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Therapeutic Ultrasound in Experimental Animal Models: Biophysical Foundations, Tissue-Specific Effects, and Translational Perspectives

Ayşe GÖLGELİ BEDİR[1]
Yasemin AKÇORA[1]

[1]Atatürk University, Faculty of Veterinary Medicine, Department of Surgery, Erzurum, Türkiye

ABSTRACT

Therapeutic ultrasound (TUS), including low-intensity pulsed ultrasound (LIPUS) and low-intensity focused ultrasound (LIFU), is increasingly investigated in experimental animal models as a non-invasive modality capable of modulating regeneration and neural function through predominantly sub-thermal mechanical mechanisms. This narrative review synthesizes the biophysical foundations of TUS and summarizes tissue-specific evidence across musculoskeletal, neural, ocular, cutaneous, and tumoral/immune contexts. Across models, reported bioeffects commonly involve mechanotransduction-driven changes in cell signaling, neurotrophic support, microcirculatory enhancement, and modulation of inflammatory pathways, with outcomes shaped by frequency, intensity metric, duty cycle, exposure duration, and treatment timing. Bone and tendon studies generally demonstrate improved matrix organization and biomechanical recovery within non-ablative ranges, while muscle and wound-healing models support accelerated structural restoration, angiogenesis, and closure, particularly when pulsed protocols are applied. In neuromodulation, LIFU can elicit excitation or suppression depending on pulse structure and targeting, and preclinical oncology studies suggest complementary roles in localized disruption and immune microenvironment modulation. However, translation is constrained by heterogeneity in parameter reporting and dosimetry, limited tissue- and depth-specific exposure windows, and organ-specific safety considerations. We emphasize harmonized reporting standards (including intensity metrics, duty cycle, coupling conditions, and calibration) and rigorous safety assessment with long-term follow-up, especially for sensitive tissues such as the eye and central nervous system. Establishing reproducible parameter windows and rational combination strategies will be critical for meaningful clinical and veterinary translation.

Keywords: Animal models, Bone healing, Therapeutic ultrasound, Tissue regeneration, Wound healing.

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Corresponding author:
Ayşe Gölgeli Bedir
E-mail: aysegolgeli@atauni.edu.tr

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Introduction

Therapeutic ultrasound (TUS) is a non-invasive biophysical modality used to modulate tissue repair, regeneration, and neuromodulation, primarily through controlled mechanical stimulation with minimal thermal effects (Jiang et al., 2019). In therapeutic settings, ultrasound refers to acoustic waves above the audible range (>20 kHz). In practice, TUS is typically delivered in the low-megahertz range (≈0.7-3.3 MHz) using piezoelectric transducers. Acoustic energy is coupled into tissue via gel or water. The delivered "dose" depends on key protocol variables, including frequency, intensity, duty cycle, exposure duration, treatment area, and coupling conditions. Experimental animal studies indicate that low-intensity pulsed or focused ultrasound (LIPUS/LIFU) can produce biomodulatory

effects across multiple tissues, including bone, skeletal muscle, peripheral nerves, skin, ocular structures, and the central nervous system (Qin et al., 2020; Sung et al., 2021; ter Haar, 2007). Reported outcomes include enhanced cell proliferation, angiogenesis, extracellular matrix organization, neurotrophic signaling, and modulation of inflammation (ter Haar, 2007).

By contrast, high-intensity focused ultrasound (HIFU) concentrates acoustic energy to induce rapid temperature elevation and coagulative necrosis, and is therefore used for ablative applications such as tumor destruction (Zhou, 2011). Although these approaches share acoustic principles, LIPUS/LIFU and HIFU differ fundamentally in mechanism and therapeutic intent. Biological responses to ultrasound depend strongly on physical parameters, particularly frequency, acoustic intensity, duty cycle, and exposure duration (Aptel & Lafon, 2012; ter Haar, 2007). As frequency increases, penetration decreases due to greater attenuation; thus, ~1 MHz is generally used for deeper targets, whereas ~3 MHz is preferred for more superficial tissues. Intensity and exposure time influence both mechanical stimulation and heat accumulation. Duty cycle (pulsed vs. continuous delivery) shapes the temporal pattern of energy deposition and the resulting bioeffects. ISPTA denotes spatial-peak temporal-average intensity, whereas ISPPA denotes spatial-peak pulse-average intensity (W/cm^2), and both metrics help standardize reporting across studies.

This narrative review synthesizes the biophysical principles, tissue-specific effects, and translational relevance of TUS in experimental animal models. It distinguishes biomodulatory LIPUS/LIFU from ablative HIFU and highlights key considerations for reproducibility and future standardization in veterinary and biomedical practice. The included studies and their main outcomes are summarized in Table 1.

Literature Search Strategy

This narrative review was informed by a structured search of PubMed/MEDLINE, Scopus, and Web of Science (January 1980-December 2025). Search terms included “therapeutic ultrasound”, “LIPUS”, “LIFU”, “HIFU”, “animal model”, and tissue-specific keywords (e.g., bone, tendon, muscle, nerve, cornea, wound). We prioritized original in vivo animal studies reporting biological outcomes of TUS and excluded clinical-only studies, diagnostic ultrasound studies, conference abstracts without full text, and non-English papers without an accessible full-text translation. Reference lists of included studies and key reviews were also screened to identify additional relevant articles. Because this is a narrative review, we did not conduct a formal risk-of-bias appraisal or meta-analysis; instead, we synthesized the evidence qualitatively, with emphasis on parameter reporting, consistency across models, and biological plausibility.

Biophysical Basis of TUS

The biological effects of TUS arise from the concurrent interaction of thermal and mechanical processes within tissues, which are also described in the literature as thermal and non-thermal effects (Baker et al., 2001; Mason, 2011; Rantanen et al., 1999). Thermal effects primarily result from acoustic energy absorption and the consequent mild temperature elevation, which can increase membrane permeability, enhance enzymatic and mitochondrial activity, and improve local blood flow, thereby supporting fibroblast proliferation, collagen synthesis, and early tissue repair (Baker et al., 2001; Gölgeli Bedir & Yanmaz, 2023; Rantanen et al., 1999). In clinical and experimental practice, these thermal effects are most closely associated with continuous ultrasound delivery, where energy deposition is sustained and heat accumulation is more likely (Baker et al., 2001; Mason, 2011).

Non-thermal effects are predominantly mechanical and include several interrelated mechanisms such as acoustic radiation force, stable and transient cavitation, and microstreaming, which can generate localized shear stresses and micro-displacements in soft tissues (Baker et al., 2001; Miller et al., 2012; Rantanen et al., 1999). Acoustic radiation force can induce micro-displacements that create a micromassage-like effect and may improve fluid dynamics and cellular transport (Miller et al., 2012; Rantanen et al., 1999). Cavitation-related phenomena and associated shear forces may increase membrane permeability and activate mechanosensitive ion channels, contributing to sonoporation and facilitating ion exchange, uptake of growth factors, and potentially improved delivery of drugs or biomaterials (Baker et al., 2001; Shang et al., 2023). These non-thermal effects are typically emphasized by pulsed (intermittent) ultrasound settings, in which duty cycle and temporal patterning reduce heat buildup while preserving mechanical stimulation (Baker et al., 2001; Mason, 2011).

Table 1.

Summary of experimental animal studies investigating therapeutic ultrasound applications across different tissues

	Animal model	Ultrasound type	Key parameters (general)	Main biological effects	Representative references
Bone	Rat, rabbit	LIPUS / TUS	1.5 MHz; 30 mW/cm²; 20 min/day; low-intensity daily sessions	Accelerated fracture healing and callus formation; enhanced osteogenic differentiation; angiogenic support	Angle et al. (2011); Duarte (1983); Fung et al. (2014); Okur & Okumuş (2022); Takikawa et al. (2001)
Tendon	Rat (Achilles repair/tendinopathy), rabbit	TUS / LIPUS	1 MHz, 1 W/cm², 5 min/day; 1.5 MHz, 30 mW/cm²; 1 MHz, 100 mW/cm²	Improved collagen organization and maturation; increased biomechanical strength; reduced inflammation	da Cunha et al. (2001); Kurtulmuş et al. (2024); Ng et al. (2003); Şekerci et al. (2025); Zheng et al. (2024)
Muscle	Rat	Therapeutic pulsed ultrasound	1 MHz; 0.5 W/cm²	Improved microcirculation and oxygenation; accelerated muscle regeneration; reduced inflammation/oxidative stress	Piedade et al. (2008); Rantanen et al. (1999); Rosa et al. (2019); Shu et al. (2012)
Wound healing	Mouse, rat	Low-frequency noncontact US / TUS / LIPUS	40 kHz; 10 Hz pulsed; 60 min/session; 3 MHz continuous, 0.1 W/cm², 1 min/day; 1 MHz, 1 W/cm²	Accelerated wound closure; increased angiogenesis (CD31+); improved collagen organization; reduced bacterial burden in infected/diabetic models	Gölgeli Bedir & Yanmaz (2023); Roper et al. (2015); Shang et al. (2023); Wakabayashi et al. (2020); Zhong et al. (2024)
Ocular tissues	Rabbit, rat; dog (post-op)	Therapeutic US / photo-mediated US (PUT) / LIPUS	20 mW/cm²; 15 min/day; first 10 postoperative days; low-intensity short exposure; safety targets: ISPTA ≤ 50 mW/cm², MI ≤ 0.23	Reduced corneal neovascularization; modulation of ocular inflammation; improved corneal wound healing	Aptel & Lafon (2012); Kong et al. (2024); Poinard et al. (2024); Qin et al. (2020); Vicenti et al. (2003)
Brain / Neuromodulation	Rat, mouse	LIFU / transcranial pulsed US	Sub-thermal low-intensity protocols; daily 10 min sessions; 500 mW/cm² (targeted midbrain application)	Neuromodulation; reduced microglial activation; increased neurotrophic signaling; functional recovery in disease models; transient BBB modulation reported in some paradigms	Kubanek et al. (2018); Sung et al. (2021); Tufail et al. (2010); Tyler et al. (2008)
Tumoral conditions	Rat (in vivo; in vitro imaging)	Therapeutic US / Focused US; high-frequency US imaging	Therapeutic protocols in carcinogenesis models; high-frequency US tomography for tumor visualization	Reduced tumor burden and histopathological alterations; modulation of tumor microenvironment/immune response in combination approaches; high-resolution visualization	Huang & Chen (2014); Lafond et al. (2022); Ram et al. (2018); Wu et al. (2023); Xia et al. (2024)

Accordingly, low-intensity pulsed ultrasound (LIPUS) exploits primarily non-thermal mechanical bioeffects without thermal ablation and is mainly used for biomodulation, including angiogenesis, osteogenesis, neurotrophic signaling, and anti-inflammatory responses (Angle et al., 2011; Baker et al., 2001). In contrast, high-intensity focused ultrasound (HIFU) concentrates acoustic energy to produce temperatures sufficient for coagulative necrosis, leading to fundamentally different therapeutic objectives despite shared acoustic principles (Mason, 2011).

2.1. Peripheral and Neural Mechanisms

Peripheral nerve regeneration is a key experimental target for low-intensity ultrasound interventions, and animal

models provide most of the current mechanistic insight. Although direct peripheral nerve studies are fewer than musculoskeletal applications, several rodent models report pro-regenerative effects after nerve injury or reconstruction. In a rat sciatic nerve autograft model, LIPUS accelerated axonal regeneration and functional recovery in an intensity-dependent manner, highlighting the importance of dose selection (Jiang et al., 2016). Similarly, in a rat sciatic nerve crush model, ultrasound delivered at an optimized intensity enhanced regeneration and was associated with suppression of pro-inflammatory and nerve growth–inhibitory gene expression (Ito et al., 2020). Earlier work also reported accelerated sciatic nerve regeneration after neurotomy with pulsed ultrasound, supporting a reproducible effect across injury paradigms (Crisci & Ferreira, 2002).

Complementary evidence from central nervous system models strengthens the biological rationale for ultrasound-mediated neural repair. In a mouse model of Parkinson's disease, repeated LIPUS increased GDNF expression while reducing microglial activation, suggesting enhanced neurotrophic support with attenuated neuroinflammation (Sung et al., 2021). Mechanistic studies further indicate that ultrasound can modulate neuronal activity through mechanically mediated effects on ion channels, providing a plausible basis for parameter-dependent differences in neural outcomes (Kubanek et al., 2018).

Across the included peripheral nerve paradigms, the direction of effect is consistent—improved regeneration and functional recovery—yet study-to-study differences in effect size are expected given the distinct injury contexts (autograft vs crush vs neurotomy) and dose selection emphasized in the primary studies (Crisci & Ferreira, 2002; Ito et al., 2020; Jiang et al., 2016). Likewise, variability across neural outcomes is biologically plausible because the dominant target can shift from neurotrophic support and neuroinflammation modulation to more direct mechanosensitive modulation of neuronal excitability depending on parameterization and tissue access (Huy Nguyen et al., 2025; Kubanek et al., 2018; Sung et al., 2021).

Bone

Bone is one of the most ultrasound-responsive tissues, with experimental animal studies showing that osteogenic cells are highly sensitive to low-intensity mechanical stimulation. Early rabbit and rat fracture models demonstrated that low-intensity pulsed ultrasound applied at approximately 1.5 MHz and intensities below 50 mW/cm² significantly accelerated callus formation and fracture healing through predominantly mechanotransductive effects (Duarte, 1983).

Subsequent rodent work also supported efficacy in challenging healing contexts. In a rat nonunion fracture model, daily LIPUS exposure (1.5 MHz, ~30 mW/cm², 20 min/day) initiated and enhanced healing responses compared with controls, indicating that low-intensity protocols can be effective even when baseline repair is impaired (Takikawa et al., 2001). Subsequent rodent studies confirmed dose-dependent osteogenic responses. In a rat tissue-engineered bone model, low-intensity pulsed ultrasound at 1.5 MHz and ~30 mW/cm² enhanced osteoblast differentiation, extracellular matrix maturation, and early vascular infiltration without thermal damage (Angle et al., 2011). Because effective acoustic dose varies with target depth and exposure geometry, experimental evidence suggests that distance from the transducer and field characteristics can influence the delivered pressure profile and, in turn, healing outcomes-an important consideration when comparing protocols across studies (Fung et al., 2014). In addition, ultrasound-mediated benefits have been reported in inflammatory musculoskeletal conditions; in a rat arthritis model, TUS at 1 MHz and 0.5 W/cm² combined with low-level laser therapy reduced synovial hyperplasia and inflammation, suggesting an indirect osteoprotective effect (Okur & Okumuş, 2022).

Overall, rodent and rabbit models indicate that low-intensity, non-ablative ultrasound can promote osteogenesis and vascularized repair, with outcomes influenced by acoustic dose and protocol design (Angle et al., 2011; Duarte, 1983; Okur & Okumuş, 2022). However, animal and translational assessments also note variability in effect size across fracture settings, highlighting that benefit is context- and protocol-dependent (Shakouri et al., 2010).

Across bone models, findings are largely convergent in direction (enhanced callus formation and osteogenic

activity), but differences in effect magnitude are biologically plausible because fracture phenotype (standard union vs nonunion), tissue environment (inflammatory context), and delivered dose are not equivalent across protocols, even when nominal frequency and intensity appear similar (Okur & Okumuş, 2022; Shakouri et al., 2010; Takikawa et al., 2001). In particular, variation in exposure geometry and target depth can alter the in situ pressure distribution and stimulation of mechanosensitive pathways, which provides a mechanistic explanation for study-to-study inconsistencies and underscores the need for depth-aware, calibrated dosing when comparing or translating protocols (Angle et al., 2011; Fung et al., 2014).

Tendon

Recent experimental studies indicate that Achilles tendon healing is responsive to therapeutic ultrasound when dose, mode, and timing are controlled. In a surgically induced rat Achilles tendon repair model, TUS at 1 MHz and 1 W/cm² for 5 min/day improved collagen organization and cellular regeneration, and the combined ultrasound–chitosan approach produced the most consistent late-phase functional and structural recovery (Şekerci et al., 2025). In a collagenase-induced rat Achilles tendinopathy model, LIPUS at 1.5 MHz and 30 mW/cm² improved biomechanical strength and collagen maturation, with earlier initiation during the proliferative phase yielding comparable outcomes even with shorter treatment durations (Kurtulmuş et al., 2024). In rabbits, LIPUS at 1 MHz and 100 mW/cm² promoted structural repair and reduced inflammatory signaling via downregulation of the JAK/STAT pathway (Zheng et al., 2024).

Earlier foundational animal work also supports parameter-sensitive benefits in tendon repair. In rats, 1 MHz continuous ultrasound at 1.0-2.0 W/cm² increased ultimate tensile strength, emphasizing that efficacy can depend on the selected endpoint (Ng et al., 2003). In tenotomized rat Achilles tendon, pulsed 1 MHz ultrasound at 0.5 W/cm² (SATA) improved collagen fiber organization more than continuous mode, underscoring the importance of duty cycle/mode selection (da Cunha et al., 2001). Overall, studies converge on improved collagen alignment and mechanical recovery within non-ablative ranges, but the magnitude and timing of benefit vary with model type (repair vs tendinopathy), outcome choice (histology vs biomechanics vs function), and acoustic dose/mode; these factors plausibly explain divergent results and highlight the need for harmonized parameter reporting in tendon research (da Cunha et al., 2001; Kurtulmuş et al., 2024; Ng et al., 2003; Şekerci et al., 2025; Zheng et al., 2024).

Across the included tendon models, apparently “mixed” findings are biologically plausible because the studies differ not only in dose but also in the phase of healing targeted (early proliferative vs later remodeling), which can change whether ultrasound primarily influences inflammatory signaling, cellularity, or matrix maturation (Kurtulmuş et al., 2024; Zheng et al., 2024). Likewise, comparisons across studies are sensitive to mode selection (pulsed vs continuous) and to the endpoint emphasized, collagen organization versus tensile strength, so protocols that look similar by frequency may still yield different conclusions depending on duty cycle and outcome prioritization (da Cunha et al., 2001; Ng et al., 2003; Şekerci et al., 2025).

Muscle

Skeletal muscle regeneration under ultrasound has been primarily investigated using rat skeletal muscle contusion and laceration models. In a standardized contusion model, pulsed ultrasound accelerated structural restoration, including reduced fiber necrosis, earlier appearance of centrally nucleated regenerating fibers, improved myofibrillar organization, and enhanced functional recovery; importantly, the regenerative response was intensity-dependent, highlighting the need for optimized acoustic parameters (Shu et al., 2012).

Complementary findings were reported by Piedade et al. (2008) in a rat gastrocnemius laceration model, where daily pulsed ultrasound at 1 MHz promoted myogenic activity (e.g., myoblast differentiation and regenerating myotube formation) and supported organized collagen deposition without impairing myogenesis (Piedade et al., 2008). Rantanen et al. (1999), in a rat gastrocnemius microcirculation model, found that 1 MHz ultrasound at 0.5 W/cm² improved perfusion and tissue oxygenation, supporting metabolic demands during repair (Rantanen et al., 1999).

More recent studies reinforce these patterns and add mechanistic depth. Therapeutic pulsed ultrasound has

been associated with improved revascularization and functional recovery after contusion injury in rats, supporting an angiogenic contribution to repair (Chongsatientam & Yimlamai, 2016). In experimentally injured rat quadriceps, ultrasound treatment was linked to reduced oxidative stress and inflammatory markers alongside improved post-trauma fiber organization, consistent with anti-inflammatory and tissue-remodeling effects (Rosa et al., 2019). Mode selection also appears relevant: in healthy rat skeletal muscle, pulsed versus continuous ultrasound produced distinct histomorphologic changes, supporting careful choice of delivery mode when the goal is biomodulation with minimal off-target alteration (Vásquez et al., 2014). Overall, these findings suggest that pulsed protocols can support regeneration through coordinated effects on myogenesis, microvascular support, and inflammatory/oxidative modulation, while between-study variability remains biologically plausible given differences in injury type, intensity, and exposure schedules (Chongsatientam & Yimlamai, 2016; Rosa et al., 2019; Vásquez et al., 2014).

Across the included muscle paradigms, outcomes are broadly convergent in direction (enhanced regeneration and functional recovery), yet differences in effect magnitude are expected because contusion and laceration models place different demands on myogenesis versus matrix remodeling and microvascular support, and because intensity-dependent responses have been explicitly demonstrated (Piedade et al., 2008; Rantanen et al., 1999; Shu et al., 2012). Moreover, the mode-dependent histomorphologic differences reported in healthy muscle provide a plausible explanation for variable “net benefit” across protocols, reinforcing that pulsed/continuous selection and exposure scheduling can meaningfully shape the balance between desired biomodulation and off-target tissue alteration (Chongsatientam & Yimlamai, 2016; Rosa et al., 2019; Vásquez et al., 2014).

Brain / Neuromodulation

LIFU has emerged as a promising non-invasive neuromodulation technique, with most mechanistic evidence derived from mouse and rat central nervous system models. These studies indicate that neuromodulatory effects can occur at acoustic exposures well below thermal injury thresholds, supporting a predominantly mechanical mechanism of action (Kubanek et al., 2018; Tyler et al., 2008). In a rat Parkinson’s disease model, Sung et al. (2021) applied daily 10-minute LIFU sessions to the midbrain of 6-hydroxydopamine-lesioned rats at approximately 500 mW/cm², resulting in increased GDNF levels, reduced microglial activation, and improved motor performance (Sung et al., 2021).

Foundational work further supports sub-thermal, pulse-driven neuromodulation. Tufail et al. (2010) showed that transcranial pulsed ultrasound can stimulate intact brain circuits without a measurable bulk temperature rise, reinforcing that neural effects can be achieved through non-ablative exposure patterns (Tufail et al., 2010). Additional mechanistic evidence comes from neuronal models. Tyler et al. (2008) demonstrated that low-intensity ultrasound can remotely excite neuronal circuits by altering membrane mechanics, while Kubanek et al. (2018) showed that ultrasound modulates neuronal activity through mechanically mediated effects on ion channels (Kubanek et al., 2018; Tyler et al., 2008). Syntheses of the field emphasize that LIFU can produce either excitation or suppression depending on pulse structure and targeting, and that standardized reporting of sonication parameters is critical for interpreting heterogeneous outcomes across studies (Baek et al., 2017; Fomenko et al., 2018).

Together, rodent and ex vivo evidence support neuromodulatory and, in some paradigms, neuroprotective effects that are consistent with mechanically mediated mechanisms under sub-thermal conditions (Kubanek et al., 2018; Sung et al., 2021; Tufail et al., 2010; Tyler et al., 2008). At the same time, variability in reported directionality and magnitude across studies is biologically plausible because target region selection, transcranial delivery constraints, and pulse structure can meaningfully alter in situ pressure fields through skull-dependent attenuation and focusing (Baek et al., 2017; Fomenko et al., 2018). This sensitivity to delivery conditions reinforces the need for calibration and consistent dosimetry to enable reliable cross-study comparison and translation (Baek et al., 2017; Fomenko et al., 2018; Tufail et al., 2010).

Tumor / Immune System

TUS has been explored in experimental oncology as a non-invasive approach for modulating tumoral tissue responses in controlled animal models. In a DMBA-induced sarcoma model in rats, TUS application reduced tumor burden and produced histopathological alterations such as hemorrhage and necrosis, suggesting a suppressive effect on tumor progression under experimental conditions (Ram et al., 2018). Beyond direct tumoral effects, preclinical evidence increasingly emphasizes immunomodulation as a relevant mechanism: focused ultrasound–induced tumor disruption can generate immunogenic debris that supports dendritic-cell priming and downstream T-cell responses, particularly when combined with immune adjuvants (van den Bijgaart et al., 2022).

In addition to therapeutic potential, ultrasound has also been developed as an imaging tool for tumor assessment in preclinical research. High-frequency ultrasound tomography has been applied for in vitro visualization of testicular tumors in rat models, supporting non-ionizing, high-resolution imaging in experimental settings (Huang & Chen, 2014). Focused ultrasound technologies have also been investigated in preclinical tumor models for their capacity to induce localized tissue effects and modulate the tumor microenvironment, forming the experimental basis for translational oncological applications (Lafond et al., 2022). In vascular- and microenvironment-targeted paradigms, low-intensity focused ultrasound combined with microbubble-based approaches has been reported to remodel perfusion and enhance response to immune checkpoint blockade in animal models, consistent with a microenvironment “sensitization” effect (Wu et al., 2023). Separate approaches also explore systemic immune activation; for example, LIPUS irradiation of immune-relevant sites (e.g., spleen) has been reported to activate anti-tumor immunity in mouse breast cancer models (Xia et al., 2024).

Taken together, the oncology literature supports two reproducible mechanistic themes: higher-energy focused exposures that induce localized tumor disruption (with potential downstream antigen presentation) and lower-intensity or microbubble-assisted strategies that remodel vasculature, perfusion, and immune accessibility within the tumor microenvironment (Labib et al., 2025; van den Bijgaart et al., 2022; Wu et al., 2023). At the same time, apparent inconsistencies across studies are biologically plausible because tumor histology/vascularity, target depth and acoustic window, and the chosen exposure paradigm strongly influence the delivered dose and the dominant endpoint (direct cytotoxicity vs microenvironmental/immune modulation) (Hu et al., 2024; Labib et al., 2025). This heterogeneity reinforces the need for careful dosimetry, standardized reporting, and rational combination-strategy design when interpreting results and considering translation (Hu et al., 2024; Labib et al., 2025).

Ocular Tissue

Ocular tissues, particularly the cornea, are highly sensitive to both mechanical and thermal stress; therefore, TUS must use carefully controlled low-intensity (typically pulsed) parameters to remain within sub-thermal safety limits (Aptel & Lafon, 2012). In a rabbit corneal neovascularization model, photo-mediated ultrasound therapy (PUT; synchronized laser + ultrasound) produced marked regression of neovascularization while preserving corneal epithelial, stromal, and endothelial integrity (Qin et al., 2020). Recent ophthalmic safety summaries emphasize conservative exposure constraints for the eye, including low ISPTA and low MI targets (e.g., ISPTA ≤ 50 mW/cm² and MI ≤ 0.23 in some ophthalmic guidance), reflecting the narrow safety margins of ocular tissues (Poinard et al., 2024).

Ultrasound has also been applied as an external activator of biomaterial-based therapies in infectious corneal disease. In a rat keratitis model, LIPUS stimulation of an ultrasound-responsive adhesive hydrogel patch accelerated epithelial resurfacing, reduced inflammatory cell infiltration, and promoted more physiologic stromal collagen organization (Kong et al., 2024). Veterinary animal data also support corneal wound-healing modulation: in dogs after lamellar keratoplasty, daily LIPUS (20 mW/cm², 15 min, first 10 postoperative days) showed histologic evidence consistent with more advanced healing and earlier graft incorporation at the graft–cornea interface (Vicenti et al., 2003).

Collectively, the available animal studies suggest that ultrasound can modulate corneal angiogenesis and repair

when delivered under strictly constrained, low-intensity conditions, but the therapeutic window remains narrow and strongly parameter-dependent (Aptel & Lafon, 2012; Poinard et al., 2024; Qin et al., 2020). Differences in outcomes across ocular paradigms are biologically plausible because small changes in intensity metrics (ISPTA/MI), exposure duration, and coupling can shift the balance between desired biomodulation and unintended mechanical/thermal stress in a tissue with limited tolerance (Aptel & Lafon, 2012; Poinard et al., 2024). Accordingly, emerging applications, such as biomaterial activation in keratitis or low-frequency phonophoresis, should be interpreted alongside MI/pressure-related considerations and standardized reporting of coupling and exposure geometry to support safe replication and translation (Kong et al., 2024; Lamy et al., 2021; Poinard et al., 2024).

Wound Healing

TUS has been evaluated in mouse and rat excisional wound models, including acute wounds and diabetic-impaired healing. In a mouse full-thickness excisional model, noncontact low-frequency ultrasound delivered as 40 kHz, 10 Hz-pulsed exposure for 60 min/session accelerated wound closure and increased CD31+ microvascular density with more organized collagen deposition (Wakabayashi et al., 2020). In a streptozotocin-induced diabetic rat model, 3 MHz continuous ultrasound at 0.1 W/cm² for 1 min/day combined with Triticum vulgare extract reduced wound area by day 21 and increased VEGF expression by day 7, consistent with enhanced mechanotransduction and potential phonophoretic delivery (Gölgeli Bedir & Yanmaz, 2023).

Mechanistic studies also support a role for ultrasound-driven mechanical stimulation in restoring impaired skin repair. In diabetic mouse wounds, ultrasonic mechanical stimulation has been reported to reverse delayed healing and improve closure trajectories, supporting a parameter-dependent pro-repair effect on skin regeneration pathways (Roper et al., 2015). More recent diabetic-wound experiments indicate that LIPUS can also augment pro-angiogenic responses and improve healing when combined with regenerative biologics, for example by enhancing therapeutic uptake/efficacy of stem cell–derived exosomes and thereby promoting angiogenesis and closure (Zhong et al., 2024).

Ultrasound-responsive biomaterials may further augment wound repair in infected or high-risk settings. In infected diabetic mouse wounds, nanozyme hydrogels activated by 1 MHz, 1 W/cm² ultrasound engaged enzyme-like catalytic pathways (e.g., SOD-, CAT-, POD-, NOS-, and GOx-mimetic activity), reduced bacterial burden, and accelerated healing (Shang et al., 2023). Across acute and diabetic/infected models, the direction of effect is largely convergent, faster closure with improved vascularization and matrix organization, but differences in wound etiology and protocol design plausibly explain variability in effect size and in the dominant endpoint affected (Roper et al., 2015; Shang et al., 2023; Wakabayashi et al., 2020; Zhong et al., 2024). In particular, low-frequency noncontact regimens, MHz-range continuous exposures, and LIPUS-based combination strategies likely engage overlapping but not identical mechanisms (perfusion/angiogenesis, migration, phonophoretic delivery, and adjunct activation), so comparable “healing” outcomes may reflect different biological pathways depending on parameterization and treatment timing (Gölgeli Bedir & Yanmaz, 2023; Wakabayashi et al., 2020; Zhong et al., 2024). This parameter sensitivity provides a mechanistic basis for divergent collagen remodeling or infection-control readouts across studies and supports standardized reporting of intensity metrics, duty cycle, coupling/delivery format, and phase-specific timing in wound-healing research (Gölgeli Bedir & Yanmaz, 2023; Shang et al., 2023; Wakabayashi et al., 2020; Zhong et al., 2024).

Potential Adverse Effects and Limitations of TUS

TUS is generally considered safe at low intensities, but outcomes depend strongly on tissue properties and application parameters, which limits reproducibility in animal studies (ter Haar, 2007). Unintended heat accumulation can occur with prolonged exposure or near highly absorptive tissues such as bone, potentially causing localized inflammation or cellular injury (ter Haar, 2007). Mechanical mechanisms can also be harmful if not controlled, as unstable cavitation at higher intensities may generate shear stress and tissue damage (Miller et al., 2012). Safety margins are particularly narrow in the eye, brain, gonads, and growth plates, where small deviations in delivered dose may translate into disproportionate risk (ter Haar, 2007).

Importantly, the absence of overt complications does not necessarily imply meaningful biological efficacy. Across animal studies, neutral or inconsistent outcomes are reported even under apparently "safe" exposure conditions, indicating that safety and effectiveness are separable considerations (Baker et al., 2001; Fu et al., 2008). Divergent findings are biologically plausible because effect size may vary with injury severity and model phenotype, treatment timing relative to inflammatory and remodeling phases, session duration, immobilization context, and the selected endpoint (histology vs biomechanics vs function) (Martinez de Albornoz et al., 2011; Palanisamy et al., 2022). In this context, null findings may reflect under-dosing at depth, suboptimal targeting, or mismatched outcome selection rather than absence of a mechanistic effect (Martinez de Albornoz et al., 2011; Palanisamy et al., 2022). Consistently, translational assessments in some clinical contexts (e.g., operative fixation) have reported no meaningful improvement in time-to-function, reinforcing that efficacy is context- and protocol-dependent (Busse et al., 2016).

Safety reporting also warrants a more critical approach. Minor adverse events have been reported in translational settings (e.g., skin irritation, swelling, discomfort), and these may be under-captured when follow-up is short or monitoring relies solely on gross observation rather than tissue-specific outcomes (Palanisamy et al., 2022). For ocular use, conservative limits (including low ISPTA and MI) are emphasized because thermal and cavitation-related effects can threaten retinal and lens structures (Poinard et al., 2024). In neuromodulation, skull attenuation and phase aberration can distort the pressure field and create unexpected hotspots, making calibration and temperature-aware interpretation essential (Kim et al., 2014; Lee et al., 2021). Accordingly, studies should report key dosimetry indices (e.g., intensity metric, duty cycle, treatment area), coupling conditions, and monitoring practices (e.g., temperature/pressure estimation where feasible) to interpret both positive and negative outcomes and to distinguish safety limitations from avoidable under-delivery of dose (Kim et al., 2014; Lee et al., 2021; Poinard et al., 2024).

Lack of Standardization and Reporting Heterogeneity

Although ultrasound outcomes are closely linked to acoustic dose, animal studies show substantial heterogeneity in reported frequency (MHz), intensity metrics (ISPTA/ISPPA), duty cycle, exposure duration, treatment area, coupling conditions, and focusing geometry. This variation can produce divergent bioeffects even within the same tissue because penetration, attenuation, heat accumulation, and mechanical stress distributions change with protocol choices, species, and target depth. As a result, reproducibility is reduced and cross-study comparison and translation are complicated. To improve interpretability, future work should report a minimum parameter set-frequency, ISPTA/ISPPA, duty cycle (and pulse structure), exposure time, treatment area, target depth, coupling medium, device/transducer specifications, and calibration/output verification-and justify settings in relation to tissue depth and the intended mechanism (biomodulation vs ablation). Even within the same tissue, differences in target depth and proximity to bone/air interfaces may shift the balance between mechanical bioeffects and inadvertent heating.

Ultrasound Dosimetry and Safety Metrics

In addition to frequency and nominal output, dosimetry should be interpreted using standardized intensity descriptors. ISPTA (spatial-peak temporal-average intensity) reflects time-averaged energy delivery and is therefore sensitive to exposure duration and duty cycle. For pulsed exposures, ISPTA is approximately ISPPA × duty cycle (fraction), whereas ISPPA (spatial-peak pulse-average intensity) represents the intensity during the "on" phase of each pulse. Because heating risk and many biological responses scale with time-averaged energy, cross-study comparison and safety assessment are strengthened when ISPTA, ISPPA, and duty cycle are reported together, alongside transducer specifications and calibration/output verification (Baker et al., 2001; Miller et al., 2012; ter Haar, 2007).

Mechanical bioeffects and safety are also influenced by peak negative pressure and cavitation. The Mechanical Index (MI) estimates the likelihood of cavitation-related effects and is defined as peak rarefactional pressure (MPa) divided by the square root of frequency (MHz). Cavitation thresholds vary with tissue, dissolved gas, available nuclei, and pulse structure; however, lower frequencies and higher peak negative pressures generally increase

cavitation probability. This may be beneficial for sonoporation and enhanced delivery, but unstable cavitation can be injurious. Protocols should therefore justify peak pressure/intensity choices and apply conservative MI/pressure limits in gas-containing regions and fragile tissues (Miller et al., 2012; ter Haar, 2007).

Tissue-specific absorption further constrains safety margins. Bone has high attenuation/absorption and can heat at bone-soft tissue interfaces, so settings that are sub-thermal in soft tissue may not remain sub-thermal near bone. In transcranial applications, skull attenuation and phase aberration can shift the focus and create hotspots, highlighting the need for depth-aware dosimetry and careful coupling/positioning. These considerations are particularly important for the eye, central nervous system, and growth plates; therefore, conservative pulsed protocols, minimized exposure duration, and explicit safety monitoring (e.g., temperature estimation/measurement and output verification) should be emphasized. (Aptel & Lafon, 2012; ter Haar, 2007; Mason, 2011)

Translational Challenges and Perspectives

Despite promising preclinical findings, several barriers limit translation to veterinary and human clinical practice. Species differences in anatomy and tissue composition can change acoustic propagation, vascular responses, and healing dynamics. Target depth and interfaces such as bone or air may further restrict the acoustic window and increase off-target risk. Experimental setups also differ from clinical devices in output accuracy, beam profile, focusing, and dosimetry; therefore, direct parameter transfer is unreliable without calibration and output verification. Finally, regulatory and safety constraints, particularly for sensitive organs such as the eye and central nervous system, require conservative exposure limits and explicit monitoring strategies in clinical protocols.

Conclusions and Recommendations

Findings from experimental animal models indicate that TUS, particularly LIPUS/LIFU, can promote regeneration and neuromodulation across musculoskeletal, neural, ocular, and cutaneous tissues when applied within appropriate sub-thermal, mechanically dominated exposure ranges. These effects are largely attributed to mechanotransduction, neurotrophic signaling, improved microcirculation, and modulation of inflammatory pathways. However, meaningful translation will require clearer definition of tissue- and depth-specific parameter windows, harmonized reporting standards to enable replication, and rigorous safety assessments with long-term follow-up, especially in the eye and central nervous system. Future directions also include combining TUS with pharmacological or regenerative strategies to enhance therapeutic magnitude and precision, particularly in complex or chronic models.

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