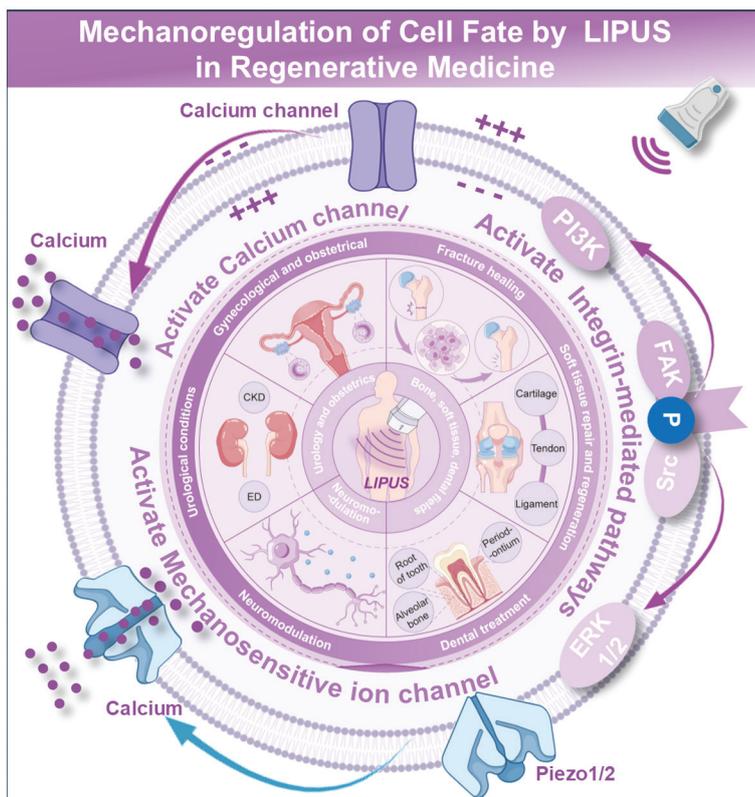


Mechanoregulation of Cell Fate by Low-intensity Pulsed Ultrasound: Mechanisms and Advances in Regenerative Medicine

Graphical abstract



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In brief

This review explores low-intensity pulsed ultrasound (LIPUS), an emerging non-invasive therapy leveraging mechanical forces for tissue regeneration. It details how LIPUS activates cellular mechanotransduction via ion channels and cytoskeletal pathways, driving proliferation and repair. We summarize translational advances across skeletal, neural, renal, and reproductive systems, while addressing key clinical challenges and future directions for precision regenerative medicine.

Highlights

- LIPUS uses non-thermal mechanical forces (radiation force, cavitation) for therapy.
- LIPUS activates cellular mechanotransduction via ion channels and cytoskeletal pathways.
- LIPUS promotes regeneration: cell proliferation, differentiation, and tissue repair.
- Clinical applications span skeletal, neural, renal, and reproductive systems.
- Review addresses LIPUS mechanisms, advances, and unresolved clinical challenges.

Mechanoregulation of Cell Fate by Low-intensity Pulsed Ultrasound: Mechanisms and Advances in Regenerative Medicine

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Abstract

As a mechanical wave capable of transmitting thermal and mechanical energy, ultrasound has emerged as a pivotal tool in regenerative medicine due to its non-invasive nature. Low-intensity pulsed ultrasound (LIPUS), a mechanoregulatory technique independent of thermal effects, delivers controlled mechanical stimuli to activate endogenous mechanotransduction pathways, such as ion channels, transmembrane proteins, and cytoskeleton-mediated signaling cascades. These pathways regulate critical cellular processes, such as proliferation, differentiation, and apoptosis, positioning LIPUS as a promising modality for targeted modulation of cell fate. Preclinical and clinical studies have demonstrated the therapeutic efficacy of LIPUS across diverse applications, including bone repair, neural regeneration, and soft tissue rehabilitation. However, optimizing stimulation parameters and advancing clinical translation remain key challenges. This review summarizes the central role of LIPUS in promoting tissue regeneration through non-thermal regulation of cellular homeostasis and explores strategies to accelerate clinical adoption of LIPUS. By integrating mechanistic insights with translational perspectives, this review provides a roadmap for advancing LIPUS-driven regenerative medicine in the era of precision bioengineering.

Keywords

Biological effects, dental treatment, fracture healing, low-intensity pulsed ultrasound, neuromodulation, regenerative medicine, urologic condition.

Introduction

Ultrasound, defined as mechanical vibrations exceeding 20 kHz without ionizing radiation, serves dual diagnostic and therapeutic roles in medicine. Originally developed for imaging, the biological effects of ultrasound, which were first observed in 1927, have propelled the evolution of ultrasound into a versatile therapeutic tool [1, 2]. Therapeutic ultrasound modalities are categorized into thermal and non-thermal types. High-intensity focused ultrasound (HIFU), an early thermal application, induces coagulative necrosis and has gained clinical approval for tumor ablation and neurosurgical interventions [3]. In contrast, low-intensity ultrasound (LIU) predominantly exploits non-thermal effects, exemplified by an ability to trigger significant biological responses in neuromodulation with minimal temperature elevation ($< 0.1^{\circ}\text{C}$), underscoring the pivotal role of mechanical forces in cellular regulation [4].

As a critical subset of LIU, low-intensity pulsed ultrasound (LIPUS) is characterized by low intensity ($< 3 \text{ W/cm}^2$), intermediate frequency (0.7–3 MHz), and pulsed-wave output. LIPUS generates mechanical signals through non-thermal mechanisms, including acoustic radiation force (steady momentum transfer in the direction of wave propagation) and cavitation effects, eliciting transient and sustained biological responses. Recent research has shown that mechanical stimuli promote cell differentiation during early embryogenesis and *in vitro* culture, which respond to morphogenetic movements, muscular activity, or substrate stiffness. Pathologic mechanical stimuli further disrupt effector cell proliferation and apoptosis, driving maladaptive remodeling and disease progression [5–7]. These mechanobiological principles now inform modern regenerative strategies, where the mechanical energy of ultrasound is harnessed to direct cell fate. Numerous studies have demonstrated its

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capacity to enhance cellular viability, stimulate proliferation, facilitate differentiation, and promote migration [8]. This mechanotransduction process occurs as LIPUS-generated mechanical waves activate ion channels or transmembrane proteins on the cell membrane, converting mechanical stimuli into biochemical signals that orchestrate cellular fate decisions. These signals subsequently regulate intracellular pathways, ultimately driving tissue repair and regeneration [9–11]. LIPUS activates intrinsic mechanotransduction networks, including ion channels and non-channel mediators. These pathways converge to regulate cellular processes critical for regeneration, including proliferation, differentiation, and apoptosis, which position LIPUS as a precision tool in regenerative medicine [11, 12].

LIPUS applications span multiple clinical domains, offering a reliable and non-invasive treatment modality for fracture healing, soft tissue repair and regeneration, dental treatment, neuromodulation, urologic conditions, as well as obstetrics and gynecologic-related diseases [9, 10, 12–20]. The non-invasive nature of LIPUS addresses key limitations of traditional surgeries by reducing hospital length of stay, complications, and systemic burden on healthcare systems. Recent advances in regenerative medicine further expand the scope of LIPUS applications, enabling targeted interventions in neurologic repair. This review delineates the mechanoregulatory paradigms underpinning LIPUS therapy, exploring the dual modulation of ion channel-driven and cytoskeletal-mediated signaling cascades. In addition, we summarized translational advances across regenerative contexts, including skeletal, neural, renal, and reproductive systems, while highlighting unresolved challenges in parameter optimization and clinical validation (Figure 1). By bridging mechanistic insights with therapeutic innovation, this work aims to chart a roadmap for LIPUS-driven regenerative medicine in the era of precision bioengineering.

Mechanoregulation of cell fate: mechanisms underlying LIPUS for regenerative medicine

Ultrasound propagates through tissues as high-frequency pressure waves, generating mechanical forces (compression, tension, shear stress, and torque) that activate cellular mechanotransduction pathways. LIPUS, defined by non-thermal and pulsed parameters, exerts therapeutic effects primarily through mechanical interactions (Figure 2). A hallmark of ultrasound is the capacity to induce bioelectric signals via deformation of piezoelectric materials within tissues [21]. These bioelectric signals regulate critical processes, such as cell migration, proliferation, differentiation, neural conduction, muscle contraction, and tissue regeneration [22]. For example, bone collagen fibrils exhibit piezoelectric properties and mechanical stress induces bending and relaxation of the non-centrosymmetric conformation, which alters axial polarization to generate electrical signals [23, 24]. These signals activate voltage-gated calcium channels on cell membranes, modulating intra- and extra-cellular ion gradients [25].

Calcium signaling in mechanotransduction

Calcium (Ca^{2+}) signaling serves as a key initiating mechanism in mechanotransduction, coordinating diverse cellular functions [26]. Mechanical stimulation elevates cytosolic Ca^{2+} through extracellular influx (via voltage-gated channels) or release from intracellular stores [27, 28]. Ca^{2+} influx activates calmodulin-dependent pathways in osteoblasts, regulating transforming growth factor (TGF)- β 1 levels and osteoblast behavior [23].

Mechanosensitive channels are multimeric membrane proteins responsive to mechanical forces that mediate ion flux (predominantly Ca^{2+}) across cell membranes within milliseconds and convert mechanical stimuli into electrochemical signals. Mammalian Piezo1/2, which are non-selective cation channels with a Ca^{2+} preference, are activated by diverse mechanical cues, including membrane tension, shear stress, and stretching. Structural studies suggest that membrane tension shifts Piezo1 from a bent to an open conformation, enabling ion permeation [29, 30]. LIPUS-generated shear stress activates Mechanosensitive channels, such as TRP-4 in *Caenorhabditis elegans* sensory neurons and Piezo1 in primary mouse neurons, inducing Ca^{2+} influx and nuclear c-Fos expression to modulate neuronal activity [31, 32].

Integrin-mediated signaling and downstream pathways

Integrins are heterodimeric transmembrane glycoprotein receptors that serve dual roles in mechanotransduction [anchoring cells to the extracellular matrix (ECM) and converting mechanical stimuli into intracellular biochemical signals] [33]. This dual functionality positions integrins as central mediators of LIPUS-induced cell proliferation [34].

LIPUS generates cyclic mechanical strain through acoustic radiation force and microstreaming, directly acting on cell membranes or indirectly transmitting stress via ECM interactions [35]. These mechanical stimuli induce conformational changes in integrins, exposing intracellular domains to initiate downstream signaling. For example, during bone repair, LIPUS enhances integrin α 5 β 1 binding to fibronectin and type I collagen by modulating mechanical strain gradients (1–10 μe) in the callus region, thereby promoting osteoprogenitor cell migration and adhesion.

Activated integrins recruit focal adhesion adaptor proteins, triggering autophosphorylation of focal adhesion kinase (FAK) at Tyr397 and forming an FAK-Src complex [36]. This complex activates downstream signaling cascades. LIPUS significantly elevates phosphorylated FAK (p-FAK) levels in synovial cells, and FAK inhibition abolishes LIPUS-induced proliferation, underscoring FAK phosphorylation as a critical signaling node [37].

LIPUS-activated integrin-FAK signaling upregulates COX-2 expression, stimulating paracrine release of PGE2. PGE2 binds EP2/EP4 receptors on adjacent osteoblasts, activating the Wnt/ β -catenin pathway to enhance bone formation. Integrin-mediated TGF- β 1 receptor upregulation promotes aggrecan synthesis in intervertebral disc

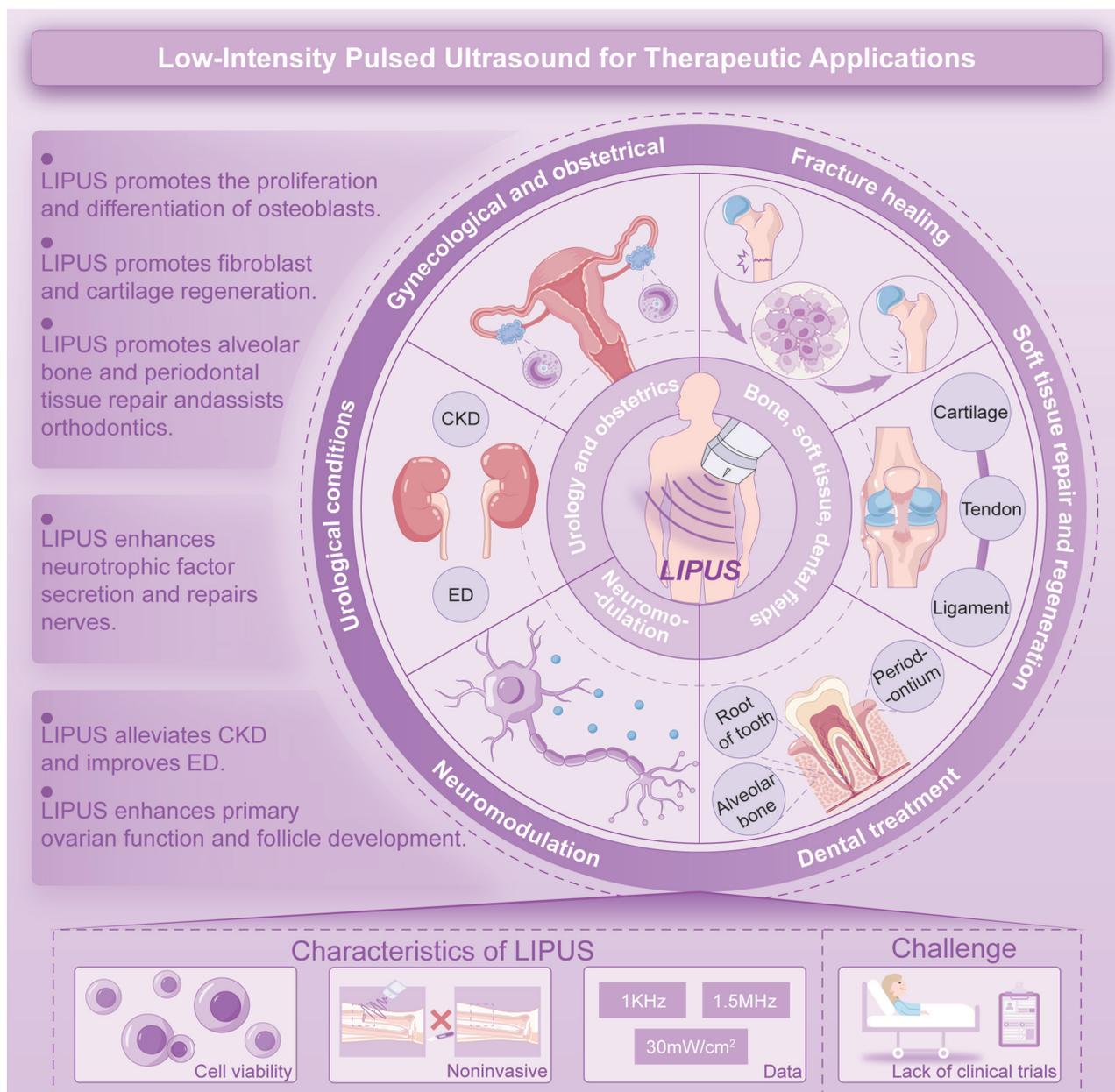


Figure 1 Schematic summary of LIPUS therapy in systemic treatments.

degeneration models and suppresses matrix metalloproteinase (MMP-3) activity, thereby preserving ECM integrity.

PI3K-Akt and MAPK pathways in proliferation and survival

The PI3K-Akt pathway, a key regulator of cell proliferation, is activated by LIPUS through multiple mechanisms [38]. Mechanical stimulation via integrins facilitates FAK/Src complex formation, which directly activates PI3K. Additionally, LIPUS enhances growth factor secretion (e.g., VEGF and TGF- β 1), enabling ligand-receptor binding to RTK. RTK phosphorylation recruits PI3K via phosphotyrosine residues or Ras-dependent pathways. Activated PI3K converts phosphatidylinositol-4,5-bisphosphate (PIP₂) to PIP₃, which binds the pleckstrin homology (PH) domain of Akt, a serine/threonine kinase with three isoforms (Akt1,

Akt2, and Akt3) expressed across tissues [39]. The PI3K/Akt axis promotes cell survival and proliferation by enhancing protein synthesis and inhibiting apoptosis. Akt phosphorylates and inactivates pro-apoptotic factors, while activating NF- κ B to further bolster survival [40].

The Ras/ERK pathway, a branch of the MAPK cascade, governs cell proliferation, differentiation, and survival [41]. LIPUS stimulates MAPK signaling via integrin-mediated mechanotransduction. The MAPK family includes p38, ERK, and JNK. ERK1/2, which is ubiquitously expressed, regulates mitotic and post-mitotic functions, whereas JNK and p38 are stress-responsive kinases [42]. JNK phosphorylates c-Jun to enhance AP-1-mediated pro-apoptotic gene expression under stress conditions.

Wang et al. demonstrated dose-dependent effects of LIPUS on human adipose-derived stem cells (ADSCs). High-intensity LIPUS (210 mW/cm²) induced apoptosis via p38 MAPK activation, while low-intensity (70 mW/cm²) enhanced cell

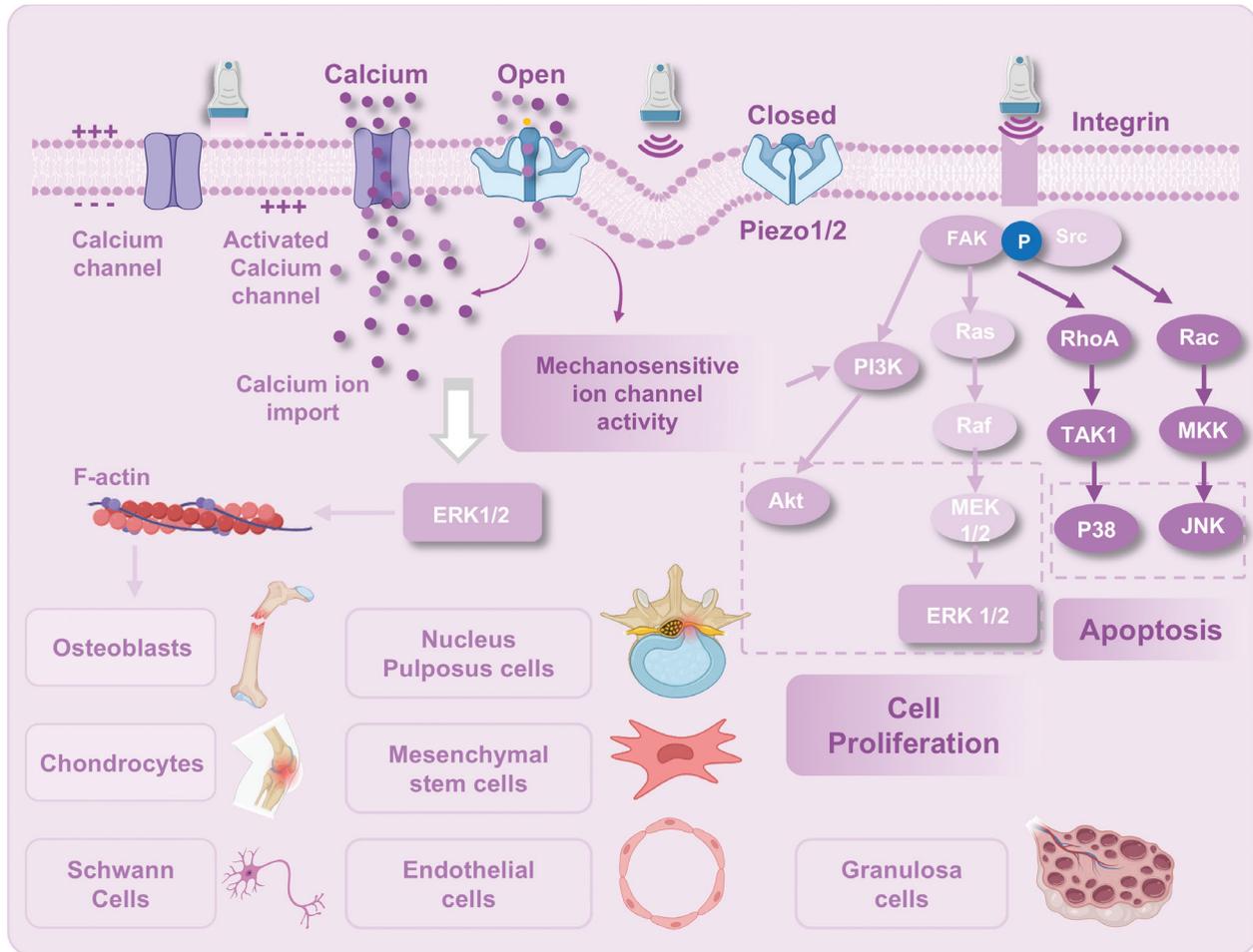


Figure 2 Schematic diagram of LIPUS-mediated cellular fate modulation.

viability by suppressing p38 phosphorylation. p38 inhibitors reversed high-intensity LIPUS-induced apoptosis, confirming pathway specificity [43]. LIPUS activated ERK1/2 signaling in dental pulp stem cells (DPSCs), whereas periodontal ligament stem cells (PDLSCs) responded via JNK/p38 pathways. ERK1/2 or JNK/p38 inhibitors abolished LIPUS-induced proliferation in respective cell types, highlighting cell- and intensity-dependent MAPK regulation [44, 45].

LIPUS advances in regenerative medicine: regulating specific cells

LIPUS-mediated skeletal system repair

Promoting fracture healing

The global incidence of non-union/bone delayed healing has been reported to be between 5% and 10%, with an increasing trend [46]. Mechanical stimulation of bones is crucial for maintaining bone mass and structural stability. When bone is subjected to mechanical loading, the movement of fluid in the spaces around bone cells generates fluid shear stress,

which stimulates osteoclasts and osteoblasts, enhancing anabolic bone remodeling activities to achieve appropriate bone resorption and subsequent new bone formation. As early as 1892, Wolff and colleagues proposed that LIPUS might induce bone formation by generating micro-mechanical stimulation in bone. Specifically, the differential absorption of LIPUS may create strain gradients in healing calluses, stimulating endosteal bone formation [47, 48]. Corradi and Cozzolino demonstrated that 500 mW/cm² of ultrasound accelerated the healing of fresh radial fractures in rabbits compared to the control group. Similarly, Klug et al. reconfirmed acceleration of fracture healing with ultrasound in rabbits with tibial fractures [49]. Chang et al. showed that limbs treated with 500 mW/cm² of ultrasound exhibited a 36% increase in new bone formation and an 80% increase in torsional stiffness compared to untreated limbs [50]. These studies collectively confirmed that LIPUS significantly stimulates and accelerates fresh fractures and in the treatment of aseptic and infected delayed unions and non-unions, improves healing rates 70%–93% [51].

The exact mechanisms underlying the therapeutic effects of fracture healing have not been completely elucidated. Histologic evidence indicates that LIPUS modulates the activity of key cellular participants in bone repair, including osteoblasts, osteoclasts, and mesenchymal stem cells (MSCs) (Figure 3). Notably, these effects appear restricted

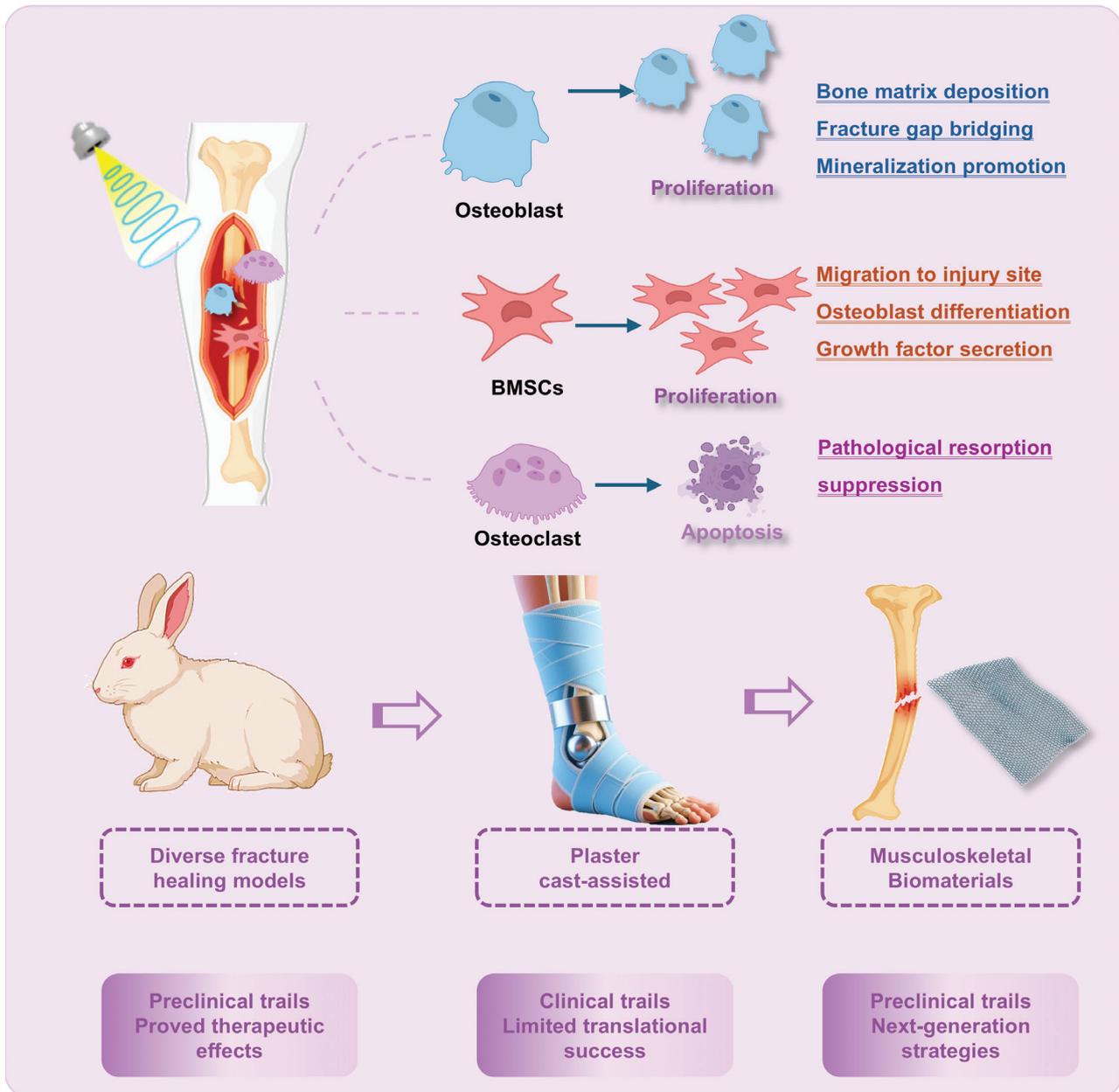


Figure 3 Mechanisms and emerging trends of LIPUS in fracture repair.

to cells within soft tissues with a minimal impact observed on cells embedded in calcified bone matrices [51]. Osteoblasts accumulate at the injury site during fracture healing, actively synthesizing and secreting bone matrix to bridge the fracture gap and ultimately restoring both structural integrity and functional capacity of the bone.

Studies have suggested osteoblast proliferation and differentiation are mechanosensitive processes that are responsive to external mechanical, electrical, and magnetic stimuli [52]. These mechanotransduction pathways enable osteoblasts to adapt bone remodeling activities in accordance with mechanical loading conditions, thereby facilitating restoration of physiologic bone morphology and function [53, 54]. This mechanoregulatory framework provides a theoretical basis for LIPUS-mediated enhancement of osteoblast activity to accelerate fracture repair.

LIPUS exhibits pro-osteogenic effects by enhancing osteoblast proliferation, activation, and mineralization with

mechanistic links to the upregulation of Sonic hedgehog (SHH) signaling. Experimental inhibition of SHH signaling significantly attenuates these effects, underscoring the critical role of SHH in mediating LIPUS therapeutic efficacy [55, 56]. Corollary studies revealed that LIPUS enhances calcium and phosphate uptake in pre-osteoblasts, facilitating mineralization processes [57]. Transcriptomic analyses performed by Tabuchi et al. identified LIPUS-induced upregulation of osteogenic regulators in murine pre-osteoblasts, including *Cd200*, *Dmp1*, *Igf2*, and *Nr4a1*, which are pivotal for osteoblast differentiation and skeletal development [58]. Notably, LIPUS stimulates COX-2 expression in osteoblasts, driving PGE₂ synthesis [34, 59]. This bioactive lipid mediates the following two distinct reparative functions: recruitment of inflammatory cells through COX-2-dependent pathways, thereby triggering cytokine release to initiate tissue repair; and activation of the cAMP/PKA signaling cascade, which enhances osteoblast proliferation and promotes woven bone formation.

While preclinical data strongly support LIPUS as a bone anabolic modality, clinical outcomes remain heterogeneous. Randomized trials report conflicting evidence regarding LIPUS efficacy in long bone regeneration, especially in recalcitrant fractures with compromised healing potential [60] [Table 1]. Current clinical guidelines assign moderate-to-high confidence to the conclusion that LIPUS constitutes an inefficient allocation of healthcare resources for bone healing, driven by the lack of clinically significant benefits in fracture repair and disproportionate treatment costs [61]. This evidence-based assessment warrants a strategic shift toward prioritizing alternative interventions with higher mechanistic plausibility and demonstrated efficacy in accelerating osseous regeneration. Furthermore, research agendas should incorporate systematic investigations into the de-implementation of LIPUS in orthopedic practice, guided by frameworks for eliminating low-value care [62].

Early investigations into LIPUS revealed dose-dependent limitations in bone healing efficacy. A seminal study by Warden et al. in 2001 demonstrated that conventional LIPUS parameters (30 mW/cm² intensity) failed to mitigate spinal cord injury-induced bone loss, although alternative dosing regimens showed promise for intact bone preservation [63]. Subsequent analyses suggested that suboptimal acoustic intensity may critically undermine therapeutic outcomes. While the protocol using 30 mW/cm² remains standard in clinical fracture management [64, 65], emerging preclinical evidence has identified 150 mW/cm² as a superior intensity threshold, yielding enhanced bone mineral density, microarchitectural preservation, and biomechanical competence in both healthy and osteoporotic models [66]. Mechanistically, high-intensity LIPUS (150 mW/cm²) accelerates murine fracture healing through dual pathways: [1] potentiation of chondrocyte hypertrophy and callus chondrogenesis; and [2] stimulation of osteoblast recruitment and *de novo* bone formation, thereby optimizing endochondral ossification kinetics [67].

The therapeutic potential of LIPUS may be further amplified through integration with biomaterial scaffolds. Bioengineered matrices approved for bone tissue engineering could synergize with LIPUS-induced mechanotransduction, creating a regenerative niche to accelerate musculoskeletal repair [68]. However, material-specific parameter optimization represents a critical gap in current research. The acoustic responsiveness of biomaterials, which is influenced by composition, porosity, and viscoelastic properties, necessitates systematic characterization of intensity-frequency-duration matrices tailored to scaffold design. For example, hydrogel-based systems may require distinct intensity thresholds compared to ceramic or polymeric substrates to maximize energy coupling efficiency [69, 70].

Stimulating oral regeneration in orthodontics and periodontics

In addition to long bone applications, LIPUS has emerged as a novel therapeutic modality in dentistry, demonstrating promising therapeutic potential in orthodontics and periodontics despite the nascent integration into routine clinical practice

[71]. Mechanistically, LIPUS suppresses pressure-induced alveolar bone resorption by downregulating Piezo1 expression while enhancing osteogenic capacity in mechanically stressed periodontal ligament cells [62]. Furthermore, LIPUS modulates the osteogenic potential of MSCs by promoting localized alveolar bone remodeling, increasing bone mineral density, mitigating vertical bone loss and gingival recession, and accelerating orthodontic tooth movement [63].

Preclinical studies have consistently indicated LIPUS efficacy in reducing orthodontically induced root resorption and enhancing osteoclast differentiation to optimize tooth displacement efficiency [54]. Clinical trials further validate these findings. El-Bialy et al. first demonstrated LIPUS potential in reducing root resorption and accelerating healing in a 2004 pilot trial [72] with subsequent studies confirming LIPUS effectiveness in human orthodontic root repair and introducing specialized low-power intraoral devices [73]. A single-blinded randomized controlled trial also established LIPUS utility in alleviating orthodontic separation pain, leveraging the safety, non-invasiveness, and cost-effectiveness of LIPUS [74, 75]. However, parameter optimization remains critical to maximize root resorption healing while minimizing potential pulpal side effects, which is a priority for future investigations.

LIPUS restores alveolar bone homeostasis by mitigating oxidative stress through PI3K-Akt/Nrf2 pathway regulation in periodontitis models [76]. The synergy of LIPUS with bone marrow-derived MSCs (BMSCs) enhances alveolar regeneration, which is partially attributed to improved BMSC homing to injury sites [77]. In addition to direct cellular effects, LIPUS amplifies the therapeutic potential of MSC-derived extracellular vesicles (EVs), which circumvent transplantation risks while delivering pro-regenerative signals. Zhang et al. demonstrated that LIPUS augments EV production and efficacy in counteracting experimental periodontitis-associated bone loss [78]. Despite robust preclinical evidence, clinical translation requires rigorous validation. Current research, while elucidating broad mechanistic frameworks, must address knowledge gaps in molecular cascades and parameter standardization to optimize LIPUS integration into precision dentistry.

Regulating cartilage degradation and soft tissue repair in osteoarthritis (OA)

LIPUS has shown promising potential in the management of OA by targeting the underlying mechanisms of cartilage degradation. OA is characterized by the progressive loss of cartilage, driven by inflammation and catabolic processes mediated by IL-1 β and NF- κ B. LIPUS has been shown to alleviate OA progression through multiple pathways, including a reduction in tissue degeneration, alleviation of pain, improved subchondral bone microstructure, and decreased sensory nerve distribution [79].

LIPUS has been shown to protect damaged cartilage in the early stage of OA by inhibiting the degradation of IL- β and stimulating the migration, proliferation, and differentiation of chondrocytes, especially in temporomandibular joint OA [80]. Furthermore, LIPUS enhances the inhibitory effect of synovial fluid on cartilage degradation, thereby worsening

Table 1 Clinical Trials of LIPUS in Fracture Healing: Outcomes and Limitations

Bone Fracture	LIPUS Protocol	Healing Results	Study Design (Population)	Year	Ref.
Closed or grade-I open tibial shaft fractures	1.5 MHz, 200 μ s burst, 1 kHz, 30 mW/cm ² , 20 min/d for 20 weeks	Clinical healing: 86 \pm 5.8 d (active) vs. 114 \pm 10.4 d (placebo), <i>P</i> = 0.01. Overall healing: 96 \pm 4.9 d (active) vs. 154 \pm 13.7 d (placebo), <i>P</i> = 0.0001.	Prospective, randomized, double-blind, placebo-controlled multi-institutional trial (/)	1994	[150]
Dorsally angulated distal radial fractures	1.5 MHz, 200 μ s burst, 1 kHz, 30 mW/cm ² , 20 min/d for 10 weeks	Time to union: 61 \pm 3 d (active) vs. 98 \pm 5 d (placebo), <i>P</i> < 0.0001	Multicenter, prospective, randomized, double-blind, placebo-controlled trial (/)	1997	[151]
Fresh midshaft clavicle fractures, non-operatively treated	1.5 MHz, 200 ms burst, 1 kHz, 30 mW/cm ² , 20 min/d for 28 d	No significant difference in clinical healing time (<i>P</i> = 0.91)	Multicenter, double-blind, randomized, placebo-controlled trial [101]	2008	[61]
Delayed union of osteotomized fibula after high tibial osteotomy	1.5 MHz, 200 μ s burst, 1 kHz, 30 mW/cm ² , 20 min/day at home	Osteoid thickness \uparrow 47%, mineral apposition rate \uparrow 27%, bone volume \uparrow 33% (all <i>P</i> < 0.05). Direct bone formation observed vs controls	Prospective, double-blind, placebo-controlled, histomorphometric trial [13]	2008	[152]
Hallux valgus deformity treated with chevron osteotomy	1.5 MHz, 200 ms burst, 1 kHz, 30 mW/cm ² , 20 min/d	No significant differences in clinical or most radiographic parameters. Distal metatarsal articular angle stable in LIPUS group vs. relapse in placebo	Randomized, placebo-controlled, double-blind trial [44]	2009	[153]
Delayed unions of the tibial shaft	1.5 MHz, 200 μ s pulse, 1 kHz, 30 mW/cm ² , 20 min/d for 16 weeks	BMD improvement 1.34x greater with LIPUS (<i>P</i> = 0.002). Bone gap area reduction favored LIPUS (<i>P</i> = 0.014)	Multicenter randomized sham-controlled trial [101]	2010	[154]
Posttraumatic tibial defects treated with callus distraction	1.5 MHz, 200 μ s impulse, 1 kHz, 30 mW/cm ² , 20 min/d	Healing index: 33 d/cm (LIPUS) vs. 45 d/cm (control), 27% faster maturation. Fixator time shortened by 95 d in LIPUS group.	Prospective, randomized controlled trial [21]	2014	[155]
Standardized corticotomy in proximal tibial metaphysis	Daily 20 min treatment	No significant difference in time to frame removal.	Prospective, multicenter, double-blind, randomized controlled trial [62]	2017	[156]
Tibial shaft fractures managed with intramedullary nailing	Daily LIPUS	No significant difference in radiographic healing, functional recovery, or return to work. Mean cost higher for LIPUS.	Prospective, multicenter, randomized controlled trial (501)	2017	[157]
Mandibular prognathism treated with bilateral intraoral vertical ramus osteotomy	1.5 MHz, 200 μ s burst, 30 mW/cm ² , 20 min/d for 3 weeks	Higher CT values in LIPUS group. Increased D1 grade area in LIPUS group on CT images.	Prospective cohort study [21]	2019	[158]

cartilage damage in the early stages of OA [81]. These findings provide valuable insights into the potential clinical application of LIPUS in the treatment of early symptomatic OA. In addition to the effects of LIPUS on cartilage, LIPUS has been shown to have a positive impact on tendon-bone healing [82]. Following LIPUS treatment at the patella-patellar tendon junction, significant increases in the histologic expression of VEGF were observed in chondrocytes and osteoblasts [83]. LIPUS also significantly increases the proportion of M2 macrophages and expression of anti-inflammatory mRNA, contributing to the therapeutic effects [84].

Sufficient vascular invasion has a critical role in the initiation of visceral ossification and soft tissue repair. LIPUS promotes tendon-bone junction healing by enhancing tissue perfusion and facilitating angiogenesis. Furthermore, MSCs are considered a promising autologous source for cartilage repair and numerous studies have demonstrated that LIPUS act as an effective stimulus to enhance MSC differentiation into chondrocytes *in vivo* [85, 86], thereby promoting tendon-bone injury healing. Compared to other external stimuli for tendon-bone healing, such as shockwaves, electrical stimulation, and combined magnetic fields, LIPUS is a safe, efficient, and cost-effective method. This feature makes the clinical application and translation of LIPUS more feasible in regenerative medicine [87].

Although numerous preclinical studies have demonstrated the therapeutic potential of LIPUS, the specific mechanisms underlying the effects of LIPUS in human musculoskeletal diseases, such as tendinopathy and OA, are incompletely understood [14]. Furthermore, translational studies have shown that the therapeutic benefits of LIPUS observed in animal models have been difficult to replicate in patients [88]. Several clinical trials have failed to demonstrate the effectiveness of LIPUS in promoting ligament and muscle regeneration or alleviating symptoms in patients with tendinopathy and OA. For example, in clinical trials involving patellar tendinopathy and chronic lateral epicondylitis, the outcomes of the LIPUS treatment group were comparable to the control group ($P > 0.05$), indicating that the additional therapeutic effects of LIPUS in clinical applications are minimal [88, 89]. These discrepancies in translational studies may stem from physiologic differences between species, insufficient modeling of human pathophysiology in preclinical models, differences in clinical trial designs, and patient heterogeneity. Future research should focus on conducting large-scale, multi-center randomized controlled trials with standardized protocols to validate the efficacy of LIPUS across different disease stages and anatomic sites. Additionally, conducting mechanism studies using human-derived tissues or organoids may help bridge these translational gaps.

Delaying disc degeneration

Demonstrating robust pro-regenerative effects across diverse biological systems. Preclinical studies have highlighted the ability of LIPUS to stimulate fibroblast proliferation and enhance regeneration of cartilage, intervertebral discs, ligaments, and tendon sheaths [12]. Notably, LIPUS enables non-invasive healing of injured or degenerative

intervertebral disc tissue by upregulating the transforming growth factor-beta 1 receptor in nucleus pulposus cells, thereby promoting cellular proliferation and ECM synthesis [44]. Mechanistic investigations have revealed that LIPUS treatment induces a 2.06-fold increase in tissue inhibitor of metalloproteinase-1 expression and a 2.3-fold elevation in monocyte chemoattractant protein-1 levels within nucleus pulposus cells, which facilitates macrophage recruitment and active participation in disc remodeling processes [90]. Taken together, these findings position LIPUS as a promising non-invasive therapeutic strategy for mitigating degenerative disc pathologies and alleviating chronic low back pain.

LIPUS-mediated neurologic repair

Accelerating peripheral nerve regeneration

LIPUS has shown significant therapeutic effects in neural modulation, especially in the peripheral nervous system [91]. Peripheral nerve injuries are common in clinical practice and often result in neuropathy, causing weakness, paralysis, sensory deprivation, neurogenic pain, and autonomic dysfunction. Repair of nerve injuries using neurotrophic factors has challenges, such as paradoxically inducing pain hypersensitivity through BDNF-mediated central sensitization and hyperexcitability of spinal WDR neurons [92]. LIPUS has demonstrated an essential role in peripheral nerve healing from multiple perspectives [18].

Mechanistically, LIPUS enhances neurotrophic factor secretion (e.g., BDNF and NGF), while suppressing inflammatory cascades *in vitro*, as demonstrated in models of peripheral neuropathy [93]. This effect is partially mediated through TrkB/Akt-CREB pathway activation, which upregulates neurotrophic expression and confers neuroprotection in rodent brains, suggesting therapeutic relevance for neurodegenerative disorders [35, 94]. LIPUS synergizes BMSC transplantation by augmenting BMSC survival and paracrine release of neurotrophic factors, yielding superior functional recovery compared to standalone cell therapy in preclinical models [95].

While a hypothesis suggests that LIPUS application directly enhances axonal regeneration, the majority of evidence supports the idea that LIPUS exerts therapeutic effects in peripheral nerve regeneration primarily through direct modulation of Schwann cell (SC) repair [19]. The mechanisms by which LIPUS positively influences nerve regeneration likely involve modulation of SCs, thereby facilitating the repair process. SCs are pivotal in peripheral nerve regeneration and have a critical role in the survival, regeneration, and reorganization of damaged axons.

It has been shown that LIPUS significantly impacts SC activity and proliferation. A seminal *in vitro* study published in 2009 demonstrated that LIPUS effectively promotes cell growth and expression of neurotrophic factor genes in SCs, highlighting the potential utility of LIPUS in peripheral nerve regeneration [96]. Subsequent work by Ren et al. validated LIPUS efficiency in enhancing SC viability and proliferation [97]. Furthermore, studies have indicated that LIPUS treatment may enhance nerve regeneration through the GSK-3 β / β -catenin/cAMP signaling pathway.

Notably, previous research has shown that the absence of cyclin D1 in SC proliferation does not impede axonal regeneration or myelin regeneration in regenerating nerves [98]. These findings suggested that SC proliferation during Wallerian degeneration is not essential for the regeneration and functional recovery of damaged peripheral nerves. Consequently, further research is warranted to fully elucidate the molecular mechanisms underlying LIPUS promotion of nerve repair.

Recent investigations have explored additional avenues through which LIPUS may exert its therapeutic effects. For example, exosomes secreted by LIPUS-pretreated SCs have been shown to enhance axonal extension. Ye et al. reported that LIPUS stimulation regulates gene expression in pelvic neurons by modulating miRNA in exosomes derived from SCs, thereby activating the PI3K-Akt-FoxO signaling pathway to enhance nerve regeneration [99].

Innovative approaches to accelerating nerve healing involve enhancing SC function. A study demonstrated that co-culturing ADSCs with SCs combined with LIPUS stimulation synergistically enhances the myelination capacity of SCs [100]. This effect is attributed to the secretion of growth factors or cytokines by ADSCs, which promote the myelination process of SCs. However, this research remains largely confined to laboratory settings and awaits validation in clinical trials. To optimize the therapeutic effects of LIPUS, further research is necessary to explore the impact of prolonged application. Such studies will contribute to a deeper understanding of the underlying mechanisms and potential applications of LIPUS in clinical settings.

The repair efficiency of LIPUS in neural modulation is strongly influenced by the dose that is administered. While low-intensity ultrasound has been shown to promote nerve regeneration, higher intensities may hinder this process, as

demonstrated in animal models [101]. Notably, there is considerable variability in the intensity parameters across studies with effective dosages for nerve regeneration ranging from 0.016 W/cm² to 1 W/cm² [102]. Additionally, the effects of LIPUS on SC proliferation and myelination depend on exposure time and duty cycle. Studies have indicated that repeated applications with higher duty cycles enhance SC proliferation with the most significant effects observed at a 50% duty cycle [103]. These findings suggest an incremental approach, starting with lower duty cycles and gradually increasing to the optimal level for maximal therapeutic benefit.

LIPUS exerts its neural regenerative effects by modulating neural cells and stem cell activity, regulating neurotrophic factors, pro-inflammatory cytokines, and activating critical signaling pathways [18]. While LIPUS has shown promising results in preclinical studies, LIPUS has not been widely implemented in clinical treatments for nerve injuries. The evidence underscores the potential of LIPUS as a non-invasive modality for neural repair. However, the variability in study protocols, such as differences in intensity, duration, and duty cycle, necessitates further investigation to establish standardized parameters for clinical use. Although progress is being made in understanding the molecular mechanisms underlying ultrasound-mediated neural modulation, a comprehensive mechanistic framework is still lacking. Addressing these gaps will be essential for translating LIPUS therapies into clinical practice, enabling a broader application in the treatment of peripheral nerve injuries (Figure 4).

Modulating central nervous system function

The translational scope of LIPUS extends to central nervous system pathologies, with emerging evidence highlighting the

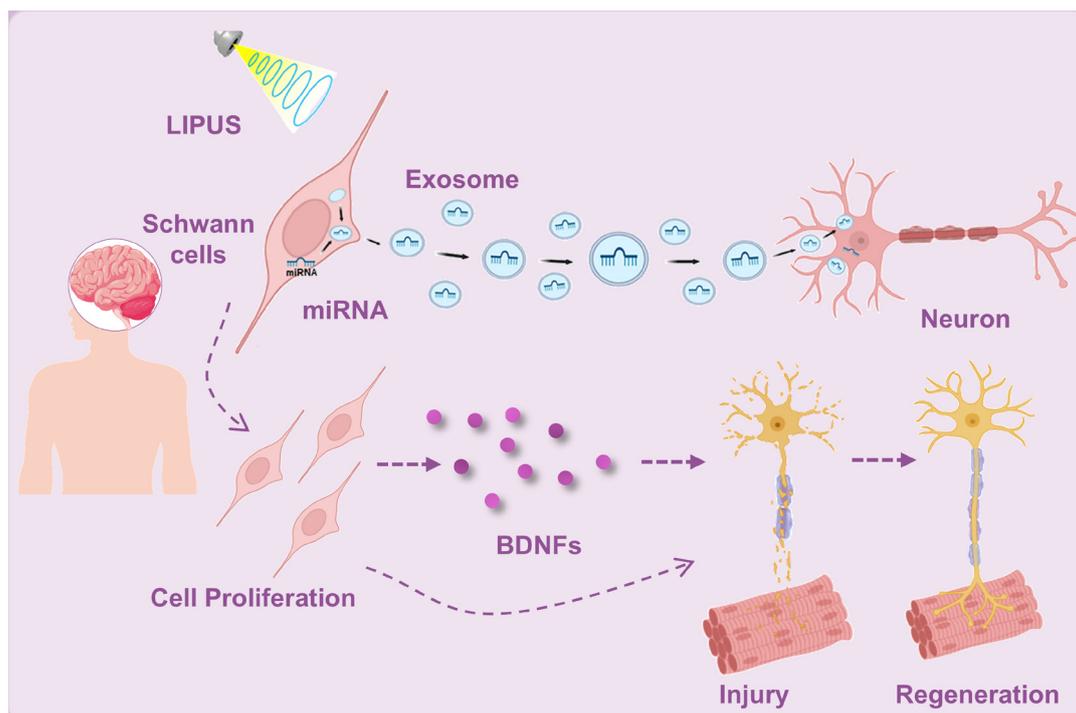


Figure 4 LIPUS-mediated repair of peripheral nerve injury.

multimodal therapeutic mechanisms. Transcranial LIPUS has demonstrated disease-modifying potential in Parkinson's disease (PD) models. Specifically, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mice exhibited improved motor performance in open-field and pole tests ($P < 0.05$) with elevated striatal antioxidant enzymes (T-SOD and GSH-PX; $P < 0.03$) post-LIPUS, while histologic analyses confirmed absence of tissue damage [104]. Parallel studies in 6-OHDA-lesioned rats revealed unilateral LIPUS stimulation upregulated glial cell line-derived neurotrophic factor (GDNF) by 1.98-fold in the substantia nigra pars compacta (SNpc) and attenuated lipidocalin-2-mediated neuroinflammation, which correlated with restored tyrosine hydroxylase density [105, 106].

By elevating endogenous BDNF levels and modulating neuroinflammatory pathways, LIPUS mitigates disease progression in Alzheimer's disease (AD) and PD models, the effects of which were corroborated by improved cognitive outcomes in a randomized, double-blind trial of AD patients [105–108]. Notably, human trials in PD with cognitive impairment (PD-CI) further validate clinical translatability. Specifically, an 8-week LIPUS regimen ($n = 28$) significantly enhanced Mini-Mental State Examination (MMSE)/Montreal Cognitive Assessment (MoCA) scores ($P < 0.05$), reduced anxiety/depression indices (Beck Anxiety Inventory [BAI]/Beck Depression Inventory [BDI]), and upregulated serotonin, norepinephrine, and dopamine levels compared to sham controls, suggesting systemic neuromodulatory effects [6]. Pioneering human studies by Shindo et al. have safely applied transcranial LIPUS (0.5 MHz and 1.3 MPa spatial peak pressure) to modulate brain circuits, although long-term efficacy remains unvalidated [109].

Mechanistically, LIPUS synergizes with nanotechnology to overcome the blood-brain barrier (BBB). LIPUS-enhanced delivery of felodipine nanodrugs redirected endoplasmic reticulum stress toward antioxidative signaling in AD models, suppressed NLRP3 inflammasome activation, and rescued anxiety-like behaviors in 5xFAD mice [110]. Similarly, LIPUS-facilitated transport of 30-nm PEG-Q10 nanoparticles reduced amyloid plaques and formaldehyde toxicity in APP/PS1 mice with combined therapy outperforming standalone drug delivery [$P < 0.05$] [111]. These advances underscore the dual role of LIPUS as a standalone neuromodulator and a facilitator of precision drug delivery, positioning LIPUS as a cornerstone for next-generation neurotherapeutics.

LIPUS-mediated renal and erectile function recovery

Renal function recovery by targeting inflammation and fibrosis

The pathogenesis of chronic kidney disease (CKD) is largely influenced by renal inflammation and fibrosis. Therefore, inhibiting renal inflammation and fibrosis to protect kidney function is crucial [112]. LIPUS has been shown to have significant anti-inflammatory and repair properties, making LIPUS a promising treatment option

for CKD [113]. Aibara et al. provided preliminary evidence indicating that daily LIPUS treatment has beneficial effects on renal fibrosis and inflammation in experimental models of hypertensive and diabetic nephropathy [114]. Moreover, it was found that LIPUS exposure directly inhibits the TGF- β 1/Smad signaling pathway. These findings suggested that LIPUS may partly alleviate the progression of renal fibrosis by suppressing inflammatory cell infiltration. Corollary studies confirmed that LIPUS treatment effectively improves body weight, the albumin/globulin ratio, serum kidney function markers, renal pathologic changes, and renal fibrosis in CKD mice. LIPUS slows the progression of CKD by inhibiting epithelial-mesenchymal transition and age-related signaling [115]. Additionally, Ouyang et al. found that LIPUS application alleviates renal inflammation and fibrosis by inhibiting ferroptosis, thus protecting kidney function in CKD [116]. Furthermore, LIPUS may have a crucial role in modulating the TGF- β 1/Smad and Nrf2/Keap1/HO-1 signaling pathways, thus inhibiting ferroptosis. The authors further confirmed that the therapeutic effects of LIPUS are related to modulation of the NF- κ B and ferroptosis signaling pathways, with Fgf23, Fgg, and Cyp2c11 identified as potential LIPUS targets in improving renal fibrosis and dysfunction [117]. Recent studies have also shown that LIPUS effectively improves muscle mass loss, muscle weakness, protein expression related to muscle atrophy, and Akt inactivation in CKD-induced muscle atrophy [118]. However, further research is needed to fully understand the complex mechanisms and determine the potential clinical applications.

Chiang et al. demonstrated that LIPUS effectively reverses the renal and splenic inflammatory responses induced by ischemia/reperfusion injury in mice. This finding suggested that LIPUS, as a non-invasive therapeutic intervention, holds significant potential for treating renal injury or as an adjunct tool for acute kidney injury (AKI) management [119]. Macrophages have a critical role in the pathogenesis of AKI and LIPUS is being explored as an immunomodulatory intervention. Laboratory studies have shown that LIPUS effectively inhibits the expression of M1 macrophage-related genes, while significantly promoting the expression of M2 macrophage-related genes. This modulation is accompanied by suppression of apoptosis and a significant decrease in heat shock protein-70 levels, ultimately leading to the improvement of AKI caused by gemcitabine [120]. However, a comprehensive understanding of the long-term effects of LIPUS in AKI or chronic kidney disease (CKD) patients and optimization of LIPUS therapy is incomplete. Therefore, long-term follow-up studies are crucial to evaluate the efficacy of LIPUS post-AKI or CKD and gain insights into its long-term benefits.

Erectile function recovery

Erectile dysfunction (ED) is a common condition that negatively affects various physiologic and psychosocial aspects. It is estimated that the prevalence of ED exceeds 50% among men 40–70 years of age [121]. The extant literature relevant to the regenerative properties of LIPUS is promising and provides potential opportunities for many men to restore

spontaneous erectile function [122]. The therapeutic effects of LIPUS in treating nerve damage and diabetes-induced ED have been explored. In 2015 Lei et al. reported that LIPUS therapy has encouraging results in improving erectile function and effectively reverses adrenal tissue lesions in diabetic rats [123]. Furthermore, damage to the corpus cavernosum endothelial cells has a crucial role in the development of diabetes-induced ED. Studies have shown that by activating mitochondrial autophagy, LIPUS effectively alleviates endothelial cell dysfunction in the corpus cavernosum caused by advanced glycation end-products [124].

Additionally, similar to other adjunct stem cell therapies utilizing LIPUS, combining MSC transplantation with LIPUS may become a synergistic therapy for diabetic ED. It has been reported that LIPUS enhances the therapeutic effects of MSCs in the treatment of diabetic ED by activating the Piezo-ERK-VEGF pathway in rats [125]. In 2019 a randomized, double-blind, placebo-controlled clinical trial conducted in a clinical setting first demonstrated the efficacy of LIPUS in treating mild-to-moderate ED induced by diabetes with no major adverse events [126]. The observed results can be attributed to the mechanical forces exerted by LIPUS and the ability of LIPUS to restore the pathologic changes in the corpus cavernosum. Chen et al. found that for younger, overall healthy patients with mild-to-moderate ED, LIPUS therapy performed 2 or 3 times a week showed similar therapeutic effects and safety [127].

Recent clinical studies suggest that LIPUS may also produce positive therapeutic effects in patients with severe ED, especially in the absence of other co-morbidities [128]. Moreover, the application of LIPUS stimulation may offer therapeutic benefits for ED induced by cavernous nerve injury [129]. Studies have shown that LIPUS effectively improves erectile function in rats with neurogenic ED, potentially by regulating YAP/TAZ-mediated mechanotransduction [130]. Further confirmation of these statements is pending. Research on the use of LIPUS in treating urinary system diseases is still in the early stages and additional multi-center randomized

placebo-controlled studies are warranted before LIPUS can be adopted as a standard clinical treatment for ED. Dose studies with supportive measures will also be required at this stage.

Targeting apoptosis and stem cell activation in ovarian regeneration

Recent advances in bio-ultrasound research have propelled the clinical translation of ultrasound therapies in obstetrics and gynecology. While high-intensity ultrasound has established clinical utility in targeted gynecologic interventions, low-intensity modalities, such as LIPUS, are emerging as promising tools for ovarian dysfunction management. Preclinical studies highlight the potential in addressing primary ovarian insufficiency (POI), a condition associated with premature ovarian failure and systemic health risks. Mechanistically, LIPUS enhances follicular development by stimulating revascularization and follicular growth in animal models [131], while concurrently increasing sex hormone secretion, reducing follicular atresia, and suppressing granulosa cell apoptosis [132]. Tang et al. showed that LIPUS restores ovarian function in POI rats through histologic repair, normalizing estrous cycles, elevating follicle counts, and improving hormonal profiles [133].

A paradigm-shifting application involves combining LIPUS with MSC therapy (Table 2). MSCs are multipotent stromal cells isolated from diverse tissue sources, including bone marrow, adipose tissue, dental pulp, amniotic membrane, placenta, umbilical cord, and umbilical cord blood. These cells exhibit self-renewal capacity and multilineage differentiation potential [134]. Under specific conditions, MSCs can differentiate into diverse cell lineages of mesodermal origin, including osteocytes, chondrocytes, and adipocytes. MSCs have been extensively utilized in tissue engineering and are recognized as pivotal therapeutic agents in regenerative medicine [135, 136]. Pretreatment with LIPUS enhances MSC viability and directional migration,

Table 2 Therapeutic Application of LIPUS in Conjunction with Stem Cells or Exosomes

Diseases	Stem Cells	Parameter (LIPUS)	Therapeutic Effects	Ref.
Orthodontic tooth movement	hBMSCs	F = 1.5 MHz; I = 30 mW/cm ² ;	Enhanced BMSCs homing and promoted alveolar bone regeneration via LIPUS stimulation	[159]
Alveolar bone loss	BMSCs	DC = 20%; T = 20 min	Reduced alveolar bone resorption and accelerated tooth movement by modulating the cytoskeleton-Lamin A/C-YAP axis	[77]
Periodontitis	Stem cells from apical papilla (SCAP)	F = 1.5 MHz; I = 90 mW/cm ² ; DC = 20%; T = 30 min	Enhanced osteogenic differentiation and anti-inflammatory effects of SCAP-EVs via LIPUS stimulation	[78]
Spinal cord injury	BMSCs	I = 50 mW/cm ² ; T = 3 min	Improved BMSCs viability, migration, and neurotrophic factor expression; promoted functional recovery	[95]
Diabetic erectile dysfunction	ADSCs	F = 1.7 MHz; I = 200 mW/cm ² ; DC = 20%; T = 5 min	Enhanced ADSC-mediated angiogenesis and improved erectile function via the Piezo-ERK-VEGF axis	[125]
Premature ovarian insufficiency	hAD-MSCs	F = 0.25 MHz; I = 30 mW/cm ² ; DC = 20%; T = 30 min	Enhanced migration and homing of hAD-MSCs to ovaries; improved ovarian function and reduced ovarian injury	[138]

potentiating the therapeutic efficacy of MSCs in ovarian repair [137]. CXCR4-mediated chemotaxis may underlie the improved homing of LIPUS-primed MSCs to inflammatory ovarian microenvironments, where MSCs attenuate inflammation and inhibit germinal center apoptosis [138].

POI-associated complications extend beyond ovarian dysfunction because estrogen deficiency in POI often precipitates osteoporosis. LIPUS demonstrates dual therapeutic utility in patients with POI. Specifically, amplitude-modulated pulsed ultrasound (a variant of LIPUS) promotes bone formation and mitigates bone loss by optimizing acoustic energy distribution to osteocytes, thereby enhancing bone microstructure and strength [139]. In addition to direct ovarian repair, LIPUS may mitigate chemotherapy-induced gonadotoxicity. Emerging evidence suggests a cytoprotective role for LIPUS in reducing ovarian damage and preserving follicular reserves during chemotherapeutic regimens [137]. While preclinical data robustly support LIPUS efficacy, clinical validation remains imperative. Future research must prioritize mechanistic elucidation of LIPUS-induced cellular responses, tissue-specific parameter optimization, and translational frameworks to bridge the gap between experimental models and human applications.

Conclusions

LIPUS represents a promising non-invasive approach to regulate cellular behavior and tissue regeneration through mechanotransduction pathways with preclinical evidence underscoring the capacity of LIPUS to enhance osteogenesis, promote neural repair, and synergize with biomaterials. Notwithstanding the potential of LIPUS, clinical translation remains contentious, marked by inconsistent outcomes in fracture healing, musculoskeletal repair, and neural regeneration. As previously mentioned, although mechanistic studies highlight the ability of LIPUS to activate critical signaling cascades and modulate neurotrophic factors, clinical trials often fail to replicate these benefits. Key controversies arise from methodologic limitations, including unblinded designs, inadequate sham controls, and suboptimal parameter selection. Furthermore, physiologic disparities between preclinical models and humans complicate extrapolation, underscoring the need for translational frameworks that reconcile interspecies differences.

Furthermore, the mechanisms underlying the differential regulatory effects of ultrasound-induced mechanical forces across tissues require further elucidation. Substantial research has emphasized the critical need for system optimization of tissue-specific acoustic parameters, including intensity, frequency, pulse repetition frequency (PRF), and duty cycle [140]. Preclinical models demonstrate that higher intensities (e.g., 150 mW/cm²) achieve superior bone regeneration compared to conventional settings [66]. Notably, ultrasound parameters exhibit dual regulatory effects (stimulatory versus inhibitory) depending on the configurations. While elevated intensities enhance blood perfusion, excessive energy

delivery risks BBB disruption [141]. This dichotomy may stem from threshold-dependent activation of cellular signaling pathways, in which lower frequencies (e.g., 350 kHz) preferentially activate TRPA1 channels, whereas higher frequencies (e.g., 43 MHz) stimulate Piezo1 channels [142]. Heterogeneous receptor distribution contributes to ultrasound-mediated tissue heterogeneity. Recent studies reveal that LIPUS activates the mechanosensitive receptor RET in colonic tissues via compressive stress waves (rather than shear strain or thermal effects), subsequently triggering the WNT/ β -catenin pathway to promote cellular proliferation [11]. This mechanism parallels LIPUS-mediated bone repair involving Piezo1 activation or MAPK/ERK pathway induction, suggesting mechanotransduction (e.g., pressure-dependent protein conformational changes) as a conserved regulatory mechanism across tissues. Nevertheless, inter-tissue variability arises from receptor diversity and differential ultrasound energy absorption between compliant soft tissues (e.g., colon) and rigid osseous structures. Additionally, skull bone density variations pose challenges for LIPUS-mediated neuromodulation [143]. Tissue-specific parameter sensitivity is evident in cerebral blood flow (CBF) regulation, which responds strongly to intensity and stimulation duration but minimally to duty cycle adjustments [144]. Intriguingly, pulse repetition tuning may enable selective modulation of excitatory versus inhibitory neurons [145]. These findings collectively underscore the necessity for systematic exploration of the ultrasound parameter space to optimize therapeutic precision across biological systems.

Equally critical is the integration of LIPUS with adjuvant therapies to amplify the therapeutic impact. Coupling LIPUS with osteoconductive biomaterials or hydrogels could spatially guide cell differentiation and callus formation, while its synergy with stem cell therapies or growth factor delivery (e.g., BMP-2, VEGF) might enhance endogenous repair mechanisms [68, 146, 147]. Innovations, such as ultrasound-responsive nanocarriers for targeted drug release in neural or musculoskeletal niches, could further unlock combinatorial potential [148]. Clinically, bridging preclinical and human studies through advanced models, such as organ-on-chip systems or human-derived 3D tissue constructs, could elucidate species-specific mechanoresponses and refine parameter selection [149]. These efforts must be paralleled by translational research to dissect the molecular underpinnings of ultrasound-mediated cellular responses, ensuring parameter optimization aligns with human biology.

Data availability

All data generated and/or analyzed during this study are included in this published article.

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Declaration of competing interest

The authors declare no competing interests.

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