Review Article

Review on experimental study and clinical application of low-intensity pulsed ultrasound in inflammation

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Abstract: Low-intensity pulsed ultrasound (LIPUS), as physical therapy, is widely used in both research and clinical settings. It induces multiple bioeffects, such as alleviating pain, promoting tissue repair, and shortening disease duration. LIPUS can also mediate inflammation. This paper reviews the application of LIPUS in inflammation and discusses the underlying mechanism. In basic experiments, LIPUS can regulate inflammatory responses at the cellular level by affecting some signaling pathways. In a clinical trial, LIPUS has been shown to alleviate inflammatory responses efficiently. As a cheap, safe, and convenient physical method, LIPUS is promising as anti-inflammatory therapy.

Keywords: Low-intensity pulsed ultrasound (LIPUS); inflammation; signaling pathway; therapeutic ultrasound

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Introduction

Inflammation is characterized by redness, swelling, heat, and pain, and is a defensive response to stimuli such as toxins and pathogens (1). Inflammation has been shown to play an important role in many diseases, such as osteoarthritis (OA), neurodegenerative disorders, and trauma (2-4). Inflammatory disorders bring great pain and financial burden to those affected. However, there are currently few suitable anti-inflammatory drugs. Clinically, nonsteroidal anti-inflammatory drugs (NSAIDs) or corticosteroids are generally used to subdue inflammatory responses, both of which have clear side-effects, such as gastrointestinal reactions, renal dysfunction, increased risk of infection, and so on (5,6).

Low-intensity pulsed ultrasound (LIPUS) is a form of ultrasound that delivers at a much lower intensity (<3 W/cm^2) than traditional ultrasound and outputs in the mode of pulse wave (7), which has therapeutic effects. However, until now, LIPUS has not been accurately defined. The following parameters are widely used: pulse frequency of 1.5 MHz, pulse repetition frequency of 1 kHz, and the spatial average temporal average intensity of 30 mW/cm^2 of the LIPUS transducer's surface area (8). As a physical therapy, LIPUS has been applied in many areas, including the musculoskeletal and nervous systems, dentofacial tissue, and others (8-10). In contrast with traditional drug therapy and invasive treatments, LIPUS works by emitting pulsed acoustic waves to specific regions, which is non-invasive and tolerable, with very minimal side effects. The therapeutic effects of LIPUS are mostly attributed to its non-thermal effects, predominantly cavitation, acoustic streaming, and acoustic radiation force. Cavitation, occurring in a liquid or liquid-like material, is considered to be associated with the changes of membrane permeability and activation of cells (11). Acoustic streaming, especially micro-streaming, is responsible for the diffusion rate and alteration of protein synthesis, cellular secretion, and sonoporation (12). Acoustic radiation force is capable of influencing the cardiovascular and nervous systems (13). Although the thermal effect of LIPUS is very limited because of its low intensity, it should still be considered when used in some temperature-sensitive situations, particularly when some enzymes, collagenase,

and the nervous system are implicated. It is reported that thermal deactivation is one of the most important mechanisms in the denaturation of enzymes induced by LIPUS (14).

In recent years, physical therapy has been widely discussed, especially in terms of inflammation (15-17). In the past, physical therapy was mainly used in rehabilitation. But with the deepening of research, researchers have revealed the potential of physical therapy in mediating inflammation. For example, Chen *et al.* reported that extracorporeal shock wave therapy attenuated cyclophosphamide-induced acute interstitial cystitis in rats, which was initially a physical treatment for kidney stones (18). Gardner *et al.* reported that exercise therapy improved circulating markers of endothelium-derived inflammation in patients with peripheral artery disease (19). These findings suggest that physical therapy is a promising anti-inflammatory method (20). The physical therapy that is LIPUS has also attracted significant attention. Initially, LIPUS was used to promote tissue repair. It could accelerate wound healing, decrease edema, and soften scar tissue (21). These changes were partly ascribed to the effect of LIPUS on the inflammatory phase of the repair process (22). To date, LIPUS has been confirmed to regulate inflammatory responses in many fields. The underlying mechanism has been shown to be related to the alteration of cytokines and signaling pathways. Here, we review the application of LIPUS in inflammatory situations, including both experimental studies and clinical applications.

Experimental study

Many basic studies, both *in vivo* and *in vitro*, have accumulated regarding the role of LIPUS in inflammation. Multiple studies have also explored the underlying mechanisms of LIPUS. In this section, we review the application of LIPUS in inflammation at the cellular level (*Table 1*).

Table 1 The application of LIPUS in experimental study

Targets cells	Sources	LIPUS parameters	Results
White cells	Nagata *et al.* (4)	A frequency of 3 MHz with a spatially averaged intensity of 30 mW/cm^2 and pulsed 1:4 (2 ms on and 8 ms off)	Decreasing the number of inflammatory infiltrate cells (lymphocytes, plasma cells, macrophages, and neutrophil leukocytes)
	Hsieh *et al.* (23)	A frequency of 1.0 MHz, irradiation intensity of 0.1 W/cm^2 and 20% duty cycle for 20 min per treatment session	Decreasing lymphocytic inflammatory infiltration
	Signori *et al.* (24)	A frequency of 1MHz, an intensity of 0.4 W/cm^2 and I_{SATA} 0.08 W/cm^2	Promoting neutrophils and monocytes to surgical incision in the biceps femoris muscle in rats at 1 hour post-surgery
	da Silva Junior *et al.* (25)	A frequency of 1 MHz and medium intensity of 0.4 W/cm^2 in 1:5 pulsed mode	Reducing the number of neutrophils one day after injury
	Montalti *et al.* (26)	A frequency of 1.5 MHz, 1:4 duty cycles and I_{SATA} 30 mW/cm^2	Increasing inflammatory infiltration on TA muscle repair after 7 days in cryoinjured rats
Macrophages	Zhang *et al.* (1)	A frequency of 1.5 MHz, a pulse duty cycle of 1:4, a pulse repetition frequency of 1.0 kHz and various intensities (10, 30, 60, and 90 mW/cm^2)	Alleviating the expression of inflammatory factors induced by LPS in macrophages.
	da Silva Junior *et al.* (25)	A frequency of 1 MHz and medium intensity of 0.4 W/cm^2 in 1:5 pulsed mode	Reducing in the number of M1 macrophages after one day and Increased the number of M2 macrophages after two days
	Zhang *et al.* (27)	A frequency of 1.5 MHz, 20% duty cycle and 30 mW/cm^2	Suppressing the production of mature 1L1β in macrophages
	Ogata *et al.* (28)	A frequency of 1.875 MHz, pulse repetition frequency 4.90 kHz, number of cycles 32, voltage applied to each transducer element, 17.67 volts (V), and I_{SPTA} 117–162 mW/cm^2	Attenuating macrophage infiltration
	Zheng *et al.* (29)	A frequency of 1 MHz, duty cycle of 20%, pulse repetition frequency of 100 Hz, output intensity of 0.5 W/cm^2, 100 mW/cm^2 I_{SATA}	Inhibiting the expression of pro-inflammatory cytokines in macrophages.

Table 1 *(continued)*

Table 1 *(continued)*

Targets cells	Sources	LIPUS parameters	Results
Vascular Endothelial Cells	Shindo *et al.* (30)	A frequency of 1.875 MHz, pulse repetition frequency 4.90 kHz, number of cycles 32, voltage applied to each transducer element, 17.67 volts (V), and I_{SATA} 117–162 mW/cm^2	Enhancing angiogenesis and ameliorates left ventricular dysfunction
	Ogata *et al.* (28)	A frequency of 1.875 MHz, pulse repetition frequency 4.90 kHz, number of cycles 32, voltage applied to each transducer element, 17.67 volts (V), and I_{SATA} 117–162 mW/cm^2	Attenuating perivascular fibrosis
	Li *et al.* (31)	A frequency of 1MHz, pulse repetition frequency of 1.5KHz, I_{SATA} 47.12 mW/cm^2, pulse width of 200 ms and intensity distribution of 1-100mW/cm^2	Suppressing the oxidative stress-induced endothelial-Mesenchymal transition
Dendritic cells(DCs)	Li *et al.* (32)	1.5MHz frequency pulses, with a pulse width of 200 ìs, repeated at 1 kHz and 30 mW/cm^2 I_{SATA}	Increasing the amount of of miRNA-16 and miRNA-21
	Wang *et al.* (33)	Center frequency: 1.1 MHz; duty factor: 10%; repetition frequency: 100 Hz	Promoting DCs mature in the tumor microenvironment
Osteoblasts	Tang *et al.* (34)	A frequency of 1.5 MHz, 200ms burst width with repetitive frequency of 1 kHz at the intensity of 30 mW/cm^2	Increasing PGE 2 formation and the protein and mRNA levels of COX-2
	Bandow *et al.* (35)	A frequency of 1.5MHz, 200-msec burst sine waves at 1.0 kHz, and an intensity of 30mW/cm^2	Increasing the expression of RANKL, MCP-1 and MIP-1β
	Nakao *et al.* (2)	A frequency of 1.5 MHz, 200 ìs burst sine waves at 1.0 kHz, and was delivered at an intensity of 30 mW/cm^2	Inhibiting LPS-induced mRNA expression of RANKL, CXCL 1 and CXCL 10
osteoclast	Feres *et al.* (36)	A square envelop with duration of 200 ms, a carrier frequency of 1.5 MHz and an intensity of 30 mW/cm2	Increasing osteoclast resorptive activity in the absence of osteoblasts
Chondrocytes	Uddin *et al.* (37)	A frequency of 1 MHz, an intensity of 30mW/cm^2 with a pulse duration of 200ìs repeated at 100Hz	Inhibiting the catabolic action of IL-1β
	Jia *et al.* (38)	A frequency of 0.6 MHz, pulse repetition frequency of 300 Hz, 120 mW/cm^2I_{SATA} and a duty cycle of 20%	Decreasing the concentration of PGE2 and NO.
Synovial cells	Nakamura *et al.* (39)	A frequency of 3 MHz with a spatial-average intensity of 30 mW/cm^2, and pulsed 1:4 (2 ms on and 8 ms off)	Inhibiting COX-2 and PGE2 expression induced by IL-1β
	Nakamura *et al.* (40)	A frequency of 3 MHz with a spatial-average intensity of 30 mW/cm^2 and pulsed 1:4 (2 ms on and 8 ms off)	Reducing Cox-2 expression and synovial hyperplasia in vivo
	Sato *et al.* (41)	A frequency of 3 MHz with a spatial-average intensity of 30 mW/cm^2, and pulsed 1:4 (2 ms on and 8 ms off).	Suppressing synovial hyperplasia and synovial cell proliferation
Glial cell	Chen *et al.* (42)	A frequency of 1.0 MHz with 528 mW/cm^2 I_{SPTA}	Reducing neutrophil infiltration, and microglial activation
	Chen *et al.* (43)	A frequency of 1.0-MHz, a duty cycle of 20% with 528 mW/cm^2 I_{SPTA}	Inhibiting the activation of astrocytes and reducing the protein levels of TNF-α, IL-1β, and IL-6 in the mice brain induced by LPS

LIPUS, low intensity pulsed ultrasound; I_{SPTA}, spatial peak temporal average intensity; $I_{SATA,}$ spatial averaged-temporal intensity; LPS, Lipopolysaccharide; IL1β, Interleukin 1β; PGE 2, prostaglandin E2 ; COX-2, cyclooxygenase-2; RANKL, Receptor Activator for Nuclear Factor-κB Ligand; MCP-1, monocyte chemotactic protein 1; MIP, macrophage-inflammatory protein; CXCL, the C-X-C motif chemokine ligand; NO, Nitric Oxide; TNF-α, Tumor Necrosis Factor α.

White blood cells

As we know, white blood cells play a key role in the repair process. After an injury, the inflammatory response is activated, and white blood cells are recruited to clean up foreign material, inhibit bacterial infection, and subsequently help orchestrate the tissue repair response (44). As time goes on, inflammation gradually subsides, and injured tissues begin to recover. If the infiltration of white blood cells is persistent, inflammation cannot subside, leading to tissue damage and fibrosis. It has been confirmed that LIPUS promotes the repair of injured tissues, and its effect is associated with the inflammatory infiltration stage of repair. On the one hand, LIPUS can accelerate tissue repair by increasing the infiltration of white blood cells. By increasing inflammatory infiltration, the inflammatory response is completed, and the injured tissue is advanced to the next phase more efficiently (22). For example, Signori el al. reported that LIPUS induced neutrophils and monocytes to the surgical incision in the biceps femoris muscle of rats at 1 h post-surgery (24), which was beneficial to form a barrier against the invasion of micro-organisms and clean necrotic parts. On the other hand, LIPUS can alleviate inflammatory infiltration, which is detrimental to repair in the late phase of inflammation. In a muscle injury model, caused by cryoinjury of the tibialis anterior (TA) muscle in rats, da Silva Junior *et al.* found LIPUS reduced the number of neutrophils 1 day after the injury, which minimized tissue damage caused by the release of reactive oxygen species, proteases, and other lysosomal constituents of these cells (25). Interestingly, Montalti *et al.* also reported that compared to untreated rats, the application of LIPUS 24 h after the surgical procedure increased inflammatory infiltration on TA muscle repair after 7 days in cryoinjured rats (26). However, granulation tissue and newly formed muscle fibers displayed better tissue structure organization in the LIPUS group after 13 days. Although the definite mechanism of LIPUS in repair is not clear, these differences possibly relate to inconsistencies in the degree of injury. In the study by Montalti *et al.*, the injury was severe, which meant it required more inflammatory cells to infiltrate and more time to repair. The application of LIPUS accelerated this process. The improvement in tissue organization in the LIPUS group confirmed that LIPUS was beneficial for repair.

Moreover, inflammatory infiltration also plays a critical role in some inflammatory diseases. Inflammatory cells are recruited in the lesion and release proinflammatory cytokines, which lead to tissue damage. It has been reported that LIPUS also affects inflammatory infiltration in inflammatory diseases. Nagata *et al.* reported that the amount of infiltrating inflammatory cells (lymphocytes, plasma cells, macrophages, and neutrophil leukocytes) on the LIPUS-treated side was decreased significantly compared with that on the control side in the TA muscle in C57BL/6 mice injured by cardiotoxin (4). They also found that LIPUS decreased cyclooxygenase-2 (COX-2) gene and protein expression at the late stage in the injured TA muscle, which was typically considered proinflammatory. Consistently, Hsieh *et al.* reported that the infiltration of inflammatory cells was less apparent in the LIPUS-treated group in a post-traumatic knee OA model established by anterior cruciate ligament transection and meniscectomy in rats (23). There was a significant reduction in cellularity and lymphocytic inflammatory infiltration observed in the knee joint synovium of the LIPUS group as compared with the control group. Inflammatory infiltration in the lesion is often considered as a sign of early disease and results in pain, edema, and other symptoms. The early inflammatory reaction is a possible target for potential therapeutic intervention (45). These findings suggest that LIPUS has the potential to regulate inflammation in the lesion. Further clinical studies are required to investigate this mechanism.

Generally, LIPUS has a bi-directional effect on white blood cells. In the early stage of inflammation in repair, LIPUS promotes the infiltration of white blood cells, which accelerates wound cleaning and is beneficial to repair. And in the last stage of inflammation in repair and in some inflammatory diseases, LIPUS alleviates the infiltration, which prevents tissue damage. However, the underlying mechanism is still not clear.

Macrophages

Macrophages participate in immune and inflammatory responses. It has been reported that macrophages and monocytes are sensitive to biomechanical stimulation and distinct mechanical impacts are likely to induce different effects on macrophages (46-48). The ultrasound pressure wave has been proven to influence the behavior of macrophages (35).

In tissue repair, macrophages have been shown to exhibit critical regulatory activity at all stages of healing and fibrosis (49), and LIPUS can regulate the activity of macrophages in the repair process. For example, in a cryoinjury rat model, da Silva Junior *et al.* reported that LIPUS led to reductions in the number of M1 (inflammatory

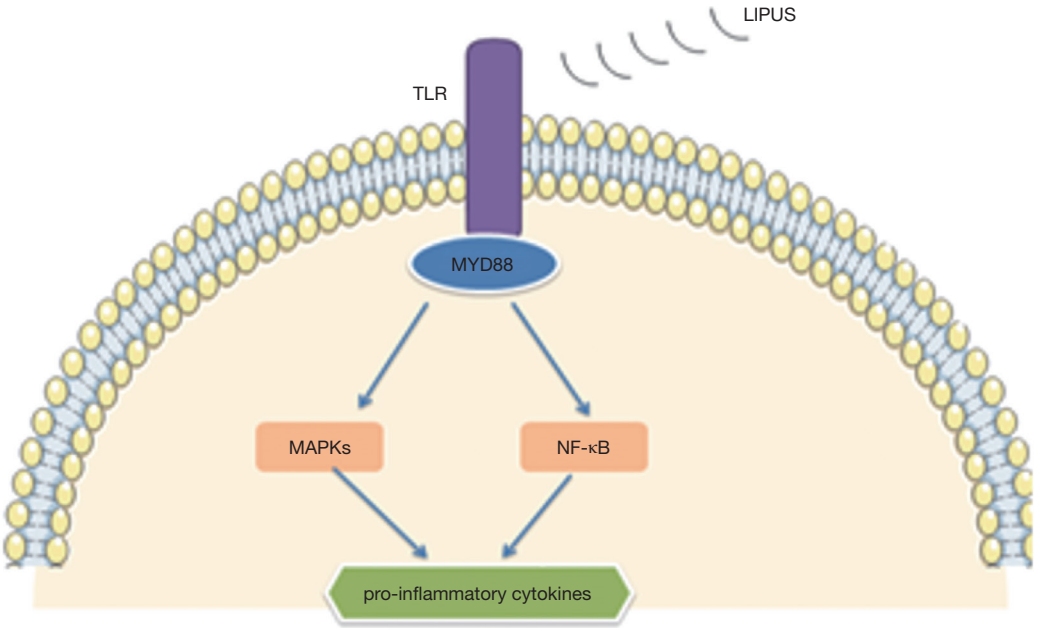

Figure 1 The MAPK signaling pathway and NF-κB signaling pathway are mediated by LIPUS through TLR to regulate inflammatory responses. When exposed to LIPUS, the TLR/MyD88 complex is inhibited. The down-streaming signaling pathways (NF-κB and MAPK signaling pathways) are also suppressed. These changes lead to the decrease of pro-inflammatory cytokines, such as TNF-α, IL-1β, IL-6, and so on. LIPUS, low intensity pulsed ultrasound; TLR, toll-like receptor; MYD88, myeloid differentiation factor 88; MAPKs, mitogen-activated protein kinases; NF-κB, nuclear factor κB; TNF-α, tumor necrosis factor α; IL-1β, interleukin-1β; IL-6, interleukin-6.

profile) macrophages after 1 day and increased the number of M2 macrophages (anti-inflammatory or reparative profile) after 2 days (25). The decrease of M1 macrophages suggested that LIPUS prevents persistent inflammatory responses in repair. The increase of M2 macrophages suggests that LIPUS is beneficial to the repair response. Generally, LIPUS can modulate the phenotype of macrophages in injured tissue to promote repair, but the specific mechanism remains unknown.

Lipopolysaccharide (LPS) is one of the primary pathogenic factors in many diseases, such as periodontitis, and macrophage cells are 1 of the target cells of LPS. Recently, in U937 macrophage cells, Zhang *et al.* reported that LIPUS alleviated the expression of inflammatory factors induced by LPS, such as tumor necrosis factor-α (TNF-α), interleukin (IL)-1β, IL-6, and IL-8 (1). Further, they revealed that this process was modulated by suppressing the toll-like receptor 4 (TLR 4) –nuclear factor κB (NF-κB) signaling pathway (*Figure 1*). Zhang *et al.* also reported that LIPUS suppressed the production of mature 1L1β in macrophages in a destabilization of the medial meniscus (DMM) mouse model made by surgery and air pouch model injected LPS (27). They found LIPUS inhibited the production of mature IL1β by enhancing autophagy flux, which was associated with the autophagy-mediated degradation of sequestosome-1 (SQSTM1), a receptor protein promoting ubiquitinated protein degradation, and the autophagic degradation of pyruvate kinase isoenzyme type M2 (PKM) in an SQSTM1-dependent manner (*Figure 2*). Zheng *et al.* also reported that LIPUS treatment alleviated LPS-induced inflammatory response on RAW264.7 macrophage cells (29). In their experiment, LIPUS treatment decreased LPS-induced elevation of pro-inflammatory cytokines (TNF-α and IL-6) and activated caveolin-1. And the phosphorylation of p38 mitogen-activated protein kinase (MAPK) and extracellular signal-regulated kinase (ERK) was inhibited by LIPUS (*Figure 3*). Caveolin-1 is 1 of 3 structural proteins of caveolae, flask-shaped plasma membrane invaginations, and takes part in cell metabolism, proliferation, and inflammatory response (50-53). It is expressed ubiquitously in all cells and can be affected by many factors, such as shear stress alteration, some proinflammatory factors, and so on. Besides, Ogata *et al.* reported that the macrophage

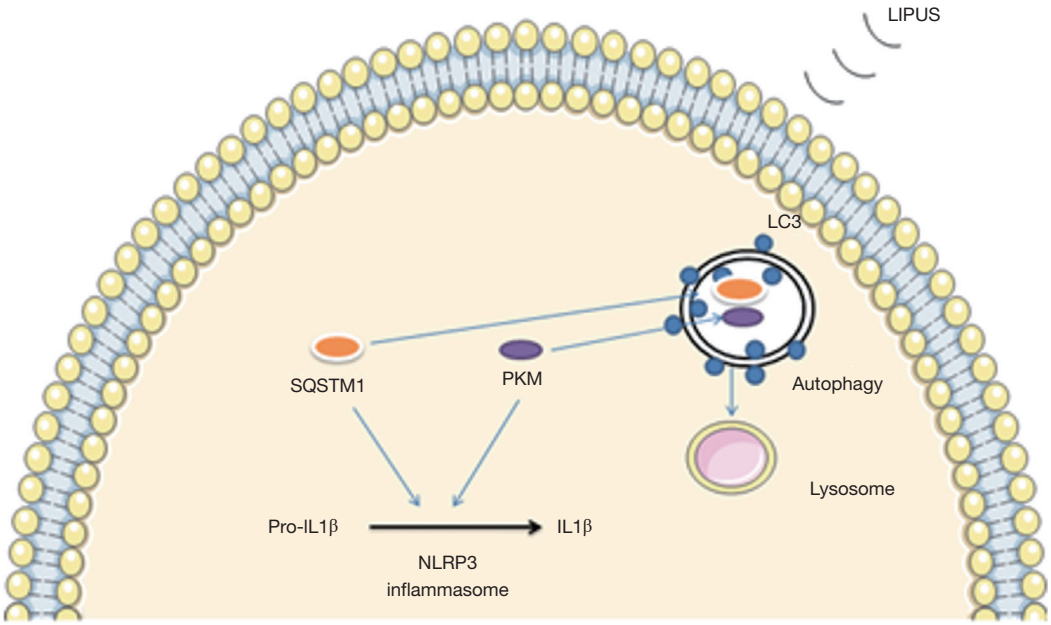

Figure 2 The production of mature IL-1β in macrophages is suppressed by LIPUS. LIPUS enhances the autophagy flux in macrophages, which promotes the degradation of SQSTM 1 and PKM in autophagy-dependent way. The decrease of SQSTM 1 and PKM inhibits the production of mature IL-1β. SQSTM 1-dependent autophagic degradation of PKM 2 in macrophages. LIPUS, low intensity pulsed ultrasound; SQSTM 1, sequestosome 1; PKM 2, pyruvate kinase M2; NLRP3, NLR family, pyrin domain containing 3; IL1β, interleukin 1β.

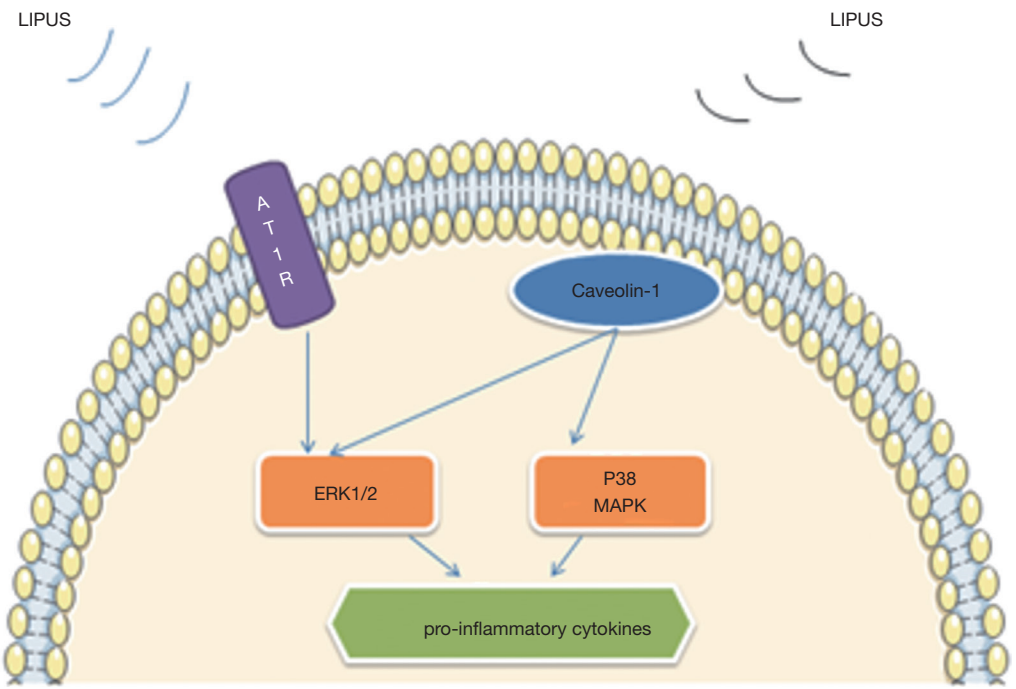

Figure 3 The MAPKs signaling pathway is affected by LIPUS through AT1 and caveolin-1 to mediate inflammation. The phosphorylation of ERK is induced by LIUPS through AT 1, and this increases the expression of inflammatory cytokines. However, the activation of caveolin-1 induced by LIPUS can suppress the expression of inflammatory kinases through MAPKs. LIPUS, low intensity pulsed ultrasound; AT 1, angiotensin type 1; MAPKs, mitogen-activated protein kinases; ERK ½, extracellular signal-regulated kinase.

infiltration was significantly attenuated after irradiation with LIPUS in left ventricle pressure-overloaded hearts in mice induced with transverse aortic constriction (28).

Overall, LIPUS can regulate the phenotype of macrophages (decrease M1 macrophages and increase M2 macrophages) in repair and prevent macrophage infiltration in inflammatory diseases. These effects are associated with signaling pathways, such as the MAPK and NF-κB signaling pathways.

Vascular endothelial cells (VECs)

VECs can sense blood flow-induced mechanical stimuli and convert these stimuli into a sequence of biological responses (54). This suggests VECs may sense other mechanism stimuli, such as LIPUS. Recently, Shindo *et al.* stated that LIPUS enhanced angiogenesis and ameliorated left ventricular dysfunction in a mouse model of acute myocardial infarction (ligation of the proximal left anterior descending coronary artery) (30). They suggested that caveolin-1 played a critical role by transmitting the mechanical stimuli to intracellular signaling pathways with subsequent phosphorylation of Fyn, focal adhesion kinase (FAK), ERK1/2, and protein kinase B (Akt), and resultantly enhanced the expression of vascular endothelial growth factor (VEGF) and angiogenesis. These findings suggest that LIPUS can possibly regulate anti-inflammatory responses in VECs. Previous studies demonstrated that shock wave therapy could improve heart function through its anti-inflammatory effects (55). The therapeutic effects of shock wave therapy and LIPUS are both caused by the mechanical energy from acoustic waves. For some diseases, shock wave therapy and LIPUS have similar therapeutic effects, such as promoting fracture repair, improving erectile function, and protecting cartilage tissue (56-58). Therefore, LIPUS seems to also exert anti-inflammatory effects in VECs. Recently, Ogata *et al.* reported myocardial perivascular fibrosis was significantly attenuated after irradiating with LIPUS in chronic left ventricular pressure overload mice (28). They indicated that LIPUS therapy attenuated perivascular fibrosis by suppressing anti-inflammatory responses; however, the mechanism is unknown. In human aortic endothelial cells, Li *et al.* reported that LIPUS produced cytoprotective effects against oxidative injuries to endothelial cells through suppressing the oxidative stress-induced endothelial-mesenchymal transition and limiting cell migration and excessive extracellular matrix deposition (31). In addition, they found the phosphatidylinositol 3-kinase (PI3K)/Akt pathway was activated by LIPUS under oxidative stress. Activation of PI3K increased the expression of endothelial markers (CD31 and VE-cadherin) and decreased the expression of mesenchymal markers (FSP-1 and α-SMA) *(Figure 4)*. Oxidative stress also exists in inflammation. It has been confirmed that the PI3K/Akt pathway plays a critical role in inflammation in vascular endothelial cells. Thus, LIPUS may exert its anti-inflammatory effects through suppressing oxidative stress and activating the PI3K/Akt pathway in VECs.

Generally, LIPUS has protective effects on vascular endothelial cells. The protective effects can partly ascribe to the anti-inflammatory effects of LIPUS. The Caveolin-1 and PI3K/Akt signaling pathways may be one of the underlying mechanisms.

Dendritic cells (DCs)

DCs are specialized antigen-presenting cells, which have an important involvement in the induction and amplification of immune responses during inflammatory reactions (59,60). DCs produce proinflammatory cytokines and exosomes, which can release some anti-inflammatory compounds, such as microRNAs (miRNAs) (61). The miRNAs regulate gene expression post-transcriptionally, function within the cells in which they are transcribed, and several specific miRNAs (miRNA-145 and miRNA-146a) have recently demonstrated an ability to suppress inflammatory immune responses (62). For example, Hui *et al.* reported that miRNA-145 attenuated high glucose-induced oxidative stress and inflammation in retinal endothelial cells (63). Exosomes have been reported as potentially promising novel therapeutics for inflammation (64,65). Previous studies have indicated that DCs can sense the stimulation of ultrasound waves (66). Recently, Yang *et al.* also reported that LIPUS enhanced DC-derived exosome biogenesis and docking (67). In an *in vitro* experiment, Li *et al.* explored the effects of LIPUS for bone marrow dendritic cells (BMDCs) in atherosclerosis (AS). They found that the exosomes derived from LIPUS-treated BMDCs contained higher amounts of miRNA-16 and miRNA-21, which possessed anti-inflammatory functions (32). Human umbilical vein endothelial cells injected with these exosomes expressed less intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) and had lower

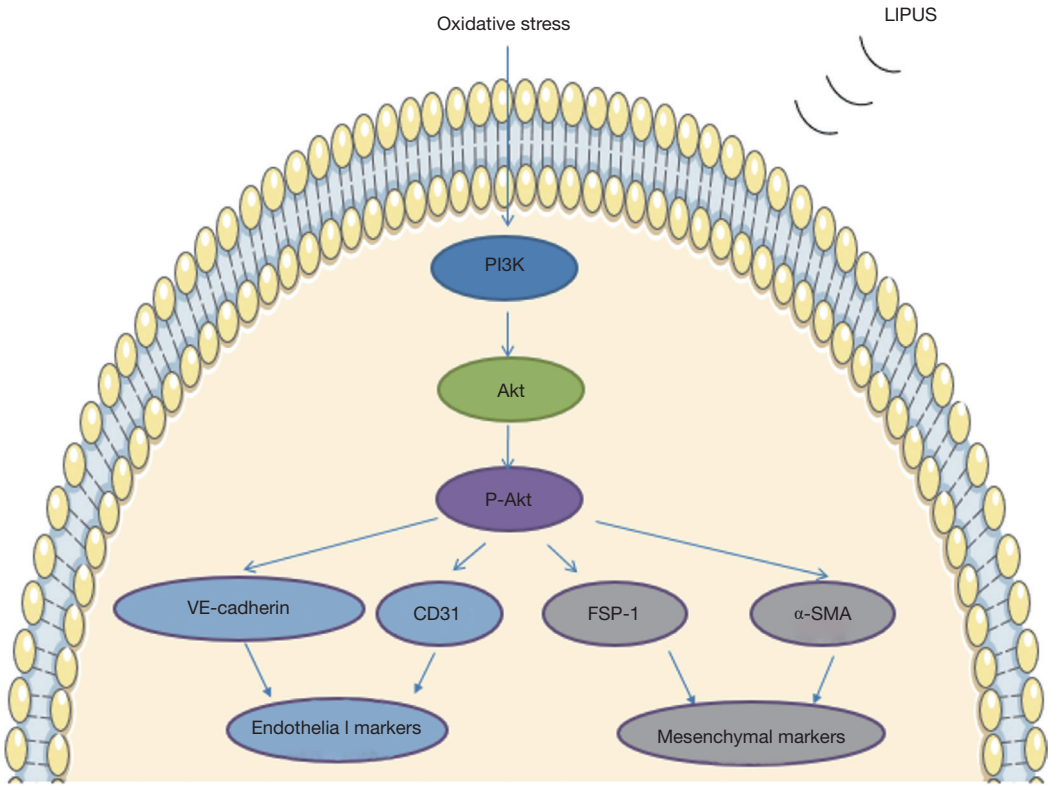

Figure 4 Oxidative injuries are alleviated by LIPUS through the activation of the PI3K-Akt signaling pathways. LIPUS can protect vascular endothelial cells from oxidative injuries. Under oxidative stress, LIPUS activates PI3K-Akt signaling pathways. Activation of PI3K increases the expression of endothelial markers (CD31 and VE-cadherin) and decreases the expression of mesenchymal markers (FSP-1 and α-SMA). LIPUS, low intensity pulsed ultrasound; PI3K, phosphatidylinositol 3-kinase; Akt, protein kinase B; FPS-1, fibroblast specific protein; α-SMA, α-smooth muscle actin.

activity of NF-kB signaling in response to TNF-α (*Figure 5*). Previous studies revealed that miRNA-16 inhibited the activity of the NF-κB signaling pathway through suppressing the expression of IκB kinase, and miRNA-21 limited the activation of the NF-κB signaling pathway through inhibiting the expression of proinflammatory factors (68,69). Consistently, Li *et al.* also found that exosomes dose-dependently attenuated TNF-α-induced p65 phosphorylation and stimulated inhibitory-kB kinase α (IKKα) degradation. These findings suggest that LIPUS can alleviate inflammations through mediating the exosome of DCs.

However, in a tumor microenvironment, DCs become immature and deprived of their abilities of antigen-presenting cells, which contributes to the state of immune equilibrium and immune escape (70). It has been reported that LIPUS can promote immature DCs to develop into mature DCs, which would enhance the inflammatory immune response in tumor tissue. The mature DCs enriched microenvironment is not favorable for the survival of tumors. Wang *et al.* reported that LIPUS facilitated DCs maturation and increased the expression of IL-10, interferon (IFN)-γ, and TNF-α, which was not conducive to the survival of tumor tissue (33). It suggests that LIPUS is harmful to the survival of tumors by enhancing the inflammatory response.

In conclusion, DCs can sense the stimuli of LIPUS and translate this stimulus into bioeffects to regulate inflammation. The DC-derived exosome and NF-κB signaling pathways may be involved in this process. In special tumor microenvironments, LIPUS promotes mature DCs to active the inflammatory immune response within tumors.

Osteoblasts

Osteoblasts, differentiated from mesenchymal stem cells

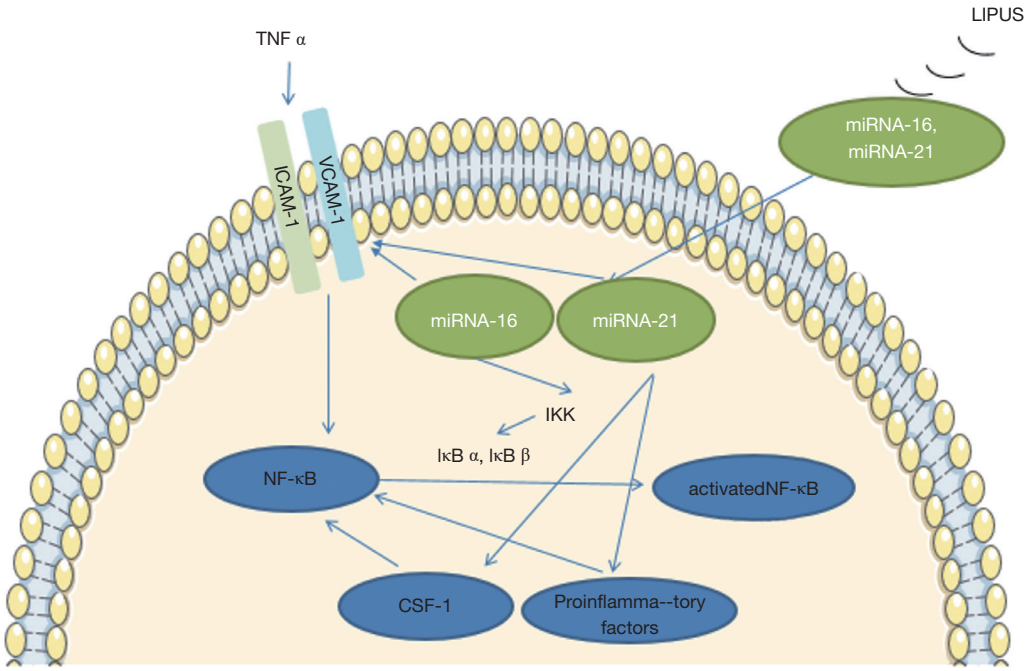

Figure 5 An anti-inflammatory effect is exerted by LIPUS by increasing miR-16 and miR-21 in exosomes. LIPUS increases miRNA-16 and miRNA-21 in exosomes derived from BMDCs. Human umbilical vein cultured endothelial cells preincubated with exosomes from LIPUS-treated BMDCs express less ICAM-1 and VCAM-1 and have lower activity of NF-κB signaling in response to TNF-α. miRNA-16 inhibitsthe activity of the NF-κB signaling pathway by suppressing the expression of IκB kinase, and miRNA-21 limits the activation of the NF-κB signaling pathway by inhibiting the expression of proinflammatory factor. BMDCs, bone marrow dendritic cells; LIPUS, low intensity pulsed ultrasound; NF-κB, nuclear factor κB; IKK, inhibitor of nuclear factor κB kinase; IκBα, inhibitor of nuclear factor κBα; VCAM-1, vascular cell adhesion molecule-1; ICAM-1, intercellular adhesion molecule-1.

(MSCs), are one of the main cells in bone. Osteoblasts have been confirmed to express some inflammatory chemokines, such as monocyte chemotactic protein 1 (MCP-1), macrophage-inflammatory protein (MIP)-1, regulated upon activation normal T cell expressed and secreted factor (RANTES), and Interleukin-8 (IL-8) (35,71). Osteoblasts are also able to sense the stimuli of LIPUS.

It is recognized that inflammatory cytokines are critical for fracture healing (72,73); they promote the proliferation and differentiation of MSCs and osteoprogenitor cells, which are important to bone remodeling (74,75). As mentioned above, osteoblasts can release inflammatory cytokines after a fracture. Findings have indicated LIPUS could enhance cyclooxygenase-2 (COX-2) gene expression and subsequently enhance endogenous prostaglandin E2 (PGE2) synthesis in various osteoblastic cell lineages (76,77). Consistently, Tang *et al.* reported that LIPUS promoted the production of COX-2 and PGE2 (34). The role of PGE 2 is essential in fracture healing and stimulates bone formation and resorption. Bandow *et al.* also reported that the expression of MIP-1 and MIP-1β mRNA was increased by LIPUS more efficiently in differentiated osteoblasts, which was caused by LIPUS-induced ERK phosphorylation through angiotensin type 1 (AT1) receptor (*Figure 3*) (35). These findings suggest that LIPUS promotes the release of inflammatory cytokines in osteoblasts and facilitates bone remodeling. It has also been confirmed that LIPUS has an impact on osteoclasts. As regulators of mineral metabolism in the case of fracture, osteoclasts resorb necrotic bone fragments and the necrotic ends of the fractured bone and initiate the process of remodeling (73). Feres *et al.* reported that LIPUS increased osteoclast resorptive activity in the absence of osteoblasts in RAW 264.7 cells (36). This result suggests that LIPUS can also promote the repair of fracture by promoting bone absorption.

Interestingly, osteoblasts also play an important role in some inflammatory bone diseases. Under the stimuli of pathogen-associated molecular patterns, osteoblasts

express inflammatory cytokines, which can aggravate inflammation in lesions. It has been reported that LIPUS has a therapeutic effect on these diseases by regulating the activity of osteoblasts. For example, Nakao *et al.* recently reported that LIPUS effectively inhibited LPS-induced mRNA expression of receptor activator for NF-κB ligand (RANKL), the C-X-C motif chemokine ligand (CXCL), 1, and CXCL 10 in mouse osteoblast cell line and calvaria-derived osteoblasts, which suggested LIPUS alleviated inflammatory responses (2). Further, they found that LIPUS inhibited the formation of toll-like receptor (TLR4)/myeloid differentiation factor 88 (MyD88) complex, which suggested that LIPUS exerted anti-inflammatory effects on LPS stimulated osteoblasts by inhibiting TLR4 signal transduction (*Figure 1*). However, it is unknown how the TLR4/MyD88 complex formation is inhibited by LIPUS. As a pathogenic factor, LPS plays an important role in some bone inflammatory diseases, such as arthritis and periodontitis. These findings suggest that LIPUS is a potential therapy for inflammatory bone diseases.

In conclusion, osteoblasts, as inflammatory regulators in bone tissue, can be regulated by LIPUS promotion through mediating the inflammatory cytokines released by osteoblasts are mediated by LIPUS to promote fracture healing and suppress inflammation in inflammatory bone diseases. The underlying mechanism is related to some signaling pathways such as the MAPK, NF-κB, and TLR signaling pathways.

Chondrocytes

Chondrocytes, the cartilage cells, can express proinflammatory cytokines under the stimulation of inflammation (78,79). These inflammatory cytokines are implicated in the damage of chondrocytes; for example, IL-1 can induce chondrocyte apoptosis (80). It has been reported that chondrocytes are highly mechanosensitive (81,82), which suggests they can sense the stimuli of LIPUS. In human cartilage explants, Uddin *et al.* found that LIPUS inhibited the expression of IL-1 receptor type 1 (IL-1R1) in the presence of IL-1β, making chondrocytes less susceptible to the catabolic and inflammatory effects of IL-1β (37). Consistently, in an OA model, excising the complete medial meniscus and both cruciate ligaments of rabbits, Jia *et al.* reported that LIPUS down-regulated apoptosis and reduced inflammatory mediators (PGE2 and nitric oxide [NO]) in chondrocytes (38). These findings suggest that LIPUS can attenuate inflammation in chondrocytes, which protects articular cartilage. Recently, Sahu *et al.* also reported that continuous low-intensity ultrasound could repair cartilage in a pro-inflammatory environment by inhibiting the activation of NF-κB induced by TNF-α and IL-1β in bovine osteochondral explant (83). Continuous ultrasound and pulsed ultrasound have been confirmed to have similar bioeffects. For example, they can both be used to improve endothelial function, alleviate pain, and improve physical disability (84-86). Continuous ultrasound produces more significant thermal effects than pulsed ultrasound when the parameters are the same (87,88). As far as we know, the therapeutic effect of low-intensity ultrasound is mainly caused by mechanical effects. But the thermal effect of ultrasound is inevitable, which increases the temperature of and may impair tissue. Therefore, LIPUS seems to be a better candidate than continuous ultrasound.

In clinical practice, there is a lack of methods to protect cartilage tissue. It has been revealed that LIPUS can prevent chondrocytes from inflammatory damage, which presents LIPUS as a promising method to ease this situation; however, the underlying mechanism is still unknown.

Synovial cells

The synovial membrane is a metabolically active tissue, which is susceptible to inflammation (89,90). Under the stimuli of inflammation, the metabolism of the synovial membrane becomes imbalanced, giving rise to the early pathogenesis of some diseases, such as rheumatoid arthritis (RA) and OA (91). It has been reported that LIPUS is able to regulate synovial cells. For example, exposing rabbit knee synovial membrane cell line (HIG-82) to LIPUS, Nakamura *et al.* found LIPUS exposure down-regulated COX-2 and PGE2 expression, and up-regulated hyaluronan synthase (HAS) 2 and HAS3 expression in IL-1β stimulated synovial membrane cells, leading to the promotion of the anti-inflammatory system (39). Interestingly, in another study, they found LIPUS suppressed the proliferation and growth of synovial cells stimulated with IL-1β or TNF-α in HIG-82 and reduced COX-2 expression and synovial hyperplasia in MRL/lpr mice, a model of RA (40). Similarly, Sato *et al.* reported that LIPUS could mediate synovial cell proliferation and apoptosis to inhibit the synovial hyperplasia via the integrin/FAK/MAPK pathway in HIG-82 (*Figure 6*) (41). We understand that abnormalities of hyaluronan metabolism and synovial hyperplasia are characteristics of synovial inflammation. These findings show that LIPUS can improve hyaluronan metabolism

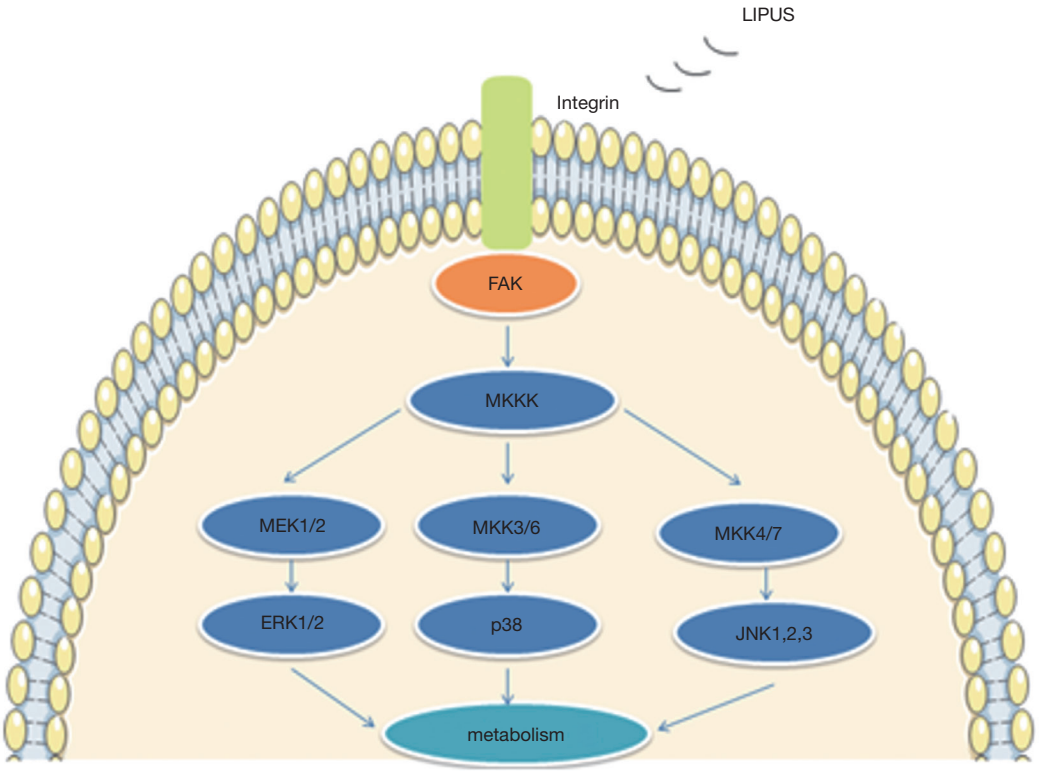

Figure 6 The influence of LIPUS on the integrin/FAK/MAPK signaling pathway to mediate inflammation. LIPUS induces the phosphorylation of integrin and FAK. The down-streaming MAPKs signaling pathways are also activated by LIPUS. These changes may result in the inhibition of synovial hyperplasia. LIPUS, low intensity pulsed ultrasound; FAK, focal adhesion kinase; MKKK, mitogen-activated protein kinase kinase kinase; MKK, mitogen-activated protein kinase kinase; JNK, c-Jun N-terminal kinase; ERK, extracellular signal-regulated kinase.

and inhibit synovial hyperplasia; thus, LIPUS appears to be a suitable therapeutic candidate for treating synovial inflammation.

Glial cells

Glial cells, an important part of the nervous system, are essential in neuroinflammatory reactions (92-94). The activation of glial cells is a characteristic of neuroinflammation, and inflammatory cytokines released by glial cells are associated with nerve injury. As a physical stimulation, LIPUS has been widely used in nervous system pathologies to modulate ion channels, promote nerve regeneration, and regulate neuronal function (95-98). It was recently discovered that LIPUS plays a role in neuroinflammation. In cortical impact injury mice, Chen *et al.* reported that LIPUS reduced neutrophil infiltration and microglial activation in the injured brain (42). They also reported LIPUS inhibited the activation of astrocytes and significantly reduced the protein levels of TNF-α, IL-1β, and IL-6 in the mice brain induced by LPS. Chen *et al.* also indicated that the anti-inflammatory effects of LIPUS might be due to the attenuation of TLR4/NF-κB-induced inflammation signaling (43). In their studies, LIPUS suppressed the expression of TLR4/NF-κB pathway-related mediators, including upstream factors (TLR4/NF-κB) and downstream factors (TNF-α, IL-1β, and IL-6) expression. Glial cells have also been shown to play a critical role in Alzheimer's disease, Parkinson's disease, and epilepsy (99-101). These findings suggest LIPUS can inhibit the activation of glial cells, so LIPUS may possibly be an effective treatment for those diseases.

Interestingly, it has also been reported that LIPUS promotes peripheral nerve regeneration and mediates the activity of Schwann cells (102). As we understand, the presence of inflammation can prevent nerve repair. It has

Table 2 The application of LIPUS in clinic

Sources	Samples	The parameters of LIPUS	Results
Samuels *et al.* (106)	20 patients	A frequency of 20 kHz or 100 kHz, 100 mW/cm^2 I_{SPTP}, a pulse repetition frequency (PRF) of 1 Hz and 500 ms pulse duration	Accelerating wound closure
Bajpai *et al.* (107)	8 patients	A frequency of 20 kHz, 100 mW/cm^2 I_{SPTP} and a pulse repetition frequency of 25 Hz	Promoting the reduction of wound size
Cui *et al.* (108)	120 patients	A pulse duration time-to-pulse rest time ratio of 1:4 (200 ìs:800 ìs) at 1,000 Hz, a frequency of 1.7 MHz and 300 mW/cm^2 I_{SATA}	Safely and effectively improving erectile dysfunction
Cruz *et al.* (84)	42 health volunteers	1MHz, 20% duty cycle (2 ms on, 8 ms off), and 0.08 W/cm^2 I_{SATA}	Improving endothelial function
Hauck *et al.* (109)	30 health volunteers	1MHz and 3Mhz, a 20% duty cycle (2 milliseconds on, 8 milliseconds off) and an intensity of 0.08 W/cm^2I_{SATA}	Improving endothelial function
Feizabadi *et al.* (110)	14 patients	1 MHz, an intensity of 1 W/cm^2 and 0.5 W/cm^2 and 10% duty cycle	Decreasing the S. aureus population in chronic rhinosinusitis patients

$I_{SPTP,}$ spatial peak temoral peak intensity; $I_{SATA,}$ spatial averaged-temporal intensity.

been found that shock wave therapy promotes peripheral nerve regeneration through its anti-inflammatory effects (103). As mentioned above, LIPUS is similar to shock wave therapy. So, it seems that the effects of LIPUS in nerve regeneration are partly achieved through an anti-inflammatory mechanism.

Generally, LIPUS prevents the activation of glial cells in the central nervous system and possibly plays an anti-inflammatory role in peripheral nerve repair. The TLR and NF-κB signaling pathways seem to be involved.

Clinical application

The Food and Drug Administration (FDA) has approved LIPUS as a physical therapy to use in accelerating the repair of fresh fractures and treating fractures at risk of non-union (7,104). Although LIPUS has been widely used in clinical research, there are few studies regarding its use in inflammation (17,105) (*Table 2*). In a pilot study, Samuels *et al.* recruited 20 patients suffering from venous ulcers, and LIPUS was used as the therapeutic tool (106). They tracked a reduction in wound size on a weekly basis. Their results showed that LIPUS was-beneficial for treating venous ulcers. Similarly, in an 8 patient pilot study, Bajpai *et al.* reported that diabetic ulcers treated with ultrasound showed a significantly faster closure rate than sham-treated ulcers (107). Moreover, they confirmed that ultrasound-induced healing was associated with a reduction in the M1/M2 score, which indicated a reduction of inflammation in the treated wounds. In their study, all healing ulcers showed a decrease in the M1/M2 score over time, while all non-healing ulcers showed an increase in the score over time. Recently, Cui *et al.* conducted a multicenter, randomized, double-blind, sham-controlled clinical study including 120 patients (108), and reported that LIPUS could safely and effectively treat patients with mild to moderate erectile dysfunction (ED) without significant adverse events. This effect was related to the mechanical force of LIPUS being able to restore the pathological changes of the corpus cavernosum. However, they did not explore the underlying mechanism of LIPUS; they simply speculated the anti-inflammatory effects of LIPUS might be involved, which required further investigation to confirm. The anti-inflammatory effect of LIPUS is also thought to be one of the mechanisms for alleviating pain, inducing dental root resorption, and promoting lumbar spondylolysis repair in patients (111-113). Interestingly, in a randomized, clinical trial, Cruz *et al.* reported that LIPUS could improve endothelial function in humans by increasing NO production, which suggested that LIPUS had anti-inflammatory vascular effects (84). Consistently, Hauck *et al.* reported both continuous or pulsed ultrasound could improve endothelial function in a randomized clinical trial of 30 patients (109).

It appears that LIPUS is also efficient at treating bacterial infection. For example, Feizabadi *et al.* reported that LIPUS decreased the population of *Staphylococcus aureus* in chronic rhinosinusitis patients (110). Karosi *et al.* exposed nasal polyps removed from a patient with chronic rhinosinusitis to low-intensity continuous ultrasound (114) and found

the inflammatory cell count was significantly decreased in the sub-epithelial layer of nasal polyps after irritation. This antibiotic effects of low-intensity ultrasound may be attributed to the destruction of the biofilm structure of bacterium caused by cavitation (115). In general, LIPUS, as a non-invasive, cheap, and convenient method, is promising in clinical settings.

Prospect and limitation

Inflammation, a defensive bodily response, aggravates damage, hinders repair, and delays recovery. As a physical tool, LIPUS continues to attract the attention of many researchers. It has been confirmed that LIPUS can regulate multiple cells to mediate inflammatory responses through the activation or inhibition of signal pathways. Some clinical studies have also confirmed that LIPUS is effective at alleviating inflammatory responses. These findings indicate that LIPUS is a promising anti-inflammatory therapy. Although the effect of LIPUS has been explored across many diseases, there are limited papers regarding metabolic diseases, such as diabetes and hyperlipidemia. It has been confirmed that inflammation plays a critical role in these diseases. For example, chronic inflammation has been demonstrated to be a key player in the development of diabetes and its complications (116-118). The cells involved in these diseases, including adipocytes, have been shown to respond under the stimuli of LIPUS (119,120). It is possible that the anti-inflammatory effects of LIPUS could play an important role in the treatment of diabetes, and future research should be conducted.

Another use of LIPUS is in tissue engineering; for example, LIPUS has been used to promote the proliferation and differentiation of MSCs (121-123). Inflammation can inhibit the ability of self-renewal and tissue reconstitution of stem cells. Basic experiments have reported no obvious inflammatory reaction when used stem cells were combined with LIPUS, but a slight inflammatory response in the absence of LIPUS (124,125). Yang *et al.* reported that LIPUS could mediate exosomes produced by MSCs by increasing exosome biogenesis, docking mediators, and enhancing extracellular vesicles and exosomes to deliver their anti-inflammatory molecules to target cells (67). These findings suggest that LIPUS can alleviate inflammation in stem cells, which can influence their proliferation and differentiation.

There are also some limitations to the use of LIPUS. First, the specific LIPUS device and parameters are different among individual experiments. Different parameters can lead to different results, as it has been confirmed in previous studies (126,127). Moreover, LIPUS does not yet have a precise definition. Generally, parameters of LIPUS include the frequency of 1–3MHz, intensity ranging from 0.02–1W/cm^2, pulse repetition rates of 0.1–1KHZ, and duty cycles of 20–50% (122). The following parameters are widely used: pulse frequency of 1.5 MHz, pulse repetition frequency of 1 kHz, spatial average temporal average intensity of 30 mW/cm^2 of the LIPUS transducer's surface area (8). Second, clinical data is lacking; although there are some clinical studies, the sample sizes have been small. Recently, the therapeutic value of LIPUS has been questioned. In 2 clinical trials, respectively, including 501 and 62 patients, it was indicated that LIPUS had no effect in bone healing (128,129). Tarride *et al.* also reported that LIPUS is not cost-effective for fresh tibial fractures (130). Further studies are required to confirm the effectiveness of LIPUS. Third, the definite mechanism of LIPUS is unknown. It has been demonstrated that LIPUS can regulate a broad range of inflammatory cytokines to mediate inflammation, irrespective of cell types and origin. Although it has been shown that some signaling pathways are affected by LIPUS stimulation, this only partially illustrates how LIPUS works. Some questions remain regarding the mechanisms of LIPUS, including how LIPUS influences specific signaling pathways. There are newly emerging theoretical models which aim to further elucidate the bioeffects of LIPUS (131).

In conclusion, LIPUS, as a safe and convenient method, is promising for clinical use, further research is required to substantiate these findings.

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Footnote

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at http://dx.doi.org/10.21037/qims-20-680). The authors have no conflicts of interest to declare.

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