REVIEW ARTICLE

Low intensity focused ultrasound: a new prospect for the treatment of Parkinson's disease

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ABSTRACT

Background: As a chronic and progressive neurodegenerative disease, Parkinson's disease (PD) still lacks effective and safe targeted drug therapy. Low-intensity focused ultrasound (LIFU), a new method to stimulate the brain and open the blood-brain barrier (BBB), has been widely concerned by PD researchers due to its non-invasive characteristics.

Methods: PubMed was searched for the past 10 years using the terms 'focused ultrasound', 'transcranial ultrasound', 'pulse ultrasound', and 'Parkinson's disease'. Relevant citations were selected from the authors' references. After excluding articles describing high-intensity focused ultrasound or non-Parkinson's disease applications, we found more than 100 full-text analyses for pooled analysis.

Results: Current preclinical studies have shown that LIFU could improve PD motor symptoms by regulating microglia activation, increasing neurotrophic factors, reducing oxidative stress, and promoting nerve repair and regeneration, while LIFU combined with microbubbles (MBs) can promote drugs to cross the BBB, which may become a new direction of PD treatment. Therefore, finding an efficient drug carrier system is the top priority of applying LIFU with MBs to deliver drugs.

Conclusions: This article aims to review neuro-modulatory effect of LIFU and the possible biophysical mechanism in the treatment of PD, summarize the latest progress in delivering vehicles with MBs, and discuss its advantages and limitations.

KEY MESSAGES
- **Neuro-modulatory effects of LIFU** at the cellular or molecular level.
- Opening the BBB through the combination of LIFU and **MBs.**
- **Biophysical mechanism** of LIFU.

1. Introduction

The permeability of the blood-brain barrier (BBB) limits many new treatment options for Parkinson's disease (PD). A new brain stimulation method, low-intensity focused ultrasound (LIFU), is receiving increasing attention due to its neuroregulatory effects and ability to open the BBB when combined with microbubbles (MBs). By using a specially designed transducer, focused ultrasound (FUS) can be formed by aiming ultrasonic (US) waves with a diameter of only a few millimeters at the focal point.

Transcranial FUS can be divided into High-Intensity Focused Ultrasound (HIFU) and LIFU based on intensity. The former refers to the use of intensity greater than 200 W/cm^2, while the latter usually refers to the use of intensity less than 100 W/cm^2 [1]. In general, 'low intensity' is regarded be the magnitude of ultrasound intensity that is similar to or lower than the intensity commonly used in diagnostic examinations in the United States [2]. Many articles refer to this technique as low-intensity pulsed ultrasound (LIPU) [3,4], which penetrates through the skull of the animal and focuses on a particular area of the brain. There are some LIPU transducers that are non-focusing, typically used for *in vitro* experiments [5]. The spatial resolution of LIFU can reach the millimeter level. It can target structures beneath the cortex down to depths of more than 10 centimeters by using the intact skull [2], which makes it a hot research topic for many

diseases such as depression [6], thrombolysis in brain ischemia [7], Alzheimer's disease (AD) [8] and brain tumors [9].Compared to HIFU, the impact of short-term burst of LIFU on neurons and neuronal circuits is reversible and there are no adverse events [2]. This article provides a review of the clinical applications of FUS and the various preclinical studies of LIFU in the treatment of PD, summarizes the different neuroregulatory effects of LIFU in improving motor symptoms and discusses the research progress in utilizing MBs to open the BBB for the delivery of various drugs.

2. Clinical application of FUS in the treatment of PD

Currently, surgical methods available for the treatment of PD include deep brain stimulation (DBS) [10,11], levodopa carbidopa intestinal gel therapy [12], HIFU ablation of the subthalamic nucleus [13], and other methods, but these methods are highly invasive. At present, HIFU is mainly used clinically for PD, such as MRgFUS (unilateral [14–16] or bilateral [17,18]), and unilateral FUS-STN [19].The clinical efficacy of non-incisional transcranial MRgFUS will ultimately rest in appropriate target selection and optimized thermolesional coverage of the target [16,20]. Evidence on different targets, such as the ventral intermediate nucleus [21,22] and putamen nucleus [16] has shown clear benefits. Other studies combining DBS with MRgFUS have found that mixed surgery improves bilateral essential tremor without any neurological deficits [23]. From the above, different invasive therapies for PD can only be tested in specific patient groups, some of which have strong scientific basis.

LIFU has become a hot spot in many research fields. For example, in the field of neural research, studies have shown that low intensity ultrasound (LIUS) has an inhibitory effect on cytotoxic cerebral edema, suggesting its therapeutic potential in reducing AQP4 localization around astrocyte feet [24]. LIFU-responsive nanomedicine can also shrink vulnerable plaques by triggering the vaporization of perfluorohexane [25] and achieve thrombolysis *via* an acoustic droplet vaporization effect [26]. In the field of tumor therapy, LIFU's disruption of microtubules as a detoxifying agent for paclitaxel cytotoxicity in cells [27]. And recently sonodynamic therapy, a combination of LIUS with a chemotherapeutic agent (sonosensitizer) has been explored as a promising alternative to cancer therapies [28]. In the field of stem cell research, LIPU promotes the expansion, differentiation, and migration of mesenchymal stem cells [29,30], and it has been reported that LIFU-induced BBB regulation can promote neurogenesis through the activation of endogenous neural stem cells [31]. In addition, LIPU can produce different biological

effects on organs, tissues, and cells, and can be applied in fracture healing, cartilage repair [32], periodontal tissue regeneration [33], and alveolar bone regeneration [34]. In human studies, transcranial FUS inhibits the brain electrical activity evoked by somatosensory evoked potentials (SEPs) induced by median nerve stimulation in the primary somatosensory area [35,36]. Combining LIFU, tLyP-1-10-HCPT-PFP NPs can undergo phase transition to phase transition to microbubbles, enhancing the tumor ultrasound molecular imaging for tumor diagnosis [37]. And LIFU has been shown to safely and noninvasively mediate BBB opening in the human hippocampus/EC region [38]. To date, a large amount of research evidences (including *in vivo* sensory and behavioral responses [39], electrophysiological records [40], and functional imaging data [41]) have emerged and confirmed that LIFU can enhance or suppress neuronal activity and modulate the oscillations of neural networks in animals and humans. LIFU under certain conditions has been shown to reversibly permeabilize the BBB, without ablation, suggesting that LIFU-induced BBB disruption might provide an avenue for the delivery of drugs, antibodies, nanoparticles, gene therapy, and even cells to the brain, providing increasing the range of options for therapeutic macromolecules that can be used to treat PD [42,43]. However, there is still a lack of clinical therapeutic evidence of non-invasive targeted drug delivery through the BBB by LIFU for the treatment of PD.

3. Optimal sonication parameters in LIFU

Ultrasound has been shown to be an effective neuromodulator in rodents [44], monkeys [45] and humans [46], ultrasound that is below FDA standards has the potential to create a noninvasive treatment for patients with PD who are unwilling or unfit for routine surgery [47]. In 2016, Lee and colleagues used ultrasound energy doses above the limits previously described by the FDA to stimulate the V1 site in humans, underscoring the safety and accuracy of FUS [48]. Downs et al. used FUS to open the BBB in the shell and caudate nucleus of primates, monitoring safety through vital signs, physiological changes, MRI analysis, and behavioral testing. No evidence of bleeding was found, the edema in a few subjects was also completely reversible [1]. Unlike HIFU, none of the studies using LIFU showed any problems with histology, the BBB, or behavioral data [49]. There were few signs of apoptosis or ischemia when LIFU destroyed the BBB, and only mild inflammatory reaction was found in the lesion. As with any emerging technology, long-term follow-up security research remains to be done. Clinically, FUS has the advantage of being non-invasive, but it also has the disadvantage that ultrasound energy will be attenuated by skull,

resulting in the difference of energy efficiency between different patients. Different acoustic parameters can induce a variety of biological effects, depending on the cell type and tissue structure [50].There is still a gap in our knowledge of the mechanism and optimal spatiotemporal parameters of ultrasound-induced stimulation *in vivo*. Currently, there are no clear display standards for the parameters used in LIFU treatment for PD as reported in the text, leading to a wide variety of types being used. The most commonly used ultrasound parameters are sound intensity, fundamental frequency (FF), pulse repetition frequency (PRF), duty cycle (DC), burst duration (TBD), and stimulation duration (SD) [5,51–53] (Table 1). There are three main types of sound intensity, namely the spatial peak pulse average intensity (Isppa), the spatial peak time average intensity (Ispta) and the spatial average time average intensity (Isata). Ispta is the best measure of heat delivered to tissue by ultrasound. The Food and Drug Administration in the United States, along with other regulatory agencies, has clearly defined the conditions for exposure to ultrasonic waves to ensure safety [54]. As follows, the thermal index (TI) should generally be maintained below 6.0, the mechanical index (MI) should not exceed 1.9, the maximum limit of Ispta should be below 720 mW/cm^2, and Isppa should be below 190 mW/cm^2. According to Kim and colleagues, the optimal 'sweet spot' for rat neuroregulatory experiments is DC of 50% and SD of 300 ms [55]. *In vivo* experiments have shown that low frequency (500KHz, 800KHz), relatively low DC voltage (5%, 10%, 20%), and greater variations in SD (50ms, 6s) are necessary to produce neuro-regulation effects in C57BL/6 mice through LIFU stimulation [51,52,56].

But Hui Zhou and her team treated PD mices with higher ultrasound frequencies (3.8 MHz) and SD (50%) to improve motor symptoms and exert neuro-modulatory effects [57]. *In vitro* studies, low intensity ultrasound (LIU) (50mW/cm^2, 100mW/cm^2) can alleviate parkinsonian mimetic MPP(+)-induced neurotoxicity in PC12 cells, using FF of 1MHz, DC of 20% [4,5,53]. The proportion of the effect of neural modulation is closely related to the intensity, frequency, SD, and DC of FUS stimulation, and further related research is needed to determine the optimal 'sweet spot' for treating PD.

4. Potential neuro-modulatory effects of LIFU at the cellular or molecular level

4.1. Release and expression of neurotrophic factors

Neurotrophic factors are a category of proteins that play important roles in the development, survival, and

Table 1. Summary of the neuro-modulatory effects of LIFU/LIPU in PD model.

Authors & Year	Species	model	Ultrasound parameters	Stimulation site	Consequence
Hui Zhou et al. 2019	C57BL/6 mice	MPTP	FF 800 kHz, PRF 100 Hz, TBD 1 ms, SD 6 s, DC 10%, ISI 10 s, TST 40 min, 7 day, 76 mW/cm^2	Motor cortex	Motor behavior ↑ Striatal T-SOD and GSH-PX ↑
Yi Yuan et al. 2020	C57/BL6 mice	MPTP	FF 500 kHz, PRF 1 kHz, DC 5%, SD 50 ms, ISI 1s, TST 5 min, 0.255 W/cm^2	Subthalamic nucleus	Motor behavior ↑
Tian Xu et al.2020	C57/BL6 mice	MPTP	FF1MHz, 5 min/day, 10 day, 0.3 W/cm^2	–	Motor behavior ↑ DA levels ↑ Dopaminergic neuron regeneration ↑
Hui Zhou et al. 2021	C57/BL6 mice	MPTP	FF 3.8 MHz, 1 kHz PRF, 50% DC, TST 30 min/day, 3.5-day intervals for 5 weeks, 430 mW/cm^2(Ispta)	Subthalamic nucleus	Motor behavior ↑ Chronic inflammatory response of microglia and astrocytes ↓
Xueying Chen et al.2021	C57/BL6 mice	MPTP/N2a cells	FF 1 MHz, 1 kHz PRF, 20% DC, TST10 min/day, 5 day	–	Motor behavior ↑ Tyrosine hydroxylase positive neurons ↑ MPP+-induced reactive oxygen species (ROS) ↑ MPP+-induced mitochondrial membrane potential ↑
Lu Zhao et al. 2016	PC12 cells	MPP+	FF 1 MHz, PRF 100 Hz, DC 20%, TST 10 min, 30 or 50 mW/cm^2	–	MPP+-induced neurotoxicity ↓ NGF-induced neurite outgrowth ↑
Lu Zhao et al. 2017	PC12 cells	MPP+	FF 1 MHz,100Hz PRF,20% DC, TST 10 min, 50 mW/cm^2	–	MPP+-induced neurotoxicity ↓ MPP+-induced oxidative stress↓
Karmacharya et al. 2017	PC12 cells	MPP+	FF 1 MHz, TST 10 min, 30, 50, or 100mW/cm^2	–	Mitochondrial complex I activity ↑ CK2 expression ↓ MPP+-induced mitochondrial ROS generation↓ phosphorylation/ aggregation of α-synuclein↓

FF: fundamental frequency PRF: pulsed repetition frequency DC: duty cycle TBD: time of burst duration SD: stimulation duration ISI: interstimulus interval TST: total stimulation time.

apoptosis of neurons, and are considered potential therapeutic targets for neurodegenerative diseases such as PD, AD, and Huntington's disease [58]. Tyler et al. showed that focusing the LIPU on deep brain regions can stimulate intact brain circuits and the activity of endogenous brain-derived neurotrophic factor (BDNF) with potential therapeutic effects [59]. Lins et al. found in an *in vivo* rat model of AD that LIPU (1 MHz FF, 1 Hz PRF, 50 ms SD, 5% DC) significantly increased the expression of BDNF, glial-derived neurotrophic factor (GDNF), and vascular endothelial growth factor in the lateral hippocampus after 15 min of stimulation [60]. Astrocytes play a protective and even regenerative role in developing neurons by secreting neurotrophic factors [61]. *In vitro* experiments have shown that LIPU (1 MHz FF, 10 Hz PRF, 50 ms SD, 50% DC) can directly stimulate astrocytes and increase the level of neurotrophic factors [3]. Moreover, *in vivo* experiments have demonstrated that ultrasound (1 MHz FF, 1 Hz PRF, 50 ms SD, 5% DC) activates NF-κB in astrocytes through the TrkB/PI3K/Akt and calcium/CaMK signaling pathways, thereby enhancing the expression of BDNF [62]. LIPU (1 MHz FF, 100 Hz PRF, 30 or 50 mW/cm^2 Ispta, 20% DC, 10 min per day) can also activate the PI3K/Akt and erk-creb-trx-1 signaling pathways through ion channels activated by stretching, enhancing Neurotrophic Factor-induced neuronal axon growth, and this method can be used to treat neurodegenerative diseases [4]. Repetitive transcranial ultrasound stimulation can modulate the human motor cortex [63], while LIFU can safely modulate the neural circuits within the central nervous system (CNS) associated with movement-related clinical targets such as the motor cortex-spinal cord and thalamocortical pathways [64]. The motor cortex, substantia nigra, and striatum participate in basal ganglia circuits that influence the modulation of behavioral responses [65]. LIFU stimulates astrocytes in the subthalamic nucleus (STN)-motor cortex to promote the expression and release of neurotrophic factors through corresponding signaling pathways. Further research is needed to determine which specific areas of the brain are stimulated by LIFU and which signaling pathways promote the expression of neurotrophic factors.

4.2. Bidirectional regulation of neuroinflammation by microglia

Neuroinflammation has long been associated with CNS disorders, including PD and multiple system atrophy in alpha-synucleosis, but its key role in pathogenicity is not yet fully understood [66]. In the rat model of cerebral ischemic injury, the use of LIPU (1.5 kHz PRF, Isata 86mW/cm^2) can alleviate brain inflammation [67]. Under low intensity and low frequency ultrasound radiation (27 kHz FF, 0.25 W/cm^2), endothelial cells can achieve vasodilation and anti-inflammatory effects by upregulating eNOS and increasing the production of NO. However, this effect is transient and reversible [68]. Microglia are resident macrophages in the brain with neuroprotective and neurotoxic functions, and their activity in CNS is considered the most important neuroinflammatory signature marker in PD [69]. Zhou et al. performed continuous ultrasound deep brain stimulation on the STN or GP for 5 weeks after MPTP formation (3.8 MHz FF, 1 kHz PRF, 50% DC, 30 min/day). The results demonstrated for the first time that US improved motor function and reduced chronic inflammatory responses of microglia and astrocytes [57]. The results were consistent with the study by Chen et al. suggesting that DBS of STN can inhibit microglial activation in SN and normalize the expression of NF-κB and IL-1β in an animal model of PD [70]. On the contrary, MRI-guided pulsed focused ultrasound (589.636 kHz FF, 1% DC) combined with ultrasound contrast agent MBs can lead to a sustained increase in IL-1, IL-18, TNFα, and heat shock protein 70 for 24 h, and activated astrocytes and microglia increased after 24 h [71]. After the ultrasound treatment combined with MBs, the expression of anti-inflammatory cytokines (IL4, IL10, and IL13) in the CNS was increased. This can limit the degree of inflammation and demonstrate a new model for regulating brain inflammation, thus promoting the survival of neurons and inducing apoptosis in microglial cells [72–74]. Interestingly, activated microglia clear dead cells from the CNS and secrete a variety of neurotrophic factors to promote neuronal survival [75]. Bobola et al. performed transcranial focused ultrasound (tFUS) (2.0 MHz FF, 40 Hz PRF, 400 ms pulse, 190W/cm2 Isppa) on 5XFAD mice in a sedated state, and the activation of microglia coexisting with Aβ plaques and the removal of nearly 50% of Aβ plaques by 5-day tFUS [76]. Therefore, it remains controversial whether FUS improves PD motor function by inhibiting or activating microglia activation.

4.3. Reduce ROS production and oxidative stress

Mitochondrial dysfunction and oxidative stress are also considered as one of the pathogenesis of PD. The pathological and clinical manifestations of MPTP in humans, monkeys and mice are consistent with clinical and idiopathic Parkinson's syndrome [77]. MPTP is converted to MPP + by the catalysis of monoamine oxidase

B, which accumulates in DAergic neurons through the DA reuptake pathway and then actively transports into mitochondria, selectively inhibiting the activity of NADHCoQ reductase complex I in the respiratory chain and interfering with ATP synthesis. In *in vivo* experiments of Parkinson's MPTP model, LIFU stimulation of the motor cortex for 7 days (800 kHz FF, 100 Hz PRF, 10%DC, 40 min/day) can reverse the reduced levels of T-SOD and GSH-PX caused by MPTP, and improve Parkinson's motor dysfunction by exerting an antioxidative effect [52]. In the rat model of brain injury induced by AlCl3, LIFU (1 Hz FF, 528 mW/cm^2 Ispta, 5% DC, 50 ms SD) also restored the antioxidant enzyme activity decreased by AlCl3, as well as the increased SOD, GSH and GSH-Px activity, thus reducing the oxidative stress in the hippocampus treated with AlCl3 [78]. According to reports, *in vitro* PC12 cells induced by MPP+, LIPU (1 MHz FF, 100 Hz PRF, 50 mW/cm^2 Ispta, 20% DC, 10 min) [5] or LIFU (30, 50 and 100 mW/cm^2) [53] can inhibit the production of ROS and mitochondrial dysfunction in PC12 cells. The mechanism of action is the regulation of antioxidant proteins or the enhancement of mitochondrial complex I activity. Mitochondrial complex I deficiency has been widely demonstrated in the brains of PD patients [79]. However, this view is also controversial. Some articles have shown that the death of dopaminergic neurons induced by rotenone, MPP+ or paraquat does not require inhibition of mitochondrial complex I [80]. Normal mitochondrial membrane potential is a prerequisite for maintaining mitochondrial function [81]. LIPU (1 MHz FF, 1000 Hz PRF, 20% DC, 10 min) also inhibits the reduction of mitochondrial membrane potential caused by MPP+ [56]. Whether LIFU protects dopaminergic neurons by reducing MPP+ inhibition of mitochondrial complex 1 activity or maintaining mitochondrial membrane potential warrants further studying.

4.4. Promotes neuron regeneration and repair

The most intuitive therapeutic effect of LIFU is the improvement of neural plasticity. This may be because, at the molecular level, ultrasound can provide a favorable microenvironment for neural regeneration and repair by promoting gradients of local cell factors, growth factors, and adhesion molecules. Many experiments have shown that low frequency ultrasound (LFU) can affect the regeneration and repair of nerves *in vitro* and *in vivo* [82,83]. Xu et al. showed that after 10-day US treatment at the intensity of 0.3 W/cm^2, neuronal repair occurred in the bilateral substantia nigra section, and the vacuoles caused by MPTP-induced

nerve necrosis were significantly reduced [47]. And they believed that the release of dopamine induced by LIU can be attributed to the combination of neuronal regeneration and improved membrane permeability produced by ultrasound mechanics [84]. Moreover, post-exposure neurite outgrowth and neuritogenesis can be respectively observed after LIFU stimulation (500 Hz PRF, 1.168 W/cm^2 Ispta, 0.84 MPa peak acoustic pressure, 100 cycle pulse duration) in neurite-bearing and neurite-less neuronal cells [85]. Some reports suggest that the application of LIPU can accelerate the regeneration of peripheral nerves, including Schwann cells (SCs) and damaged nerves, promote the expression of the NT-3 gene, and involve changes in the expression of the BDNF gene [86,87]. Random treatment of autologously transplanted peripheral nerves with LIPU of 250 mW/cm^2, 500 mW/cm^2, or 750 mW/cm^2, functional and pathological results showed that LIPU of 250 mW/cm^2 significantly induced faster axonal regeneration rates [88]. LIPU (1 MHz FF, 0.3 W/cm^2 Ispta, 5 min daily) can also promote the permeability and selectivity of cell membranes and cell walls, increase the absorption of nutrients, thereby improving the survival and proliferation rate of SCs, which is crucial for peripheral nerve regeneration [82]. In addition, researchers have found that LIPU can induce nerve growth and regulate the production of nitric oxide, promote the differentiation of neural stem/progenitor cells into neurons, enhance the vitality and proliferation of iPSCs-derived neural crest stem cells (iPSCs-NCSC), and affect the multipotent differentiation of mesenchymal stem and progenitor cell lineages through the ROCK-Cot/Tpl2-MEK-ERK signaling pathway [89]. Lv et al. indicated that at an intensity of 500 mW/cm^2, LIPU promoted the differentiation of iPSCs-NSCs into neurons and Schwann cells, while also promoting gene and protein expression [90]. Similarly, LIU and LIPU increased mesenchymal stem cell proliferation by activating the MAPK/ERK and PI3K/Akt signaling pathways, resulting in upregulation of multiple cell cycle proteins [91,92].

5. Opening the BBB to deliver drug vehicles through the combination of LIFU and MBs

The site of the lesion in PD is focal, and early intervention through LIFU-mediated opening of the BBB and MBs is an important breakthrough in PD research [93]. Targeted gene therapy aimed at specific brain regions could potentially halt the spread of Lewy bodies, while changes in early PD biomarkers could assist in early diagnosis [94]. As a forward-thinking concept in early treatment of PD, the combined use of LIFU and MBs can help deliver drugs or
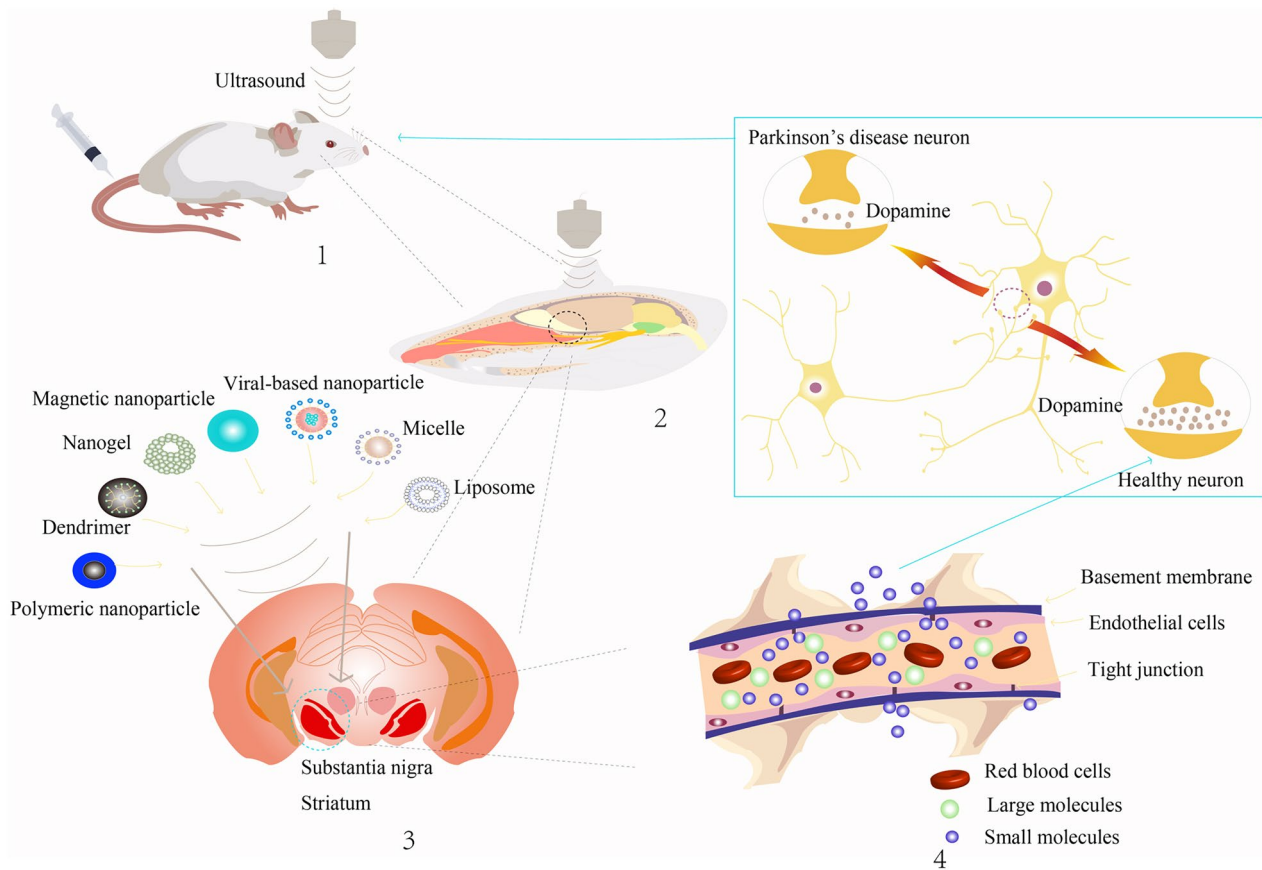
Figure 1. The strategy and effect of low intensity focused ultrasound in the treatment of PD. 1. LIFU combined with MBs opens the BBB. 2. LIFU acts on specific parts of the mice brain, such as the motor cortex, substantia nigra, striatum and other specific sites. 3. Microvesicles or nanoparticles deliver relevant genes or therapeutic drugs such as polymeric nanoparticle, dendrimer, nanogel, magnetic nanoparticle, viral-based nanoparticle, micelle and liposome. 4. Therapeutic substances typically pass through an open BBB in two ways: the paracellular route and the transcellular route.

carriers to the correct brain region for enhancing neuronal function. Specific mechanisms depend on the diversity of drugs and carriers used (Figure 1).

5.1. Pharmacological therapies

The molecular weight of BDNF (27 kDa) exceeds the ~180 Da limit for BBB permeable ionized water-soluble drugs [95], and Baseri et al. showed for the first time that exogenous BDNF can cross the BBB for noninvasive destruction, maintain biological activity, and trigger neuronal downstream signaling effects in highly localized regions of the brain [96], and GDNF family member Neurturin was also detected in the caudate putamen and SN within 1 h after intravenous administration [97]. However, the administration of neurotrophic factors *via* intravenous injection has not fully achieved therapeutic levels to reverse the disease. Current issues include shortening the half-life in the bloodstream and generating a large amount of systemic side effects [98]. Attaching GDNF to the MB surface by a biotin-affinin method allows the highest aggregation in brain tissue, with better results

than bare GDNF [99]. It has also been shown that FUS-induced active pumping enhanced BDNF release with intranasal administration in combination with FUS [100].

In addition to neurotrophic factors, other large molecules can also cross the BBB when opened by LIFU. Lipid-based PLGA nanoparticles encapsulated with curcumin are combined with LIFU to deliver the curcumin locally to deep brain nuclei, enhancing its efficacy [101]. Fibroblast growth factor-20 (FGF20) is a paracrine member of the FGF family and is preferentially expressed in the dense fraction of the substantia nigra (SNpc). Compared to rhFGF20 or rhFGF20 liposomes, FUS-liposome-rhFGF20 significantly improved apomorphine-induced behavioral disorders and prevented the loss of dopaminergic neurons in SNpcs [102]. The BBB opening mediated by FUS can also non-invasively transport neurotransmitter chemical substances to the target location in the brain, such as inhibitory neurotransmitter γ-aminobutyric acid transported to the right somatosensory cortex area of the rat brain, thereby further reducing the blood oxygen level-dependent signal [103]. Using MRI-guided

ultrasound beam positioning, neural stem cells expressing green fluorescent protein (GFP) were specifically delivered to the striatum and hippocampus of rats, and these cells were able to express doublecortin [104]. From the above, we can believe that the combination of ultrasound and minimally invasive ultrasound system will provide greater prospects for the treatment of PD.

5.2. Gene therapies

The combined application of LIFU and MB to help efficiently deliver gene vectors to specific brain regions (Figure 1) is currently a viable therapeutic strategy for patients with PD [105]. These vectors can be broadly divided into four types: DNA-loaded MBs, DNA-NPs, gene-liposome-MBs complexes, and gene-AAV vectors (Table 2).

5.2.1. DNA-loaded MBs
DNA-loaded MBs are a more novel way of administration than MBs and DNA injections separately. Studies have shown that the combination of ultrasound and pBDNF-EGFP loaded MB significantly enhances BDNF expression [106]. Cationic methyl bromide (cMB) has a

better binding ability to GDNF plasmids than neutral methyl bromide. A cationic MB platform loaded with GDNF plasmids triggered by focused ultrasound has a higher therapeutic effect than injecting high-titer GDNF genes into the brain [107].

5.2.2. DNA-NPs
It has long been reported that the use of LIFU is able to activate stable MB oscillations to trigger the release of drugs in specific target regions such as pons [108]. MRI-guided FUS delivery of nano-MBs improved the transfection efficiency of the Nrf2 gene [109]. Brain penetration of nanoparticles (BPNs) coated with dense polyethylene glycol corona can also overcome BTB/BBB. Elizabeth Nance et al. demonstrated that systemic administration of FUS and DNA-BPN in areas treated with FUS can result in dose-dependent transgene expression [110]. Research has shown that GDNF-BPN can achieve therapeutic levels in the rat striatum localization area through the FUS delivery pathway, with a duration of up to 10 weeks [111]. Currently, there is limited research on combining nanosystems with FUS for gene delivery, mainly because many nanosystems can directly penetrate the BBB, reducing damage to

Table 2. Summary of different strategies for FUS-mediated Permeabilization of the BBB in PD model.

Authors & Year	Species	Model	Ultrasound parameters	Delivery vehicle	Deliverable	Dose&time	Consequence
Gesthimani Samiotaki et al. 2015	C57BL/6 mice	–	FF 10 MHz, PRF 10 Hz, TST 60s	Microbubbles	NTN	5 μg/g	NTN bioavailability↑
Chung-Yin Lin et al. 2016	Balb/c mice	MPTP	FF 500 kHz, TBD 10 ms, DC 1%, RPF 1 Hz, TST 60 s	gene-liposome-microbubbles	GFP/GDNF	27μg；21days	LpDNA-MBs complexes administration↑
Ling Long et al. 2017	SD rats	6-OHDA	FF 0.5 MHz, TST 30 s	pDC315 nanoparticles	Nrf2	0.01 mg/kg; 14 days	Nrf2 gene transfection efficiency↑ Oxygen species levels↓
Brian P. Mead et al. 2017	SD rats	6-OHDA	FF 1.15 MHz, PNP 0.6 MPa	Brain penetrating nanoparticles	GDNF	0.5 μg/g body weight	GDNF protein content ↑
Peijian Yue et al.2018	SD rats	6-OHDA	FF 1 MHz, DC 20%, 2 W/cm^2	PEGylated liposomes-coupled microbubbles	GDNF	5μg; 3 weeks	PLs-GDNF-MBs delivery↑ Behavioral deficits ↓ Neuron loss ↓
Kristiana Xhima et al. 2019	Transgenic mice	–	FF 1.68 MHz, TBD 10 ms, PRF 1 Hz, TST 120 s.	AAV9 vectors	shRNA sequence targeting human α-syn	1.25×10^{10} VG/g	Viral vectors targeting α-synuclein delivery↑
Maria Eleni Karakatsani et al. 2019	C57BL/6 mice	MPTP	FF 1.5 MHz, PRF 10 Hz, TST 60 s, PNP 0.45 MPa.	Microbubbles, AAV1-CAG-eGFP-GDNF vectors	GDNF, NTN	Neurturn 20μg/g of body mass);100μl diluted AAV; 12 weeks	Intravenous neurotrophic delivery↑ Nigrostriatal dopaminergic pathway's damage↓
Chung-Yin Lin et al. 2020	Balb/c mice	MPTP	FF 1.0 MHz, TBD 10 ms, PRF 10 Hz, TST 3 min.	Microbubble (MB)-liposome complex	GDNF, BDNF, and concurrent GDNF/BDNF gene delivery	60μg; twice a week for 3 weeks	BDNF or GDNF gene delivery↑ Behavioral deficit↓ Dopaminergic neuronal loss↓ Calcium influx, GFAP and caspase 3 expression↓

FF: fundamental frequency PRF: pulsed repetition frequency DC: duty cycle TBD: time of burst duration. SD: stimulation duration ISI: interstimulus interval TST: total stimulation time.

dopamine neurons in PD models and reversing their neurological behavioral deficits. For example, gold nanoparticles [112], gelatin nanoparticles loaded with neuropeptide substance P [113], GDNF genes packaged with vascular iopep-2 modified NP [114], and brain-targeted peptide rabies virus glycoprotein 29 modified polymer nanoparticle systems [115]. Perhaps these nanosystems combined with LIFU to achieve gene transmission can further improve the level of drug targeting and therapeutic effect, which has broad prospects.

5.2.3. Gene-liposome-MBs complexes
Polyethylene glycol (PEG) liposomes (PLs) are promising and valuable drug carriers [116,117]. Using MRI-guided FUS to deliver PLs-GDNF-MBs to the brain is beneficial in reducing neuronal loss and behavioral deficits in the PD mouse model [118]. Liposomal plasmid DNA-MBs (gene-liposome-MBs) complexes are superior to LpDNA-MBs administration alone in terms of gene delivery and expression [119]. Lin et al. combined liposomes carrying BDNF or GDNF genes with MB to restore normal secretion of FUS and restore motor behavior in a PD model [120].

5.2.4 gene-AAV vectors
Bartus et al. reported that the clinical gene therapy trial using viral vectors to deliver neurotrophic factors did not achieve the primary therapeutic outcome, primarily due to incomplete delivery to the target structure and invasive surgical strategies [121]. MRI-guided FUS efficiently and noninvasively delivers gene viral vectors (including adeno-associated virus 9 (AAV9) [94,122], AAV2/1 [123], rAAV [124]) to neurons, astrocytes, and oligodendrocytes in multiple brain regions to express transgenes. Clinically scalable microbubble drug complexes (MDCs) used to carry viral genes, such as AAV.SIRT3-myc (AAV expressing myc-tagged SIRT3), have potential as disease-modifying strategies for treating PD and other neurodegenerative diseases [125]. It is anticipated that the binding of MDC to other viral genes may be useful in the treatment of PD, although significant limitations exist, such as safety issues, high production costs, and limited packaging capabilities of the virus [126].

6 Biophysical mechanism of LIFU

6.1. Biophysical mechanism of LIFU exerting neuro-modulatory effects

Currently, the debate on the biophysical mechanisms of LIFU is ongoing, and three possible physical mechanisms - heat generation, cavitation, and mechanical agitation - represent ways in which it can exert its biological effects. We are all aware that the biological effect of HIFU mainly involves thermal ablation of tissue [127], whereas the neural modulation effect of LIFU is only observed to cause mild temperature increase (below 0.1 °C) [128]. Surprisingly, Darrow et al. demonstrated that LIFU primarily reversibly inhibits somatic sensory evoked potentials (SSEPs), resulting in neuro-suppression [129], which is achieved at temperature changes of approximately 2 °C [64], depending on ultrasonic intensity rather than pressure wave amplitude or DC. However, further studies have shown that the effect of LIFU is mechanical agitation. Mechanosensitive channels are a type of transmembrane protein that can respond to mechanical force or stress [130]. The mechanical forces induced by the US on the plasma membrane activate the mechanosensitive channels in the CNS, leading to extensive morphological changes [131]. These mechanosensitive ion channels are typically present in potassium and sodium mechanosensitive ion channels [132–134]. Mechanically induced stress by LIFU converts the stimulated permeation stress into an ion flux on the voltage-gated channel, further depolarizing and producing an action potential [135]. For example, LIFU attenuated evoked potentials in rat hippocampus [136]. Mihran et al. also found in their study that short-term (0.5 ms) focused ultrasound (FUS) can excite the sciatic prepatellar nerve in frogs while lowering its action potential [137].Neural regulation induced by LIFU may also occur through mechanical stretching of lipid bilayers, and activated voltage-gated sodium channels can trigger exocytosis and synaptic transmission in the hippocampal circuit [133]. Therefore, the mechanical forces generated by LIFU not only affect the conformational activity of ion channels [138], but also on action potentials and synaptic vesicle release [139]. It is believed that the cell membrane is capable of absorbing the mechanical energy of ultrasound and converting it into contractions and expansions within the intracellular space [44]. This refers to the acoustic cavitation effect, also known as the 'dual-tone sound carrier', which is another method of US neuro-regulation [140]. It is able to regulate the influx and efflux of Ca2+, K+, and Na + ions, as well as produce action potentials in neuronal axons [141]. However, Wall and his colleagues' early research suggests that cavitation is not the primary cause of ultrasound-induced neuromodulation [142], but that the BBB can be temporarily opened [143]. The biophysical mechanisms

underlying low-intensity focused ultrasound have not been fully understood, and it may not be possible to attribute it to just one mechanism as they may interact with each other.

6.2. Routes that cross BBB mediated by LIFU and MBs

The combination of LIFU and MB induces targeted drug delivery through two main pathways: one is through the intracellular pathway, while the other is through the extracellular pathway. On one hand, using sulforhodamine B and rhodamine B hexyl ester as markers of intercellular permeation in the stratum corneum, it has been shown that intercellular permeation pathways are utilized in the delivery of LIU and LIU/SLS treated skin [144]. When the FUS wave is activated, MBs with a diameter of 1-4 μm expand or contract under low pressure, and this is stable cavitation [145]. The mechanically stretched local blood vessel walls of brain tissue from rapidly expanding but not yet ruptured bubbles produce fluid shear forces and circumferential stress on capillary walls, thereby enlarging the tight junctions between various microcirculation endothelial cells, increasing endothelial cell membrane pores, and triggering intercellular phagocytosis [146]. The joint stimulation of LFU and MBs is able to upregulate the expression of caveolin-1 and caveolin-2, which triggers an active transport process and creates a brief window of time (approximately 4-6 h) that can be activated by the fluctuation of microvesicles to promote intercellular transport through the blood-tumor barrier [147] and activate endothelial cell receptors to promote molecular intercellular transport [148]. Microfluidic and shear stress can also promote fluid-phase endocytosis in cells [149]. The mechanical force generated by MBs can cause deformation of the cell membrane or rearrangement of the cell cytoskeleton, thereby changing the tension of the cell membrane and stimulating the mechanical sensors located on the cell membrane, thereby regulating endocytosis [150]. The presence of vesicles and cytoplasmic channels within cerebral endothelial cells also implies an increase in endocytic activity [151]. On the other hand, studies have shown that within several hours after the combination of ultrasound and MBs (USMB), the bypass diffusion of compounds increases [152,153].

In an *in vitro* transwell cell model, USMB increased the cell paracellular permeability of anti-CXCR4 nanobodies by two-fold [154]. It is speculated that the downregulation of tight junction proteins or damage to the endothelial cell wall may allow for the transport of substances through the BBB *via* transcellular transport [155]. The combined application of LIFU and MBs has opened up a new approach for the treatment of PD, but its mechanism still poses numerous questions. Therefore, controlling the energy of LIFU to stably excite MBs in a cavitation state is particularly crucial.

7. Discussion

PD is one of the most common neurodegenerative diseases, with an annual incidence of 1.1-1.9‰ and prevalence of 10.8-25.7‰ in Europe [156]. This disease not only has a significant negative impact on the quality of life of patients, but also places a heavy burden on society and the economy. However, due to the complex etiology, there is currently a lack of safe and effective treatment options for PD.

As a new intervention method, LIFU can exert a wide range of neuroregulatory effects on various cascading reactions caused by thermogenesis [129], cavitation [131], and mechanical agitation [44], including promoting the generation of neurotrophic factors, regulating microglia [68], reducing oxidative stress [52], and promoting the regeneration and repair of neurons [83]. More importantly, the safety profile of MRI-guided FUS enables multiple treatments, and the duration and spatial properties of BBB opening can be adjusted by changing ultrasound parameters and the size and dose of MBs (MB) so that multiple pharmacological substances or gene vectors can be delivered to the region of interest [157]. The above technical characteristics of LIFU provide a theoretical basis for the formulation of a new therapy for PD. Compared with traditional drug or surgical therapies, LIFU combined with MBs auxiliary specific pharmacological/gene therapies has predictable advantages in terms of targeting specificity, safety, and efficacy. Moreover, these therapeutic methods can also be widely applied to other neurodegenerative diseases, such as AD [38] and motor neuron diseases [158]. Although the broad prospect of LIFU in clinical application is close at hand some key problems still need to be solved. Firstly, the use of low-frequency piezoelectric transducers in LIFU entails significant spatial limitations in terms of the excitation volume, typically with a diameter exceeding several millimeters, thereby curtailing its application in wearable devices. According to reports, using a new photoacoustic transducer (with a diameter of 600 μm) can activate neurons within a radius of 500 μm around the tip of the optical fiber, providing better spatial resolution than traditional piezoelectric low-frequency sensors [159]. In addition, the skull weakens ultrasound energy which leads to variations in energy

efficiency among different patients. Through the use of 3D printing technology, the correlation between skull composition and ultrasound energy can be determined and compared to actual skull components for determining the optimal ultrasound parameters required for clinical research [160]. Furthermore, there is potential hidden danger associated with LIFU when opening the BBB. 'Highly repetitive' LIFU treatment may cause edema, microbleeds, and acute inflammation in fragile brain tissue.

8. Conclusion

Therefore, long-term longitudinal safety studies are still needed for the clinical application of LIFU, with a focus on examining various parameters (including intensity, frequency, SD, and DC) to ensure its safety. Undoubtedly, in the clinical development of LIFU, developing a procedural and standardized ultrasound paradigm and improving personalized treatment strategies will become the inevitable research focus.

Authors contributions

Ling Long directed the conception and drafting of the review; Yun-Xiao Zhong and Jin-Chi Liao participated in the conception and drafting of the review; Xv Liu, Hao Tian and Li-Ren Deng made critical revisions to the knowledge content; Long Ling finally approved the version to be published; and that all authors agree to be accountable for all aspects of the work.

Disclosure statement

The authors have no conflicts of interest to declare.

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. Data sharing is not applicable to this article as no new data were created or analyzed in this study. The source of the figure and table provided in the article were original.

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