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The effects of LIPUS on soft-tissue healing: a review of literature

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Introduction: Ultrasound is widely used for imaging purposes and as an adjunct to physiotherapy. Low-intensity pulsed ultrasound (LIPUS), having removed the thermal component found at higher intensities, is used to improve bone healing. However, its potential role in soft-tissue healing is still under investigation.

Material and methods: We searched on Medline using the keywords: low-intensity pulsed ultrasound, LIPUS and LIPUS and soft-tissue healing. Thirty-two suitable articles were identified.

Results: Research, mainly pre-clinical, so far has shown encouraging result, with LIPUS able to promote healing in various soft tissues such as cartilage, inter-vertebral disc, etc. The effect on the bone-tendon junction, however, is primarily on bone. The role of LIPUS in treating tendinopathies is questionable. Adequately powered human studies with standardisation of intensities and dosages of LIPUS for each target tissue are needed.

Keywords: low intensity pulsed ultrasound/soft tissue healing

Introduction

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Ultrasound is widely used for imaging purposes and as an adjunct to physiotherapy. Low-intensity pulsed ultrasound (LIPUS), having removed the thermal component found at higher intensities, is used to improve bone healing. However, its potential role in soft-tissue healing is still under investigation.

Ultrasound is widely used as an imaging modality[1] at intensities of 0.05–0.5 W/cm^2. At intensities of 0.2–100 W/cm^2, the surgical and therapeutic benefits of ultrasound have also been explored.[1] In orthopaedic practice, intensities of ≤ 0.1 W/cm^2 have been shown to improve bone healing.[1,2]

For the management of ligament, muscle and tendon injuries high-intensity (>0.1 W/cm^2) continuous ultrasound has been used

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therapeutically for >50 years.[3,4] However, studies on the effects of therapeutic ultrasound in muscle and joint healing have been contradictory. Generally, there seem to be insufficient evidence to support the clinical use of ultrasound at these doses.[5] Following the success of ultrasonic waves applied at low intensity ($\leq$0.1 W/cm^2), in a constant frequency (1–1.5 MHz) and in a pulsatile manner (LIPUS) on osteoid tissue, the potential benefit in soft-tissue healing is being investigated.[6–9]

We thus review the currently available literature on the role of LIPUS in soft-tissue healing, investigating its effect both at cellular and tissue level, and summarising the evidence available so far.

Methods

For the purposes of this review, scientific articles were identified on Medline using the primary keywords: low-intensity pulsed ultrasound, LIPUS, tendon, soft tissue and healing. We also used the keywords: LIPUS and tendon, LIPUS and tendon healing, LIPUS and soft tissue, LIPUS and soft-tissue healing, low-intensity pulsed ultrasound and tendon, low-intensity pulsed ultrasound and tendon healing, low-intensity pulsed ultrasound and soft tissue and low-intensity pulsed ultrasound and soft-tissue healing. Additional publications were identified searching manually the references sections of each of the articles identified using the above-mentioned keywords.

Exclusion criteria: Publications in language other than English, articles not discussing the subject matter, studies where low-intensity ultrasound was used continuously and studies where intensity of ultrasound was >0.1 W/cm^2 were excluded. Also, studies where primary action of LIPUS was on osteoid tissue [e.g. bone-tendon junction (BTJ)] were excluded to specifically focus on the effect of LIPUS in soft-tissue healing.

We thus identified 32 articles fulfilling the inclusion criteria.

Biophysics of ultrasound therapy

Ultrasound, an acoustic radiation, is a form of mechanical energy that can be transmitted into the body as a high-frequency pressure wave. The acoustic energy generated from ultrasound is produced from a piezoelectric crystal within a transducer, which emits high-frequency acoustic pressure waves (1–12 MHz) transmitted through body tissues by molecular vibrations and collisions.[10] The micromechanical strains produced by these pressure waves in body tissues can result in biochemical events at the cellular level.[11] This ultrasonic energy can be

divided into two categories: high-intensity ultrasound with peak intensities from 5000 to 15 000 W/cm^2, and low-intensity ultrasound with intensities of 0.5–3000 mW/cm^2. This low-intensity ultrasound is used in physical therapy, whereas the high intensities are used in treating tumours by selectively heating tissue and causing necrosis.[12]

Biological effects of LIPUS

Passing through the tissue, the ultrasonic energy is absorbed at a rate proportional to the density of the tissue. Thus, the radical changes in density inherent in a healing tissue may well establish the gradients of mechanical strain.[13] Absorption of the ultrasound signal also results in energy conversion to heat.[14] While this heating effect is extremely small for low frequency ultrasonic waves, well below 1°C, some enzymes, such as matrix metalloproteinase-1, or collagenase, are exquisitely sensitive to small variations in temperature.[15] Therefore, ultrasound may serve to re-establish or normalize effective metabolic temperatures in tissue-healing regions; this effect, although subtle, may be biologically profound.[16] Furthermore, at interfaces of distinct densities, much of the incident radiation energy will be reflected, resulting in complex gradients of acoustic pressure through the tissue.[17]

Having removed the high thermal component of the higher intensities while maintaining the transmission of acoustic mechanical stress to the target tissue, LIPUS stimulates the union of non-uniting fractures, promotes the healing in a congenital pseudoarthrosis of the tibia, increase the matrix synthesis in chondrocytes, stimulates the differentiation in osteoblast precursor cells and induces the expression of growth factors, proteins and prostaglandins within osteoblasts, chondrocytes and fibroblasts.[1,8–27] Furthermore, cartilage healing assisted with LIPUS produces fewer degenerative changes in the long term, as compared with controls.[28]

In this review, the role of LIPUS in a soft-tissue healing will be discussed at cellular level first, giving a background of laboratory-based evidence before dwelling into various animal and human studies focusing on different soft tissues individually.

Effect of LIPUS on fibroblasts, myoblasts, epithelial cells and chondrocytes

In 2004, Zhou *et al.*[29] investigated the effects of daily exposure to LIPUS at an intensity of 0.03 W/cm^2 on primary human foreskin fibroblasts.

LIPUS activates RhoA (a GTPase protein) which induces the formation of F-actin stress fibres which in turn cause the recruitment of paxillin, a signal transduction adaptor protein—by a Rho-associated coiled—coil-containing protein kinase (ROCK)-dependent pathway. Furthermore, LIPUS exposure promoted a 2-fold increase in extracellular signal-related kinase (ERK) 1/2 activation, triggering DNA synthesis, and thus, cell proliferation. RhoA/ROCK is an upstream regulator of the LIPUS-induced ERK pathway. Similarly, LIPUS triggered Src, a tyrosine kinase, which further regulates the ERK cascade. ERK pathways are typically activated by mechanical stress, although, in this case, LIPUS activated a pathway, with no evidence of epidermal growth factor receptor (EGFR) involvement (EGFR is a tyrosine kinase, usually expressed in ERK pathways, triggered by direct mechanical stress). Thus, LIPUS produced results similar to mechanical stress at cellular level.[18]

LIPUS involvement in the ERK 1/2 system was also demonstrated by in a mice model. Here, the differentiation pathway of C2C12 (a subclone of C2 myoblasts from a C311 mouse) to the lineage of the osteoblast and chondroblast was observed.[25]

The effects of LIPUS appear to not only be limited to the ERK pathways. Ikai *et al.* noted a locally increased expression of heat shock protein-70 (hsp-70) in LIPUS-exposed canine gingival epithelium. The heat shock protein family is associated with the healing process and, more specifically, with the assembly of molecules utilized for endothelial cell migration and proliferation (Table 1).[30–32]

Inconsistent effects have been observed in various *in vitro* studies regarding the exact role of LIPUS in cartilage healing (Table 1). LIPUS stimulates the aggrecan m-RNA expression and proteoglycan synthesis, but it did not influence cell proliferation of rat chondrocytes.[33] However, LIPUS influences the cell proliferation in an intensity-dependent manner but did not increase the expression or synthesis of aggrecan and type II collagen of chick embryo chondrocytes.[34] In an *in vitro* atelocollagen gel-embedded culture model, LIPUS exposure promotes the synthesis of chondroitin sulfate, although it does not significantly enhance the cell number of rabbit chondrocytes.[35] From these studies, it can be concluded that exposure to LIPUS could significantly affect chondrocytes proliferation, phenotype expression and matrix production. In consistencies in these studies may be attributable to many factors, including culture media, culture models or cell senescence.

Effects of LIPUS on soft tissues

(1) *Inter-vertebral discs (IVDs)*: Results following the exposure of IVDs to LIPUS appear to feature a distinct LIPUS-activated pathway.

Table 1 The effects of LIPUS on fibroblasts, myoblasts, epithelial cells, chondrocytes and inter-vertebral discs.

Reference	Author & year	Cell type	LPUS dosage	Result(s)
29	Zhou *et al.* (2004)	Primary human foreskin fibroblasts	1 MHz, 30 mW/cm^2	LIPUS promotes extracellular receptor kinase (ERK) 1/2 expression (also known to be activated by mechanical stress), via a Rho-associated coiled-coil-containing protein kinase-dependent mechanism
25	Ikeda *et al.* (2006)	C2C12 cells, a subclone of C2 Myoblasts, from the thigh of C311 mouse	1.5 MHz, 70 mW/cm^2	LIPUS-exposed group expressed higher levels of Runx2, Msx2, Dlx5, AJ18 and Sox9, decreased levels of MyoD, C/EBP and PPARy. LIPUS stimulation involved with ERK 1/2 pathway, and converts the differentiation pathway of C2C12 cells into osteoblast and chondroblast lineage
30	Ikai *et al.* (2007)	Canine gingival epithelium	1.5 MHz, 30 mW/cm^2	Localized heat shock protein 70-positive cells found only in LIPUS-exposed epithelium
22	Mukai *et al.* (2005)	Distal femoral chondrocytes	1.5 MHz, 30 mW/cm^2	Anti-TGF-B1 neutralizing antibody reversed all changes made by LIPUS. LIPUS increased expression of type II collagen and aggrecan mRNA—increased DNA content, decreased alkaline phosphatase activity maintained
33	Parvizi *et al.* (1999)	Rat chondrocytes	1.0 MHz ultrasound signals with spatial and temporal average intensities of 50 or 120 mW/cm^2	LIPUS stimulates aggrecan mRNA expression and proteoglycan synthesis by chondrocytes
34	Zhang *et al.* (2003)	Chondrocytes from distal part of the sternum of 16-day-old chick embryos	1.0 MHz ultrasound signal applied at and 30 mW/cm^2 intensities	LIPUS influenced chondrocyte proliferation in an intensity-dependent manner
35	Nishikoli *et al.* (2002)	Cultured chondrocytes embedded in atelocollagen gel	1.5 MHz with a 30 mW/cm^2 intensity	LIPUS exposure promoted synthesis of chondroitin sulfate, especially chondroitin 6-sulfate
31	Hiyama *et al.* (2007)	Canine nucleus pulpous cells	1.5 MHz, 30 mW/cm^2	In LIPUS-exposed groups—(i) TGF-BR1 gene expression increased, (ii) proteoglycan synthesis, and hence, water content increased, (iii) TGF-B1 promotes IVD cell proliferation and matrix synthesis
36	Iwashina *et al.* (2005)	Nucleus pulposus cells and annulus fibrosus cells from rabbits	0.5 MHz, 60 mW/cm^2	LIPUS increases sensitivity of NP cells to cytokines. Ca^{2+} ion signalling necessary for LIPUS to stimulate aggrecan synthesis - Ca^{2+} channels known to be mechanically-gated

Continued

Table 1 *Continued*

Reference	Author & year	Cell type	LPUS dosage	Result(s)
37	Omi *et al.* (2008)	Rabbit intervertebral disc cells	1.5 MHz, 30 mW/cm^2	LIPUS stimulation significantly activated tissue inhibitor of metalloproteinase-1 and monocyte chemotaxis protein-1 in nucleus pulposus cells and macrophages at both the protein and gene levels suggesting that LIPUS may be a promising supplemental treatment for intervertebral disc herniation
38	Iwabuchi *et al.* (2005)	Herniated disc resorption (HDR)	1.5 MHz, 30 mW/cm^2	LIPUS enhanced the HDR via matrix metallo proteinases-3 activation through tumor necrosis factor-alpha and macrophage chemo attractant protein-1 pathways
39	Miyamoto *et al.* (2005)	Bovine intervertebral disc cells	1.5 MHz, 30 mW/cm^2	The application of pulsed low-intensity ultrasound increased proteoglycan content in alginate beads containing inner and outer annulus fibrosus cells ($P < 0.05$). Collagen synthesis by cells isolated from the intervertebral disc was increased by the application of pulsed low-intensity ultrasound (16–19% increase, $P < 0.05$–0.0001)

In nucleus pulposus (NP) cells, Hiyama *et al.* reported an increased expression of transforming growth factor-beta type 1 (TGF-BR1) receptor gene on exposure to LIPUS. Transforming growth factor-beta 1 (TGF-B1) promotes IVD cell proliferation and matrix synthesis, as well as playing a part in the regulation of chondrocytes.[31] This is attributed to the structural similarities between chondrocytes and the NP and annulous fibrosus cells of the IVDs.[36] Exposure of these cells to recombinant TGF-B1 mimicked the effects produced by LIPUS, whereas the exposure of LIPUS-treated cells to anti-TGF-B1 would reverse all the beneficial effects induced by LIPUS.[31] Also, a marked increase in proteoglycan synthesis within the NP cells encouraging the uptake of water was observed, resulting in the potential regression of disc herniation.

Also, investigating the effect of LIPUS on NP cells, Omi *et al.*[37] noted not only a 2.06-fold up-regulation of tissue inhibitor of metallo-proteinase-1, but also a 2.3-fold up-regulation in local macrophage monocyte chemotaxis protein-1 compared with controls. Similar findings have been presented by other studies,[38] suggesting that LIPUS-induced macrophage migration may play an important role in IVD remodelling and possibly IVD hernia regression, proposing that macrophages could enter IVDs following the increased capillary blood flow and cell-membrane permeability caused by LIPUS stimulation of fine vibrations in local tissue.[37] Studies by Miyamoto *et al.*[39] demonstrated increased collagen synthesis by cells isolated from the intervertebral disc by the application of LIPUS (16–19% increase, $P < 0.05–0.0001$), suggesting that LIPUS may prove useful for tissue engineering of intervertebral disc tissue in future (Table 1).

(2) *Ligaments*: Several studies investigated the effect of LIPUS in comparison to non-steroidal anti-inflammatory drugs (NSAIDs) in relation to ligamentous healing. They concluded that LIPUS accelerated but did not improve ligament healing, whereas NSAIDs delayed but did not impair healing. When used in combination, the beneficial LIPUS effect was not impaired by the detrimental NSAID effect, suggesting that LIPUS does not influence the cyclo-oxygenase pathway which is normally inhibited by NSAIDs.[40,41] Other studies found LIPUS effective in accelerating rat medial collateral ligament injury healing at an intensity of 0.03 W/cm^2.[42,43] (Table 2).

(3) *Tendons*: Therapeutic ultrasound has been found to have no effect over and above placebo on tendon healing.[44] Research focused on the role of LIPUS on tendon healing use BTJ as the main area of investigation. A closer look into the finding of these studies, however, reveals that LIPUS improves BTJ healing mainly by enhancing bony in-growth and osteo-integration.[44–48] The study by Warden *et al.*[49] demonstrates no benefit of LIPUS therapy over and above placebo in

Table 2 The effects of LIPUS on ligament, tendon and cartilage healing.

Reference	Author & year	Cell type	US dosage	Result(s)
40	Li *et al.* (2007)	Rat bilateral ulna stress fracture induction model	1.0 MHz, 100 mW/cm^2	LIPUS facilitates repair of stress fractures, while NSAID's delay tissue-level repair. In combination, NSAID's do not impair the beneficial effects of LIPUS
41	Warden *et al.* (2006)	Rat bilateral medial collateral ligamentotomy model	1.0 MHz, 100 mW/cm^2	LIPUS accelerates healing, while NSAID's delay healing, however in groups treated with both LIPUS and NSAID's, healing was still accelerated
42	Takakura *et al.* (2002)	Male Sprague–Dawley rats with surgical transaction of the bilateral medial collateral ligaments	1.0 MHz, 30 mW/cm^2	On the 12th day, the LIPUS-treated side exhibited significantly superior mechanical properties when compared with the control side in ultimate load, stiffness and energy absorption ($P < 0.05$)
43	Sparrow *et al.* (2005)	Rat bilateral medial collateral ligamentotomy model	1.0 MHz, 30 mW/cm^2	Increased proportion of type-I collagen in LIPUS-exposed groups, giving superior early-structural properties - absorbing more energy before failure
45	Walsh *et al.* (2007)	A single digital extensor tendon autograft from the right hoof was used as the graft in 89 adult sheep for ACL reconstruction	1.5 MHz, 30 mW/cm^2	Increased integration between tendon and bone and a biologically more active interface was seen in LIPUS-treated group
46	Qin *et al.* (2006)	Rabbit partial patellectomy model	1.5 MHz, 30 mW/cm^2	LIPUS group showed significantly higher BMD at week 8 than controls. LIPUS enhanced osteogenesis at the healing bone-tendon junction (BTJ)
47	Lu *et al.* (2008)	Rabbit partial patellectomy model	1.5 MHz, 30 mW/cm^2	LIPUS-exposed cells demonstrated greater VEGF expression, with accelerated healing
48	Qin L *et al.* (2006)	Rabbit partial patellectomy model	1.5 MHz, 30 mW/cm^2	LIPUS-induced acceleration of healing at the BTJ
49	Warden *et al.* (2008)	Volunteers with clinically and radiologically confirmed patellar tendinopathy (PT)	1.5 MHz, 30 mW/cm^2	LIPUS does not provide any additional benefit over and above placebo in the management of symptoms associated with PT

50	Cook *et al.* (2001)	Osteochondrites from male rabbits	1.5 MHz, 30 mW/cm^2	Accelerated healing in LIPUS-exposed groups, with fewer long-term degenerative changes
51	Cook *et al.* (2008)	18 dogs, two autologous plugs separated from host cartilage by a 1.5 mm gap were created on the medial femoral condyle in both knees of each dog	1.5 MHz, 30 mW/cm^2	Ultrasound-treated sites had significantly improved gross appearance at 6 weeks and histologic appearance at 6 and 12 weeks
52	Jia *et al.* (2005)	Adult New Zealand rabbits with bilateral full-thickness osteochondral defects	1.5 MHz, 30 mW/cm^2	LIPUS accelerated the repair of injured articular cartilage
53	Tien *et al.* (2008)	Human articular chondrocytes isolated from young children's articular ablated polydactylia	1.0 MHz; 18 mW/cm^2, 48 mW/cm^2, 72 mW/cm^2 and 98 mW/cm^2	LIPUS was found to increase aggrecan synthesis in a time-dependent manner
54	Choi *et al.* (2006)	Human articular chondrocytes isolated from osteoarthritis patients	1.0 MHz; 0, 100, 200, and 300 mW/cm^2	Histological analysis revealed an increase in the number and size of glycosaminoglycan-positive lacunae and cellular organelles, appearing as rough endoplasmic reticulum and mitochondria by LIPUS-treated group
55	Duda *et al.* (2004)	Hyaline-like cartilage specimens generated in vitro and subcutaneously implanted in the backs of nude mice	1.5 MHz, 30 mW/cm^2	There was no significant difference between ultrasound-treated and sham-treated groups. The mechanical stability of the neocartilage specimens increased with treatment time and reached values of native cartilage after 6 weeks *in vivo*. LIPUS stimulation showed no stimulatory effect on tissue maturation

the management of symptoms associated with patellar tendinopathy. Thus, LIPUS does not seem to have direct effect on healing of tendon tissue itself.

The mechanism of action pertaining LIPUS at the BTJ revolves around the acoustic mechanical stimulation of osteoblasts and fibrocytes, acoustic vibrational aligning of collagen fibres, promoting local influx of calcium and the increased expression of angiogenic factors [i.e. vascular endothelial growth factor (VEGF)].[45–47] The increase in VEGF expression of 7% from LIPUS-treated rabbit patellar-tendon samples over control samples ($P < 0.05$) suggests that a reason for the acceleration in BTJ healing was increased early vascularity and osteoblastic differentiation[45] (Table 2).

(4) *Cartilage*: *In vivo* studies[50,51] in New Zealand rabbit and canine models with full-thickness osteochondral defects have demonstrated that exposure to LIPUS significantly improves the morphologic features and histologic characteristics of repaired cartilage. Jia *et al.*[52] studied the effect of LIPUS on 10 adult New Zealand rabbits with bilateral full-thickness osteochondral defects. The scores of the gross appearance grades, histological grades and the optical density of toluidine blue of the tissues in the experimental group were significantly higher than those of the controls at 8 weeks after injury ($P < 0.05$), suggesting that LIPUS can accelerate the repair of injured articular cartilage.

Chondrocytes isolated from young children's articular cartilage of ablated polydactylia, showed that LIPUS was found to increase aggrecan synthesis in a time-dependent manner,[53] with a maximal response observed at an intensity of 48 mW/cm^2. After 14 days of exposure at this intensity, the aggrecan and type II collagen synthesis were significantly higher than control group. However, LIPUS treatment revealed no significant influence on cell proliferation, confirming that the stimulation of aggrecan and type II collagen synthesis by LIPUS was not the result of an increase in chondrocyte cell proliferation. In addition, human chondrocytes harvested from older donors were less responsive to LIPUS.[53] Similar studies on human articular chondrocytes isolated from osteoarthritis patients demonstrated an increase in the number and size of glycosaminoglycan-positive lacunae and cellular organelles, appearing as rough endoplasmic reticulum and mitochondria in LIPUS exposed group, suggesting that viability and metabolism of human articular chondrocytes in alginate culture were induced by LIPUS treatment.[54] Studies on both animal and human tissues have shown that LIPUS enhance the healing of cartilage defects (Table 2). Whether this acceleration of chondrogenesis *in vitro* will lead to the maturation of tissue-engineered neo cartilage in a clinical setting has been questioned.[55]

Economical aspects of LIPUS therapy

Every fracture or soft-tissue injury is accompanied by direct and indirect implication on the economy of an individual and the establishment for which the individual works. The goal of modern orthopaedic surgery is to minimize this financial strain while maximizing the functional recovery after injury.[56,57] Study on potential economical benefit of LIPUS therapy in tibial fractures demonstrated substantial cost savings for third party payers, employers and government agencies after the use of LIPUS therapy, as there was lesser need for secondary procedures after LIPUS treatment thereby substantial reduction of the amount of Workers' Compensation payments.[58] The literature, however, is deficient on the economical benefits of LIPUS on soft-tissue healing.

Conclusions

Research, mainly pre-clinical, so far have shown encouraging result with LIPUS in promoting healing in various soft tissues such as cartilage, inter vertebral disc, etc. The effect on BTJ is primarily on osseous tissue. The role of LIPUS in treating tendinopathies is questionable . These preclinical studies that support the positive effect of LIPUS therapy on soft-tissue healing could be translated into human use. However, poor methodological standards in animal studies mean that positive results rarely translate to the clinical domain[59] suggesting that sufficient supporting evidence in the 'clinically justifiable' role of LIPUS in soft-tissue healing is lacking. Adequately, powered human studies together with the standardization of intensities and dosages for each target tissue are needed to build a stronger clinical database for routine clinical use. Furthermore, if the aforementioned results with LIPUS concerning IVD's, tendon and cartilage healing are repeatable and consistent in humans, they could change the future of the management of soft-tissue injuries.

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