non-union human fractures (Fajardo et al., 2009; Kloen et al., 2002; Kwong et al., 2009a; Kwong et al., 2009b). The application of LIPUS has been previously shown to enhance expression of BMP-2, BMP-4 and BMP-7 and their receptors in osteoblasts-like cells (Gleizal et al., 2006; Suzuki et al., 2009a; Suzuki et al., 2009b), which might help to compensate for this imbalance.

**Fig. 2. Can LIPUS help regenerate a non-union?** Biological signatures of non-union bone (left) and hypothetical effects of LIPUS-stimulation on non-union regeneration (right).

Mechanical loading in the properly stabilized fracture induces nitric oxide (NO) production, which in turn modulates bone adaptation to the applied stimulus (Klein-Nulend et al., 2014). NO signaling is especially deregulated in patients with atrophic non-unions (Wijnands et al., 2012). The LIPUS stimulation of osteoblasts augmented NO release via Nuclear Factor-κB signaling pathway (Hou et al., 2009). The NO signaling induced expression of Vascular Endothelial Growth Factor-A (VEGF-A) and hypoxia-inducible factor 1α (HIF-1α) in the LIPUS-treated osteoblasts (Wang et al., 2004). This promoted tube formation by endothelial cells, which is crucial for angiogenesis and is often debilitated in the pathological fractures. The NO release also activates other pathways, such as canonical Wnt/β-catenin signaling in osteoblasts and osteocytes, which is known to influence bone mass (Krishnan et al., 2006). The secretion of Dickkopf-1 (DKK-1) protein, antagonizing Wnt-signaling (Pinzone et al., 2009) was enhanced in culture media of MSCs isolated from patients with atrophic non-unions (Bajada et al., 2009). LIPUS may be able to counteract this effect, since the Wnt-signaling have been demonstrated to be enhanced in the stimulated osteoblasts and osteoprogenitors (Khan, 2010; Olkku et al., 2010).
The expression of Matrix Metalloproteinases (MMPs), regulating cell attachment, migration, release of biologically active molecules, and the invasion of newly formed blood vessels into the callus is also alleviated in non-union fractures (Ortega et al., 2003). The decrease in expression of MMP-2, -9 and -13 in the non-union fractures results in the impaired bone remodeling (Ding et al., 2018). The LIPUS mechanical stimulus enhanced expression of MMP-13 in long-term cultured osteoblasts (Unsworth et al., 2007), which could potentially improve the extracellular matrix (ECM) turnover that is critical for successful tissue regeneration.

The key biological signatures of a non-union fracture and the hypothetical LIPUS effects influencing them are summarized in Fig. 2. Despite the positive evidence of the LIPUS stimulation, most of the reports described in this section revolve around cell-lines or cells isolated from bones with uncomplicated healing scenario. Whether LIPUS can have similar effects on cells from atrophic and hypertrophic non-unions is a question worth further investigation that needs to be addressed in vitro and in appropriate preclinical models. To our knowledge, only two preclinical in-vivo studies, investigating the effects of LIPUS on a hypertrophic non-union, have been published so far, demonstrating contradicting findings (Takikawa et al., 2001; Volpon et al., 2010).

**LIPUS for aged and osteoporotic patients**

With progressing age, the human skeleton undergoes cortical bone thinning, increased trabecular spacing and expansion of the medullary cavity (Javaheri and Pitsillides, 2019). These morphological changes and overall bone homeostasis are results of systemic changes in biochemical signaling pathways of the human body, eventually leading to impaired mechanoadaptation and compromised fracture regeneration (Haffner-Luntzer et al., 2016). Aged individuals experience decreased number of osteoprogenitors (Kasper et al., 2009), with reduced osteogenic potential (D’Ippolito et al., 1999; Ross et al., 2000) and altered response to mechanical stimulation (Kasper et al., 2009). Additionally, changes in shape of osteocytes and the number of canaliculi per lacuna were found in the aged organism, which dampens their mechanosensitivity and could result in an inefficient interaction between the osteoblasts and osteoclasts (Hemmatian et al., 2017). The mechanical stimulation of chronic non-unions with LIPUS in aged patients has shown certain promise, although the fracture-healing rate declined moderately with the increasing age (Zura et al., 2015a). The MSCs isolated from aged rats experienced enhanced expression of osteogenic markers, i.e., Runx-2 transcription factor and osteocalcin, when stimulated with higher acoustic LIPUS-intensity, in comparison to the cells from young rats (Puts et al., 2016a). This might imply that due to changes in mechano-responsiveness of the osteoprogenitors with increasing age, the adjustment of the LIPUS-stimulation protocol is required. The accelerated fracture healing after the LIPUS exposure was also confirmed in the in-vivo studies with aged rodents (Aonuma et al., 2014; Katano et al., 2011), however, the relevance of these results for the clinical setting remain
questionable, due to the animal size in relation to the area of the transducer (see section ‘Importance of LIPUS acoustic dose based on preclinical studies’).

Osteoporosis is a chronic metabolic bone disorder, which more commonly affects postmenopausal women and, given the increasing life expectancy, is becoming a global health challenge (Cauley, 2017). Medication-free therapies for the management of this disease represent a very appealing research topic (Kasturi and Adler, 2011b; Yadollahpour and Rashidi, 2017). Application of LIPUS as a treatment option for postmenopausal bone loss has been investigated previously and no positive effects on the bone mineral density (BMD) were observed (Leung et al., 2004a; Ozdemir et al., 2008) (Table 3). Another study in young male patients with spinal cord injury, experiencing up to 70 % bone loss, comparable to 5-year-long bone depletion during osteoporosis, found that the LIPUS stimulation of the calcaneus bone did not influence its bone mineral content (Warden et al., 2001). In this study, shorter pulses were used for ultrasound stimulation and the frequency of the sine wave was 1 MHz in comparison to the 1.5 MHz conventional stimulation frequency (Table 3). In contrast, several in-vivo reports in the ovariectomized rat osteoporosis model have shown beneficial effects of the LIPUS exposure on improvement of the disease markers (Carvalho and Cliquet Junior, 2004; Ferreri et al., 2011; Wu et al., 2009). Given the size of the LIPUS-probe, the anabolic effects of ultrasound in rodents might partially mimic a low-magnitude high-frequency whole body vibration therapy, which shows promising results in improvement of BMD in postmenopausal women (Kasturi and Adler, 2011a; Lai et al., 2013; Rubin et al., 2004; Verschueren et al., 2004).

Table 3 is here

Table 3. LIPUS and osteoporosis. ‘LIPUS’ and ‘C’ stand for ultrasound-treated and control groups, respectively, and the number next to them designates a number of patients per each group. PRF, DC, $I_{SATA}$ stand for pulse repetition frequency, duty cycle, spatial average temporal average acoustic intensity, respectively.

Although stimulation with LIPUS represents an appealing medication-free treatment for osteoporosis, this chronic metabolic disorder has a systemic nature and will not likely succumb to the local stimulation with ultrasound. As it was discussed in Warden et al. study (Warden et al., 2001), the losses associated with the ultrasound propagation, constrain the acoustic stimulation to a very restricted volume. Although the current clinical LIPUS set-up and protocol most likely have limiting potential for the treatment of osteoporosis, the investigation of the LIPUS application for regeneration of fractures in aged, osteoporotic patients and patients with other co-morbidities is of great interest.
**LIPUS and patient compliance**

Patient compliance with the treatment regimen can profoundly affect the outcome of a clinical trial. As it was evidently demonstrated by Czobor and Skolnick (Czobor and Skolnick, 2011), noncompliant patients can disguise efficacy of a tested therapy. In this report, the compliant patients were screened out via detection of the drug metabolite in their blood over the course of treatment. A comparison of the compliant patients, which comprised 70%, to the placebo group confirmed the drug’s efficacy, whereas noncompliant group did not differ from the control. Moreover, the same compliance assessed via counting consumed pills was greater than 92%. The adherence to the study protocol carries even a bigger challenge for the lasting treatments outside the medical facility, resulting in a biased data interpretation (Pounder et al., 2016; Pullar et al., 1989). The LIPUS application is usually prescribed to the patients as a long-term treatment and requires a 20-minute time window every day. Therefore, motivation and dedication of the patients plays an indispensable role in the study outcome. It has been shown that certain factors, such as age and fracture site, could significantly affect the adherence to the prescribed LIPUS protocol (Matsubara, 2015). The detailed description of patient compliance of the reviewed reports are summarized in Tables 1 (a-h), 2 (a-d) and 3.

There is a considerable variation in documentation of compliance in the LIPUS clinical trials. Some studies report the number of patients available at the end of the treatment out of the whole sample, whereas others additionally supply the number of successful LIPUS-days and LIPUS-minutes accomplished by the subjects. It is not always clear though, whether the active minutes were counted only when the device was in the direct skin contact, as it was described in some studies (Emami et al., 1999; Zacherl et al., 2009). Overall there is a trend towards positive regenerative outcomes of the LIPUS application in clinical trials with the increasing patient compliance to the device application (Gopalan et al., 2020; Maurya et al., 2019; Namera et al., 2020; Nolte et al., 2001; Roussignol et al., 2012; Santana-Rodriguez et al., 2019; Schofer et al., 2010; Tsumaki et al., 2004). Studies, where around 30% of patients applied less than 50% of the LIPUS-courses, found the LIPUS application ineffective (Emami et al., 1999; group et al., 2016; Simpson et al., 2017). As an example, exclusion of the non-compliant patients in a study of LIPUS-treated non-unions, as reported by the recordings of the device, revealed pro-healing effects of sonication comparable to the surgical intervention (Bawale et al., 2020). Reports, where the compliance is not descriptively documented decide both for and against the LIPUS therapy (Tables 1 (a-h), 2 (a-d) and 3).

A stringent weekly control of adherence to the prescribed protocol, requiring a minimum 15-minute long skin contact with the device through a coupling gel, resulted in an excellent compliance in 44 patients after chevron osteotomy for hallux valgus (Zacherl et al., 2009). A profound impact on bone formation was observed in the LIPUS-active group, whereas a relapse in a first distal metatarsal articular angle 6 weeks after the treatment was reported in the placebo group. The active support of
patients and communication with the medical personnel seem to improve the compliance significantly, favoring the LIPUS therapy (Arimoto et al., 2019; Gopalan et al., 2020; Maurya et al., 2019; Namera et al., 2020; Patel et al., 2015; Santana-Rodríguez et al., 2019; Tsumaki et al., 2004; Zacherl et al., 2009). This should be taken into account during the planning of a clinical trial. New generation Exogen devices might also help raising patients’ awareness on the treatment progress and support their motivation via direct feedback of an integrated calendar (Pounder et al., 2016). In summary, an inclusion of the detailed information on the number of completed days and successful LIPUS-minutes of the treatment in the scientific reports, along with a population size that was intended to be treated and actually adhered to the protocol, can aid an adequate judgment of the LIPUS therapy.

Importance of LIPUS acoustic dose based on preclinical studies

The clinically most used LIPUS parameters (1.5 MHz frequency, 1 kHz PRF, 20% DC, and 30 mW/cm² ISATA (Exogen®)) have originated from a preclinical rabbit model (Duarte, 1983). Since then, little effort has been done to optimize this acoustic dose. With the exception of nine reports (see Materials and Methods, and Tables 1a, 1c, 1f, 1g and 3), the rest of the studies applied Exogen-like parameters. As of today, the current evidence on LIPUS-induced pro-regenerative potential in bone show pronounced positive effects in cell culture (Padilla et al., 2016; Pounder and Harrison, 2008) and in animal studies (Azuma et al., 2001; Shakouri et al., 2010; Wang et al., 1994). However, it seems that these reports hyperbolize the degree of the LIPUS pro-regenerative potential, which does not coincide with the clinical findings (Emami et al., 1999; Poolman et al., 2017; Schandelmaier et al., 2017a).

Two most described in-vitro LIPUS set-ups, transmitting ultrasound through gel from the bottom of the tissue culture plate or through the media from the top of the cells, exposes them to a near field of the transducer, which is prone to large spatial and temporal intensities variations (described in detail in (Padilla et al., 2014) (Puts et al., 2014)). Although Harrison et al. (Harrison et al., 2016) argue that the near-field ultrasound-stimulation represents a closest configuration to the clinical setting, the cells and the transducer in those in-vitro experiments are usually separated by several millimeters. This exposes the cells to the most heterogeneous proximal near-field of the transducer (Padilla et al., 2014), whereas the clinical device stimulates the fracture site in the mid or far near-field of the transducer (Harrison et al., 2016), where the amplitude differences are dampened. The in-vitro configurations with focused transducers or far-field stimulation (Horne et al., 2020; Puts et al., 2016b; Subramanian et al., 2013) can help to account for these variables. Additionally, the most described in-vitro set-ups (Padilla et al., 2014) can subject the cells to physical artefacts, such as multiple reflections and standing waves (Hensel et al., 2011; Mortazavi et al., 2016), and, especially for the gel-coupled configurations, to temperature elevation (Leskenen
These are, most likely, hardly present in the clinical configurations, and should be further evaluated starting with in-silico analyses.

The widely used in preclinical studies the Exogen LIPUS-probe has a diameter of 22 mm, which exposes the stimulated site to the effective area of 3.88 cm². If the probe is applied to the femur of a laboratory Wistar rat for example, whose average femur length is 39 mm (Prodinger et al., 2018), more than 50 % of the bone is then coupled with the transducer. In contrast, a human femur is on average 440 mm long (Polguj et al., 2013), which results in a 5-%-overlap between the bone and the LIPUS-probe. The femur length of a white New Zealand rabbit, another animal often used in in-vivo studies showing positive influence of LIPUS (Pilla et al., 1990; Shakouri et al., 2010), is around 94 mm (Polguj et al., 2013), and more than 20 % of the bone overlaps with the gel-coupled stimulating probe. These in-vivo reports apply LIPUS in a manner exactly opposite to the proportional adjustment of the mechanical dose. Subsequently, the smaller the bone treated with LIPUS is, the bigger and more diverse resident-cell populations embraced by the mechanical stimulation are, including the ones in the bone epiphyses, where large cancellous bone area, rich in stem cells and vasculature, is observed (Gurevitch et al., 2007). This, in turn, can intensively promote migration of the osteoprogenitors to the fracture site, attract immune cells and induce angiogenesis, promoting osteogenesis (Filipowska et al., 2017; Lancerotto and Orgill, 2014). Additionally, thin soft-tissue layers and small bone circumferences of a rat, results in stimulation of the fracture in the most heterogeneous near field of the transducer. Fig. 3a, depicting the numerical simulation of ultrasound field, generated by the Exogen® probe, evidently demonstrates how big the stimulation area of a fractured rat femur with LIPUS is and how high the intensity fluctuations in the near field of the transducer are. When the same femur was positioned in the simulated field of a focused transducer (Fig. 3b), the geometrically confined and acoustic dose-controlled exposure of the bone gap region was achieved. The geometry of the simulated field in Fig. 3b is similar to the one created by a custom-made scanning acoustic microscope (SAM200 Ex, Q-Bam, Halle, Germany) (Rohrbach et al., 2013).

**Fig. 3. Schematic drawing of a fractured rat femur positioned in a simulated sound field produced by (a) a clinically used Exogen® probe (Bioventus LLC, Durham, NC) and (b) a 5-MHz focused probe producing a -6 dB spot of 7.4 x 0.6 mm. In (a), the fracture or osteotomy gap region is exposed to a highly inhomogeneous near field of the transducer and almost the entire femur receives the acoustic stimulation. In (b), the acoustic energy is deposited in the gap region only. The simulations were performed using Field II program and show transmit temporal peak intensity. The pin locations of a typically used external fixation device (Rohrbach et al., 2013) are also shown.**
In contrast to the unproportioned scaling down of acoustic dose from clinical setting to in vivo and in vitro, application of Bone Morphogenetic Protein-2 (BMP-2), a potent growth factor for regeneration of complex bone injuries and non-unions (Schlundt et al., 2018). The induction of bone healing by BMP-2 in clinic is performed at concentration of 1 mg/mL or 1.5 mg/mL (Carter et al., 2008; Govender et al., 2002; Hwang et al., 2016), whereas the same growth factor is used in rats and rabbits in vivo at concentrations ranging from 200 ng/mL to 37.5 µg/mL (Chen et al., 2018; Hyun et al., 2005; Koolen et al., 2019; Seong et al., 2020; Zara et al., 2011; Zhao X, 2016). The cells in vitro are usually stimulated by 50 ng/mL - 5 µg/mL of BMP-2 (Chen et al., 2018; Chen et al., 2019; Kim et al., 2013; Ning H, 2019). Although supraphysiological doses of the growth factor are used in clinics, the reports elucidating the mechanisms attempt to adjust the concentration of BMP-2 to the size of the stimulated biological system. Exactly the opposite is done with the LIPUS stimulation experiments. This might explain the significant difference in results from the small animal long bones fixed with intramedullary nail and ultrasound-stimulated with ultrasound and fixed with the intramedullary nail long bones from small animals, where pronounced bone-healing effects were observed (Azuma et al., 2001; Wang et al., 1994) and the unsuccessful clinical cases (Busse et al., 2014; Emami et al., 1999; group et al., 2016). In order to compare adequately the influence of LIPUS on bone regeneration in vivo in small animals and translate these findings to the clinical setting, the set-ups with well-controlled physical effects need to be applied (Horne et al., 2020; Puts et al., 2016b; Subramanian et al., 2013). Further optimization of the reproducible clinical acoustic dose might be then required (Warden, 2003; Warden et al., 2000). Until one can decipher the essential mechanisms of bone regeneration by the defined acoustic stimulation, using the spatially adjusted set-ups, which were translated from human to in vivo preclinical models, and in vitro and back, are deciphered, LIPUS is going to remain underestimated in clinic.

Discussion

Upon the onset of a long-bone fracture, the orthopedic surgeon has to make quick and efficient decisions on what the best treatment option for the patient is. The new generation of surgeons more frequently refer to invasive treatments with fixation even for uncomplicated fractures (Courtney et al., 2011; Fernandez, 2005; Schmidt et al., 2003). This, on one hand, provides the desired mechanical stability and ensures adequate conditions for bone regeneration. On the other hand, surgical interventions are prone to infections, which ultimately impair bone healing and result in bone non-unions (Coles and Gross, 2000). Not only these are economically burdensome (Hak et al., 2014; Heckman and Sarasohn-Kahn, 1997; Majeed et al., 2020; Teoh et al., 2018), but also the established non-union bone is often hard to diagnose, because the blood inflammatory markers in up to 20% of those cases remain within the reference levels (Bishop et al., 2012; Nauth et al., 2018). Given these and other risks that the surgical
procedures have, it cannot be used as a one-fits-all treatment solution: elderly, individuals with chronic metabolic disorders and other underlying health conditions, and people with certain lifestyles, where the long recovery time is not desired, are the audience for the alternative methods (Anderson et al., 2019; Bawale et al., 2020; Berber et al., 2020; Cook et al., 1997; Leighton et al., 2017; Nolte et al., 2001; Zura et al., 2015a).

Within the process of bone healing, a miscommunication between the components of the “diamond concept” (Fig. 4), essential for successful bone regeneration, could result in a complicated healing scenario (Andrzejowski and Giannoudis, 2019; Giannoudis et al., 2007). When all four facets of the concept, i.e., cells, matrix, growth factors and mechanical stability, are in balance (Busse et al., 2014; Emami et al., 1999; group et al., 2016), the LIPUS stimulation will, most likely, not have an additional effect. Furthermore, if an atrophic non-union is established and substantial biological inertness in bone is observed, the fracture deterioration might not be efficiently compensated by mechanical stimulation with LIPUS (Malizos et al., 2006; Moghaddam et al., 2016; Watanabe et al., 2010). The exposure to micromotion generated by LIPUS (Greenleaf, 2003), might, however be beneficial for fractures healing with a delay, where biological phenomena is still in place and LIPUS can help supporting to overcome the detrimental biomechanical environment (Leighton et al., 2017; Majeed et al., 2020; Watanabe et al., 2013). These hypotheses, however, require further evaluation in valid in-vitro and preclinical models, followed by clinical research.

Fig. 4. Role of LIPUS with respect to the “diamond concept” of bone regeneration. Given the fracture stability, LIPUS stimulation might mimic the mechanical cues induced by interfragmentary motion, crucial for successful healing.

Conclusions

This review attempts to emphasize our limited knowledge on the principal mechanisms of the LIPUS technique and on the lack of adequate clinical evaluation. The in-vitro and in-vivo biological and physical mechanisms are in need to be researched using set-ups, where an adequate translation of the acoustic dose from the clinical setting is ensured. The conduction of double-blind, randomized, placebo-controlled clinical trials for various bone fractures (fresh, delayed- and non-union) in cast and fixed with implants for large patient cohorts is is-desired. Moreover, these studies should ideally be non-industry funded to eliminate potential bias. The clinical trials need to be supplied with regular follow-up appointments and easy access to communication with the medical personnel. Patient compliance needs to be documented in great detail, including the population that was intended to be treated originally, the individuals that followed the protocol properly, the number of days
LIPUS was applied and for how long amongst those who adhered. It also should be specified whether the active-minutes recorded by the LIPUS device were counted only when the probe was in the direct skin contact. Additionally, investigation and optimization of the LIPUS-treatment protocols for fractures in aged individuals and patients with chronic metabolic disorders, where complementary methods could be of choice, is worthy. The application of microenergy stimulations has a great potential for regeneration of a number of tissues and an abandonment of the LIPUS devices from the clinical routine, might in fact be “throwing the baby with the bathwater”.
References


Khan YL, K. W. H.; Veronick, J. Low Intensity Pulsed Ultrasound Increases Wnt Signaling in Mouse Osteoprogenitor Cells In: Proceedings of the 56th Annual Meeting of the Orthopaedic Research Society, New Orleans, LA USA.


List of Figure Legends

Fig. 1. Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Diagram of search inclusion and exclusion criteria. The research yielded 62 scientific reports published from December 1950 until April 2021, which were analyzed in this review.

Fig. 2. Can LIPUS help regenerate a non-union? Biological signatures of non-union bone (left) and hypothetical effects of LIPUS-stimulation on non-union regeneration (right).

Fig. 3. Schematic drawing of a fractured rat femur positioned in a simulated sound field produced by (a) a clinically used Exogen® probe (Bioventus LLC, Durham, NC) and (b) a 5-MHz focused probe producing a -6 dB spot of 7.4 x 0.6 mm. In (a), the fracture or osteotomy gap region is exposed to a highly inhomogeneous near field of the transducer and almost the entire femur receives the acoustic stimulation. In (b), the acoustic energy is deposited in the gap region only. The simulations were performed using Field II program and show transmit temporal peak intensity. The pin locations of a typically used external fixation device (Rohrbach et al., 2013) are also shown.

Fig. 4. Role of LIPUS with respect to the “diamond concept” of bone regeneration. Given the fracture stability, LIPUS stimulation might mimic the mechanical cues induced by interfragmentary motion, crucial for successful healing.
List of Table Legends

Table 1 (a-h). LIPUS for fresh fractures and distraction osteogenesis. ‘LIPUS’ and ‘C’ stand for ultrasound-treated and control groups, respectively, and the number next to them designates a number of patients per each group. IM, PRF, DC, I_sata stand for intramedullary nail, pulse repetition frequency, duty cycle, spatial average temporal average acoustic intensity, respectively.

Table 2 (a-d). LIPUS for delayed- and non-union bones. ‘LIPUS’ and ‘C’ stand for ultrasound-treated and control groups, respectively, and the number next to them designates a number of patients per each group.

Table 3. LIPUS and osteoporosis. ‘LIPUS’ and ‘C’ stand for ultrasound-treated and control groups, respectively, and the number next to them designates a number of patients per each group. PRF, DC, I_sata stand for pulse repetition frequency, duty cycle, spatial average temporal average acoustic intensity, respectively.
Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) Diagram of search inclusion and exclusion criteria. The research yielded 62 scientific reports published between December 1950 and April 2021, which were analyzed in this review.

216x174mm (150 x 150 DPI)
Can LIPUS help regenerate a non-union? Biological signatures of non-union bone (left) and hypothetical effects of LIPUS-stimulation on non-union regeneration (right).

247x123mm (150 x 150 DPI)
Schematic drawing of a fractured rat femur positioned in a simulated sound field produced by (a) a clinically used Exogen® probe (Bioventus LLC, Durham, NC) and (b) a 5-MHz focused probe producing a -6 dB spot of 7.4 x 0.6 mm. In (a), the fracture or osteotomy gap region is exposed to a highly inhomogeneous near field of the transducer and almost the entire femur receives the acoustic stimulation. In (b), the acoustic energy is deposited in the gap region only. The simulations were performed using Field II program and show transmit temporal peak intensity. The pin locations of a typically used external fixation device (Rohrbach et al., 2013) are also shown.
Role of LIPUS with respect to the “diamond concept” of bone regeneration. Given the fracture stability, LIPUS stimulation might mimic the mechanical cues induced by interfragmentary motion, crucial for successful healing.

164x150mm (150 x 150 DPI)
Table 1a. LIPUS for fresh fractures and distraction osteogenesis.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type of Clinical Study</th>
<th>Fracture Details</th>
<th>Patients Mean Age ± STD or Range</th>
<th>LIPUS Parameters</th>
<th>Sham Device</th>
<th>Compliance</th>
<th>Outcome</th>
<th>Follow-ups</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Akiyama et al., 2014)</td>
<td>Retrospective comparative</td>
<td>Femoral reconstruction with cortical onlay strut allograft</td>
<td>35 patients LIPUS 14 Mean: 63 y.o. 23 to 79 y.o. C 21 Mean: 65.8 y.o. 45 to 84 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not reported</td>
<td>Early and complete radiographic bridging was 60 - 65 % faster in US group</td>
<td>LIPUS mean 29 months Control mean 75 months No complications</td>
<td>Retrospective study, without sham control. Small patient cohort</td>
</tr>
<tr>
<td>(Arima et al., 2017)</td>
<td>Retrospective comparative</td>
<td>Pediatric lumbar spondylosis treated conservatively (brace)</td>
<td>13 patients LIPUS 6 14.7 ± 2.2 y.o. C 7 14.6 ± 2.9 y.o.</td>
<td>1.5 MHz 200 ms at 1 kHz and $I_{ATA} = 60$ mW/cm$^2$</td>
<td>No</td>
<td>Follow up rate 86.7%. LIPUS application performed by medical staff. Compliance is not specified</td>
<td>66.7% of defects healed in LIPUS group vs 10% in control group. Time to healing was shorter in the active group</td>
<td>Every 1.5 months CT scans were performed</td>
<td>Retrospective study, without sham control. Small patient cohort</td>
</tr>
<tr>
<td>(Arimoto et al., 2019)</td>
<td>Prospective randomized patients' distribution and blind assessment of images</td>
<td>Intraoral vertical ramus osteotomy, mandibular</td>
<td>21 patients LIPUS 12 C 9 16 to 54 y.o. not specified between groups</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Patients were assisted 3 weeks on LIPUS. Beyond 3 weeks was not assessed.</td>
<td>LIPUS improves bone density</td>
<td>At 1 month, 6 months and 1 year postoperatively</td>
<td>Small patient cohort. No sham treatment</td>
</tr>
<tr>
<td>(Brand et al., 1999)</td>
<td>Prospective observational</td>
<td>Tibial stress fractures</td>
<td>8 patients High school or college students</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>All but one fractures healed.</td>
<td>4 weeks</td>
<td>Lack of any controls. Small patient cohort</td>
</tr>
</tbody>
</table>
Table 1b. LIPUS for fresh fractures and distraction osteogenesis (cont.).

<table>
<thead>
<tr>
<th>Source</th>
<th>Type of Clinical Study</th>
<th>Fracture Details</th>
<th>Patients Mean Age ± STD or Range</th>
<th>LIPUS Parameters</th>
<th>Sham Device</th>
<th>Compliance</th>
<th>Outcome</th>
<th>Follow-ups</th>
<th>Limitations</th>
</tr>
</thead>
</table>
| (Busse et al., 2014) | Prospective multicenter double-blind randomized placebo-controlled trial | Tibial fracture fixed with reamed IM (pilot study) | 51 patients  
LIPUS 23  
39.0 ± 13.6 y.o.  
C 28  
39.6 ± 13.6 y.o. | Exogen/  
Bioventus | Yes | 76% fully compliant and 24% more than 50% compliant | No improvement with LIPUS therapy | At 1 year, follow-up rate 84% | IM provides optimal mechanical conditions. |
| (Busse et al., 2016) | Prospective multicenter double-blind randomized placebo-controlled | Tibial fracture fixed with reamed intramedullary nail | 501 patients  
LIPUS 250  
37.1 ± 13.2 y.o.  
C 251  
39.1 ± 14.6 y.o. | Exogen/  
Bioventus | Yes | 73% administered 50% of treatments | Addition of LIPUS did not improve healing rate | At 52 weeks | IM provides optimal mechanical conditions. Inadequate compliance |
| (Coughlin et al., 2008) | Prospective comparative | Hindfoot undergoing subtalar arthrodesis, cast fixed | 15 patients compared retrospectively to 15 patients without LIPUS. No patients' demographics. | Exogen/  
Bioventus | No | Not specified | Accelerated healing at 9 weeks (radiographically) | At 6 and 12 months | Study without sham control. Small patient cohort |
| (Dudda et al., 2011) | Prospective randomized comparative | Distraction osteogenesis of long bones (Ilizarov fixator) | 36 patients  
LIPUS 16  
34.9 ± 14.7 y.o.  
C 20  
42.2 ± 13.3 y.o. | Exogen/  
Bioventus | No | Not specified | LIPUS group had shorter healing time, despite bigger distraction gaps | Every 3-4 weeks until healing | No sham control, small patient cohort, unblinded design |
| (El-Mowafi and Mohsen, 2005) | Prospective randomized comparative | Distraction osteogenesis of tibia (Ilizarov fixator) | 20 patients  
LIPUS 10  
C 10  
Mean: 35 y.o.  
18 to 45 y.o.  
Age distribution between groups is not specified | Exogen/  
Bioventus | No | Not specified | LIPUS shortened time for bone consolidation | Every week until healing | No sham control, small patient cohort |
<table>
<thead>
<tr>
<th>Source</th>
<th>Type of Clinical Study</th>
<th>Fracture Details</th>
<th>Patients Mean Age ± STD or Range</th>
<th>LIPUS Parameters</th>
<th>Sham Device</th>
<th>Compliance</th>
<th>Outcome</th>
<th>Follow-ups</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Emami et al., 1999)</td>
<td>Prospective randomized double-blind placebo-controlled</td>
<td>Tibial fracture fixed with statically locked or reamed intramedullary nail</td>
<td>32 patients LIPUS 15 39.9 ± 16.2 y.o. C 17 34.3 ± 14.1 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>Yes</td>
<td>Average 91.4% compliance recorded by device. LIPUS applied only 53% of the time until healing.</td>
<td>No effect of LIPUS on healing time</td>
<td>Every 3 weeks until healing and at weeks 26 and 52</td>
<td>IM provides optimal mechanical conditions. Inadequate compliance. Small patient cohort</td>
</tr>
<tr>
<td>(Gan et al., 2014)</td>
<td>Prospective randomized double-blind placebo-controlled</td>
<td>Lower limb bone stress injuries</td>
<td>23 patients LIPUS 10 32.7 ± 10.6 y.o. C 13 28.6 ± 13.3 y.o.</td>
<td>1.5 MHz 1 kHz PRF 200 ms pulses $I_{\text{SATA}} = 30 \text{ mW/cm}^2$</td>
<td>Yes</td>
<td>Not measured</td>
<td>No effect of LIPUS</td>
<td>At 4, 8, 10 and 12 weeks. LIPUS applied for only 4 weeks</td>
<td>Good spontaneous healing rate of bone stress injuries. Small patient cohort</td>
</tr>
<tr>
<td>(Gold and Wasserma, 2005)</td>
<td>Prospective comparative</td>
<td>Distraction osteogenesis of tibia (large bone defect via Ilizarov fixator)</td>
<td>20 patients LIPUS 8 Mean: 34 y.o. 18 to 50 y.o. Compared retrospectively C 12</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>The external fixation index was reduced by 17.2% as a result of LIPUS therapy (statistically non-significant)</td>
<td>Weekly for 4 weeks, twice a month for 2 months and once a month until healing</td>
<td>Lack of any controls. Small patient cohort</td>
</tr>
<tr>
<td>(Gopalan et al., 2020)</td>
<td>Prospective randomized single-blind comparative</td>
<td>Mandibular fracture</td>
<td>40 patients LIPUS 20 28.0 ± 7.3 y.o. C 20 26.8 ± 8.7 y.o.</td>
<td>1.5 MHz and $I_{\text{SATA}} = 30 \text{ mW/cm}^2$ (rest not specified), on days 4, 8, 14 and 20</td>
<td>No</td>
<td>100%, LIPUS applications performed by medical staff</td>
<td>LIPUS reduced pain. Improved fracture healing (radiographically)</td>
<td>Pain: on days 5, 9, 15 and 21. Images: at weeks 4, 8 and 12</td>
<td>No sham control</td>
</tr>
</tbody>
</table>
Table 1d. LIPUS for fresh fractures and distraction osteogenesis (cont.).

<table>
<thead>
<tr>
<th>Source</th>
<th>Type of Clinical Study</th>
<th>Fracture Details</th>
<th>Patients Mean Age ± STD or Range</th>
<th>LIPUS Parameters</th>
<th>Sham Device</th>
<th>Compliance</th>
<th>Outcome</th>
<th>Follow-ups</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Handolin et al., 2005a)</td>
<td>Prospective randomized double-blind placebo-controlled</td>
<td>Screw-fixed lateral malleolar fracture</td>
<td>22 patients LIPUS 11 Mean: 37.5 18 to 54 y.o. C 11 Mean: 45.5 y.o. 26 to 59 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>Yes</td>
<td>Not specified</td>
<td>No effect of LIPUS on bone healing (radiographically)</td>
<td>At weeks 2, 6, 9 and 12</td>
<td>Small patient cohort. Possibility of early weight-bearing</td>
</tr>
<tr>
<td>(Handolin et al., 2005b)</td>
<td>Prospective randomized double-blind placebo-controlled</td>
<td>Screw-fixed lateral malleolar fracture</td>
<td>30 patients LIPUS 15 Mean: 41.4 y.o. 19 to 65 y.o. C 15 Mean: 39.4 y.o. 18 to 59 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>Yes</td>
<td>Not specified</td>
<td>LIPUS did not speed up fracture healing. However, more frequent callus formation was observed in LIPUS group</td>
<td>At weeks 2, 6, 9 and 12</td>
<td>Small patient cohort. Possibility of early weight-bearing</td>
</tr>
<tr>
<td>(Heckman et al., 1994)</td>
<td>Prospective multicenter randomized double-blind placebo-controlled</td>
<td>Tibial fracture fixed with cast</td>
<td>66 patients with 67 fractures LIPUS 33 36 ± 2.3 y.o. C 34 31 ± 1.8 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>Yes</td>
<td>89.5% of patients returned to follow-ups. Exact device usage is not specified</td>
<td>LIPUS accelerated bone healing, when assessed both clinically and radiographically</td>
<td>At weeks 10, 12, 14, 20, 33 and 52. Final follow-up at 24 months.</td>
<td>Compliance is not descriptively specified, but seems rather low</td>
</tr>
<tr>
<td>(Kinami et al., 2013)</td>
<td>Multicenter retrospective comparative</td>
<td>Femur or tibia managed surgically</td>
<td>141 patients LIPUS 78 Mean: 48.7 y.o. 16 to 95 y.o. C 63 Mean: 46.9 y.o. 16 to 94 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>LIPUS accelerated by 30% healing of stable comminuted fractures, but not of simple and wedge ones</td>
<td>Every month until bone union. LIPUS therapy administered at least for 3 months.</td>
<td>Retrospective design</td>
</tr>
</tbody>
</table>
Table 1e. LIPUS for fresh fractures and distraction osteogenesis (cont.).

<table>
<thead>
<tr>
<th>Source</th>
<th>Type of Clinical Study</th>
<th>Fracture Details</th>
<th>Patients Mean Age ± STD or Range</th>
<th>LIPUS Parameters</th>
<th>Sham Device</th>
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<tbody>
<tr>
<td>(Kristiansen et al., 1997) Prospective multicenter randomized double-blind placebo-controlled</td>
<td>Distal radius fracture fixed with cast</td>
<td>61 fractures in 60 patients LIPUS 30 54 ± 3 y.o. C 31 58 ± 2 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>Yes</td>
<td>By device: average for LIPUS 62 days (29-77); average for placebo 65 days (39-76)</td>
<td>LIPUS accelerated healing by 30%</td>
<td>At weeks 1, 2, 3, 4, 5, 6, 8, 10, 12 and 16</td>
<td>Compliance is not descriptively specified, but seems rather low</td>
<td></td>
</tr>
<tr>
<td>(Leung et al., 2004b) Prospective randomized single-blind placebo-controlled</td>
<td>Complex open tibial fractures surgically fixed</td>
<td>28 patients with 30 fractures LIPUS 16 C 14 Mean: 35.3 y.o. 22 to 61 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>Yes, differs from active</td>
<td>Not specified</td>
<td>LIPUS improved fracture healing, when assessed clinically, radiographically and biochemically</td>
<td>At weeks 3, 6, 9, 12, 18, 24, 32, 40 and 48</td>
<td>Unblinded study design. Small patient cohort per group</td>
<td></td>
</tr>
<tr>
<td>(Liu et al., 2014) Prospective randomized single-blind comparative</td>
<td>Distal radius fixed with cast</td>
<td>81 patients LIPUS 41 67.9 ± 5.6 y.o. C 40 65.7 ± 6.1 y.o.</td>
<td>Most likely Exogen/ Bioventus, PRF is not specified, 15 min/day</td>
<td>No</td>
<td>Not specified</td>
<td>LIPUS accelerated fracture healing</td>
<td>Every week until healing</td>
<td>No sham group, single-blinded design</td>
<td></td>
</tr>
<tr>
<td>(Lubbert et al., 2008) Prospective multicenter randomized double-blind placebo-controlled</td>
<td>Midschaft clavicle fracture treated non-operatively</td>
<td>101 patients LIPUS 52 C 49 Age distribution between groups is not specified</td>
<td>Exogen/ Bioventus</td>
<td>Yes</td>
<td>Not specified</td>
<td>LIPUS does not accelerate fracture healing when assessed clinically</td>
<td>At weeks 1, 2, 4, 6 and 8</td>
<td>Good spontaneous healing of clavicle fractures</td>
<td></td>
</tr>
<tr>
<td>(Maurya et al., 2019) Prospective randomized double-blind placebo-controlled</td>
<td>Temporoman dibular joint (TMJ) with a fixed functional appliance</td>
<td>40 patients LIPUS 20 14.1 y.o. C 20 Mean: 14 y.o.</td>
<td>Exogen/ Bioventus 10 days in a raw and 3 times a week after</td>
<td>Yes</td>
<td>100% compliance, LIPUS applications performed by medical staff</td>
<td>LIPUS improved TMJ remodeling and condylar head position and joint space, assessed via CT scans</td>
<td>Not specified. Assumed to be on days of LIPUS application</td>
<td>Small patient cohort per each group</td>
<td></td>
</tr>
</tbody>
</table>
Table 1f. LIPUS for fresh fractures and distraction osteogenesis (cont.).

<table>
<thead>
<tr>
<th>Source</th>
<th>Type of Clinical Study</th>
<th>Fracture Details</th>
<th>Patients Mean Age ± STD or Range</th>
<th>LIPUS Parameters</th>
<th>Sham Device</th>
<th>Compliance</th>
<th>Outcome</th>
<th>Follow-ups</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Namra et al., 2020)</td>
<td>Prospective randomized single-blind placebo-controlled</td>
<td>Temporomandibular joint (TMJ) with a functional Twin-Block (TB) appliance</td>
<td>45 patients LIPUS 15 (TB) TB 15 C 15 (untreated) 10.5 to 14 y.o. Age distribution between groups is not specified</td>
<td>Exogen/ Bioventus</td>
<td>Yes, medical staff - unblind</td>
<td>100% compliant, LIPUS applications performed by medical staff</td>
<td>LIPUS reduces functional treatment and stimulated growth during correction</td>
<td>Every 3 weeks</td>
<td>Unblinded study design. Small patient cohort per group</td>
</tr>
<tr>
<td>(Nolte et al., 2016)</td>
<td>Retrospective observational</td>
<td>Metatarsal fractures treated either with cast and LIPUS or surgery</td>
<td>Patients evaluated via propensity matching using registry of 594 LIPUS-treated fractures</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>LIPUS accelerated healing of fractures younger than 1 year. These results were comparable to surgery</td>
<td>Not specified</td>
<td>Retrospective study without sham control</td>
</tr>
<tr>
<td>(Ota et al., 2017)</td>
<td>Retrospective comparative</td>
<td>Surgically fixed with IM radius or ulna in children</td>
<td>44 patients LIPUS 25 8.9 ± 3.1 y.o. C 19 9.7 ± 3.2 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>No loss to follow-ups. Compliance is not specified</td>
<td>LIPUS reduced healing time. All fractures achieved functional recovery</td>
<td>Every week until healing</td>
<td>Retrospective study without sham control</td>
</tr>
<tr>
<td>(Ota et al., 2018)</td>
<td>Retrospective comparative</td>
<td>Displaced mallet finger fractures either LIPUS-stimulated or pinned</td>
<td>19 patients LIPUS 8 Mean: 13 y.o. 11 to 15 y.o. C 11 (pinned) Mean: 13.5 y.o. 11 to 15 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>LIPUS provided excellent functional recovery, although at cost of longer application, when compared to pinning via Ishiguro’s method</td>
<td>Every week until bone union and every 2 weeks until functional recovery</td>
<td>Retrospective study without sham control. Small patient cohort</td>
</tr>
<tr>
<td>(Patel et al., 2015)</td>
<td>Prospective comparative</td>
<td>Minimally displaced mandibular fracture via intermaxillary fixation</td>
<td>28 patients LIPUS 14 C 14 15 to 35 y.o. Not mentioned between groups</td>
<td>1.0 MHz ISATA = 1.5 W/cm², PRF is not specified.</td>
<td>No</td>
<td>Performed by medical staff. Compliance is not specified</td>
<td>LIPUS accelerated healing and improved clinical mobility was observed in the sonicated group</td>
<td>Every week</td>
<td>Study without sham control. Small patient cohort in each group</td>
</tr>
<tr>
<td>Source</td>
<td>Type of Clinical Study</td>
<td>Fracture Details</td>
<td>Patients Mean Age ± STD or Range</td>
<td>LIPUS Parameters</td>
<td>Sham Device</td>
<td>Compliance</td>
<td>Outcome</td>
<td>Follow-ups</td>
<td>Limitations</td>
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<tr>
<td>(Raza et al., 2016)</td>
<td>Prospective randomized double-blind placebo-controlled</td>
<td>Torque on tooth root during orthodontic procedure</td>
<td>10 patients LIPUS 10 and C 10 (left or right) 15.5 ± 5.5 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>Yes</td>
<td>Not specified</td>
<td>LIPUS decreased root damage (lower number of resorption lacunae)</td>
<td>At 4 weeks evaluated by µ-CT</td>
<td>Very small patient cohort</td>
</tr>
<tr>
<td>(Salem and Schmelz, 2014)</td>
<td>Prospective randomized comparative</td>
<td>Distraction osteogenesis of tibia (Ilizarov fixator)</td>
<td>21 patients LIPUS 12 Mean: 32 y.o. C 9 Mean: 29 y.o. Rest is not specified</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>LIPUS shortened healing time clinically and radiographically</td>
<td>Every 2 weeks clinical follow-ups, and every 4 weeks radiographic evaluation</td>
<td>Unblinded study design. Lack of sham control. Small patient cohort</td>
</tr>
<tr>
<td>(Santana-Rodríguez et al., 2019)</td>
<td>Prospective randomized double-blind comparative</td>
<td>Rib fracture</td>
<td>47 patients LIPUS 24 Mean: 40.0 ± 13.1 y.o. C 23 Mean: 28.9 ± 17.3 y.o.</td>
<td>1 MHz at 0.5 W/cm² at DC 10% 1 min/day. PRF is not specified</td>
<td>No</td>
<td>100% compliance, LIPUS applications performed by medical staff</td>
<td>LIPUS decreased pain and intake of pain medication, Accelerated callus healing and return to life activities</td>
<td>At months 1, 3 and 6</td>
<td>No sham control</td>
</tr>
<tr>
<td>(Simpson et al., 2017)</td>
<td>Prospective multi-center randomized double-blind placebo-controlled</td>
<td>Distraction osteogenesis of tibia (Ilizarov fixator)</td>
<td>55 patients LIPUS 30 Mean: 37.2 ± 12.9 y.o. C 25 Mean: 38.4 ± 12.0 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>Yes</td>
<td>75% of patients were 50%-compliant</td>
<td>LIPUS did not accelerate bone healing</td>
<td>Every 4 weeks until healing radiographically and via weight-bearing</td>
<td>Inadequate compliance</td>
</tr>
<tr>
<td>(Song et al., 2019)</td>
<td>Retrospective comparative</td>
<td>Bilateral tibial lengthening over nail (also fixed via Ilizarov fixator)</td>
<td>30 patients LIPUS 15 Mean: 22.1 y.o. 17.5 to 34.0 y.o. C 15 Mean: 20.6 y.o. 17.9 to 25.4 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>LIPUS enhanced callus formation and accelerated bone healing assessed radiographically</td>
<td>At weeks 1, 2, 3 and 4, and monthly until healing</td>
<td>Retrospective study without sham control</td>
</tr>
</tbody>
</table>
Table 1h. LIPUS for fresh fractures and distraction osteogenesis (cont.).

<table>
<thead>
<tr>
<th>Source</th>
<th>Type of Clinical Study</th>
<th>Fracture Details</th>
<th>Patients Mean Age ± STD or Range</th>
<th>LIPUS Parameters</th>
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<th>Outcome</th>
<th>Follow-ups</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Tsumaki et al., 2004)</td>
<td>Prospective randomized comparative</td>
<td>Bilateral one stage opening-wedge high tibial osteotomy by hemicallotasis</td>
<td>21 patients Left or right were randomly with/without LIPUS Mean: 68 y.o. 53 to 78 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>100% compliance, LIPUS applications performed by medical staff</td>
<td>LIPUS accelerates callus maturation in elderly patients assessed radiographically Earlier removal of pins in active group</td>
<td>Every week</td>
<td>No placebo control and unblinded study design</td>
</tr>
<tr>
<td>(Urita et al., 2013)</td>
<td>Prospective randomized single-blind comparative</td>
<td>Shortening osteotomy of ulnar or radius</td>
<td>27 patients LIPUS 14 Mean: 52 y.o. 34 to 70 y.o. C 13 Mean: 44 y.o. 20 to 56 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>LIPUS accelerated bone healing assessed radiographically. Clinical parameters were not improved</td>
<td>At weeks 2, 4, 6, 8, 12, 16 and 24</td>
<td>No placebo control and unblinded study design</td>
</tr>
<tr>
<td>(Zacherl et al., 2009)</td>
<td>Prospective randomized double-blind placebo-controlled</td>
<td>Chevron osteotomy for hallux valgus</td>
<td>52 osteotomies in 44 patients LIPUS 26 Mean: 51 y.o. 20 to 77 y.o. C 26 Mean: 54 y.o. 28 to 77 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>Yes</td>
<td>Checked weekly. Noncontact with device produced sound. 92.3% completed &gt;78.6% of treatments</td>
<td>LIPUS had no effect on radiographic and clinical healing. Placebo group had more frequent relapse (statistically significant) in distal metatarsal articular angle at 6 weeks</td>
<td>At 6 weeks and 1 year</td>
<td>None</td>
</tr>
<tr>
<td>(Zura et al., 2015b)</td>
<td>Retrospective observational</td>
<td>Fractures at various locations</td>
<td>4190 patients 43.3 ± 18.2 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Only compliant patients were included in the study. Details are not specified</td>
<td>96% of fresh fractures healed. Shorter time to treatment correlated with positive outcome</td>
<td>Not specified</td>
<td>Retrospective study without any controls</td>
</tr>
</tbody>
</table>
Table 2a. LIPUS for delayed- and non-union bones.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type of Clinical Study</th>
<th>Fracture Details</th>
<th>Patients Mean Age ± STD or Range</th>
<th>LIPUS Parameters</th>
<th>Sham Device</th>
<th>Compliance</th>
<th>Outcome</th>
<th>Follow-ups</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Adukia et al., 2021)</td>
<td>Retrospective observational</td>
<td>Non-unions at various locations. Mostly atrophic</td>
<td>46 patients 47.0 ± 19.7 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>8 patients were lost to follow-ups. Not specified how it was measured</td>
<td>Union was achieved in 57.89% of the cases. Small inter-fragmentary gap was a predictor of success</td>
<td>At 6 weeks, and 3 and 6 months, and 1 year.</td>
<td>Retrospective study, without sham control</td>
</tr>
<tr>
<td>(Anderson et al., 2019)</td>
<td>Retrospective observational</td>
<td>Metatarsal fractures with delayed healing (&gt;14 days)</td>
<td>256 patients 65.8 ± 11.5 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not measured</td>
<td>Delayed healing: in younger; with obesity, psychosis, anemia, chronic lung disease. Surgery rather prescribed to patients, who first saw specialist</td>
<td>Not specified</td>
<td>Retrospective study, without sham control. If person did not seek treatment after LIPUS, the fracture was assumed to be healed</td>
</tr>
<tr>
<td>(Bawale et al., 2020)</td>
<td>Prospective observational</td>
<td>Various locations</td>
<td>66 patients Mean 49.2 y.o. 19 - 85 y.o.</td>
<td>Not specified</td>
<td>No</td>
<td>4 patients excluded due to poor compliance. Not specified how it was measured</td>
<td>67% of compliant patients healed. Post-ORIF scaphoid fracture and post-ankle joint fusion non-union did not heal</td>
<td>At 6 months minimum</td>
<td>Study without sham control</td>
</tr>
<tr>
<td>(Biglari et al., 2016)</td>
<td>Prospective observational</td>
<td>Long Bones non-unions</td>
<td>61 non-unions from 60 patients 45.0 ± 9.8 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>32.4 % healed successfully, the rest had to undergo revision surgery</td>
<td>At 6, 12 weeks, 4, 5, 6, and 12 months</td>
<td>Study without sham control</td>
</tr>
<tr>
<td>(Carlson et al., 2015)</td>
<td>Retrospective observational</td>
<td>Scaphoid non-union treated surgically</td>
<td>14 patients 15.3 ± 1.3 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>13 out 14 non-unions healed successfully within a range 61 - 217 days</td>
<td>Every 4 to 6 weeks until healing</td>
<td>Without sham control and without non-surgically treated controls. Heterogeneous surgical treatments. Small patient cohort</td>
</tr>
</tbody>
</table>
Table 2b. LIPUS for delayed- and non-union bones (cont.).

<table>
<thead>
<tr>
<th>Source</th>
<th>Type of Clinical Study</th>
<th>Fracture Details</th>
<th>Patients Mean Age ± STD or Range</th>
<th>LIPUS Parameters</th>
<th>Sham Device</th>
<th>Compliance</th>
<th>Outcome</th>
<th>Follow-ups</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Elvey et al., 2020)</td>
<td>Retrospective observational</td>
<td>Hand and wrist non-unions</td>
<td>26 patients 27.7 ± 9.8 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not measured</td>
<td>62.5% of non-unions healed after LIPUS therapy within 12 months</td>
<td>At 12 months</td>
<td>Retrospective study, without any controls</td>
</tr>
<tr>
<td>(Farkash et al., 2015)</td>
<td>Retrospective observational</td>
<td>Scaphoid delayed-union fixed with cast</td>
<td>29 patients 18 to 22 y.o. One patient 34 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>76% of delayed-union healed assessed via X-ray and CT-scans. LIPUS success was higher in younger fractures</td>
<td>Heterogeneous within cases</td>
<td>Retrospective study, without any controls</td>
</tr>
<tr>
<td>(Gebauer et al., 2005)</td>
<td>Prospective observational</td>
<td>Various locations</td>
<td>67 non-unions in 66 patients 46.0 ± 1.9 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Based on device recording, it was used on average 89% of the time</td>
<td>In 1- to 2-month intervals until complete healing</td>
<td>No comparison group, no sham treatment</td>
<td></td>
</tr>
<tr>
<td>(Gebauer and Correll, 2005)</td>
<td>Prospective observational</td>
<td>Non-unions after long bones lengthening</td>
<td>17 non-unions in 13 children 7.9 ± 2.2 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>All cases healed fully</td>
<td>Every 6 weeks until healing and 4 years later</td>
<td>Small patient cohort. No comparison group, no sham treatment</td>
</tr>
<tr>
<td>(Hemery et al., 2011)</td>
<td>Retrospective observational</td>
<td>Long bones non-unions</td>
<td>14 patients 39.1 ± 13.8 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>79% of non-unions healed</td>
<td>Every 3 months</td>
<td>Small patient cohort. No comparison group, no sham treatment</td>
</tr>
<tr>
<td>(Jones et al., 2006)</td>
<td>Prospective observational</td>
<td>Hindfoot non-unions after revision surgery with internal fixation</td>
<td>13 patients Mean: 51 y.o. 15 to 71 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>12 out of 13 cases healed</td>
<td>Radiographs at weeks 3, 6 and 12, and CT scans routinely 3 months after surgery</td>
<td>Small patient cohort. No comparison groups: “surgery only”, “LIPUS only”</td>
</tr>
</tbody>
</table>
Table 2c. LIPUS for delayed- and non-union bones (cont.).

<table>
<thead>
<tr>
<th>Source</th>
<th>Type of Clinical Study</th>
<th>Fracture Details</th>
<th>Patients Mean Age ± STD or Range</th>
<th>LIPUS Parameters</th>
<th>Sham Device</th>
<th>Compliance</th>
<th>Outcome</th>
<th>Follow-ups</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Lerner et al., 2004)</td>
<td>Retrospective observational</td>
<td>Long bones high-energy fractures</td>
<td>17 patients with 18 fractures 32.1 ± 12.2 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>16 out of 18 non-unions healed</td>
<td>Not specified</td>
<td>Small patient cohort. Lack of any controls</td>
</tr>
<tr>
<td>(Majeed et al., 2020)</td>
<td>Prospective observational</td>
<td>Foot and ankle post-trauma and post-surgery non-unions</td>
<td>Mean: 56.6 y.o. 23 to 76 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>No losses to follow-ups, all patients completed the treatment</td>
<td>37 out 47 non-unions healed, assessed clinically. 26 of healed cases were atrophic</td>
<td>Not specified</td>
<td></td>
</tr>
<tr>
<td>(Mayr et al., 2000)</td>
<td>Retrospective observational</td>
<td>Delayed unions and non-unions at various locations</td>
<td>1317 patients 20 to 70 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>91% of delayed-unions and 87% of non-unions healed</td>
<td>Not specified</td>
<td>Retrospective study without any controls</td>
</tr>
<tr>
<td>(Moghaddam et al., 2016)</td>
<td>Prospective observational</td>
<td>Long bones non-unions</td>
<td>23 patients 43.0 ± 13.5 y.o. Before and after LIPUS therapy</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>Healed and failed cases - no differences in cytokine concentrations in blood. Decrease in TGF-ß1 was observed in healed group on week 1</td>
<td>At weeks 1 and 2, and months 1, 2 and 3</td>
<td>Lack of any controls</td>
</tr>
<tr>
<td>(Nolte et al., 2001)</td>
<td>Retrospective observational</td>
<td>Non-unions at various locations</td>
<td>28 patients with 29 non-unions 47.0 ± 18.2 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>72% of cases used device for more than 75% (recorded by device)</td>
<td>86% of non-unions healed assessed clinically and radiographically</td>
<td>Every 6 to 8 weeks until healing</td>
<td>Retrospective study without any controls</td>
</tr>
<tr>
<td>(Roussignol et al., 2012)</td>
<td>Retrospective observational</td>
<td>Long bones non-unions</td>
<td>59 patients Mean: 43 y.o. 17 to 85 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Checked at each follow-up. Compliance measured &gt;95%</td>
<td>88% of non-unions healed</td>
<td>Up to 6 weeks, and at 3 and 6 months</td>
<td>Retrospective study without any controls</td>
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</table>
Table 2d. LIPUS for delayed- and non-union bones (cont.).

<table>
<thead>
<tr>
<th>Source</th>
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<th>Compliance</th>
<th>Outcome</th>
<th>Follow-ups</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Rutten et al., 2007)</td>
<td>Retrospective observational</td>
<td>Tibia non-unions</td>
<td>71 patients Mean: 40 y.o. 17 to 89 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>73% of non-unions healed assessed via radiographic and clinical assessment</td>
<td>Average long-term follow-up 2.7 years</td>
<td>Retrospective study without any controls</td>
</tr>
<tr>
<td>(Rutten et al., 2008)</td>
<td>Prospective randomized double-blind placebo-controlled</td>
<td>Delayed-union of osteotomized fibula</td>
<td>13 patients LIPUS 7 52.3 ± 9.0 y.o. C 6 52.8 ± 6.1 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>Yes</td>
<td>Not-specified</td>
<td>LIPUS increased osteoid thickness, mineral apposition and bone volume established by histology</td>
<td>Biopsies taken 2 to 4 months after start of therapy</td>
<td>Very small patient cohort</td>
</tr>
<tr>
<td>(Rutten et al., 2009)</td>
<td>Prospective randomized double-blind placebo-controlled</td>
<td>Delayed-union of osteotomized fibula</td>
<td>7 patients LIPUS 3 54.3 ± 10.3 y.o. C 4 50.8 ± 5.9 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>Yes</td>
<td>Not-specified</td>
<td>LIPUS reduced number of Runx2-positive cells in soft tissue established by histology</td>
<td>Biopsies taken 2 to 4 months after start of therapy</td>
<td>Very small patient cohort</td>
</tr>
<tr>
<td>(Schofer et al., 2010)</td>
<td>Prospective multi-center randomized double-blind placebo-controlled</td>
<td>Delayed-union of tibia</td>
<td>101 patients LIPUS 51 42.6 ± 14.6 y.o. C 50 45.1 ± 11.9 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>Yes</td>
<td>91% compliance if evaluate only ‘completers’</td>
<td>LIPUS accelerated healing: improved BMD and reduced gap accessed via CT. No clinical effect at 16 weeks.</td>
<td>At 1, 2, 3 and 4 months</td>
<td>Larger (but non-significantly) amount of older fractures in LIPUS group</td>
</tr>
<tr>
<td>(Teoh et al., 2018)</td>
<td>Retrospective observational</td>
<td>Delayed union of fifth metatarsal</td>
<td>30 patients Mean: 39.3 y.o. 14 to 76 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>90% of delayed unions healed after LIPUS therapy assessed both clinically and radiographically</td>
<td>Every 4 weeks</td>
<td>Retrospective study without any controls</td>
</tr>
<tr>
<td>(Zura et al., 2015a)</td>
<td>Retrospective observational</td>
<td>Chronic non-unions (&gt;1 year) at various locations</td>
<td>764 patients 45.8 ± 16.5 y.o.</td>
<td>Exogen/ Bioventus</td>
<td>No</td>
<td>Not specified</td>
<td>86.2% of cases healed after LIPUS. Patient age - a negative factor for healing. Failed mostly in non-compliant patients</td>
<td>Not specified</td>
<td>Retrospective study without any controls</td>
</tr>
</tbody>
</table>
Table 3. LIPUS and osteoporosis.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type of Clinical Study</th>
<th>Location of Application</th>
<th>Patients Mean Age ± STD or Range</th>
<th>LIPUS Parameters</th>
<th>Sham Device</th>
<th>Compliance</th>
<th>Outcome</th>
<th>Follow-ups</th>
<th>Limitations</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Leung et al., 2004a)</td>
<td>Prospective randomized comparative</td>
<td>Postmenopausal osteoporosis. LIPUS applied at distal radius</td>
<td>20 females 69.1 ± 7.6 y.o. Control – contralateral part</td>
<td>Exogen/Bioventus 5 times a week for 3 months</td>
<td>No</td>
<td>Not specified. LIPUS applied by medical staff</td>
<td>LIPUS had no effect on trabecular and integral BMD assessed by peripheral quantitative CT</td>
<td>At 3 and 6 months</td>
<td>Small patient cohort. Short follow-up period</td>
</tr>
<tr>
<td>(Ozdemir et al., 2008)</td>
<td>Retrospective comparative</td>
<td>Postmenopausal osteoporosis. Ultrasound applied at neck and dorsal, shoulders and knees</td>
<td>74 females LIPUS 36 59.6 ± 5.0 y.o. C 38 56.9 ± 6.8 y.o. Not specified</td>
<td>No</td>
<td>Not specified</td>
<td>Ultrasound had no effect on BMD assessed by DXA</td>
<td>Not specified</td>
<td>Heterogeneous locations of US application: limited number of patients per group</td>
<td></td>
</tr>
<tr>
<td>(Warden et al., 2001)</td>
<td>Prospective randomized double-blind placebo-controlled</td>
<td>Osteoporosis via spinal cord injury. LIPUS applied at calcaneus</td>
<td>15 males 23.9 ± 7.3 y.o. Control – contralateral part</td>
<td>1 MHz 3.3 kHz PRF 3.3% DC ISATA = 30 mW/cm² 5 times a week for 2 months</td>
<td>Yes</td>
<td>LIPUS applied by medical staff</td>
<td>LIPUS had no effect on BMD and assessed by DXA and quantitative ultrasound</td>
<td>At 6 weeks</td>
<td>Small patient cohort. Not clear whether staff is blinded towards treatment. Short follow-up period</td>
</tr>
</tbody>
</table>