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 studies, however, have questioned the usefulness of the LIPUS treatment for the regeneration of long bones, including those with a 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 the suboptimal parameters of the clinical set-up. This raises the question of whether the so-called “acoustic dose” requires a thorough characterisation to reveal the mechanisms of the therapy. The adequate definition of the acoustic dose is especially important in the elderly population and patients with underlying medical conditions, where distinct biological signatures lead to a delayed regeneration. Non-industry-funded, randomised, double-blind, placebo-controlled clinical trials of the LIPUS application alone and as an adjuvant treatment for bones with complicated healing, where consistent control of patient compliance is ensured, are required.

Keywords: Low-intensity pulsed ultrasound, bone regeneration, surgery, acoustic dose, non-union, age, osteoporosis, compliance.

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TB | Twin-Block
---|---
TMJ | temporomandibular joint
TRUST | trial to re-evaluate low-intensity pulsed UltraSound in treatment of tibial fractures
VEGF-A | vascular endothelial growth factor A

### Introduction

According to the USA National Health Interview Survey, more than half of all chronic medical conditions reported in 2012 were associated with musculoskeletal problems (Hauser et al., 2016). The 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 was calculated to be USD 25,556 (Antonova et al., 2013). With progressing age, the odds of a complicated bone healing abruptly increase (Clark et al., 2017). Since the proportion of ageing 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 osteotomised rabbit fibula and femur bones with LIPUS enhanced callus formation (Duarte, 1983). Currently, a device employing LIPUS is manufactured under the brand name of Exogen® (Bioventus LLC, Durham, NC, USA), which emits pulsed sine waves at an ultrasound frequency of 1.5 MHz, a PRF of 1 kHz and a 20% DC, generating a $I_{SATA}$ of 30 mW/cm² (Pounder and Harrison, 2008). Exogen® 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 its operation. The treatment can be applied by the patient at home and lasts 20 min/d 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; Poolman et al., 2017; Schandelmaier et al., 2017a; Tarride et al., 2017; TRUST Investigators writing group et al., 2016).

Once a bone fracture occurs, the orthopaedic surgeon has 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 the present review is to provide the reader with an impartial opinion on the above questions.

### Materials and Methods

Search and retrieval of scientific studies was conducted in accordance with the PRISMA (Moher et al., 2009). Studies published between December 1950 and April 2021 were collected from PubMed and Web of Science databases using as keywords “low-intensity pulsed ultrasound” and “bone fracture”. Search duplicates were first identified using EndNote software. Then, these were verified and further removed manually. Articles, that were not peer-reviewed, without a full-text option or written in a language other than English were excluded. Studies describing in vitro findings and studies in animal models were not retained for the main data analysis. Additionally, articles irrelevant to ultrasound, using ultrasound for other purposes than LIPUS stimulation or describing LIPUS application in other organs than bone were excluded.

### 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 using PubMed and Web of Science databases, respectively. 6 publications, meeting all the inclusion criteria, were found in a Google Scholar free search and designated in the PRISMA chart as “other sources”. EndNote software identified 134 duplicates and an additional 95 were excluded upon manual verification, resulting in 583 search results. A restriction of the search results based on full-text peer-reviewed articles in English language excluded 43 additional studies. LIPUS application in vitro, in silico and in animal models accounted for 88, 2 and 139 entries, respectively. These were identified following thorough screening of the full-text articles. Studies, irrelevant to ultrasound techniques (27), irrelevant to bone fracture stimulation (10) or describing other ultrasound methods (111) were screened out manually and excluded from the analysis. Finally, 163 articles met all the set criteria. Out of them, 77 and 24 were review articles and case studies (data not shown), respectively. Finally, 62 articles (Table 1-3) reporting original findings were included in the present review. Most of the clinical studies identified employ Exogen® or Exogen®-like stimulation devices, with the clinical acoustic parameters of 1.5 MHz, 1 kHz PRF, 20% DC and 30 mW/cm² $I_{SATA}$. These are summarised in Table 1-3. 9 studies use LIPUS parameters that are different from the conventionally used ones or are not clearly specified (Arima et al., 2017; Bawale et al., 2020; Gan et al., 2014; Gopalan et
LIPUS and fresh fractures: surgery vs. cast
There are several hurdles that the application of LIPUS in a clinical setting encounters. The first is the definition of a fresh fracture, which discriminates cases older than 1 week (Heckman, 2017; Zura et al., 2017). This might prevent some potential candidates from receiving non-invasive treatment strategies such as LIPUS. Furthermore, a large number of studies dedicated to LIPUS stimulation of fresh fractures are either based on case studies (data not shown), 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), challenging the credibility of the LIPUS therapy. Additionally, the small size of patient cohorts of several prospective, randomised, 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).

The discussion on whether LIPUS should be used as an alternative or an adjuvant therapy to surgical intervention has become more intense recently, especially since the results of the multicentre randomised, blinded, sham-controlled clinical trial TRUST was published in 2016 (Busse et al., 2014; TRUST Investigators writing group et al., 2016). The study enrolled 501 patients with tibial fractures treated surgically and fixed with an IM nail. No effect of LIPUS stimulation on the radiographically indicated healing time and restoration of full bone-functionality was observed. The data were published soon after as a BMJ Rapid Recommendations article (Poolman et al., 2017), advising the removal of LIPUS from clinical practice. A systematic review (Schandelmaier et al., 2017a) further analysed 26 randomised trials on the use of LIPUS therapy in all types of fracture, concluding that only 3 unbiased studies (Busse et al., 2014; Emami et al., 1999; TRUST Investigators writing group et al., 2016) have been published, with two of them being the results of the TRUST study. LIPUS treatment in these studies was not found to accelerate bone healing. The high risks of bias were defined as i) the lack of a blinded expert, ii) non-identically looking sham device, iii) a
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<tr>
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<th>Sham device</th>
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<th>Outcome</th>
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<tbody>
<tr>
<td>Akiyama et al., 2014</td>
<td>Retrospective, comparative</td>
<td>Femoral reconstruction using a cortical-only strut allograft</td>
<td>35 patients</td>
<td>Femoral reconstruction using a cortical-only strut allograft</td>
<td>LIPUS 14, mean: 63 years old (23–79 years old)</td>
<td>Exogen® No</td>
<td>Follow-up rate 86.7%</td>
<td>Early and complete radiographic bridging was 60–65% faster in LIPUS group</td>
<td>No complications</td>
</tr>
<tr>
<td>Arima et al., 2017</td>
<td>Retrospective, comparative</td>
<td>Paediatric lumbar spondylosis treated conservatively (brace)</td>
<td>13 patients</td>
<td>Paediatric lumbar spondylosis treated conservatively (brace)</td>
<td>LIPUS 6 (14.7 ± 2.2 years old)</td>
<td>Exogen® No</td>
<td>Compliance not specified</td>
<td>Follow-up rate 86.7%</td>
<td>Not reported</td>
</tr>
<tr>
<td>Arimoto et al., 2019</td>
<td>Prospective, randomised</td>
<td>Intraoral vertical ramus osteotomy (mandible)</td>
<td>21 patients</td>
<td>Intraoral vertical ramus osteotomy (mandible)</td>
<td>LIPUS 12</td>
<td>Exogen® No</td>
<td>Patients treated for 3 weeks with LIPUS</td>
<td>Time to healing was shorter in the active group</td>
<td>No LIPUS application performed by medical staff</td>
</tr>
<tr>
<td>Brand et al., 1999</td>
<td>Prospective, observational</td>
<td>Tibial fracture distribution of blind images</td>
<td>8 patients</td>
<td>Tibial fracture distribution of blind images</td>
<td>LIPUS 23 (39.0 ± 13.6 years old)</td>
<td>Exogen® Yes</td>
<td>Compliance not specified</td>
<td>Lack of any controls; small patient cohort</td>
<td>Not assessed beyond 3 weeks</td>
</tr>
<tr>
<td>Busse et al., 2014</td>
<td>Prospective, multicentre, randomised, placebo controlled</td>
<td>Tibial fracture fixed using a reamed IM (pilot study)</td>
<td>51 patients</td>
<td>Tibial fracture fixed using a reamed IM (pilot study)</td>
<td>LIPUS 28 (47.1 ± 13.2 years old)</td>
<td>Exogen® Yes</td>
<td>No</td>
<td>All but 1 fractures healed</td>
<td>76% fully compliant and 24% more than 50% compliant</td>
</tr>
<tr>
<td>Busse et al., 2016</td>
<td>Prospective, multicentre, randomised, placebo controlled</td>
<td>Tibial fracture fixed using an intramedullary nail</td>
<td>501 patients</td>
<td>Tibial fracture fixed using an intramedullary nail</td>
<td>LIPUS 26 (39.1 ± 14.6 years old)</td>
<td>Exogen® Yes</td>
<td>Yes</td>
<td>LIPUS did not improve healing rate</td>
<td>73% administered 50% of treatments</td>
</tr>
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Table 1. LIPUS for fresh fractures and distraction osteogenesis.
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<tr>
<td>Coughlin et al., 2008</td>
<td>Prospective, comparative</td>
<td>Hindfoot undergoing subtalar arthrodesis, fixed using a cast</td>
<td>15 patients compared retrospectively to 15 patients without LIPUS No patients' demographics</td>
<td>Exogen®</td>
<td>No</td>
<td>Not specified</td>
<td>Accelerated healing at 9 weeks (measured radiographically)</td>
<td>At 6 and 12 months</td>
<td>Study without sham control; small patient cohort</td>
</tr>
<tr>
<td>Dudda et al., 2011</td>
<td>Prospective, randomised, comparative</td>
<td>Distraction osteogenesis of long bones (Ilizarov fixator)</td>
<td>36 patients</td>
<td>Exogen®</td>
<td>No</td>
<td>Not specified</td>
<td>LIPUS group had shorter healing time, despite bigger distraction gaps</td>
<td>Every 3-4 weeks until healing</td>
<td>No sham control; small patient cohort; unblinded design</td>
</tr>
<tr>
<td>El-Mowafi and Mohsen, 2005</td>
<td>Prospective, randomised, comparative</td>
<td>Distraction osteogenesis of tibia (Ilizarov fixator)</td>
<td>20 patients</td>
<td>Exogen®</td>
<td>No</td>
<td>Not specified</td>
<td>LIPUS shortened time needed for bone consolidation</td>
<td>Every week until healing</td>
<td>No sham control; small patient cohort</td>
</tr>
<tr>
<td>Emami et al., 1999</td>
<td>Prospective, randomised, double-blind, placebo controlled</td>
<td>Tibial fracture fixed using statically locked or reamed intramedullary nails</td>
<td>32 patients LIPUS 15 (39.9 ± 16.2 years old) Control 17 (34.3 ± 14.1 years old)</td>
<td>Exogen®</td>
<td>Yes</td>
<td>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 provided optimal mechanical conditions; inadequate compliance; small patient cohort</td>
</tr>
<tr>
<td>Gan et al., 2014</td>
<td>Prospective, randomised, double-blind, placebo controlled</td>
<td>Lower limb, bone stress injuries</td>
<td>23 patients LIPUS 10 (32.7 ± 10.6 years old) Control 13 (28.6 ± 13.3 years old)</td>
<td>1.5 MHz, 1 kHz PRF, 200 ms pulses, $I_{SATA} = 30 \text{ mW/cm}^2$</td>
<td>Yes</td>
<td>Not measured</td>
<td>No effect of LIPUS</td>
<td>At 4, 8, 10 and 12 weeks</td>
<td>Good spontaneous healing rate of bone stress injuries; small patient cohort</td>
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<tr>
<td>Gold and Wasserman, 2005</td>
<td>Prospective, comparative</td>
<td>Distraction osteogenesis of tibia (large bone defect; Ilizarov fixator)</td>
<td>20 patients</td>
<td>LIPUS 8 Control 12</td>
<td>No</td>
<td>Not specified</td>
<td>The external fixation index was reduced by 17.2 % (statistically non-significant) as a result of LIPUS therapy</td>
<td>Weekly for 4 weeks, twice a month for 2 months and once a month until healing</td>
<td>Lack of any control; small patient cohort</td>
</tr>
<tr>
<td>Gopalan et al., 2020</td>
<td>Prospective, randomised, single-blind, comparative</td>
<td>Mandibular fracture</td>
<td>40 patients</td>
<td>LIPUS 20 (28.0 ± 7.3 years old) Control 20 (26.8 ± 8.7 years old)</td>
<td>No</td>
<td>100 % LIPUS applications performed by medical staff</td>
<td>LIPUS reduced pain and improved fracture healing (measured 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>
<tr>
<td>Handolin et al., 2003a</td>
<td>Prospective, randomised, double-blind, placebo controlled</td>
<td>Screw-fixed, lateral malleolar fracture</td>
<td>22 patients</td>
<td>LIPUS 11, mean: 37.5 years old (18-5 years old 4) Control 11, mean: 45.5 years old (26-59 years old)</td>
<td>Exogen®</td>
<td>Yes</td>
<td>No effect of LIPUS on bone healing (measured radiographically)</td>
<td>At weeks 2, 6, 9 and 12 Small patient cohort possibility of early weight bearing</td>
<td></td>
</tr>
<tr>
<td>Handolin et al., 2005b</td>
<td>Prospective, randomised, double-blind, placebo controlled</td>
<td>Screw-fixed, lateral malleolar fracture</td>
<td>30 patients</td>
<td>LIPUS 15, mean: 41.4 years old (19-65 years old) Control 15, mean: 39.4 years old (18-59 years old)</td>
<td>Exogen®</td>
<td>Yes</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 Small patient cohort possibility of early weight bearing</td>
<td></td>
</tr>
<tr>
<td>Heckman et al., 1994</td>
<td>Prospective, multicentre, randomised, double-blind, placebo controlled</td>
<td>Tibial fracture, fixed using a cast</td>
<td>66 patients with 67 fractures</td>
<td>LIPUS 33 (36 ± 2.3 years old) Control 34 (31 ± 1.8 years old)</td>
<td>Exogen®</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 Compliance was not descriptively specified but seemed rather low</td>
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<tr>
<td>Kinami et al., 2013</td>
<td>Multicentre, retrospective, comparative</td>
<td>Femur or tibia, managed surgically</td>
<td>LIPUS 78, mean: 48.7 years old (16-95 years old)</td>
<td>Exogen®</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</td>
<td>Retrospective design</td>
</tr>
<tr>
<td>Kristiansen et al., 1997</td>
<td>Prospective, multicentre, randomised, double-blind, placebo controlled</td>
<td>Distal radius fracture, fixed using a cast</td>
<td>61 fractures in 60 patients</td>
<td>Exogen®</td>
<td>Yes</td>
<td>By device: average for LIPUS 62 d (29-77); average for placebo 65 d (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 was not descriptively specified but seemed rather low</td>
</tr>
<tr>
<td>Leung et al., 2004b</td>
<td>Prospective, randomised, single-blind, placebo controlled</td>
<td>Complex, open tibial fractures, surgically fixed</td>
<td>28 patients with 30 fractures</td>
<td>Exogen®</td>
<td>Yes, differs from active device</td>
<td>Not specified</td>
<td>LIPUS improved fracture healing, as 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>
</tr>
<tr>
<td>Liu et al., 2014</td>
<td>Prospective, randomised, single-blind, comparative</td>
<td>Distal radius fixed using a cast</td>
<td>81 patients</td>
<td>Most likely Exogen®, PRF not specified, 15 min/d</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-blind design</td>
</tr>
<tr>
<td>Lubbert et al., 2008</td>
<td>Prospective, multicentre, randomised, double-blind, placebo controlled</td>
<td>Midshaft clavicle fracture treated non-operatively</td>
<td>101 patients</td>
<td>Exogen®</td>
<td>Yes</td>
<td>Not specified</td>
<td>LIPUS did not accelerate fracture healing when accessed clinically</td>
<td>At weeks 1, 2, 4, 6 and 8.</td>
<td>Good spontaneous healing of clavicle fractures</td>
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<td>Maurya et al., 2019</td>
<td>Prospective, randomised, double-blind, placebo controlled</td>
<td>TMJ with a fixed functional appliance</td>
<td>40 patients</td>
<td>LIPUS 20, mean: 14.1 years old</td>
<td>Exogen® 10 d in a row and 3 times a week after</td>
<td>Yes</td>
<td>LIPUS improved TMJ remodeling and condylar head position and joint space, and as assessed by CT</td>
<td>Every 3 weeks</td>
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<td>Namera et al., 2020</td>
<td>Prospective, randomised, single-blind, placebo controlled</td>
<td>TMJ with a functional TB appliance</td>
<td>45 patients</td>
<td>LIPUS 15 (TB)</td>
<td>Exogen® 21 d in a row and every 3 weeks after</td>
<td>Yes, medical staff unblind</td>
<td>LIPUS reduced functional treatment and stimulated growth during correction</td>
<td>LIPUS accelerated healing of fractures less than 1 year old, these results were comparable to surgery</td>
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<tr>
<td>Nolte et al., 2016</td>
<td>Retrospective, observational</td>
<td>Metatarsal fractures treated either with cast and LIPUS or surgery</td>
<td>44 patients</td>
<td>LIPUS 25 (8.9 ± 3.1 years old)</td>
<td>Exogen®</td>
<td>No</td>
<td>LIPUS provided excellent functional recovery</td>
<td>Every week until bone union and every 2 weeks until functional recovery</td>
<td></td>
</tr>
<tr>
<td>Ota et al., 2017</td>
<td>Retrospective, comparative</td>
<td>Surgically fixed with IM nail; radius or ulna in children</td>
<td>19 patients</td>
<td>LIPUS 8, mean: 13 years old (11-15 years old)</td>
<td>Exogen®</td>
<td>No</td>
<td>LIPUS reduced healing time all fractures achieved functional recovery</td>
<td>Every week until bone union and every 2 weeks until functional recovery</td>
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<tr>
<td>Ota et al., 2018</td>
<td>Retrospective, comparative</td>
<td>Displaced mallet finger fractures treated either with LIPUS or surgery</td>
<td>19 patients</td>
<td>LIPUS 8, mean: 13 years old (11-15 years old)</td>
<td>Exogen®</td>
<td>No</td>
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<td>LIPUS provided excellent functional recovery</td>
<td>Every week until bone union and every 2 weeks until functional recovery</td>
<td></td>
</tr>
<tr>
<td>Ota et al., 2017</td>
<td>Retrospective, comparative</td>
<td>Surgically fixed with IM nail; radius or ulna in children</td>
<td>19 patients</td>
<td>LIPUS 8, mean: 13 years old (11-15 years old)</td>
<td>Exogen®</td>
<td>No</td>
<td>LIPUS reduced healing time all fractures achieved functional recovery</td>
<td>Every week until bone union and every 2 weeks until functional recovery</td>
<td></td>
</tr>
<tr>
<td>Ota et al., 2018</td>
<td>Retrospective, comparative</td>
<td>Displaced mallet finger fractures treated either with LIPUS or surgery</td>
<td>19 patients</td>
<td>LIPUS 8, mean: 13 years old (11-15 years old)</td>
<td>Exogen®</td>
<td>No</td>
<td>LIPUS reduced healing time all fractures achieved functional recovery</td>
<td>Every week until bone union and every 2 weeks until functional recovery</td>
<td></td>
</tr>
</tbody>
</table>

<table>
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<tr>
<th>Source</th>
<th>Type of clinical study</th>
<th>Fracture details</th>
<th>Patients</th>
<th>LIPUS parameters</th>
<th>Sham device</th>
<th>Compliance</th>
<th>Outcome</th>
<th>Limitations</th>
<th>Follow-ups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maurya et al., 2019</td>
<td>Prospective, randomised, double-blind, placebo controlled</td>
<td>TMJ with a fixed functional appliance</td>
<td>40 patients</td>
<td>LIPUS 20, mean: 14.1 years old</td>
<td>Exogen® 10 d in a row and 3 times a week after</td>
<td>Yes</td>
<td>LIPUS improved TMJ remodeling and condylar head position and joint space, and as assessed by CT</td>
<td>Every 3 weeks</td>
<td></td>
</tr>
<tr>
<td>Namera et al., 2020</td>
<td>Prospective, randomised, single-blind, placebo controlled</td>
<td>TMJ with a functional TB appliance</td>
<td>45 patients</td>
<td>LIPUS 15 (TB)</td>
<td>Exogen® 21 d in a row and every 3 weeks after</td>
<td>Yes, medical staff unblind</td>
<td>LIPUS reduced functional treatment and stimulated growth during correction</td>
<td>LIPUS accelerated healing of fractures less than 1 year old, these results were comparable to surgery</td>
<td></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>44 patients</td>
<td>LIPUS 25 (8.9 ± 3.1 years old)</td>
<td>Exogen®</td>
<td>No</td>
<td>LIPUS provided excellent functional recovery</td>
<td>Every week until bone union and every 2 weeks until functional recovery</td>
<td></td>
</tr>
<tr>
<td>Ota et al., 2017</td>
<td>Retrospective, comparative</td>
<td>Surgically fixed with IM nail; radius or ulna in children</td>
<td>19 patients</td>
<td>LIPUS 8, mean: 13 years old (11-15 years old)</td>
<td>Exogen®</td>
<td>No</td>
<td>LIPUS reduced healing time all fractures achieved functional recovery</td>
<td>Every week until bone union and every 2 weeks until functional recovery</td>
<td></td>
</tr>
<tr>
<td>Ota et al., 2018</td>
<td>Retrospective, comparative</td>
<td>Displaced mallet finger fractures treated either with LIPUS or surgery</td>
<td>19 patients</td>
<td>LIPUS 8, mean: 13 years old (11-15 years old)</td>
<td>Exogen®</td>
<td>No</td>
<td>LIPUS reduced healing time all fractures achieved functional recovery</td>
<td>Every week until bone union and every 2 weeks until functional recovery</td>
<td></td>
</tr>
</tbody>
</table>
Table 1. 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>Patel et al., 2015</td>
<td>Prospective, comparative</td>
<td>Minimally displaced mandibular fracture through intermaxillary fixation</td>
<td>28 patients</td>
<td>1 MHz, I_{rup} = 1.5 W/cm², PRF not specified</td>
<td>No</td>
<td>Performed by medical staff, compliance is not specified</td>
<td>LIPUS-accelerated healing and improved clinical mobility were 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>Raza et al., 2016</td>
<td>Prospective, randomised, double-blind, placebo controlled</td>
<td>Torque on tooth root during orthodontic procedure</td>
<td>10 patients</td>
<td>Exogen®</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 micro-CT</td>
<td>Very small patient cohort</td>
</tr>
<tr>
<td>Salem and Schmelz, 2014</td>
<td>Prospective, randomised, comparative</td>
<td>Distraction osteogenesis of tibia (Ilizarov fixator)</td>
<td>21 patients</td>
<td>Exogen®</td>
<td>No</td>
<td>Not specified</td>
<td>LIPUS shortened healing time, as measured both 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-Rodriguez et al., 2019</td>
<td>Prospective, randomised, double-blind, comparative</td>
<td>Rib fracture</td>
<td>47 patients</td>
<td>1 MHz, 0.5 W/cm², DC 10 %, 1 min/d, PRF 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-centre, randomised, double-blind, placebo controlled</td>
<td>Distraction osteogenesis of tibia (Ilizarov fixator)</td>
<td>55 patients</td>
<td>Exogen®</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, as measured radiographically and by weight-bearing</td>
<td>Inadequate compliance</td>
</tr>
</tbody>
</table>
Table 1. LIPUS for fresh fractures and distraction osteogenesis.

<table>
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<tr>
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</tr>
</thead>
<tbody>
<tr>
<td>Song et al., 2019</td>
<td>Retrospective, comparative</td>
<td>Bilateral tibial lengthening over nail (also fixed using an Ilizarov fixator)</td>
<td>30 patients</td>
<td>LIPUS 15, mean: 22.1 years old (17.5-34.0 years old)</td>
<td>Exogen®</td>
<td>No</td>
<td>LIPUS enhanced callus formation and accelerated bone healing, as assessed radiographically</td>
<td>At weeks 1, 2, 3 and 4, and monthly until healing</td>
<td>Retrospective study without sham control</td>
</tr>
<tr>
<td>Tsumaki et al., 2004</td>
<td>Prospective, randomised, comparative</td>
<td>Bilateral one stage opening–wedge high tibia osteotomy by hemicallotasis</td>
<td>21 patients</td>
<td>Left or right were randomly with/without LIPUS, mean: 68 years old (53 to 78 years old)</td>
<td>Exogen®</td>
<td>No</td>
<td>100 % compliance; LIPUS applications performed by medical staff</td>
<td>Every week</td>
<td>No placebo control and unblinded study design</td>
</tr>
<tr>
<td>Urita et al., 2013</td>
<td>Prospective, randomised, single-blind, comparative</td>
<td>Shortening osteotomy of ulnar or radius</td>
<td>27 patients</td>
<td>LIPUS 14, mean: 52 years old (34-70 years old)</td>
<td>Exogen®</td>
<td>No</td>
<td>LIPUS accelerated bone healing, as assessed radiographically</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, randomised, double-blind, placebo controlled</td>
<td>Chevron osteotomy for hallux valgus</td>
<td>52 osteotomies in 44 patients</td>
<td>LIPUS 26, mean: 51 years old (20-77 years old)</td>
<td>Exogen®</td>
<td>Yes</td>
<td>LIPUS had no effect on radiographic and clinical healing</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 years old)</td>
<td>Only compliant patients were included in the study; details not specified</td>
<td>Exogen®</td>
<td>No</td>
<td>96 % of fresh fractures healed</td>
<td>Not specified</td>
<td>Retrospective study without any controls</td>
</tr>
</tbody>
</table>
less than 90% compliance without the appropriate sensitivity analyses. The present review excluded two well-controlled studies, in which fresh tibial 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 immobilised in a cast and treated by LIPUS. Both studies reported that the radiographically assessed healing time was significantly decreased by the LIPUS treatment; however, they were excluded based on a 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; TRUST Investigators writing group et al., 2016), as defined by Schandelmaier et al. (2017a), investigated the healing of fresh tibial fractures fixed using only a reamed IM nail. 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 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 immobilised in the cast, on the other hand, might have a 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 present several challenging tasks for the orthopaedic 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 RCT involving alternative treatments such as 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 standardised definition of delayed- and non-union fractures, including the absence of a universal agreement on whether radiographic, clinical or both criteria should be used to characterise those bones (Bhandari et al., 2012; Corrales et al., 2008; Özkan et al., 2019). Surgical intervention is a first-line treatment for most 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 orthopaedic 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 for LIPUS effects on delayed- and non-unions (Table 2) 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).

As far as it can be ascertained, only one multicentre, randomised, placebo-controlled clinical trial evaluating the effects of LIPUS on delayed bone healing (minimal fracture age 4 months) and enrolling a total of 101 subjects with a 91% final compliance has been performed (Schofer et al., 2010). The study reported an increase in bone-mineral density and a decrease in fracture gap for the LIPUS-active group at the 16-week follow-up, although no statistically significant difference in the number of healed fractures between the groups was found. As it was mentioned by 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 not statistically significant (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 have evaluated biopsies of fibulae with delayed healing within a randomised double-blind, placebo-controlled trial, revealing that LIPUS increased osteoid thickness and bone mineralisation (Rutten et al., 2008), which, most likely, occurred through the locally enhanced osteogenic differentiation of cells (Rutten et al., 2009). However, both studies were based on very small patient cohorts.
<table>
<thead>
<tr>
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<th>Compliance</th>
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</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 years old</td>
<td>Exogen®</td>
<td>No</td>
<td>8 patients were lost during follow-up Not specified how it was measured</td>
<td>Union was achieved in 57.89 % of the cases A small inter-fragmentary gap was a predictor of success</td>
<td>At 6 weeks; 3 and 6 months; 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 d)</td>
<td>256 patients, 65.8 ± 11.5 years old</td>
<td>Exogen®</td>
<td>No</td>
<td>Not measured</td>
<td>Delayed healing in young patients with obesity, psychosis, anaemia, chronic lung disease Surgery 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 years old (19-85 years old)</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 years old</td>
<td>Exogen®</td>
<td>No</td>
<td>Not specified</td>
<td>32.4 % healed successfully, the rest had to undergo revision surgery</td>
<td>At 6 and 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 years old</td>
<td>Exogen®</td>
<td>No</td>
<td>Not specified</td>
<td>13 out 14 non-unions healed successfully within a range of 61-217 d</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>
<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>
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</tr>
<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 years old</td>
<td>Exogen®</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; 18-22 years old</td>
<td>Exogen®</td>
<td>No</td>
<td>Not specified</td>
<td>76 % of delayed-union healed as assessed by X-ray and CT scans</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 years old</td>
<td>Exogen®</td>
<td>No</td>
<td>Not specified</td>
<td>85 % of non-unions healed radiographically and clinically</td>
<td>In 1- to 2-month intervals until complete healing</td>
<td>Small patient cohort; no comparison group; no sham treatment</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, 79 ± 22 years old</td>
<td>Exogen®</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 years old</td>
<td>Exogen®</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 (two-centre)</td>
<td>Hindfoot non-unions after revision surgery with internal fixation</td>
<td>13 patients Mean: 51 years old (15-71 years old)</td>
<td>Exogen®</td>
<td>No</td>
<td>Not specified</td>
<td>12 out of 13 cases healed</td>
<td>Radiographs at 3, 6 and 12 weeks; CT scans 3 months after surgery</td>
<td>Small patient cohort; no comparison groups: surgery only, LIPUS only</td>
</tr>
<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 years old</td>
<td>Exogen®</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>
</tbody>
</table>
## Table 2. LIPUS for delayed- and non-union bones.

<table>
<thead>
<tr>
<th>Source</th>
<th>Type of study</th>
<th>Fracture details</th>
<th>Patients</th>
<th>Mean age ± SD or range</th>
<th>LIPUS parameters</th>
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</tr>
</thead>
<tbody>
<tr>
<td>Majeed et al., 2020</td>
<td>Prospective, observational</td>
<td>Foot and ankle post-trauma and post-surgery non-unions</td>
<td>47 patients</td>
<td>Mean: 56.6 years old (23-76 year-old)</td>
<td>Exogen®</td>
<td>No</td>
<td>Not specified</td>
<td>77 out of 77 non-union healed, assessed clinically. 26 of healed cases were atrophic, 91% of delayed-unions and 87% of non-unions healed. Hol &amp; failed cases, no differences in cytokine concentrations in blood. Decrease in TGF-β1 observed in healed group at week 1.</td>
<td>Retrospective study without any controls</td>
<td>Lack of any controls</td>
</tr>
<tr>
<td>Mayer et al., 2000</td>
<td>Retrospective, observational</td>
<td>Delayed unions and non-unions at various locations</td>
<td>1317 patients</td>
<td>20-70 years old</td>
<td>Exogen®</td>
<td>No</td>
<td>Not specified</td>
<td>91% of cases more than 75% used device for more than 75% recorded by device. 86% of delayed-unions and 87% of non-unions healed.</td>
<td>Retrospective study without any controls</td>
<td>Lack of any controls</td>
</tr>
<tr>
<td>Moghaddam et al., 2016</td>
<td>Prospective, observational</td>
<td>Long bones non-unions at various locations</td>
<td>23 patients</td>
<td>43.0 ± 13.5 years old</td>
<td>Exogen®</td>
<td>No</td>
<td>Not specified</td>
<td>72% of cases used device for more than 75% recorded by device. 88% of non-unions healed.</td>
<td>Retrospective study without any controls</td>
<td>Not specified</td>
</tr>
<tr>
<td>Nolte et al., 2001</td>
<td>Retrospective, observational</td>
<td>Non-unions at various locations</td>
<td>71 patients</td>
<td>Mean: 40 years old (17-89 year-old)</td>
<td>Exogen®</td>
<td>No</td>
<td>Not specified</td>
<td>73% of non-unions healed by radiographic and clinical assessment.</td>
<td>Retrospective study without any controls</td>
<td>Very small patient cohort</td>
</tr>
<tr>
<td>Rutten et al., 2007</td>
<td>Retrospective, observational</td>
<td>Tibia non-unions</td>
<td>13 patients</td>
<td>Mean: 74 years old (74-79 years old)</td>
<td>Exogen®</td>
<td>No</td>
<td>Not specified</td>
<td>86% of non-unions healed.</td>
<td>Retrospective study without any controls</td>
<td>Not specified</td>
</tr>
<tr>
<td>Rutten et al., 2008</td>
<td>Prospective, randomised, double-blind, placebo controlled</td>
<td>Delayed union of osteotomised fibula</td>
<td>13 patients</td>
<td>Mean: 57 years old (52.3-59 year-old)</td>
<td>Exogen®</td>
<td>Yes</td>
<td>Not specified</td>
<td>88% of non-unions healed.</td>
<td>Retrospective study without any controls</td>
<td>Very small patient cohort</td>
</tr>
</tbody>
</table>

Note: LIPUS = Low-Intensity Pulsed Ultrasound; Exogen® = Manufacturer's branded term.
<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>Rutten et al., 2009</td>
<td>Prospective, randomised, double-blind, placebo controlled</td>
<td>Delayed union of osteotomised fibula</td>
<td>7 patients LIPUS 3 (54.3 ± 10.3 years old) Control 4 (50.8 ± 5.9 years old)</td>
<td>Exogen®</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-centre, randomised, double-blind, placebo controlled</td>
<td>Delayed union of tibia</td>
<td>101 patients LIPUS 51 (42.6 ± 14.6 years old) Control 50 (45.1 ± 11.9 years old)</td>
<td>Exogen®</td>
<td>Yes</td>
<td>91 % compliance if evaluate only ‘completers’</td>
<td>LIPUS accelerated healing: improved BMD and reduced gap, as observed by CT No clinical effect at 16 weeks</td>
<td>At 1, 2, 3 and 4 months</td>
<td>Larger (but non-significantly) number 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 years old (14-76 years old)</td>
<td>Exogen®</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 years old</td>
<td>Exogen®</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, randomised, comparative</td>
<td>Postmenopausal osteoporosis LIPUS applied at distal radius</td>
<td>20 females, 69.1 ± 7.6 years old</td>
<td>Exogen®, 5 times a week for 3 months</td>
<td>No</td>
<td>Not specified</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 years old) Control 38 (56.9 ± 6.8 years old)</td>
<td>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 application (within USA): limited number of patients per group</td>
</tr>
<tr>
<td>Warden et al., 2001</td>
<td>Prospective, randomised, double-blind, placebo controlled</td>
<td>Osteoporosis following spinal cord injury LIPUS applied at calcaneus</td>
<td>15 males, 23.9 ± 7.3 years old Control: contralateral part</td>
<td>1 MHz 3.3 kHz PRF 3.3 % DC $I_{SATA}=30 \text{ mW/cm}^2$ 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, as assessed by DXA and quantitative ultrasound</td>
<td>At 6 weeks</td>
<td>Small patient cohort; not clear whether staff was blinded towards treatment; short follow-up period</td>
</tr>
</tbody>
</table>
The lack of positive evidence for the LIPUS treatment in fixed fresh fractures, based on the three unbiased studies highlighted above (Schandelmaier et al., 2017a), also advised against the ultrasound technique for 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 summarised 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 remodelling, the fractured bone is reconstituted ad integrum (Løi et al., 2016; Marsell and Einhorn, 2011). If one or more phases of this well-orchestrated process are compromised, a non-union occurs. Based on radiographic and histological assessments, these non-unions can be further categorised 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 remodelling. 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 stabilisation 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 shown in animal studies (Grundnes and Reikeras, 1993a; Grundnes and Reikeras, 1993b; Park et al., 2002). It is usually the strongest within several days to a week and declines with time in a normal healing scenario (Løi 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 secrete exosomes with enhanced anti-inflammatory potential, which alleviates TNF-α-induced inflammation of endothelial cells (Li et al., 2019). The LIPUS treatment also supports the transition of inflammatory to resident macrophages, enhances gene expression of anti-inflammatory factors and improves spinal fusion in a rat animal model (Zhang et al., 2019). The anti-inflammatory potential of ultrasound stimulation has been as well described in several other studies (da Silva Junior et al., 2017; Li et al., 2003; Nakao et al., 2014; Yang et al., 2017).

When MSCs are isolated from hypertrophic non-union fractures, they show strong differentiation potential into all three lineages in vitro, i.e. chondrogenic, adipogenic and osteogenic (Iwakura et al., 2009). The same cell type isolated from atrophic non-unions not only undergo senescence and growth arrest but also have a significantly lower osteogenic differentiation potential (Bajada et al., 2009). The co-stimulation of mesenchymal cells isolated from patients with different non-union types with BMP-7 and LIPUS significantly enhances the osteogenic potential of these cells (Koga et al., 2013). Unfortunately, the effect of LIPUS alone is not described. The expression and activation of BMPs and their antagonists are 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 enhances 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.

Mechanical loading in the properly stabilised fracture induces NO production, which in turn modulates bone adaptation to the applied stimulus (Klein-Nulend et al., 2014). NO signalling is especially deregulated in patients with atrophic non-unions (Wijnands et al., 2012). LIPUS stimulation of osteoblasts augments NO release via nuclear factor-kB signalling pathway (Hou et al., 2009). NO signalling induces expression of VEGF-A and HIF-1α in LIPUS-treated osteoblasts (Wang et al., 2004). This promotes tube formation by endothelial cells, which is crucial for angiogenesis and is often debilitated in pathological fractures. NO release also activates other pathways, such as canonical Wnt/β-catenin signalling in osteoblasts and osteocytes, which is known to influence bone mass (Krishnan et al., 2006). The secretion of DKK-1, antagonising Wnt-signalling (Pinzone et al., 2009), is enhanced in the culture medium of MSCs isolated from patients with atrophic non-unions (Bajada et al., 2009). LIPUS may be able to counteract this effect, since Wnt-signalling is enhanced in stimulated osteoblasts and osteoprogenitors (Olkku et al., 2010).

The expression of MMPs, regulating cell attachment, migration, release of biologically active molecules and 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 non-union fractures results in impaired bone remodelling (Ding et al., 2018). LIPUS mechanical stimulus enhances MMP-13 expression in long-term cultured osteoblasts (Unsworth et al., 2007), which could potentially improve ECM turnover, critical for successful tissue regeneration.

The key biological signatures of a non-union fracture and the hypothetical LIPUS effects influencing
them are summarised in Fig. 2. Despite the positive evidence of LIPUS stimulation, most of the studies 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 the authors’ knowledge, only two preclinical in vivo studies, investigating the effects of LIPUS on a hypertrophic non-union, have been published so far, demonstrating contradictory 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 to biochemical signalling pathways of the human body, eventually leading to impaired mechanoadaptation and compromised fracture regeneration (Haffner-Luntzer et al., 2016). Aged individuals experience a reduction in osteoprogenitor cells (Kasper et al., 2009), with a reduced osteogenic potential (D’Ippolito et al., 1999; Ross et al., 2000) and an altered response to mechanical stimulation (Kasper et al., 2009). Additionally, changes in shape of osteocytes and the number of canaliculi per lacuna are found in aged organisms, which dampens their mechanosensitivity and could result in an inefficient interaction between 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 declines moderately with increasing age (Zura et al., 2015a). MSCs isolated from aged rats experience enhanced expression of osteogenic markers, i.e. Runx-2 transcription factor and osteocalcin, when stimulated with high intensity LIPUS, in comparison to cells isolated from young rats (Puts et al., 2016a). This might imply that due to changes in mechano-responsiveness of the osteoprogenitors with increasing age, an adjustment of the LIPUS-stimulation protocol is required. The accelerated fracture healing following LIPUS exposure was also confirmed in in vivo studies performed with aged rodents (Aonuma et al., 2014; Katano et al., 2011); however, the relevance of these results for the clinical setting remains 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 that 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 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 years of bone depletion due to osteoporosis, found that LIPUS stimulation of the calcaneus bone did not influence its bone mineral content (Warden et al., 2001). In this study, shorter pulses of ultrasound stimulation were used 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 studies using an ovariectomised rat osteoporosis model have shown the beneficial effects of 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

---

**Pathogenesis of non-union**

- Inflammation ↑
- Osteogenesis of MSCs ↓
- BMPs vs. antagonists imbalance
- NO signalling ↓
- Angiogenesis ↓
- Wnt signalling ↓
- ECM remodelling ↓

**Possible effects of LIPUS**

- Inflammation ↓
- + BMP-7 → ↑ osteogenesis of MSCs
- BMPs and BMP receptors ↑
- NO signalling ↑
- Angiogenesis ↑
- Wnt signalling ↑
- ECM remodelling ↑

---

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).
effects of ultrasound in rodents might partially mimic a low-magnitude high-frequency whole-body vibration therapy, which shows promising results in improving BMD in postmenopausal women (Kasturi and Adler, 2011a; Lai et al., 2013; Rubin et al., 2004; Verschueren et al., 2004).

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 local stimulation with ultrasound. As discussed by 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 has limited 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 was demonstrated by Czobor and Skolnick (2011), non-compliant patients can disguise the efficacy of a tested therapy. In this study, the compliant patients were screened out based on the detection of the drug metabolite in their blood over the course of treatment. A comparison of the compliant patients, which comprised 70% of the patients, to the placebo group confirmed the drug’s efficacy, whereas the non-compliant group did not differ from the control. Moreover, the same compliance assessed by counting consumed pills was more than 92%. Adherence to the study protocol carries even a bigger challenge for treatments outside the medical facility, resulting in a biased data interpretation (Pounder et al., 2016; Pullar et al., 1989). LIPUS application is usually prescribed to the patients as a long-term treatment and requires a 20 min time window every day. Therefore, motivation and dedication of the patients plays an indispensable role in the study outcome. Certain factors, such as age and fracture site, could significantly affect the adherence to the prescribed LIPUS protocol (Matsubara et al., 2015). The detailed description of patient compliance in the reviewed studies is summarised in Table 1-3.

There is a considerable variability in documentation regarding patients’ compliance in LIPUS clinical trials. Some studies reported the number of patients available at the end of the treatment out of the whole sample, whereas others additionally supplied the number of days and min/d of LIPUS application accomplished by the patients. It is not always clear, though, whether the active minutes were counted only when the device was in 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 increasing patient device-application compliance (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 the patients performed less than 50% of LIPUS applications found LIPUS ineffective (Emami et al., 1999; TRUST Investigators writing group et al., 2016; Simpson et al., 2017). As an example, exclusion of non-compliant patients (as reported by the recordings on the device) in a study of LIPUS-treated non-unions revealed pro-healing effects of sonication comparable to surgical intervention (Bawale et al., 2020). Studies, where the compliance is not descriptively documented are ambiguous regarding the efficacy of LIPUS therapy (Table 1-3).

A stringent weekly control of adherence to the prescribed protocol, requiring a minimum 15 min-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 treatment was reported in the placebo group. The active support of patients and communication with the medical personnel seem to improve the compliance significantly, favouring LIPUS therapy (Arimoto et al., 2019; Gopalan et al., 2020; Maurya et al., 2019; Namera et al., 2020; Patel et al., 2015; Santana-Rodriguez et al., 2019; Tsumaki et al., 2004; Zacherl et al., 2009). This should be considered when planning a clinical trial. New generation Exogen® devices might also help raising patients’ awareness on the treatment progress and support their motivation through direct feedback of an integrated calendar (Pounder et al., 2016). In summary, an inclusion in the scientific studies of the detailed information on the number of completed days and minutes of LIPUS treatment, along with a population size that was intended to be treated and actually adhered to the protocol, can aid an adequate judgment of 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 I_SAVA (Exogen®)] originate from a preclinical rabbit model (Duarte, 1983). Since then, little effort has been made to optimise this acoustic dose. With the exception of 9 studies (see Materials and Methods, and Table 1 and 3), the rest of the studies applied Exogen®-like parameters.

The current evidence for LIPUS-induced pro-regenerative potential in bone shows 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 studies hyperbolise 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).

The two most described in vitro LIPUS set-ups, transmitting ultrasound through gel from the bottom of the tissue culture plate or through the medium from the top of the cells, exposes them to the near field of the transducer, which is prone to large spatial and temporal intensity variations (described in detail by Padilla et al., 2014). Although Harrison et al. (2016) argued that the near-field ultrasound-stimulation represents the closest configuration to the clinical setting, the cells and transducer, in those in vitro experiments, are usually separated by several mm. 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 (Leskinen and Hynynen, 2012). These are, most likely, hardly present in the clinical configurations and should be further evaluated starting with in silico analyses.

The Exogen® LIPUS-probe, widely used in preclinical studies, has a diameter of 22 mm, which exposes the stimulated site to an 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 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 studies 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 larger and more diverse resident cell populations embraced by the mechanical stimulation are – including the ones in the bone epiphyses where a 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, the thin soft-tissue layers and small bone-circumferences of a rat result in a stimulation of the fracture in the most heterogeneous near-field of the transducer. Fig. 3a, depicting the numerical simulation of the ultrasound field generated by the Exogen® probe, shows how large the stimulation area of a fractured rat femur with LIPUS is and how high are the intensity fluctuations in the near field of the transducer. 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).

In contrast to the unproportional scaling down of

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**Fig. 3.** Schematic drawing of a fractured rat-femur positioned in a simulated sound field produced by (a) a clinically used Exogen® probe and (b) a 5 MHz focused probe producing a ~6 dB spot of 7.4 × 0.6 mm. (a) The fracture or osteotomy gap region was exposed to a highly inhomogeneous near field of the transducer and almost the entire femur received the acoustic stimulation. (b) The acoustic energy was deposited in the gap region only. The simulations were performed using Field II program and showed transmit temporal peak intensity. The pin locations of a typically used external fixation device (Rohrbach et al., 2013) are also shown.
the acoustic dose from the clinical setting to in vivo and in vitro, is the application of 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 the clinic is performed at a concentration of either 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 vivo in rats and rabbits 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 et al., 2016). In vitro, cells are usually stimulated using 50-5,000 ng/mL of BMP-2 (Chen et al., 2018; Chen et al., 2019; Kim et al., 2013; Ning et al., 2019). Although supraphysiological doses of the growth factor are used in clinics, the studies 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 obtained from small-animal long bones fixed with an IM nail and stimulated with ultrasound, 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; TRUST Investigators writing group et al., 2016). To compare adequately the influence of LIPUS on in vivo bone regeneration in small animals and translate these findings to the clinical setting, set-ups with well-controlled physical effects need to be applied (Horne et al., 2020; Puts et al., 2016b; Subramanian et al., 2013). Then, further optimisation of the reproducible clinical acoustic dose might be required (Warden, 2003; Warden et al., 2000). Until it is possible to decipher the essential mechanisms of bone regeneration by the defined acoustic stimulation, using the spatially adjusted set-ups translated from human to preclinical models, in vitro and back, the potential benefits of LIPUS will remain underestimated in the clinic.

Discussion

Upon the onset of a long-bone fracture, the orthopaedic surgeon has to make rapid and efficient decisions as to what are the best treatment options for the patient. 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 are these 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 remain within the reference levels in up to 20 % of those cases (Bishop et al., 2012; Nauth et al., 2018). Given these and other risks that the surgical procedures have, they cannot be used as a universal treatment solution: elderly individuals with chronic metabolic disorders and other underlying health conditions as well as people with certain lifestyles where the long recovery time is not desired, are the candidates for 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 4 facets of the concept, i.e. cells, matrix, growth factors and mechanical stability, are in balance (Busse et al., 2014; Emami et al., 1999; TRUST Investigators writing 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 for 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 are still in place and LIPUS can help supporting the biomechanical environment (Leighton et al., 2017; Majeed et al., 2020; Watanabe et al., 2013). However, these hypotheses 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

The present review attempted to emphasise the limited knowledge on the principal mechanisms of the LIPUS technique and on the lack of adequate clinical evaluation. Research is needed to better understand the in vitro and in vivo biological and physical mechanisms involved, using set-ups ensuring an adequate translation of the optimal acoustic dose to the clinical setting. Conducting double-blind, randomised, placebo-controlled clinical trials is required for various bone fracture types (fresh, delayed- and non-union), in cast and fixed with implants, for large patient cohorts. Moreover, these studies should ideally be non-industry funded so as to eliminate potential bias. Clinical trials need to be supplied with regular follow-up appointments and easy access to communication with the medical personnel. Detailed documentation of patient compliance is needed, 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 the duration of treatment. It should also be specified whether the active minutes recorded by the LIPUS device were counted only when the probe was in direct skin contact. Additionally, investigation and optimisation of LIPUS-treatment protocols for fractures in aged individuals and patients with chronic metabolic disorders, where complementary methods could be used, is worth considering.

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Hurdles and outcomes to LIPUS application in clinic


Discussion with Reviewers

Reviewer 1: Do you think an advanced design of an \textit{in vitro} set-up might improve the comparability of the LIPUS stimulation? Would a “tissue-mimicking” \textit{in vitro} approach be an option?

Authors: Due to the complexity of physical phenomena induced by LIPUS, which are highly dependent on the structural and material properties of the interrogated material, the physical sub-mechanisms differ \textit{in vivo} vs. \textit{in vitro}. A better understanding of which sub-mechanisms are encountered in the clinical setting and their proper translation into advanced \textit{in vitro} and \textit{in vivo} set-ups, supported by \textit{in silico} studies can indeed help to decipher the resulting biological
phenomena. Most of the existing in vitro set-ups do not allow for controlled transfer of the acoustic dose and, furthermore, introduce physical artefacts, as discussed by Padilla et al. (2014). This produces misleading results that most likely do not reflect the clinical reality. Creating tissue constructs, mimicking as closely as possible the material properties of bone and other surrounding tissues could be an excellent way to study the physico-biological mechanisms of ultrasound stimulation and could be object of future research. This research could yield a re-optimisation of the LIPUS acoustic parameters originated from a rabbit animal model (Duarte, 1983). Such studies should be performed using advanced in vitro and in vivo set-ups.

**Reviewer 1:** Based on all the information given in the present review, LIPUS might be effective but a good clinical trial is still missing. What could be the main reasons why a well-designed trial was not conducted even through LIPUS has been used since the early 1990s?

**Authors:** We do not have a clear explanation on why a well-designed trial has not yet been conducted. We can only speculate that clinical trials with non-unions, for example, are unlikely to include LIPUS as a first-line treatment. Patients might be referred to it only after the failure of other type of treatments (e.g. surgery). However, for more “simple” fracture types, we believe that the hurdles in conducting such trials might be more related to the difficulty of finding a funding source, especially if companies are not willing to sponsor them. We can only speculate that LIPUS-device manufacturers, principally Bioventus, who sells the Exogen® system, do not see the need of sponsoring further a long and costly clinical trial to improve acceptance and/or rentability of their operations. The device is already approved by several regulatory agencies worldwide and it seems to be commercially successful. Additionally, provider-sponsored trials raise questions of bias, diminishing the concluded findings. We purposely decided not to contact manufacturers on this issue to remain neutral and propose an objective review of published data.

**Reviewer 2:** What is/are the main future research direction(s) of LIPUS on bone regeneration?

**Authors:** A thorough characterisation of acoustic dose in preclinical models, followed by its translation to human is an important first step towards the reproducibility and acceptance of the LIPUS therapy. This dose should be further optimised for “special conditions”, such as bones with impaired healing, elderly individuals and patients with underlying health conditions. The defined parameters should be tested in preclinical models and verified in well-controlled clinical studies. LIPUS has been also shown to have synergistic effects in vitro and in vivo, when used together with other therapies, e.g. growth factors such as BMP-2 (Angle et al., 2014, additional reference) and BMP-7 (Koga et al., 2013; Lee et al., 2013, additional reference) and mesenchymal stromal cells (Carina et al., 2017; Chen et al., 2019; Polo-Corrales et al., 2018, additional references), enhancing effects of those treatments. This could be another direction towards exploration of the LIPUS capabilities for tissue regeneration.

**Reviewer 2:** Is LIPUS scientifically sound for clinical application for bone regeneration?

**Authors:** There is no doubt that stimulation with LIPUS induces pro-regenerative processes in biological tissues, such as bone, and that this therapy has potential to be used for clinical treatment of bone fractures. However, at this point, randomised double-blind clinical trials with defined and characterised acoustic doses, enrolling large patient cohorts and ensuring patients compliance following support of the medical personnel, are necessary to draw definitive conclusions.

**Melanie Haffner-Luntzer:** What lessons can we learn from animal models regarding LIPUS application during fracture healing and what might be the limitations?

**Authors:** Preclinical models are crucial for evaluation of a therapy’s efficacy, determination of the underlying mechanisms and optimisation of conditions for its improvement. The use of LIPUS in small animal models, such as rats and rabbits, has shown profound pro-regenerative effects in bone fractures at various locations. However, translation of those findings to the clinical setting, unfortunately, has not always been found successful. One of the biggest limitations to translate preclinical results to the clinical setting could be the fact that the same probes and stimulation parameters were used in most of the preclinical and in the clinical studies, although animal and human proportions, including the soft tissue amount or the bone defect size, differ greatly. This brings us to the question of whether the LIPUS acoustic parameters are directly translatable from preclinical models to patients, or if there is a so-called “acoustic dose” that is suitable for a small animal and which should be then appropriately scaled for a human. Depending on type of fracture, fracture location, patients’ characteristics and their medical history, this acoustic dose needs to be standardised and further tested in preclinical models and clinical studies.

**Additional References**


Editor’s note: The Guest Editor responsible for this paper was Anita Ignatius.