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REVIEW

Male Health

Low-intensity pulsed ultrasound for regenerating peripheral nerves: potential for penile nerve

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Peripheral nerve damage, such as that found after surgery or trauma, is a substantial clinical challenge. Much research continues in attempts to improve outcomes after peripheral nerve damage and to promote nerve repair after injury. In recent years, low-intensity pulsed ultrasound (LIPUS) has been studied as a potential method of stimulating peripheral nerve regeneration. In this review, the physiology of peripheral nerve regeneration is reviewed, and the experiments employing LIPUS to improve peripheral nerve regeneration are discussed. Application of LIPUS following nerve surgery may promote nerve regeneration and improve functional outcomes through a variety of proposed mechanisms. These include an increase of neurotrophic factors, Schwann cell (SC) activation, cellular signaling activations, and induction of mitosis. We searched PubMed for articles related to these topics in both *in vitro* and *in vivo* animal research models. We found numerous studies, suggesting that LIPUS following nerve surgery promotes nerve regeneration and improves functional outcomes. Based on these findings, LIPUS could be a novel and valuable treatment for nerve injury-induced erectile dysfunction.

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INTRODUCTION

Peripheral nerves are often damaged by compression, stretch, avulsion, or division. For example, radical prostatectomy, the gold standard for surgical treatment of prostate cancer, may damage the cavernous nerves, causing neurogenic erectile dysfunction. The prevalence of erectile dysfunction secondary to nerve damage during radical prostatectomy is estimated to be 14%–90%.[1] In a recent study, Jo *et al.*[2] showed that early penile rehabilitation with sildenafil after robot-assisted laparoscopic prostatectomy significantly improved erectile dysfunction compared with delayed treatment. However, the treatment options for nerve injury after radical prostatectomy remain limited,[3] and the prognosis remains poor if treatment is delayed. Fortunately, previous studies have shown that low-intensity pulsed ultrasound (LIPUS) has the potential to induce nerve regeneration by stimulating neurotrophic factors and reducing neuroinflammation.[4,5]

Peripheral nerve damage is a significant clinical challenge, which leads to long-lasting morbidity, disability, and economic costs.[6–8] When identified, peripheral nerve injuries are typically reconstructed by primary repair (direct reconnection between damaged nerve stumps), by interposition of an artificial conduit, or by autologous nerve graft if tension-free coaptation is not possible. In cases of severe nerve injury, the long distance between the lesion and the end organ may represent a limiting factor for reinnervation.[9–12] One approach to accelerate peripheral nerve regeneration is to stimulate the physiological processes that occur following nerve injury thereby promoting nerve regeneration.

There are many proposed methods of speeding nerve regeneration, including physical methods (such as electric stimulation,[13] magnetic field stimulation,[14] and laser stimulation[15]) and biological methods (such as administration of neurotrophic factors,[16] vitamins,[17] and medications[18]). However, there are some disadvantages to the clinical application of many of these therapies, and their clinical efficacy is unproven in many cases. Therefore, a novel and effective therapeutic approach to stimulate the physiological processes involved in nerve regeneration is needed.

Very recently, LIPUS has been successfully employed to promote tissue healing;[19,20] to inhibit inflammation and reduce pain;[20] to provoke differentiation of stem cells;[21] and to stimulate tissue regeneration of muscle, nerve, bone, ligament, and articular cartilage in intervertebral discs.[22–26] It is believed that ultrasound waves stimulate tissue regeneration by transmitting mechanical energy which induces mechanical motion of molecules in periodically alternating phases of compression and rarefaction. Although no clinical studies examining the effects of LIPUS on nerve regeneration exist, several experimental studies have investigated the application of LIPUS treatment following peripheral motor nerve injury and report positive outcomes. This article presents a systematic review of the available preclinical literature reporting on the effects of LIPUS in peripheral nerve regeneration and discusses the potential clinical applications of LIPUS.

PATHOGENESIS OF NERVE INJURY AND REGENERATION

Peripheral nerves are particularly vulnerable to injury, but the nerves of the peripheral nervous system have the ability to regenerate. This

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is in contrast to the nerves of the central nervous system, which cannot regenerate. Currently, the pathophysiology of peripheral nerve injuries and the mechanisms involved in spontaneous regeneration are relatively well understood. There is some evidence that a conditioning lesion primes the peripheral nerve for regeneration.[27] Despite this structural recovery, functional recovery is often incomplete.

The process of spontaneous nerve regeneration starts with the initial response to an injury such as a complete nerve transection.[28] After nerve transection, the distal nerve ending undergoes Wallerian degeneration, which is a unique and structured form of axon degeneration.[29] At first, axonal and myelin debris are produced, and resident macrophages in the nerve tissue then differentiate into activated macrophages to phagocytose the cellular debris. In the proximal stumps of axons, activation of mRNA translation is observed. This stimulates the formation of the protein complex, importin-phosphorylated extracellular regulated protein kinase 1/2 vimentin. This complex is transported by the motor protein dynein in a retrograde direction to the cell body, and this signal informs the neuron of the axonal damage.[30] The neuron of soma then reacts by increasing its volume and breaking up Nissl bodies to promote protein synthesis.[28,31] Within a few hours of the nerve injury, the growing axonal extremity extends filopodia which are randomly oriented at first but thereafter gain unidirectionality. Next, the proximal stump sprouts processes that sample the environment for neurotrophic factors to guide them to their target.[32–34]

Successful peripheral nerve regeneration after injury relies on both injured axons and nonneuronal cells, including Schwann cells (SCs), endoneurial fibroblasts, and macrophages, which produce a supportive microenvironment to promote successful regrowth of the proximal nerve endings.[35] SCs play an important role in the axonal regeneration. They secret chemokines, such as monocyte chemoattractant protein-1, which recruit circulating macrophages to remove myelin and axonal debris.[36,37] SCs also produce neurite-promoting proteins, such as fibronectin, laminin, tenascin, heparin sulfate, and collagen, which are incorporated to replace the extracellular matrix (ECM) lost secondary to injury.[38] In addition, proliferating SCs align into columns and form "bands of Büngner," which provides a physical guide for new axonal regrowth.[39,40] To further support neuronal regeneration, SCs express cell adhesion molecules that interact with matrix proteins to modulate axonal outgrowth and pathfinding.[38,41,42] SCs also express neurotrophic factors, such as ciliary neurotrophic factor (CNTF), brain-derived neurotrophic factor (BDNF), glial cell line-derived neurotrophic factor (GDNF), and nerve growth factor (NGF), which increase cell survival and promote nerve regeneration.[35] Furthermore, it was very recently reported that SCs regulate peripheral nerve regeneration by secreting exosomes.[43]

THE PHYSICAL CHARACTERISTICS OF ULTRASOUND

Ultrasound is defined as sound waves with frequencies above the human hearing threshold. Ultrasound is clinically divided into two main categories: imaging ultrasound and therapeutic ultrasound. Both preclinical and clinical studies have shown that LIPUS stimulates tissue regeneration by transmitting mechanical energy. The beneficial biological effects of LIPUS likely result from the biomechanical conduction of the ultrasound vibration, which produces microturbulence within the intercellular and intracellular fluids in the vicinity of the wave. Recent developments in the science of ultrasound have improved and refined the technology, making ultrasound a promising therapy for various diseases.[44–47]

An ultrasound wave is a high-frequency wave that is generally 1–12 MHz. Depending on the level of ultrasonic energy, therapeutic ultrasound can be classified into two categories: high-intensity ultrasound with peak intensities of 5000–15 000 W cm^{-2} and low-intensity ultrasound with peak intensities of 0.5–3000 mW cm^{-2}. LIPUS is delivered at low intensities (<0.1 W cm^{-2}) and at a constant frequency (1–1.5 MHz).[48,49] LIPUS is both nonthermogenic and nondestructive to tissues.[50] This is in direct contrast to high-intensity continuous ultrasound.

LIPUS has been found to have a wide range of biological effects on tissues, including promoting bone fracture healing,[51] accelerating soft-tissue regeneration,[52,53] and inhibiting inflammatory responses.[54] However, the potential mechanisms producing the above biological effects are still unclear and are under continuing investigation. Low-intensity extracorporeal shock wave therapy (Li-ESWT) is similar to LIPUS, but features a single, mainly positive pressure wave with high amplitude, short duration, and fast rise time.[55]

EFFECT OF LIPUS ON PERIPHERAL NERVE REGENERATION

LIPUS promotes peripheral nerve regeneration

It has been reported that peripheral nerves are very sensitive to ultrasound stimulation and that ultrasound can reversibly regulate nerve conduction.[56] It has also been found that LIPUS can promote functional recovery of oppressive neuropathy, which suggests that LIPUS simulates damaged nerves to regenerate.[57,58] Further, a study found that LIPUS stimulates the growth of SCs, thereby accelerating the recovery of damaged nerves.[59]

Autologous nerve grafts, widely used to bridge peripheral nerve defects, serve as a standard repair technique when primary suture anastomosis is impossible.[60] However, the limited availability of donor nerves and donor-site morbidity are major limitations of this technique. In addition, the outcomes of autologous nerve transplantation are far from ideal.[61] With this in mind, researchers have investigated whether LIPUS can improve the outcomes of autologous nerve transplantation. In addition, significant effort has been made to generate synthetic nerve conduits,[62,63] which may promote axonal proliferation by developing a scaffold, recruiting support cells (*i.e.*, SCs and macrophages), and producing induction factors and extracellular matrices.[64] Multiple groups have investigated the effect of LIPUS on sciatic nerve regeneration after interposition of autologous nerve or synthetic nerve conduits. In 2016, Jiang *et al.*[65] used a rat sciatic nerve defect model with a right-sided 10 mm sciatic nerve reversed autologous nerve transplantation and treated with LIPUS (1 MHz, 0.25 W cm^{-2} for 5 min). Functional results showed that sciatic functional index (SFI) and electrophysiological evaluation were significantly increased with LIPUS. Histologic results showed that LIPUS increased the rate of axonal regeneration significantly. These results suggested that autograft nerve regeneration was improved. The authors hypothesized that LIPUS provides appropriate mechanical stimulus to promote local neovascularization, to stimulate nerve sprouting, and to provoke the release of more neurotrophic factors. In 2004, Chang and Hsu[66] found that LIPUS can improve peripheral nerve regeneration on poly(DL-lactic acid-*co*-glycolic acid) (PLGA) nerve guidance conduits seeded with SCs. These authors interposed the seeded conduit into rats' sciatic nerve gaps, then they treated the site with LIPUS (1 MHz, 0.2 W cm^{-2} for 5 min). The results showed that LIPUS stimulated nerve regrowth, and the LIPUS-treated rats exhibited considerably more myelinated axons with a larger mean area at the mid-conduit than the control group. These results suggest that LIPUS may stimulate the SCs within the PLGA conduits to regenerate nerves. In 2010, Park *et al.*[67] used a rat sciatic nerve defect model to explore the effect of LIPUS as a simple, noninvasive stimulus at the poly(lactic-*co*-glycolic acid) and

Pluronic F127 (PLGA/F127) nerve guide site. The results showed that animals treated with LIPUS (1 MHz, 0.4 W cm^{-2} for 2 min) displayed more rapid nerve regeneration (0.71 mm per day) than the group without LIPUS treatment (0.48 mm per day). The LIPUS group also showed greater neural tissue area as well as larger axon diameter and thicker myelin sheaths than the group without LIPUS treatment, indicating improved nerve regeneration. These effects of LIPUS may be due to both the physical stimulation of SCs and the activation of the neurotrophic factors.

A recent meta-analysis[55] reviewed ten preclinical *in vivo* LIPUS studies which included a total of 445 animals. The authors included four studies with sciatic nerve crush injury, one study with reverse sciatic autograft, and five studies with a conduit. The results showed that repetitive LIPUS with intensities between 200 mW cm^{-2} and 500 mW cm^{-2} significantly promoted axonal regeneration and muscle reinnervation, increased the number and myelination of axons distal to the lesion site, and improved nerve conduction velocity after nerve injury. In addition, there were no negative side effects noted. Overall, there is significant experimental evidence that LIPUS promotes both functional and structural peripheral nerve regeneration after nerve injury.

Dosage of LIPUS for peripheral nerve regeneration

In 1988, Lowdon *et al*.[68] investigated the effects of therapeutic ultrasound for regeneration of the tibial nerve following a compressive lesion in a rat model. These authors demonstrated that the nerve conduction velocity recovered significantly earlier with the lower intensity of 0.5 W cm^{-2} and significantly later with the higher intensity of 1 W cm^{-2}, as compared to the control group. They concluded that low-intensity therapeutic ultrasound promoted nerve regeneration, but high-intensity ultrasound delayed nerve regeneration. A similar study in 2001 used a rat sciatic nerve crush injury model followed with therapeutic ultrasound of different intensities and frequencies. These authors applied LIPUS three times a week for 1 month, and they found that nerve regeneration was enhanced with an intensity of 0.25 W cm^{-2} and a frequency of 2.25 MHz.[57] Over the following decade, more and more researchers utilized the sciatic nerve injury rat model to explore the effects of LIPUS on peripheral nerve regeneration. In 2002, Crisci and Ferreira[58] found that LIPUS (16 mW cm^{-2}, 1.5 MHz) stimulated faster regeneration of peripheral nerves following neurotomy. These authors suggested that the numerous thick fibers in the nerves of LIPUS-treated animals were a result of amplified SC activity, which led to earlier recovery of their myelin sheaths. In 2005, Raso *et al*.[69] found that LIPUS (1 MHz, 0.4 W cm^{-2}, 2 min duration) increased SFIs and prompted nerve regeneration after sciatic nerve crush injury. Three weeks after nerve crush injury, the SFI improved more significantly for the LIPUS-treated nerves (73%) than the control (55%). The small-diameter, thin myelin sheath fibers typical of nerve regeneration were predominant in the LIPUS-treated group, as opposed to large-diameter, thin myelin sheath fibers in the control group. This suggested that LIPUS enhanced nerve regeneration.

In 2010, Chen *et al*.[70] utilized LIPUS (0.25 W cm^{-2}, 1.0 MHz for 1 min) to treat a sciatic nerve crush rat model. Their results showed that the density of nerve fibers with myelin sheaths and the SFI of the treatment group were significantly higher than those of the control group. This suggested that LIPUS accelerated the regeneration and functional recovery of injured sciatic nerves. In 2017, another group used similar LIPUS (0.2 W cm^{-2}, 1.0 MHz for 1 min) to treat the sciatic nerve crush injury rat model. These authors found that the LIPUS-treated rats had higher SFIs, compound muscle action potentials, wet weight ratios of the target muscle, and mRNA expression of BDNF in the crushed nerve and ipsilateral dorsal root ganglia as compared with the control group. This suggested that LIPUS might promote injured nerve regeneration by stimulating BDNF release.[71] Overall, in these studies, the effective dosage of LIPUS for nerve regeneration ranged from 0.016 W cm^{-2} to 1 W cm^{-2}.

LIPUS promotes recovery of erectile function

In 2015, Lei *et al*.[4] used an erectile dysfunction (ED) rat model with streptozotocin-induced diabetes mellitus (DM) to explore the effect of LIPUS on erectile function. After 2 weeks of LIPUS treatment with different low energy levels (100, 200, and 300 mW cm^{-2}; 3 times per week), intracavernous pressure (ICP) was measured, and neuronal nitric oxide synthase (nNOS) expression in penile tissue was examined by histology and Western blot. The results showed that LIPUS enhanced the ICP levels and increased the nNOS expression in both dorsal and cavernous nerves. These results indicated that LIPUS can significantly improve erectile function in diabetic rats. In 2019, Chiang and Yang[72] hypothesized that LIPUS could have a therapeutic effect on erectile function deriving from cavernous nerve injury based on its neuroregenerative and protective effects. Thus far, little research has been done to further investigate this hypothesis or the potential efficacy of LIPUS for the treatment of ED.

LIPUS ACTIVATES SCHWANN CELLS

The effects of LIPUS on peripheral nerve regeneration are positive. However, the potential mechanisms producing the effects remain unclear and are under further investigation. As SCs play a predominant role in the processes of peripheral nerve regeneration,[73–76] many researchers have focused on the effects of LIPUS on SCs.

In *in vivo* studies, many researchers have demonstrated that LIPUS can activate SCs at the site of nerve injury. In 2002, Crisci and Ferreira[58] found increased numbers of SCs exhibiting morphological characteristics consistent with increased metabolic activity in LIPUS-treated animals after sciatic nerve neurotomy as compared with the control group. This indicated that LIPUS stimulated SCs during the regeneration of the sciatic nerve and that the increased SC activity accelerated the recovery of myelin sheaths. This study was the first to describe that LIPUS activated SCs *in vivo*. A similar result was subsequently found by Raso *et al*.[69] in 2005. These authors found that LIPUS stimulated increased SC activity and an increase in SC nuclei with the characteristic reactional appearance of synthesis activity as compared to the control group. Moreover, in 2010, Chen *et al*.[70] found that LIPUS improved SC proliferation at an earlier stage (first 4 weeks) after nerve injury.

As activation of SCs by LIPUS *in vivo* is proposed to be one of the primary mechanisms by which LIPUS promotes nerve regeneration, many groups have investigated the effect of LIPUS on SCs *in vitro*. In 2005, Chang *et al*.[59] cultured SCs in serum deprivation culture medium to simulate an environment of mechanical trauma on a nerve injury site. They then treated the cells with LIPUS, and the results showed that LIPUS reduced the level of lactate dehydrogenase (LDH) in comparison with the sham group. This indicated a protective effect of SCs. At the same time, the 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) assay showed increased numbers of living cells after LIPUS treatment, suggesting that LIPUS enhanced the activity of SCs. In 2009, Zhang *et al*.[77] cultured rat SCs to explore how the SCs respond to *in vitro* LIPUS (1 MHz, 100 mW cm^{-2} for 5 min). The results revealed that LIPUS increased SC proliferation, indicating that SCs may become mitogenic in response to LIPUS *in vitro*. A

similar result was found by Tsuang *et al.*[61] who reported lower levels of LDH and increased values of MTT. These authors concluded that LIPUS promoted SC proliferation and prevented cell death, which is consistent with results of previous studies. In 2018, Ren *et al.*[78] once again confirmed that LIPUS promoted SC viability and proliferation and explored the mechanisms of LIPUS. These authors asserted that the effects of LIPUS were a result of activation of the glycogen synthase kinase 3 beta (GSK-3β)/β-catenin signaling pathway.

Extensive studies provide evidence that LIPUS activates and promotes SC proliferation both *in vivo* and *in vitro* and that LIPUS promotes SC survival in serum deprivation culture medium, which simulates the environment of mechanical trauma on sites of injury nerve. This is of great significance for peripheral nerve regeneration and may be one of the mechanisms by which LIPUS promotes peripheral nerve regeneration after injury. Very recently, it was reported that LIPUS enhanced the secretion of exosomes from bone marrow dendritic cells (BMDCs). This may be another mechanism by which LIPUS promotes peripheral nerve regeneration.

LIPUS INDUCES NEUROTROPHIC FACTORS

A class of secreted proteins called neurotrophic factors (NFs) are essential during the development and differentiation of the central nervous system (CNS) and the peripheral nervous system (PNS). NFs consist of NGF, BDNF, and neurotrophin-3 (NT-3), among others.[79] Since the discovery of NFs in the 1950s by Levi-Montalcini,[80] *in vitro* and *in vivo* animal experiments have elucidated their ability to elicit positive survival and functional responses in neurons of the CNS and PNS.[79] After nerve injury, NFs are essential in controlling the survival, proliferation, and differentiation of neural and nonneural cells involved in nerve regeneration.[14,81] NGF was the first identified NF and is the dominant NF in the PNS. During peripheral nerve regeneration, NGF promotes the proliferation and differentiation of neurons and the repair of injured nerves.[82,83] Upregulation of NGF leads to SC differentiation and proliferation to form regenerating neurites.[84] In a sciatic nerve injury rat model, Chen *et al.*[70] found that *in vivo* LIPUS increased NGF expression compared to the control group throughout the entire postinjury period (2–8 weeks). Ren *et al.*[78] found that *in vitro* LIPUS promoted SCs secretion of NGF at both the mRNA and protein levels. In 2017, Xia *et al.*[85] found that LIPUS upregulated the expression of NGFR in cultured induced pluripotent stem cell-derived neural crest stem cells. NGFR can bind to NGF, BDNF, NT-3, and NT-4 and mediate both the survival and the death of neural cells.[86] In summary, LIPUS can promote NGF secretion both *in vivo* and *in vitro* and enhance the effects of NGF, which may be one of the mechanisms through which LIPUS enhances nerve regeneration.

BDNF plays an important role in the survival of existing neurons and in the differentiation of new neurons.[87] BDNF is associated with axonal regeneration, myelinogenesis of medullated nerve fibers,[88] and SC regeneration during the repair of nerve injury.[89] In a rat sciatic nerve injury model, Ni *et al.*[71] found that *in vivo* LIPUS increased mRNA expression of BDNF in the crushed nerve and the ipsilateral dorsal root ganglia. In 2017, in a mouse model of traumatic brain injury, Su *et al.*[90] found that LIPUS increased BDNF protein levels and inhibited the progression of apoptosis. Thus, investigators have found that LIPUS can promote the expression of BDNF in both the PNS and CNS. Ren *et al.*[78] found that *in vitro* LIPUS can promote SCs to secrete BDNF at both the mRNA and protein levels. Contrary to these findings, a study in 2009[77] showed that the mRNA expression of BDNF at the mRNA level was very slightly decreased after LIPUS treatment; these results seem implausible when compared to all other findings. In summary, we suggest that LIPUS can promote BDNF secretion both *in vivo* and *in vitro* and that the increase in BDNF may be beneficial for nerve regeneration after injury.

NT-3, a key NF in the PNS, is an important regulator of neural survival, development, function, and differentiation[91] and an important autocrine factor supporting SC survival and differentiation in the absence of axons.[92] As recent studies have shown, NT-3 has a strong effect on neurite outgrowth,[93,94] and SCs which overexpress NT-3 induce a significantly increased number of axons at the site of injury. In 2009, Zhang *et al.*[77] treated cultured SCs with LIPUS and found that the mRNA expression of *NT-3* was significantly upregulated compared with the control 14 days after LIPUS stimulation. These authors postulated that the increased expression of NT-3 induced by LIPUS might establish an environment that promotes axonal sprouting and SC migration after peripheral nerve injury.

EFFECT OF LIPUS ON CELLULAR SIGNALING FOR CELL ACTIVATION AND MITOSIS

It is well established that ultrasound therapy can promote cultured SC survival and proliferation. To define the mechanisms by which LIPUS activates SCs and promotes nerve regeneration after injury, cellular signaling was the focus of recent investigation (**Figure 1**).

Phosphatidylinositol 3-OH kinase (PI3K)/Akt pathway

In 2008, Takeuchi *et al.*[95] found that LIPUS activates PI3K/Akt pathway via an integrin in cultured chondrocytes. These authors hypothesized that LIPUS transmits signals into the cell via an integrin acting as a mechanoreceptor on the cell membrane and promotes the attachment of various focal adhesion adaptor proteins. Focal adhesion kinase (FAK) and Paxillin are then phosphorylated to initiate signal transduction to PI3K/Akt, which is known to be involved in various cellular functions including cell survival, proliferation, motility, control of cell size, and metabolism.[96,97]

Wnt/β-catenin pathway

Additional studies revealed that the activation of FAK stimulates the phosphorylation of GSK3β and stabilizes the Wnt/β-catenin protein

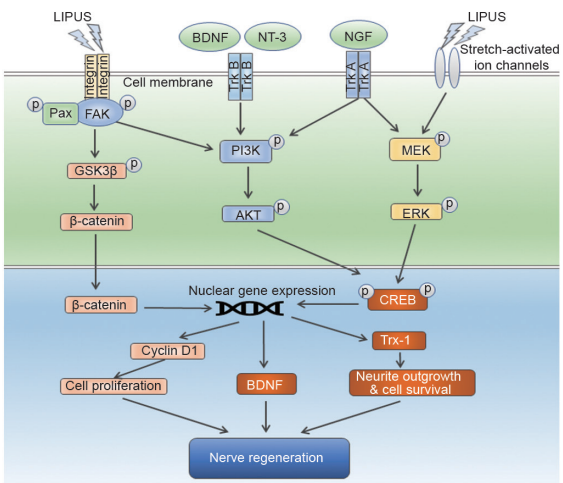

Figure 1: Cellular signaling pathways regulated by low-intensity pulsed ultrasound for peripheral nerve regeneration. LIPUS: low-intensity pulsed ultrasound; Pax: Paxillin; FAK: focal adhesion kinase; GSK3β: glycogen synthase kinase 3 beta; BDNF: brain-derived neurotrophic factor; NT-3: neurotrophin-3; PI3K: phosphatidylinositol 3-OH kinase; NGF: nerve growth factor; Trk: tyrosine kinase; MEK: mitogen-activated protein kinase/ERK kinase; ERK: extracellular signal-regulated protein kinase; CREB: cAMP-regulated enhancer B.

to promote its nuclear translocation and to activate target-gene expression.[98,99] Ren *et al*.[77] also investigated the GSK-3β/β-catenin/cyclin D1 signaling pathway to investigate the mechanisms of improved SC proliferation after LIPUS. Their results indicated that LIPUS promotes phosphorylated GSK3β at serine-9, which regulates the nuclear accumulation of β-catenin to control cell biofunctions, such as gene expression, protein synthesis, and cell viability. The subsequent increased expression of cyclin D1 stimulated SC proliferation.

Extracellular signal-regulated protein kinase (ERK1/2)–cAMP-regulated enhancer B (CREB)–Trx-1 pathway

In 2016, Zhao *et al*.[100] found that LIPUS activates the ERK1/2–CREB–Trx-1 pathway to promote neurite outgrowth in cultured PC12 cells. LIPUS significantly increased the levels of both phosphorylated ERK1/2 through stretch-activated ion channels and phosphorylated AKT through activation of tyrosine kinase A (TrkA) by increasing NGF. The activation of AKT and ERK1/2 phosphorylated CREB and increased expression of Trx-1. Trx-1 has several biological functions, including antioxidant, neurotrophic cofactor, cell growth promoter, and cellular apoptosis suppressor.

In 2017, Su *et al*.[90] found that LIPUS treatment increased BDNF levels in a mouse model of traumatic brain injury and that BDNF mediates its effect through its high affinity for the TrkB receptor. The activation of TrkB triggers the downstream PI3K/Akt signaling pathway and increases the phosphorylation of CREB, a key transcription factor for neuroprotection and BDNF production.[101]

CONCLUSIONS

There is significant evidence supporting the application of LIPUS to promote nerve regeneration and improve functional outcomes after surgery or trauma. The benefits of LIPUS in peripheral nerve regeneration are likely secondary to increased production of neurotrophic factors, activation of SCs, and stimulation of cellular signaling pathways for cell activation and mitosis. Given the preclinical benefits of LIPUS in the absence of any negative side effects, LIPUS shows promise as a potential clinical therapy following nerve surgery or trauma.

AUTHOR CONTRIBUTIONS

TFL and SJX generated the concept. DYP and GTL collected the information and DYP drafted the manuscript. GTL, ABRM, SJX, and TFL reviewed and edited the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declared no competing interests.

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