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REVIEW ARTICLE

Intracerebral drug delivery using microbubble/nanodroplet-assisted ultrasound to address neurodegenerative diseases

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Abstract

Background: The blood–brain barrier (BBB) is a selective and semi-permeable barrier essential for protecting the brain's parenchyma against pathogens and toxic molecules present in the bloodstream. It consists of a monolayer of brain capillary endothelial cells, pericytes, astrocytic end-feet, and neurons. The tight junctions between endothelial cells prevent paracellular transport, further reinforcing its selectivity. However, this high level of selectivity represents a significant challenge for the delivery of therapeutic molecules to the central nervous system. **Aim:** Microbubble-assisted ultrasound (US) is a promising strategy for transiently permeabilizing the BBB to enable safe, non-invasive, localized, and efficient drug delivery to the brain. This approach enhances drug extravasation and bioavailability. Recently, nanodroplets (NDs) have emerged as good candidates to replace MBs. The aim of this review is to provide an updated overview of the rapidly expanding field of MB/ND-assisted US for the treatment of neurodegenerative diseases. This exciting field bridges research in biology and chemistry (MBs, NDs), US technology and the development of new drugs, small molecules, and biomedicines. The review begins with an update on MBs and NDs and discusses laboratory-manufactured and clinically approved devices such as Sonocloud®, NaviFUS®, and ExAblate Neuro®. It then focuses on the potential use of MB/ND-assisted US in treating neurodegenerative diseases, particularly Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, and Huntington's disease (HD). **Relevance for patients:** Acoustically mediated BBB opening is an innovative and rapidly advancing strategy that holds great promise for improving the efficacy of existing treatments for neurodegenerative diseases. It also facilitates the discovery of new therapeutic molecules by enhancing their delivery to the brain.

Keywords: Blood–brain barrier opening; Sonoporation; Ultrasound; Microbubbles; Nanodroplets; Neurodegenerative diseases

1. Introduction

The large family of neurodegenerative diseases includes Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and HD. Over 33 million people worldwide are affected by these conditions and, with the rapid aging of the population; they have become a major public health problem and a heavy socio-economic burden.[1] In developed countries, the population over 65 has grown considerably over the past 50 years, coinciding with a rise in the incidence of neurodegenerative diseases. These diseases are now among the leading causes of mortality. Their management is often challenging and complex for caregivers and imposes substantial costs on healthcare systems. These diseases are characterized by a loss of neurons, leading to deficits in memory, cognition, and motor behavior.

At present, most treatments are either palliative or ineffective, offering no curative solutions. They mainly alleviate the clinical symptoms of these diseases but rarely target the underlying pathophysiological mechanisms. Some patients do not respond to pharmacological treatments, while others develop drug resistance. In addition, several of these diseases lack any form of treatment altogether.

In this context, the scientific and medical community has made significant efforts to design and validate pharmacological treatments that target the brain regions affected by these diseases. However, many systemically administered therapeutics show limited or no accumulation in the brain parenchyma and often cause off-target effects due to non-specific accumulation in healthy tissues. One of the main barriers to the delivery of therapeutics from the vascular compartment to the brain parenchyma is the blood–brain barrier (BBB).

The BBB is one of the most selective and semi-permeable endothelial barriers. It consists of a monolayer of brain capillary endothelial cells, surrounded by a basal lamina, astrocytic perivascular end-feet, pericytes, and neurons.[2] The presence of tight junctions (TJs) between adjacent endothelial cells reinforces the selective permeability of the BBB, preventing paracellular diffusion of molecules (Figure 1). The primary function of BBB is to physically and metabolically control the transport of endogenous and exogenous molecules, thereby maintaining brain homeostasis and function while protecting the brain microenvironment from systemic neurotoxic substances and pathogens (e.g., bacteria, viruses, etc.).[3] The transport of molecules at the level of brain and blood vessel cells is governed by two main pathways: One passive and the other active. The passive pathway is the paracellular pathway, which allows water-soluble molecules to pass through TJs. The active pathways are the transcellular pathways, which depend on the physicochemical properties of the transported molecules and include the transcellular lipophilic pathway (e.g., lipid-soluble molecules), transport proteins for specific molecules (e.g., glucose, amino acids, etc.), receptor-mediated transcytosis (e.g., insulin, transferrin, etc.), and adsorptive transcytosis (e.g., albumin and other plasma proteins). These pathways are responsible for transporting the nutrients and gases required to control brain homeostasis and functions.[2]

Because of its vital physiological roles, the BBB poses a significant challenge to treating brain diseases by severely limiting or completely blocking the intracerebral (i.c.) bioavailability of therapeutics. Indeed, this BBB excludes nearly 100% of large neurotherapeutics (*e.g.* monoclonal antibodies, recombinant proteins, nucleic acids) and over 98% of small molecules (<400 Da),[4] largely due to their physicochemical properties. This barrier explains the limited efficacy of many therapies for brain disorders.[5] In addition, the presence of active efflux transporters (e.g., ATP-binding cassette transporters) at the BBB

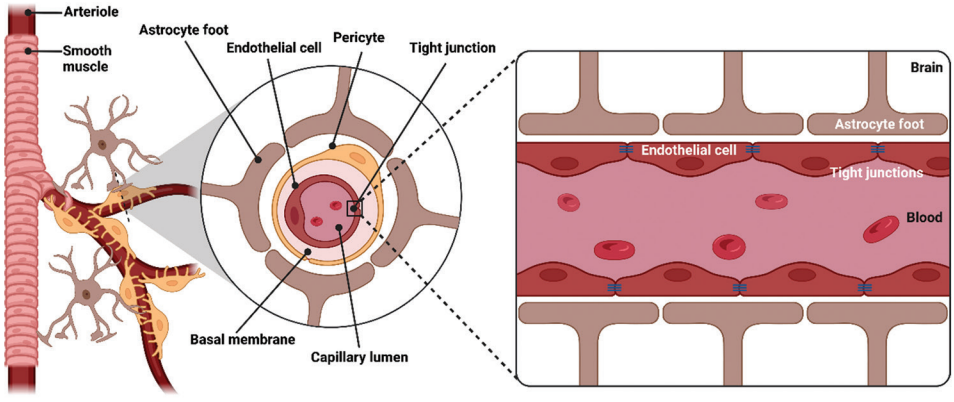

Figure 1. Schematic diagram of the blood–brain barrier and tight junctions. Adapted from "Brain vascular system". Retrieved from https://app.biorender.com/biorender-templates.

further contributes to the exclusion of these therapeutics by transporting them out of the brain tissue and back into the bloodstream.[6]

The integrity and various functions of the BBB are often compromised in many brain diseases, for example, due to neuroinflammation.[7-9] Such BBB disruptions are the consequences of disease progression. The increase in BBB permeability observed in Alzheimer's[10] and PDs[11] is positively correlated with improved i.c. bioavailability of therapeutic molecules, although it still fails to reach an efficient therapeutic dose. The intra-individual and inter-individual heterogeneity of these BBB disruptions might explain this observation.[12] In this context, the design and validation of targeted drug delivery systems are needed to increase the i.c. dose of therapeutic molecules while minimizing off-target effects.

For several decades, the scientific community has been developing safe and efficient methods for i.c. delivery of therapeutic molecules. These methods can be classified into two categories: (1) the invasive methods, which require surgical interventions to insert i.c. implants or microchips or to perform intraventricular and intrathecal infusions; (2) the non-invasive methods, which rely on either biochemical agents (e.g., mannitol, vasoactive agents, etc.) or physical agents (*e.g.*, electric field, magnetic field, etc.) to transiently disrupt the BBB, or on genetic/chemical modifications (e.g., fusion proteins, cell-penetrating peptides, etc.) of therapeutics or the use of biopharmaceutical vectors (e.g., nanoparticles, Trojan horses, viral vectors, etc.) to deliver molecules through the BBB's native transport pathways.[13-16] Among these drug delivery methods, acoustically mediated drug delivery using microbubbles (MBs) or nanodroplets (NDs) is a promising modality for the non-invasive and targeted delivery of therapeutic molecules into brain tissues.[17-21]

In this review, we will first discuss the different approaches using MBs and NDs in combination with ultrasound (US). We will then review pre-clinical and clinical studies employing this strategy for the treatment of neurodegenerative diseases, including its efficacy, safety, limitations, and future prospects.

2. Methods

The electronic databases PubMed® and ClinicalTrials.gov were screened using pre-defined search dates (January 1995 – July 2024) and terms related to i.c. drug delivery using microbubbles/NDs-assisted US for brain diseases. The search terms for the PubMed® database were: (BBB opening [MeSH terms]) AND (drug delivery [MeSH terms]) AND (US [MeSH terms]) AND (microbubbles [MeSH terms] OR NDs [MeSH terms]) AND (neurodegenerative diseases [MeSH terms]) AND ('English'[language]). The search terms for the ClinicalTrials.gov database were: 'blood–brain barrier opening' AND 'drug delivery' AND 'ultrasound' AND 'microbubbles OR nanodroplets' AND 'neurodegenerative diseases'. The inclusion and exclusion criteria are summarized in Table 1. The results of our database analysis are presented in Figure 2.

3. Acoustically mediated drug delivery using microbubbles and NDs

The great interest in acoustically mediated drug delivery using MBs and NDs for the treatment of brain disorders, particularly neurodegenerative diseases, is clearly reflected in the increasing number of publications in this field, as shown in the histogram chart in Figure 3. This US modality induces transient, efficient, and safe permeabilization of the BBB, thereby enhancing the extravasation and the i.c. bioavailability of therapeutics (Figure 4).[22-25]. The resulting increase in the i.c. dose of therapeutics improves their therapeutic efficacy while minimizing their off-target effects on healthy tissues.[25] Hynynen *et al.*[26,27] were the first to report BBB disruption in a rabbit model without causing neuronal damage.

As described below, MBs and NDs can also act as sonoresponsive drug carriers, releasing their payload specifically in the target tissue under US action. Furthermore, these sonoresponsive particles can be functionalized with targeting agents to bind membrane biomarkers that are overexpressed on target cells (e.g., endothelial and cerebral cells, etc.), thereby improving the specificity of therapeutic delivery.[28] This US modality is non-invasive, easy to apply, and cost-effective, making it a viable method for i.c. delivery of a wide range of therapeutics, including chemotherapeutics, monoclonal antibodies, nucleic acids, viral vectors, stem cells, and immune cells. The technique is typically guided by magnetic resonance imaging (MRI) and monitored using passive cavitation detection devices (PCD).[29] Therapeutic delivery can be triggered on demand and precisely controlled spatially and temporally by US focusing and directed propagation. In this section, we will describe MBs and NDs, including their composition,

Table 1. Inclusion and exclusion criteria used to select studies

Inclusion criteria	Exclusion criteria
In vivo	*In silico, In vitro*
Original article	Review papers, comments, and letters
Efficacity, bioavailability, safety	Neuropsychiatric disorders,
BBB opening and drug delivery	Neurooncological diseases
Neurodegenerative diseases	Drug delivery with US only
MB/ND-assisted US	Other languages
English	

Abbreviations: MB: Microbubble; ND: Nanodroplet; US: Ultrasound.

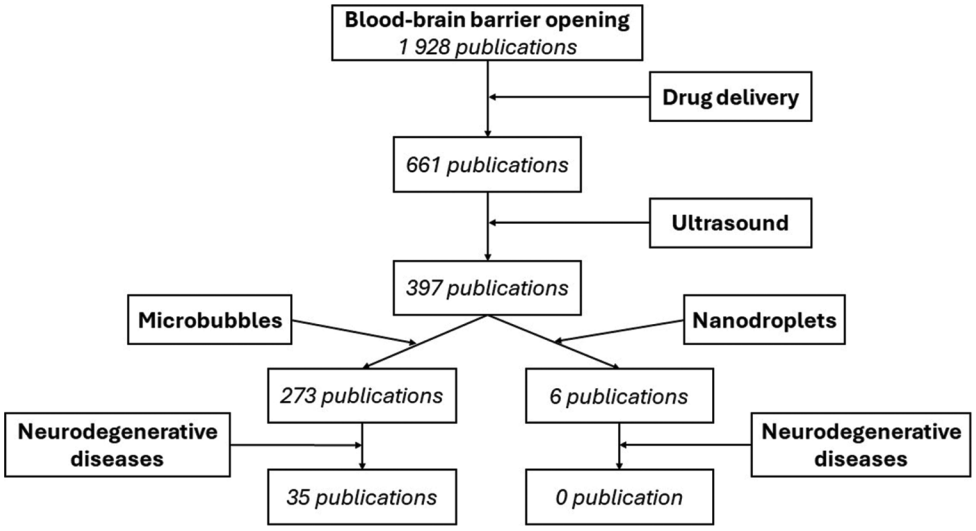

Figure 2. Flow diagram detailing the search and selection process applied during the review

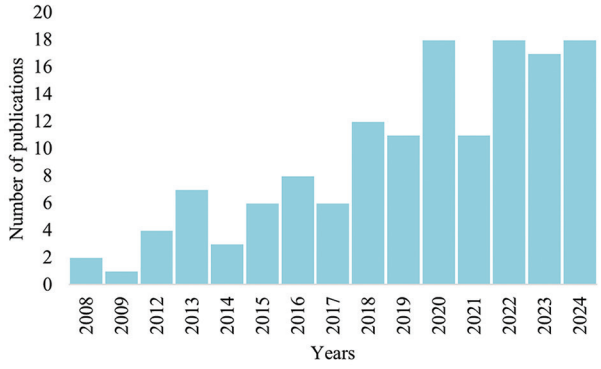

Figure 3. The literature of microbubble/nanodroplet-assisted ultrasound for drug delivery into the brain.

applications, advantages, and limitations, as well as the biophysical mechanisms underlying BBB disruption.

3.1. Description of sonoresponsive agents

3.1.1. Microbubbles

Common ultrasound contrast agents consist of an aqueous solution of micrometer-sized bubbles (MBs) filled with a heavy-weight hydrophobic gas (e.g., perfluorocarbon [PFC], sulfur hexafluoride) and encapsulated by a biocompatible shell (e.g., lipids, polymers).[30] These purely vascular agents are administered intravenously to enhance US image contrast, thus improving diagnostic accuracy[31,32] in fields, such as cardiology and radiology. At present, four MB formulations have received clinical approval (Tables 2 and 3).

For over 30 years, the combination of high-frequency US (0.5 – 10 MHz) and MBs – often referred to as MB-assisted US, sonoporation, or sonopermeabilization, which induces pore in the tissue – has emerged as a promising approach to improving the therapeutic efficacy of drugs by increasing local delivery to brain tissue while minimizing side effects on healthy tissues.[33] MBs are typically co-administered or injected sequentially with therapeutics through the intravenous (i.v.) route. These two strategies enable the use of clinically approved MBs and therapeutics, facilitating a rapid clinical translation of this drug delivery method. The doses of MBs[34,35] and therapeutics can be easily adjusted to achieve the desired therapeutic outcomes. However, the main limitations of these strategies lie in the differing spatiotemporal biodistributions of MBs and therapeutics due to their distinct physicochemical properties, their rapid degradation, and the non-specific accumulation of free therapeutics in healthy tissues.

To overcome these limitations, MBs have been designed to function not only as cavitation nuclei but also as carriers for therapeutics. Lipophilic therapeutics can be incorporated into the lipid monolayer shell of MBs or dissolved in an oil cavity situated between the gas core and the MB shell. Hydrophilic therapeutics, on the other hand, are typically loaded into the aqueous lumen of nanoparticles (e.g., liposomes, polyplex) which are then attached to the MB surface. However, identifying the optimal MBs for a specific therapeutic molecule – those with the most suitable physicochemical and pharmacological properties – can sometimes be challenging. These various approaches are shown in Figure 5.[28]

The main limitation of drug-loaded MBs is their low drug-loading capacity. To overcome this, several strategies have been developed to enhance drug loading. In addition, the i.v. administration of higher doses of drug-loaded MBs or the application of consecutive treatment sessions is an alternative solution. Moreover, MBs can be functionalized

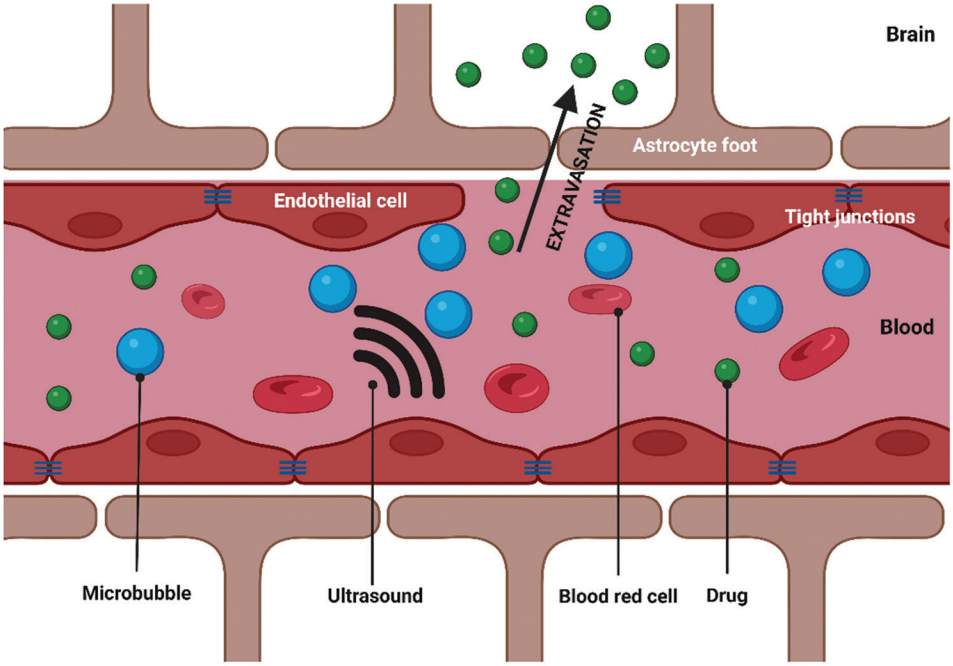

Figure 4. Intracerebral drug delivery using ultrasound contrast agent-assisted ultrasound. Adapted from "Blood–brain barrier (simple longitudinal)". Retrieved from https://app.biorender.com/biorender-templates.

Table 2. Characteristics of different clinically approved MBs

Name	Optison®	Lumason®/SonoVue®	Definity®/Luminity®	Sonazoid®
1st approved for clinical use	1998	2001/2014	2001/2006	2007
Diameter (μm)	3 – 5	2.5	1.5	2.6
Shell's composition	Hydrogenated egg PS	Phospholipid	Phospholipid	Hydrogenated egg PS
Charge	-	-	-	-
Gas	C_3F_8	SF_6	C_3F_8	C_4F_{10}
Provider	GE HealthCare	Bracco	Lantheus	GE HealthCare
Country	Norway	Europe, China, USA	USA	Japan, South Korea

Abbreviation: PS: phosphatidylserine.

Table 3. Comparative table between the composition of MBs and NDs

Particule	Shell composition	Core	Size	Functionality	Load	Administration	Extravasation	Lifespan
MB	Lipids, polymers	SF_6 C_3F_8 C_4F_{10}	1.5 – 5 μm	Intravenous	Yes	Co-administration or sequential	No	Few minutes
ND	Lipids, surfactants, polymers, proteins	PFC	20 – 200 nm	Intravenous	Yes	Co-administration or sequential	Yes	Few hours

Abbreviations: MB: Microbubble; ND: Nanodroplet: PFC: Perfluorocarbon.

with targeting agents to bind specific overexpressed biomarkers on cerebral microvasculature (e.g., transferrin receptor, which is expressed on vascular endothelial cells).[36,37] This targeting strategy can increase the accumulation of MBs in target brain regions, enhance BBB permeabilization, and improve the i.c. bioavailability of therapeutics.

The use of MBs for acoustically mediated i.c. delivery of therapeutics faces two main limitations. First, most clinically approved or custom-made MBs used for this purpose exhibit polydispersity in size. Since the effectiveness of MBs depends on the relationship between the central frequency of US waves and the size of MBs, only a fraction of MBs

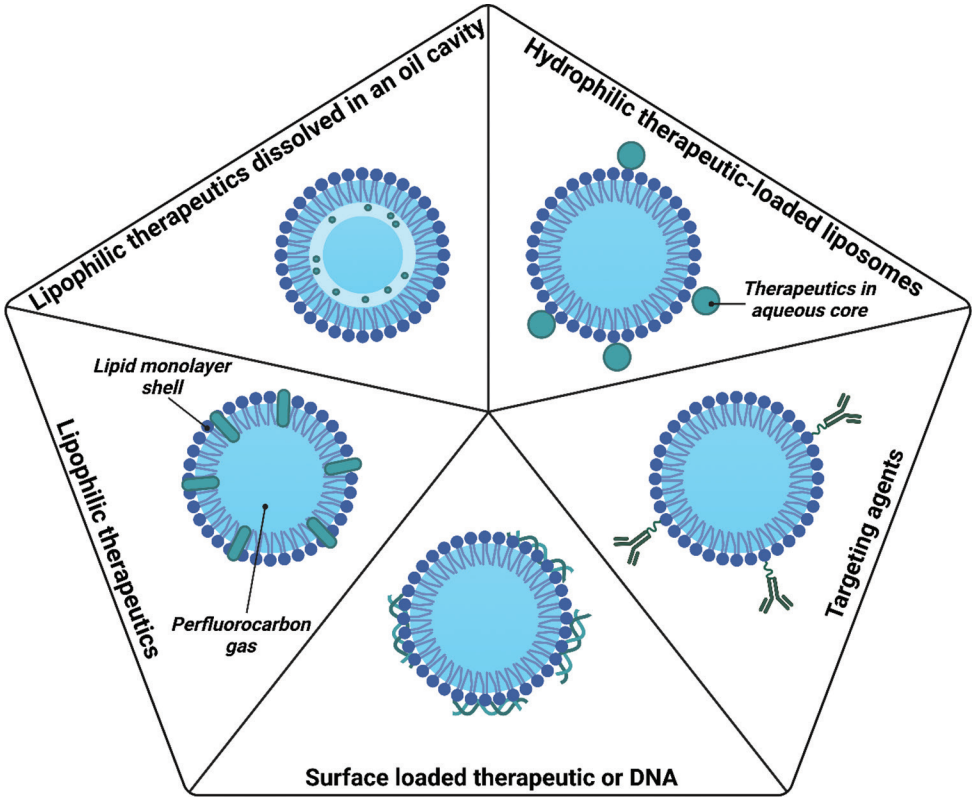

Figure 5. Schematic representation of various drug-delivery vehicle designs. Created with BioRender.com

responds to a given frequency and contributes to BBB permeabilization. This relationship, as described by Shapk *et al.*[38] using the Rayleigh-Plesset equation, highlights the need for a population of MBs with uniform size, which would be more effective in permeabilizing the BBB.[39] Second, MBs have a limited lifespan in the bloodstream, ranging from 5 to 15 min.[40,41] This relatively short lifespan necessitates either continuous infusion of MBs or repeated bolus injections to achieve efficient permeabilization of the BBB. These requirements increase the complexity of the protocol and the overall cost of the procedure.

3.1.2. NDs

NDs have recently emerged as phase-changing sonoresponsive agents, attracting significant interest in biomedical applications for both imaging and therapeutic purposes. These NDs consist of a liquid core (e.g., PFC) stabilized by a biocompatible shell (e.g., surfactants, lipids, proteins, polymers, etc.) (Table 3).[42] Their size typically ranges from 20 to 200 nm, and they generally exhibit narrower size distribution compared to MBs. In addition, NDs have a prolonged systemic lifespan of up to 4 – 5 h.[43] Similar to MBs, NDs can be co-injected, administered sequentially with therapeutics, or used as drug nanocarriers. Moreover, the liquid core of NDs remains in its liquid state at body temperature but can vaporize into MBs in a controlled, non-invasive, and localized manner under the effect of an acoustic process known as acoustic droplet vaporization (ADV).[44,45] During ADV, the applied US disrupts the vapor pressure equilibrium of the saturated PFC liquid, causing it to vaporize and form MBs, which in turn induces cavitation and opens the BBB (Figure 6).[46]

Under specific US conditions, this process can promote the reversible permeabilization of the BBB as well as the plasma membrane of cerebral cells when NDs are located in the vascular and brain compartments, respectively. Unlike MBs, NDs can easily extravasate and accumulate in target tissues due to their nanometric size and the enhanced penetration and retention effect (EPR effect). Consequently, their acoustic activation not only induces the transient permeabilization of cerebral cells but also facilitates the release of therapeutics loaded into NDs and their intracellular uptake when NDs are used as drug nanocarriers (Figure 7). This strategy holds significant potential for improved tissue targeting, particularly in the treatment of brain tumors.[43] At present, these NDs have not yet received clinical approval, despite offering clinical prospects comparable to, or even exceeding, those of MBs. Nevertheless, further research is needed to clearly establish the efficacy and the safety of acoustically mediated drug delivery using NDs.

3.2. Mechanisms of acoustically mediated drug delivery

The effectiveness of i.c. delivery of therapeutics dependents heavily on: (1) The presence of sufficient amounts of sonoresponsive agents (i.e., MBs and NDs) and therapeutics near the biological targets (i.e., BBB and cerebral cells). This is influenced by their physiochemical properties (e.g., size, composition, etc.) and pharmacological characteristics (i.e., pharmacokinetics, pharmacodynamics, bioavailability), as well as their mode of administration (i.e., i.v. bolus vs. perfusion, co-administration versus sequential administration) and the physiological state of biological targets (e.g., healthy versus pathological cells/tissues, microenvironment, etc.); (2) The US setup, including the type of probe (e.g., mono-element US transducer versus transducer array, focused versus unfocused transducer, etc.), the device used (i.e., laboratory-made device, US imaging scanner, clinically approved therapeutic US device), and the parameters applied (e.g., frequency, acoustic pressure, pulse duration, etc.), which must be optimized to ensure safe and efficient activation of the sonoresponsive agents near the biological targets; (3) The treatment protocol including the time interval between the administration of sonoresponsive agents and therapeutics on one hand and the subsequent US exposure on the other, the number of treatments, and the intervals between sessions.[47]

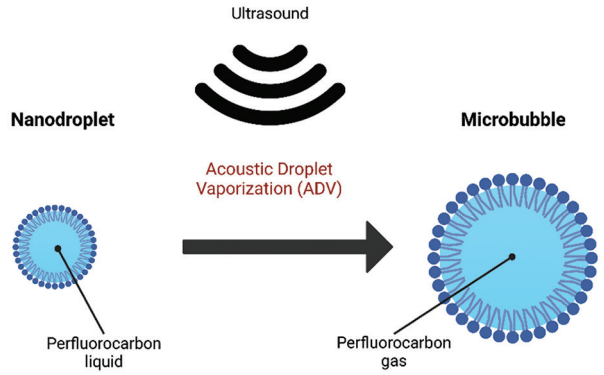

Figure 6. Schematic representation of the acoustic droplet vaporization process. Vaporization of perfluorocarbon droplets following exposure to ultrasonic pulses leads to the formation of gas bubbles. Created with BioRender.com.

3.2.1. Microbubbles

As described above, the properties of US and MBs, along with *in vivo* environmental conditions (e.g., hydrostatic pressure and dissolved gas saturation) influence the response of MBs to US waves. The high compressibility and the low density of the gas core of MBs create a significant impedance mismatch with the surrounding medium, making MBs highly responsive to US waves. During the rarefaction and compression phases of the wave, MBs alternately expand and contract, a phenomenon referred to as MB oscillation.[40] At low acoustic pressures, MBs oscillate in a symmetrical and linear manner, a process known as stable cavitation.[25,30,48] When in close proximity to biological barriers (e.g., cell membrane and BBB), these oscillations can induce "cell massage" (i.e., a pushing and pulling effect)

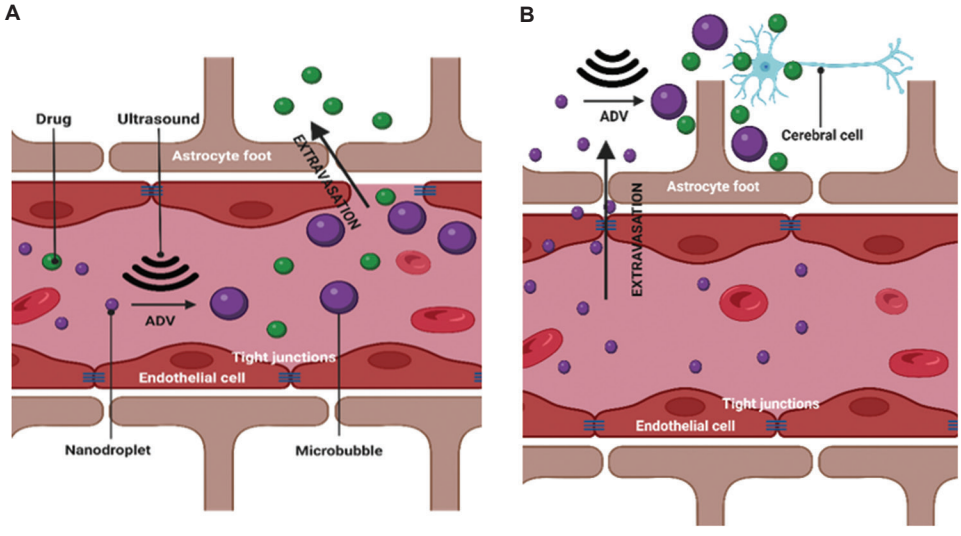

Figure 7. Acoustic activation of NDs. (A) Transient permeabilization of the BBB promoted by ADV under specific US conditions. (B) Upon extravasation of NDs through the EPR effect, ADV facilitates the release of therapeutics loaded into NDs and reversible permeabilization of cerebral cells. Adapted from "Blood–brain barrier (simple longitudinal)". Retrieved from https://app.biorender.com/biorender-templates.
Abbreviations: ADV: Acoustic droplet vaporization; BBB: Blood–brain barrier; EPR: Enhanced penetration and retention; NDs: Nanodroplets; US: Ultrasound.

and generate fluid flows around the MBs, known as acoustic microstreaming. Both of these biophysical processes exert shear stress on biological barriers, enhancing their natural permeability to therapeutics.[35]

At much higher acoustic pressures, MBs exhibit a nonlinear acoustic behavior, characterized by larger expansion amplitude relative to compression. This more violent oscillation often leads to the collapse and destruction of MBs, a phenomenon termed inertial cavitation. This disruption of MBs during inertial cavitation generates shock waves in the surrounding medium, producing greater shear stress on biological barriers and thereby increasing their permeability.[30,48,49] In addition, the asymmetrical collapse of MBs can create high-velocity jets (i.e., microjets) that transiently damage biological barriers, further enhancing the permeability of tracers (e.g. MR contrast agent, fluorescent dyes) and/or therapeutic molecules (e.g. monoclonal antibodies, recombinant proteins, nucleic acids). These biophysical processes promote the permeabilization of the BBB by stimulating paracellular pathways (i.e., disruption of TJs) and/or transcellular pathways (i.e., transcytosis), thereby facilitating the extravasation of therapeutics.[47] They also improve the intracellular uptake of therapeutics by forming membrane pores[50] and/or stimulating endocytosis pathways.[51,52] At present, scientific and medical consensus favors the use of stable cavitation of MBs over inertial cavitation due to the potential tissue damage associated with the latter.

3.2.2. NDs

As previously mentioned, the core of NDs remains in a liquid state at body temperature but vaporizes into MBs through the process of ADV. Vaporization occurs when the vapor pressure of volatile liquids in the liquid state (e.g., PFCs) exceeds the surrounding gas phase pressure.[44,45] This phenomenon is presented in Shpak *et al.*[38] US reduces the pressure around the NDs below the vapor pressure of the volatile liquid in their core, triggering vaporization and the subsequent formation of MBs.[45,53,54] In recent years, PFCs have become the primary volatile candidates for ND cores due to their low solubility in aqueous formulations, low toxicity, and suitability as low-boiling-point liquids.[55]

The ADV process depends on various factors, including ND properties (e.g., the type of volatile liquid and ND size), acoustic parameters (e.g., pressure and frequency), and ambient parameters (e.g., pressure and temperature).[53] Following i.v. administration, the ADV process can occur in the vascular compartment, enabling the release of therapeutics from NDs when used as drug nanocarriers.[56] Subsequently, the stable or inertial cavitation of newly formed MBs can transiently permeabilize the BBB, enhancing the i.c. bioavailability of therapeutics. For instance, Chen *et al.*[57] demonstrated transient BBB permeabilization using ND-assisted US in the rat model, showing more homogeneous dextran delivery to the targeted hippocampus without inducing inertial cavitation or compromising safety. In addition, the ADV process may occur within the cerebral parenchyma due to ND extravasation. In this case, therapeutics are released in close proximity to targeted cerebral cells. The stable cavitation of MBs can then further permeabilize these cells, facilitating the intracellular uptake of therapeutics.

One significant limitation of NDs is that they cannot be imaged before ADV, unlike MBs. To address this, multimodal imaging NDs have been developed by incorporating imaging tracers for various modalities, such as gadolinium for MRI,[58,18]-F for positron emission tomography,[59] or DiR fluorescent dye for fluorescence imaging.[60]

4. US devices

Several pre-clinical and clinical investigations have demonstrated significant progress in the development, optimization, and validation of acoustic sequences to achieve efficient and safe i.c. delivery of therapeutics. Among these studies, two main categories of US devices have been highlighted: lab-made US devices and clinically approved US devices specifically designed for therapeutic delivery.

4.1. Lab-made US devices

The lab-made US devices typically consist of three main components: a generator, an amplifier, and a commercial or custom-built single-element US transducer. The US waves are generated by the US transducer which operates at a fixed center frequency (ranging from 0.250 to 1 MHz). The transducer is driven by an electrical signal produced by an arbitrary waveform generator and subsequently amplified using a power amplifier. To ensure effective coupling with the animal's head and precise targeting of the focal point within the brain region of interest, the transducer can either be placed in direct contact with the animal's head using US gel, or inserted into a dedicated degassed water-filled adaptor, allowing for proper alignment and positioning of the focal point within the targeted brain area (Figure 8).[61]

Spherically focused US (FUS) transducers are commonly used to significantly increase US intensity within a small, targeted brain area. These transducers are typically calibrated in a separate setup using a calibrated hydrophone.[62] Lab-made US devices provide flexibility to control various US parameters (i.e., center frequency, pulse repetition frequency, duty cycle, acoustic pressure, and total exposure time), allowing these parameters to be optimized

for therapeutic delivery. The exposure of brain tissue to FUS is generally guided by MRI or neuronavigation systems, enabling more precise and safe treatments.[63] For several years, lab-made US devices have been paired with PCD devices to monitor and manage acoustic intensity in real-time during FUS exposure, ensuring safe and effective treatment within the brain tissue.[29]

4.2. Clinically approved devices

At present, three clinically approved US devices—SonoCloud®, NaviFUS®, and ExAblate Neuro®—are available for i.c. delivery of therapeutics. Below, their distinct characteristics are described.

4.2.1. SonoCloud®

CarThera© (Lyon, France) has designed innovative therapeutic US-based medical devices known as SonoCloud® for delivering therapeutics into the brain. SonoCloud® is an intracranial US implant that transmits US waves to a target brain region, bypassing the skull and transiently opening the BBB before or after i.v. administration of therapeutics. The device is implanted in a skull window, positioned beneath the skin, and remains invisible externally. After an i.v. injection of MBs, SonoCloud® is activated through a transdermal needle connected to an external control unit. Low-intensity pulsed US exposure to the targeted brain area disrupts the BBB for several hours (typically 4 – 6 h), thereby increasing the effective concentration of therapeutics in this brain area. This acoustically mediated BBB disruption can be repeated with each cycle of pharmacological therapy.

CarThera© has developed and validated two MRI-compatible SonoCloud® devices: SonoCloud-1® and SonoCloud-9®. SonoCloud-1® is an 11.5-mm diameter biocompatible implant containing a 1-MHz planar US transducer,[64,65] while SonoCloud-9® consists of nine 1-MHz planar US transducers arranged on an implantable grid (Figure 9). SonoCloud-9® safely and efficiently disrupts the BBB over a large volume of brain tissue and significantly increases the i.c. bioavailability of therapeutics in the brain compared to SonoCloud-1®. This US protocol is fully compatible with conventional pharmacological treatments and does not require patient anesthesia. In addition, BBB disruption can be monitored using dynamic contrast-enhanced MRI (DCE-MRI) following the i.v. administration of an MRI contrast agent. These SonoCloud® devices are currently being evaluated in clinical trials for treating brain tumors[66-68] and AD.[69]

4.2.2. NaviFUS®

NaviFUS® Corp. (Taipei, Taiwan) has developed a neuronavigation-guided transcranial FUS system known as NaviFUS®. This custom-designed system features a multichannel hemispherical FUS-phased array operating at a frequency of 0.5 MHz. It delivers FUS to brain tissue in a transcranial, non-invasive manner (Figure 9). Before the FUS intervention, a personalized treatment plan is designed for each patient based on cranial bone data obtained from their MRI and/or CT scans. Physicians determine the target brain regions and specify the positioning of the transducers. The intervention is conducted on an outpatient basis, with the patient remaining awake and seated in a chair for the duration of the procedure which typically lasts <30 min. After i.v. administration of MBs, a neuronavigation tracking device guides the FUS to the targeted brain regions, ensuring precise treatment delivery.[70]

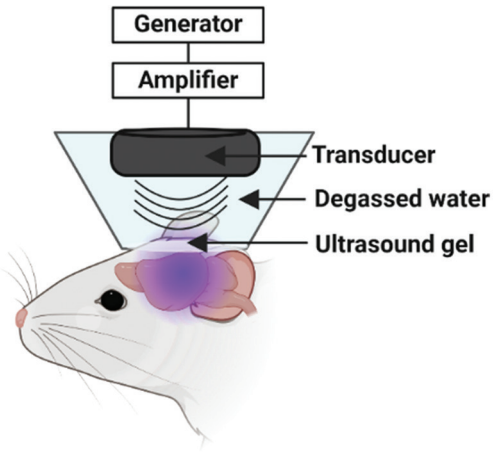

Figure 8. An example of lab-made ultrasound device. Created with BioRender.com.

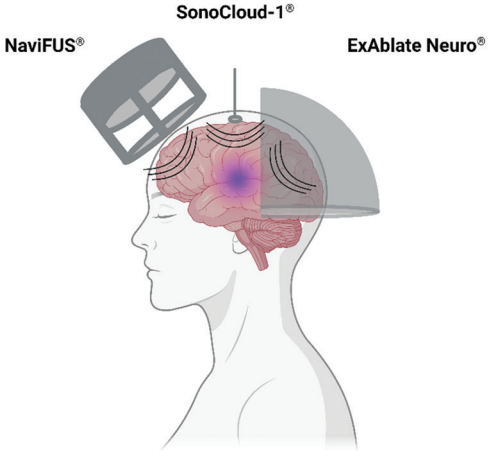

Figure 9. Illustration of the different models of clinically approved US devices—NaviFUS®, SonoCloud-1® and ExAblate Neuro®. Created with BioRender.com.

The NaviFUS® system also integrates a real-time PCD device to manage the acoustic energy in real-time during the procedure. Pre-clinical studies have demonstrated the safety and efficacy of BBB disruption using NaviFUS® in both small and large animal models.[70,71] Notably, the BBB disruption is reversible within 24 h. At present, the NaviFUS® system is under clinical investigation specifically for the treatment of recurrent glioblastoma.[57,71,72]

4.2.3. ExAblate Neuro®

InSightec© Ltd. (Israel) has developed an MRI-guided FUS (MRgFUS) system known as ExAblate Neuro®. Similar to the NavisFUS® system, this extracorporeal MRgFUS device delivers noninvasive acoustic energy into targeted brain tissues through the intact skull. The ExAblate Neuro® Type 1.0 system was originally designed to treat essential tremor and PD by partially ablating the thalamus through acoustically mediated thermal ablation at a center frequency of 0.65 MHz. Later, this system was adapted to enhance the native BBB permeability for therapeutic delivery to targeted brain tissues at a center frequency of 0.220 MHz, now referred to as ExAblate Neuro Model 4000 Type 2.0.

The ExAblate Neuro® system consists of a high-field 3T MRI scanner and a hemispherical 1,024-element phased array US transducer, which is integrated with computer systems. These systems utilize computed tomography (CT) scan data to align, steer, and control the transducer array (Figure 9).[73] Before FUS exposure, the treatment parameters are precisely planned for each patient based on anatomical and functional data of cranial bone and target brain tissues provided by MRI and CT scans. During the intervention, the awake patient lies on an MRI-compatible robotic positioning table, with the patient's head is immobilized in a stereotactic frame to prevent any movement during the procedure, which lasts between 2 and 4 h.[74] The stereotactic frame enables precise target selection and intraoperative MRI confirmation, ensuring electronic steering of the FUS beam with submillimeter accuracy (<1 mm) to one or multiple brain targets, as defined by the planned volume geometry. US sequences are initiated immediately after the i.v. injection of MBs. The MRI scanner precisely guides the US beam to the targeted brain regions during the procedure, and the BBB opening is monitored using DCE-MRI after the i.v. administration of gadolinium-based contrast agents. Notably, the BBB disruption is reversible within 20 h.

Clinical investigations have demonstrated the safety and efficacy of BBB disruption using the ExAblate Neuro® system. At present, this system is undergoing clinical trials for the treatment of brain tumors (e.g., recurrent glioblastoma and brain metastases[75]) and neurodegenerative diseases (*e.g.*, ALS, AD, and PD[74,76]).

After reviewing the state-of-the-art of acoustically mediated therapeutic delivery using MBs or NDs – including the biophysical mechanisms, sonoresponsive agents, and US devices – we will now turn our focus to the application of this US modality for the treatment of neurodegenerative diseases. First, we will provide the definition of these diseases, followed by a detailed discussion of pre-clinical and clinical investigations of these diseases using this US-based approach.

5. Applications in AD

5.1. AD

AD is the most common neurodegenerative disorder, characterized by its progressive and fatal nature.[77] In 2000, AD affected 15.3 million people worldwide, a number projected to rise to 63 million by 2030.[78] It represents a growing global health challenge, with an annual incidence of 1.8 million cases in the USA and Europe.[79] AD progresses as a continuum, with stages that vary in duration for each patient: (1) The pre-clinical or prodromal stage, where no clinical symptoms are apparent, but biomarkers such as amyloid-tau-neurodegeneration can be detected; (2) The mild cognitive impairment stage, during which symptoms, such as memory, language, and thinking difficulties emerge but do not significantly interfere with daily life; and (3) The dementia stage, marked by a loss of autonomy, further categorized into mild, moderate, and severe levels.

The progression from the pre-clinical stage to the onset of dementia can span 15 – 25 years. AD is characterized by the extracellular accumulation of beta-amyloid protein fragments, forming clumps known as beta-amyloid plaques, and the intracellular accumulation of an abnormal form of the tau protein known as tau tangles. Amyloid-β (Aβ) is believed to play roles in synaptic homeostasis, immunity, and lipid processing.[77] However, abnormal cleavage of the amyloid pre-cursor protein by β- and γ-secretases results in the production of Aβ peptides, which form the core of plaques.[80] Tau protein is crucial for microtubule stabilization, axonal transport, and signaling pathway modulation. Abnormal phosphorylation of tau leads to its aggregation, disrupting pre-synaptic and post-synaptic compartments by altering signaling cascades, mitochondrial function, and axonal transport, ultimately causing neurotoxicity.[77] The presence of Aβ plaques and tau tangles is linked to chronic neuroinflammation and progressive synaptic and neuronal loss.

These aggregates remain the main targets for the development of imaging tracers and therapeutic molecules.

Present pharmacological treatments do not address the underlying causes of the disease (curative) but instead alleviate the symptoms (palliative) by acting primarily on acetylcholinesterase and N-methyl-D-aspartate (NMDA) receptors. Numerous experimental molecules, including those targeting Aβ or tau accumulation, are under investigation for AD.[81,82] However, the results of recent clinical trials have been less promising than pre-clinical findings, with many therapeutics failing to reach their brain targets. This is due not only to their physicochemical and pharmacological properties but also to the presence of biological barriers. To overcome these challenges, specialized US protocols have been designed and validated in pre-clinical and clinical studies to facilitate the delivery of therapeutics using MB/ND-assisted US.

5.2. Pre-clinical phase

Several pre-clinical studies have demonstrated the feasibility, safety, and efficacy of MB-assisted US for reversible BBB opening in various transgenic murine models of AD, including APP/PS1dE9,[63,83-86] PDAPP,[83] TgCRND8,[87-89] 5xFAD,[61,90] pR5,[91,92] and K3.[93] For instance, Choi *et al.*[63] investigated BBB opening in three transgenic APP/PS1 mice and three wild-type mice using an acoustically mediated US approach. After an i.v. injection of SonoVue® (25 μL), the left hippocampus was exposed to US waves (1.525 MHz, 20% duty cycle (DC), 20 ms pulse duration, 0.6 MPa) for 1 min using a lab-made MRgFUS device. DCE-MRI revealed that MB-assisted MRgFUS successfully induced reversible BBB opening in the hippocampus of both wild-type and AD mice without causing any tissue damage. Similarly, Burgess *et al.*[87] validated these findings in TgCRND8 transgenic mice. Collectively, these studies underscore that MB-assisted US can safely and reversibly open the BBB in mouse models of AD.

In addition, MB-assisted MRgFUS has facilitated the delivery of various therapeutics in AD mouse models, including full-size antibodies and antibody fragments targeting Aβ,[61,83,85,86,88-90] or tau,[91-93] as well as antioxidant and anti-inflammatory molecules.[84] Regardless of the US protocol or the MBs used, MB-assisted MRgFUS significantly increased the i.c. the concentration of anti-Aβ antibodies (e.g., BAM-10, IVIg, Aducanumab, anti-pGlu3 Aβ, anti-$Aβ_{1-40}$,) in the hippocampus of AD mice compared to antibody treatment alone.[61,83,85,86,88-90] These antibodies bound to Aβ plaques, activated phagocytic microglia, and increased the number of astrocytes associated with Aβ plaques,[61,85,86] leading to a significant reduction in Aβ plaque load in the hippocampus.[61,85,88,89] Furthermore, this therapeutic strategy significantly enhanced hippocampal neurogenesis.[61,85,88,89] As a result, cognitive functions in treated mice were notably improved compared to antibody treatment alone.[61,85]

Preclinical studies have shown the potential of MB-assisted US for delivering therapeutics in AD. For instance, MB-assisted US using a lab-made device (1 MHz, 10 Hz PRF, 0.7 MPa, 10 ms pulse duration, DC 10%, for 6 s) with lab-made lipid-shelled MBs effectively delivered the 2N tau isoform-specific single chain antibody fragment (RN2N) across the forebrain of P301L human tau transgenic pR5 mice.[91,92] This approach improved the i.c. bioavailability of RN2N compared to the RN2N treatment alone, resulting in reduced anxiety-like behavior. Similarly, a novel tau-specific monoclonal antibody (RNF5) was delivered by MB-assisted US to the forebrain of the K369I tau transgenic K3 mouse model.[93] Although i.c. bioavailability was improved with the US, no behavioral improvement was observed in these mice. In addition, Liu *et al.*[84] evaluated the delivery of quercetin, an antioxidant and anti-inflammatory molecule, using MB-assisted US. Quercetin was loaded onto lab-made MBs, which were intravenously injected, followed by exposure of the parietal cortex in APP/PS1 mice to US waves (1 MPa for 5 min) using a lab-made US device. MB-assisted US facilitated BBB opening, quercetin release, and its i.c. accumulation. Quercetin treatment significantly reduced neuronal apoptosis, neuroinflammation, calcium homeostasis variation, and oxidative stress, ultimately enhancing learning and memory capacities in AD mice.

Furthermore, Gouveia *et al.*[62] explored ND-assisted US (lab-made US device; 2 MPa, 10 ms bursts, 1 Hz, for 180 s) to deliver the anesthetic pentobarbital into the amygdala of TgCRND8 transgenic mice to achieve neuromodulation (Table 4). The NDs were composed of Definity® MB shells and loaded with pentobarbital. ND vaporization did not disrupt the BBB, but the released lipophilic pentobarbital crossed the BBB, localizing the therapeutic effect to the target area. Pentobarbital-loaded NDs significantly improved agitation and aggressive behavior in AD mice compared to unloaded NDs. However, only behavioral tests were conducted. Further studies assessing the brain drug bioavailability with or without ND-assisted US are needed.

Collectively, these findings underscore the promise of MB/ND-assisted MRgFUS as an innovative modality for delivering therapeutics to treat AD.

5.3. Clinical phase

Recent clinical investigations have explored the feasibility, safety, and efficacy of acoustically mediated BBB opening and therapeutic delivery in AD patients. Three clinical trials demonstrated the feasibility, reproducibility, safety, and

Table 4. Drug delivery with MB/ND-assisted US for pre-clinical studies in Alzheimer's disease

References	Drug, dye, particle	Animal model	US devices/parameters	Targeted area	MB/ND	Therapy duration
Choi *et al*., 2008[63]	Gadolinium	APP/PS1dE9 mouse	Lab-made MRgFUS device; 1.525 MHz, DC 20%, 20 ms pulse duration, 0.6 MPa, for 1 min	Left hippocampus	SonoVue® MBs (25 μL)	1 sonication
Raymond *et al*., 2008[83]	Anti-Aβ antibodies	APP/PS1dE9 and PDAPP mouse	Lab-made US device; 0.69 MHz, 10 ms burst length, 1 Hz PRF, 0.67 – 0.8 MPa, for 40 – 45 s	Hippocampus	Optison® MBs (30 – 50 μL)	1 sonication
Jordão *et al*., 2010[88]	BAM-10	TgCRND8 mouse	Lab-made US device; 0.3 MPa, 120 s, 10 ms bursts/Hz	Right hemisphere	Definity® MBs (160 mL/kg)	1 sonication
Burgess *et al*., 2014[87]	Gadolinium	TgCRND8 mouse	Lab-made US device; 10-ms bursts, 1 Hz burst repetition frequency, for 120 s	Hippocampus	Definity® MBs (0.02 mL/kg)	1 sonication per week for 3 weeks
Nisbet *et al*., 2017[91]	RN2N	pR5 mouse	Lab-made US device; 1 MHz, 10 Hz PRF, 0.7 MPa, 10 ms pulse duration, DC 10%, for 6s	Whole brain	Lab-made lipid-shelled MBs (30 μL)	1 sonication per week for 4 weeks
Janowicz *et al*., 2019[92]	RN2N	pR5 mouse	Lab-made US device; 1 MHz, 10 Hz PRF, 0.7 MPa, 10 ms pulse duration, DC 10%, for 6 s;	Whole brain	Lab-made lipid-shelled MBs (40 μL)	1 sonication
Dubey *et al*., 2020[89]	IVIg	TgCRND8 mouse	Lab-mad US device; 1 Hz burst repetition frequency, 10 ms bursts, for 120 s	Hippocampus	Definity® MBs (0.02 mL/kg)	1 sonication
Liu *et al*., 2020[84]	Quercetin	APP/PS1dE9 mouse	Lab-mad US device; 1 MPa for 5 min using a	Parietal cortex	Lab-made lipid-shelled MBs	1 sonication per week for 5 weeks
Sun *et al*., 2021[85]	Anti-pGlu3 Aβ	APP/PS1dE9 mouse	Lab-mad US device; 2 Hz, 10 ms bursts, for 100 s	Hippocampus	Optison® MBs (100 μL/kg)	1 sonication per week for 3 weeks
Bathini *et al*., 2022[86]	Anti-pGlu3 Aβ	APP/PS1dE9 mouse	Lab-mad US device; 2 Hz, 10 ms bursts, for 100 s	One or two hemispheres	Lab-made lipid-shelled MBs	1 sonication
Kong *et al*., 2022[61]	Aducanumab	5×FAD mouse	Lab-mad US device; 1 Hz burst, 0.25 MPa, 10 ms bursts, for 120 s	Hippocampus	Definity® MBs (0.04 mL/kg)	1 sonication every 2 weeks for a total of 3
Bajracharya *et al*., 2022[93]	RNF5	K3 mouse	Lab-made US device; 1 MHz, 10 Hz PRF, 0.7 MPa, 10 ms pulse duration, DC 10%, for 6s;	Whole brain	Lab-made lipid-shelled MBs (1 μL/g)	1 sonication per week for 12 weeks
Gouveia *et al*., 2023[62]	Pentobarbital	TgCRND8 mouse	Lab-made US device; 2 MPa, 10 ms bursts, 1 Hz, for 180 s	Amygdala	Lab-made NDs (0.1 mL/injection)	1 sonication
Antoniou *et al*., 2024[90]	Anti-Aβ1 – 40	5×FAD mouse	Lab-made US device; 1 MHz, 0.5 MPa *in situ*, 10 ms bursts, DF 1%, for 100 s	Left hemisphere	SonoVue® MBs (5 μL)	1 sonication

Abbreviations: DC: Duty cycle; MB: Microbubble; MRgFUS: Magnetic resonance imaging-guided focused ultrasound; ND: Nanodroplet; PRF: Pulse repetition frequency; US: Ultrasound.

reversibility of repeated BBB opening in the frontal lobe[74] and hippocampus[94,95] of early-stage AD patients using the ExAblate Neuro® Type 2 and Definity® MBs. In a study by Rezai *et al.,*[96] aducanumab was delivered through this US device, resulting in a significant reduction in Aβ plaque load. However, no neurological, cognitive, or behavioral changes were observed during the follow-up phase. The authors explained that the study primarily assessed safety due to insufficient statistical power to detect clinical changes. The study reported one case of cognitive decline following aducanumab treatment and at least one case of severe adverse effects, which were deemed unrelated to

the trial intervention. Headaches were the most frequently reported adverse events. Interestingly, the effects of the US in this study might be pleiotropic, involving actions of the therapeutic molecule, blood-borne factors entering cerebral tissue due to BBB opening, and neuromodulatory effects of the US itself through associated radiation forces.[97]

In addition, a pilot study demonstrated the safety and repeatability of BBB opening in the left supramarginal gyrus of mild AD patients using the SonoCloud®-1 device and SonoVue® MBs [69]. Recently, Bae *et al.* [98] designed and validated a portable clinical neuronavigation-guided US device for BBB opening using Definity® MBs. This device successfully opened the BBB in the right frontal lobe of AD patients without severe side effects (Table 5)

6. Applications in PD

6.1. PD

PD is the second most common neurodegenerative disease after AD. It affects approximately 4 million people worldwide, with an average onset age of 60 years. While rare cases occur in individuals under 40 years, 3% of people over 80 years are affected by PD.[99] The disease has a prevalence of 1 – 2/1000 and an incidence of 13.5/100,000 people/year.[100] PD is an idiopathic neurodegenerative disorder primarily characterized by the degeneration of dopaminergic neurons in the pars compacta of the substantia nigra in the midbrain, leading to impaired motor control. A hallmark of PD is the presence of Lewy bodies, which are intracellular aggregates of misfolded proteins, including α-synuclein (synucleinopathy).

The clinical presentation of PD primarily includes motor symptoms, such as bradykinesia, cogwheel rigidity, resting tremor, a slow shuffling gait, and imbalance. However, non-motor symptoms are also present and include orthostatic hypotension, rapid eye movement sleep behavioral disorder, and hallucinations.[101,102] Present pharmacologic treatments focus on increasing dopamine levels to address the dopamine deficiency observed in PD patients. The most common treatment is levodopa (L-DOPA), a dopamine pre-cursor that crosses the BBB, unlike dopamine itself. Dopamine agonists and enzyme inhibitors, such as DOPA decarboxylase inhibitor (carbidopa), catechol-O-methyltransferase inhibitors, and monoamine oxidase B (MAO-B) inhibitors, are often prescribed as adjuvants to levodopa to manage motor complications. In addition, numerous molecules with therapeutic potential are under investigation for the treatment of PD.[103,104]

6.2. Pre-clinical phase

MB-assisted US has been demonstrated to effectively deliver therapeutics to neurotoxic-lesioned (i.e., 6-hydroxydopamine, 6-OHDA; 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine,

Table 5. Drug delivery with MB-assisted US for clinical studies in Alzheimer's disease

References	Drug, dye, particle	Patients	US devices/parameters	Targeted area	MBs	Therapy duration
Lipsman *et al.*, 2018[74]	Gadolinium	5 patients with early AD	ExAblate Neuro® Type 2 300 ms burst length, repetition interval of 2.7 s, DC 0.74%, for 50 s	Frontal lobe	Definity® MBs (4 μL/kg)	2 sonications separated by 1 month
Rezai *et al.*, 2020[95]	Gadolinium	6 patients with early AD	ExAblate Neuro® Type 2	Hippocampus	Definity® MBs	3 sonications separated by 2 weeks
Mehta *et al.*, 2021[94]	Gadolinium	3 patients with early AD	ExAblate Neuro® Type 2 2.6 ms pulses spaced by 30.4 ms, 10 cycles, 1550 ms rest period, 4 – 11.5 W, for 90 s	Hippocampus	Definity® MBs	3 sonications separated by 2 weeks
Epelbaum *et al.*, 2022[69]	Gadolinium	9 patients with mild AD	SonoCloud-1® device 25,000-cycle pulse, every second, for 4 min	Left supra-marginal gyrus	SonoVue® MBs (0.1 mL/kg)	1 sonication every 2 weeks for a total of 7
Bae *et al.*, 2024[98]	Gadolinium	6 patients	Portable clinical neuronavigation-guided US device PNP 200 kPa, MI 0.4, center frequency 0.25 MHz, pulse length 10 ms, 2 Hz PRF, for 2 min	Right frontal lobe	Definity® MBs (0.1 mL/kg)	1 sonication
Rezai *et al.*, 2024[96]	Aducanumab	3 patients	ExAblate Neuro® Type 2	Left frontal, parietal, temporal lobes, hippocampus	Definity® MBs	1 sonication per month for 6 months

Abbreviations: AD: Alzheimer's disease; DC: Duty cycle; MB: Microbubble; PRF: Pulse repetition frequency; US: Ultrasound.

MPTP)[105-112] and transgenic (i.e., overexpression of α-synuclein gene).[113-116] rodent or non-human primate models of PD. These therapies primarily aim to protect dopaminergic neurons from neurotoxicity by activating cell growth and survival, autophagy, clearance of alpha-synuclein, or by inhibiting oxidative stress, neuroinflammation, and apoptosis. Therapeutics tested include glial cell line-derived neurotrophic factor (GDNF),[105-107,109] brain-derived neurotrophic factor,[110] neurturin,[109] curcumin,[108] triptolide (T10),[113] gastrodin,[112] and a short hairpin RNA (shRNA) against α-synuclein.[114]

Preclinical studies have investigated MB-assisted MRgUS protocols for delivering the *GDNF* gene (through plasmid DNA or AAV vectors) to various brain regions including the striatum, substantia nigra, caudate-putamen, and ventral midbrain in rodent models of PD.[105-107,109,110] Plasmids encoding GDNF were generally loaded onto MBs to protect them from enzymatic degradation in the bloodstream.[105-107,110] In contrast, the AAV-GDNF vector was co-administered with MBs before US exposure.[109] These studies demonstrated successful GDNF expression in targeted brain regions, attenuating damage to nigrostriatal dopaminergic pathways[109] and even rescuing dopaminergic neuronal loss.[107,109,110] This neuroprotective strategy also improved motor-related behavioral deficits.[105-107,109,110]

Due to its critical role in PD pathology, α-synuclein has been a primary target for therapeutic strategies such as shRNA against α-synuclein[114] and triptolide.[113] Xhima *et al.*[114] used MB-assisted US to deliver an AAV9 vector encoding shRNA targeting α-synuclein into the hippocampus, substantia nigra, olfactory bulb, and dorsal motor nucleus in a transgenic PD mouse model. After i.v. administration of Definity® MBs (0.02 mL/kg), the targeted brain regions were exposed to US waves (lab-made MRgFUS device; 10 ms bursts, 1 Hz PRF) for 120 s. This approach significantly reduced α-synuclein expression in the targeted areas, although no changes were observed in other neuronal biomarkers (e.g., tyrosine hydroxylase, synaptophysin), glial activation, or cell death. However, this study did not analyze behavioral or cognitive outcomes, which warrants further investigation. As for the tripolide treatment, Feng *et al.*[113] explored MB-assisted FUS for delivering this drug to the substantia nigra in a transgenic PD mouse model. Triptolide, an autophagy inducer, alleviates autophagic dysfunction associated with the accumulation of α-synuclein in PD. MBs loaded with triptolide targeted the BBB and accumulated at the endothelial wall of cerebral vessels. US waves (lab-made US device; 10 ms burst length, 0.3, 0.45, and 0.8 MPa, 1 Hz PRF) were applied to the substantia nigra for 60 s after i.v. MB injection. This resulted in increased triptolide concentrations in the targeted brain region, facilitating the clearance of various forms of α-synuclein, reducing neuronal loss, restoring dopamine secretion, and improving motor deficits in PD mice (Table 6).

In conclusion, MB-assisted MRgFUS is an effective and safe modality for delivering innovative therapeutics for the treatment of PD.

6.3. Clinical phase

The first two clinical studies investigated the feasibility and safety of BBB opening in PD patients using a bolus of Luminity® MBs (4 μL/kg) and the ExAblate Neuro® MRgFUS system.[117,118] In the study of Gasca-Salas *et al.*,[117] patients underwent two treatments, separated by a 3 – 3-week interval, to permeabilize the BBB at the level of the right parieto-occipito-temporal cortex. BBB opening was monitored through DCE-MRI for 24 h and 7 days post-treatment. Neuropsychological and motor evaluations, as well as 18F-FDG and 18F-FMT PET imaging, were conducted 3 – 4 weeks after the final treatment. This study demonstrated that BBB opening in PD patients was both reversible and safe, with no reported side effects. Similarly, Pineda-Pardo *et al.*[118] investigated uni- and bilateral BBB opening in the posterior putamen using the same MB-assisted MRgFUS protocol as Gasca-Salas *et al*. Similar conclusions were drawn, confirming the safety and feasibility of the approach.[117]

In 2024, Gasca-Salas *et al.*[119] expanded on their earlier work by demonstrating that BBB opening in the substantia nigra and putamen in PD patients was well tolerated, reversible, and feasible. In this study, Luminity® MBs (2.5 mL/min) were infused using the same protocol. In addition, Huang *et al.*[120] further evaluated the ExAblate Neuro® MRgFUS system's cavitation feedback controller for active power modulation during unilateral targeting of the putamen in PD patients. Definity® MBs (4 μL/kg/5 min) were infused, and a cavitation emission-based feedback controller automatically adjusted the acoustic power to maintain the desired cavitation dose levels. The efficacy of BBB opening was assessed with DCE-MRI and hemorrhages were monitored with T2*-weighted MRI. Results demonstrated that such a device enabled efficient and safe BBB opening by dynamically modulating acoustic power.

In another study, Meng *et al.*[121] investigated the safety and feasibility of delivering recombinant glucocerebrosidase (GCase) to the putamen of PD patients with *GBA1* mutations using MB-assisted MRgFUS. The GCase enzyme, encoded by the *GBA1* gene, is inversely related to α-synuclein oligomer accumulation. Its cerebral deficit in *GBA1*-related and idiopathic PD is associated with disease severity. In this study, patients underwent

Table 6. Drug delivery with MB-assisted US for pre-clinical studies in Parkinson's disease

References	Drug, dye, particle	Animal model	US devices/ parameters	Targeted area	MBs	Therapy duration
Fan *et al*., 2016[105]	GDNF	6-OHDA rat	Lab-made US device; 1-MHz, 1 Hz PRF, 0.7 MPa, 5,000 cycles)	Substantia nigra and striatum	Lab-made lipid-shelled MBs	1 sonication
Lin *et al*., 2016[107]	GDNF	MPTP mouse	Lab-made US device; 0.3 – 0.8 MPa, 10 ms burst length, 1 Hz PRF, for 60 s	Substantia nigra	Lab-made lipid-shelled MBs	Sonication twice a week for 3 weeks
Zhang *et al*., 2018[108]	Curcumin	MPTP mouse	Lab-made US device; 60s	Corpus striatum in medial forebrain bundle (MFB)	Lab-made lipid-shelled MBs	1 sonication every 2 days for 4 times
Xhima *et al*., 2018[114]	shRNA against α-synuclein	Transgenic mouse overexpression α-synuclein gene	Lab-made US device; 10 ms bursts, 1 Hz PRF, for 120 s	Hippocampus, substantia nigra, olfactory bulb, and dorsal motor nucleus)	Definity® MBs (0.02 mL/kg)	1 sonication
Yue *et al*., 2018[106]	GDNF	6-OHDA rat	Lab-made US device; 1 MHz, DC 20%, 2 W/cm^2 intensity	Right substantia nigra	Lab-made lipid-shelled MBs (0.01 mL/kg)	Once every 3 days, sacrificed at 3 weeks after treatment
Karakatsani *et al*., 2019[109]	GDNF	MPTP mouse	Lab-made US device; 10 Hz PRF, 0.45 MPa, for 60 s	Caudate-putamen and ventral midbrain region	Lab-made lipid-shelled MBs (0.1 μL/g)	1 sonication
Lin *et al*., 2020[110]	BDNF	MPTP mouse	Lab-made US device; 1 MHz, 10 ms burst length, 10 Hz PRF, for 3 min	Substantia nigra	Lab-made lipid-shelled MBs (10 μL)	Sonication twice a week for 3 weeks
Feng *et al*., 2022[113]	Triptolide	Transgenic mouse overexpression α-synuclein gene	Lab-made US device; 10 ms burst length, 1 Hz PRF, 0.3 – 0.8 MPa, for 60 s	Substantia nigra	Lab-made lipid-shelled MBs (100 μL)	Sonication twice a week for 3 weeks
Wang *et al*., 2022[112]	Gastrodin	MPTP mouse	Lab-made US device; 10 ms pulse width, 1 Hz, PRF, for 60 s	Left striatum	SonoVue® MBs (1.125 μL/g)	Sonication once every 3 days for 6 times
Blesa *et al*., 2023[111]	AAV9-hSyn -GFP	6 male macaque monkeys	Lab-made US device; for 60 s	Striatum and midbrain	Luminity® MBs (4 μL/kg/mL, 0.02 mL/s)	1 sonication

Abbreviations: DC: Duty cycle; MB: Microbubble; PRF: Pulse repetition frequency; US: Ultrasound; BDNF: brain-derived neurotrophic factor.

three treatment sessions every 2 weeks over 5 weeks. GCase was administered at escalating doses (15, 30, and 60 lU/kg) through i.v. infusion over 1 h, followed by the infusion of Definity® MBs (4 μL/kg/5 min). US waves were applied using the ExAblate Neuro® MRgFUS system (10 ms pulses, DC 1%, 0.5 MPa, for 2 min). The efficacy of acoustically mediated BBB opening was monitored using DCE-MRI. Motor performance was evaluated between treatments and 1, 3, and 6 months post-treatment, with 18F-FDG PET imaging and mental examination performed 1 month and 3 months after the final treatment. Results supported the safety and feasibility of this approach. BBB opening facilitated the targeted delivery of GCase, leading to reduced putaminal hypermetabolism 1 month after treatment, reflecting improved striatal dopaminergic metabolism, as well as significant improvement in movement disorders in PD patients (Table 7).

7. Applications in ALS

7.1. ALS

ALS, also known as Charcot's disease, is a neurodegenerative disease that affects both upper and lower motor neurons (UMN and LMN) in the motor cortex, brainstem, and spinal cord. This degeneration is irreversible and progressive, leading to a relentless decline in motor functions. The prevalence of ALS is approximatively 1 – 2/100,000 individuals and the incidence is 6 – 8/100,000 people/year.[76,122] ALS manifests in different forms depending on the location of the

Table 7. Drug delivery with MB-assisted US for clinical studies in Parkinson's disease

References	Drug, Dye, Particle	Patients	US devices/ parameters	Targeted area	MBs	Therapy duration
Gasca-Salas *et al.*, 2021[117]	Gadolinium	5 patients	ExAblate Neuro® MRgFUS system	Right parieto-occipito-temporal cortex	Luminity® MBs (4 µL/kg)	2 sonications separated by 2 – 3 weeks
Pineda-Pardo *et al.*, 2022[118]	Gadolinium	7 patients	ExAblate Neuro® MRgFUS system	Posterior putamen	Luminity® MBs (4 µL/kg)	2 sonications separated by 2 – 4 weeks
Huang *et al.*, 2022[120]	Glucocerebrosidase (GCase)	4 patients with GBA1 mutations	ExAblate Neuro® MRgFUS system; 10 ms pulses, DC 1%, 0.5 MPa, for 2 min	Putamen	Definity® MBs (4 µL/kg/5 min)	1 sonication every 2 weeks for a total of 3 sonications
Meng *et al.*, 2022[121]	Glucocerebrosidase (GCase)	4 patients with GBA1 mutations	ExAblate Neuro® MRgFUS system; 10 ms pulses, DC 1%, 0.5 MPa, for 2 min	Putamen	Definity® MBs (4 µL/kg/ 5 min)	1 sonication every 2 weeks for a total of 3 sonications
Gasca-Salas *et al.*, 2024[119]	Gadolinium	3 patients	ExAblate Neuro® MRgFUS system	Substantia nigra and putamen	Luminity® MBs (2.5 mL/min)	2 sonications separated by 2 – 3 weeks

Abbreviations: DC: Duty cycle; MB: Microbubble; MRgFUS: Magnetic resonance imaging-guided focused ultrasound; US: Ultrasound.

initial symptoms: (1) bulbar-onset ALS, characterized by dysarthria and dysphagia; (2) spinal-onset ALS, marked by spasticity, muscle weakness, and progressive atrophy of the limbs. About 90% of ALS cases are sporadic, while 10% are familial, involving at least two affected family members. Up to 50% of ALS patients experience cognitive and/or behavioral impairments, and 13% develop frontotemporal lobar dementia.[123]

To date, more than 30 causal genes have been implicated in the disease.[124,125] The most frequently involved genes are *C9ORF72*, Superoxide dismutase 1 (*SOD1*), *TARDBP* (TDP-43), and *FUS*. A key neuropathological hallmark of ALS is the accumulation and aggregation of TDP-43 in the cytoplasm of motor neurons, which is observed in nearly all ALS patients. However, it remains unclear how pathogenic variants in different genes converge to result in the same TDP-43 aggregation.[126] The pathophysiology of ALS involved multiple mechanisms, including neuroinflammation, glutamate-mediated excitotoxicity, oxidative stress, mitochondrial dysfunction, and alterations in mRNA metabolism and protein homeostasis.[126-128] These processes represent critical targets for the development of therapeutic molecules aimed a mitigating the disease's progression.[129-131]

7.2. Pre-clinical phase

Recently, Shen *et al.*[132] explored the use of edaravone in the *SOD1*^{G93A} mouse model of ALS, leveraging the MB-assisted US for enhancing drug delivery. SOD1 is a key antioxidant enzyme that protects cells from the deleterious effects of superoxide radicals. Dysfunction or aggregation of SOD1 protein contributes to the pathogenesis of ALS. The *SOD1*^{G93A} transgenic mouse model typically develops severe motor impairments by approximately 120 days of age and succumbs to the disease around 160 days.[133] In this study, Shen *et al.* targeted the motor cortex of *SOD1*^{G93A} mice with a US sequence (1.1 MHz, 1 Hz PRF, 9.09 ms burst length, 0.52 MPa) for 60 s using a lab-made US device after an i.v. injection of lab-made lipid-shelled MBs (0.2 µL/g). The treatment involved four intermittent, non-overlapping FUS exposures, with a 15-min interval between each application. Edaravone was administered alternately through i.v. and intraperitoneal routes (15 mg/kg) every 2 days, starting when the mice were 13 weeks old, for a duration of 6 weeks. The results demonstrated that the MB-assisted US achieved a two-fold increase in edaravone concentration within the motor cortex compared to the control condition. This acoustically mediated drug delivery significantly improved neuromuscular function and reduced muscle atrophy compared to ALS mice treated with edaravone without US. Importantly no tissue damage was observed, underscoring the safety of this approach. This proof-of-concept study highlights the potential of MB-assisted US for targeted drug delivery in the treatment of ALS (Table 8).[132]

7.3. Clinical phase

At present, a single clinical trial is investigating the feasibility, reversibility, and safety of transient BBB opening using transcranial MRgFUS in ALS patients.[76] Abrahao *et al.*[76] targeted the primary motor cortex, specifically the precentral gyrus, marking the first attempt to target subcortical white matter regions in humans. The study involved four right-handed participants (two women and

two men, with a median age of 61 years), assigned to either the arm (n = 2) or leg (n = 2) target groups based on which limb exhibited greater weakness on the left side. On the day of the experiment, patients temporarily discontinued their usual ALS treatment (riluzole or edaravone) but resumed them immediately after the procedure. Definity® MBs (4 μL/kg) were administered intravenously, followed by exposure of the target region to US waves (center frequency: 220 kHz) using the ExAblate Neuro® 4000 system. An acoustic power ramp test was conducted, followed by one to two 90-s sonication cycles to achieve BBB opening. During each cycle, the targeted brain tissue received a US sequence (center frequency of 220 kHz, pulse repetition period of 300 ms, DC 0.88%) for each of the four spots within the target region. Each cycle coincided with an i.v. injection of MBs. Abrahao *et al.,* successfully demonstrated the transient and safe acoustically mediated BBB opening in ALS patients. Importantly, no serious adverse effects (i.e., hemorrhage, edema, inflammation, or tissue damage) were observed 30- or 60-day post-treatment.

However, to date, no study has explored whether combining the US with therapeutic molecules could improve motor symptoms in ALS. Similarly, research on the therapeutic impact of US/therapeutic molecule combinations in AD and PD has yet to address symptom improvement comprehensively. This promising clinical trial lays the foundation for further investigations into MB-assisted US for therapeutic delivery in ALS treatment (Table 9).[76]

8. Applications in Huntington's disease (HD)

8.1. HD

HD, also known as Huntington's chorea, is a progressive dominantly inherited neurodegenerative disorder. Its prevalence is 5 – 10/100,000 in North America and Europe, with a higher rate observed in Western Europe.[136] The disease typically begins between the fourth to fifth decades of life and progressively worsens over 10 – 20 years, ultimately leading to death; however, it can also manifest before the age of 20.[136-138]

HD is characterized by a combination of psychiatric, cognitive, and motor symptoms. Clinical signs include involuntary movement disorders (e.g., chorea and dystonia) and voluntary movement impairments (e.g., clumsiness, dysarthria, swallowing disorders, falls, bradykinesia, rigidity). Cognitive impairments often involve issues with memory, attention, judgment, reasoning, and comprehension, with dementia frequently developing over time. The hallmark symptom of HD is Huntington's chorea, which involves involuntary jerking and muscle twitching.

Neuropathological findings reveal significant neurodegeneration, with the selective loss of neurons in the caudate and putamen of the basal ganglia. The disease is caused by an abnormal expansion of a CAG trinucleotide repeat in the coding region of the *HTT* gene.[137] This mutation leads to an expanded polyglutamine stretch in the N-terminal region of the Huntingtin protein, resulting in a toxic gain of function.[134,136] Despite the clear understanding of the genetic basis of HD, no curative treatment is currently available. Existing therapeutic approaches focus primarily on managing chorea, but several novel treatments are under active investigation.[103,139,140]

8.2. Pre-clinical phase

Despite the lack of a clinically approved treatment for HD, promising pharmacological approaches aimed at reducing HTT protein levels have been explored to increase neuronal

Table 8. Drug delivery with MB-assisted US for pre-clinical studies in amyotrophic lateral sclerosis

References	Drug, dye, particle	Animal model	US devices/parameters	Targeted area	MBs	Therapy duration
Shen *et al.*, 2023[132]	Edaravone	*SOD1*^{G93A} mouse	Lab-made US device; 1.1 MHz, 1 Hz PRF, 9.09 ms burst length, 0.52 MPa for 60 s	Motor cortex	Lab-made lipid-shelled MBs (0.2 μL/g)	4 intermittent non-overlapping sonication with a 15-min interval between each other

Abbreviations: DC: Duty cycle; MB: Microbubble; PRF: Pulse repetition frequency; US: Ultrasound.

Table 9. Drug delivery with MB-assisted US for clinical studies in amyotrophic lateral sclerosis

References	Drug, Dye, Particle	Patients	US devices/parameters	Targeted area	MBs	Therapy duration
Abrahao *et al.*, 2019[76]	Gadolinium	2 women and 2 men	ExAblate Neuro® 4000 system; center frequency of 220 kHz, pulse repetition period of 300 ms, DC of 0.88% for 90 s	Primary motor cortex	Definity® MBs (4 μL/kg)	1 sonication

Abbreviations: DC: Duty cycle; MB: Microbubble; US: Ultrasound.

survival and improve motor functions.[141] To date, there are three pre-clinical studies and no clinical trials investigating i.c. drug delivery using MB-assisted US for HD. Burgess *et al.*[134] examined the therapeutic potential of delivering cholesterol-conjugated anti-Htt siRNA (cc-siRNA-Htt) into the striatum of an HD mouse model through MB-assisted US (lab-made MRgFUS device; center frequency of 558 kHz, burst length of 10 ms, PRF of 1 Hz, 0.3 MPa). Definity® MBs (0.02 mL/kg) were administered intravenously, followed by immediate US exposure to the striatum for 120 s. Then, cc-siRNA-Htt was injected through either a tail vein catheter or an intra-carotid catheter. BBB opening was monitored using DCE-MRI. This protocol involved two US exposures separated by a 1-h interval, and the mice were sacrificed 48 h later. The results demonstrated that MRgFUS successfully delivered the cc-siRNA-Htt to the striatum, resulting in a significant 32% reduction in *Htt* gene expression, regardless of the route of administration.[134] However, this pre-clinical study did not assess whether the enhanced bioavailability of cc-siRNA-Htt led to increased neuronal survival or improved motor functions in HD mice.

In another study, Lin *et al.*[115] utilized the MB-assisted US to boost i.c. production of GDNF in the R6/2 mouse model of HD. A liposomal formulation of GDNF-encoding pDNA was administered intravenously, followed by the injection of SonoVue® MBs (0.1 mg/kg). The striatum was then exposed to a US sequence (lab-made US device; 1Hz PRF, 10 ms burst length, DC 1%, 0.33 MPa) for 30 s in the contralateral hemisphere and for 60 s in the ipsilateral hemisphere. This approach significantly increased i.c. GDNF protein levels, enhancing neuronal plasticity and cell numbers. It also delayed symptom onset and improved motor impairments in the treated group compared to the control group.[115] Similarly, Owusu-Yaw *et al.*[135] investigated the efficacy of MB-assisted US for the delivery of an AAV9 viral vector encoding the miR10150, a microRNA targeting HTT transcripts for degradation, along with green fluorescent protein (GFP) as a reporter gene. Following the i.v. injection of Optison® MBs (100 µL/kg), US waves (1 Hz, 10 ms, 0.34 MPa) were applied to the striatum (right caudate putamen) of zQ175 mice for 120 s using a lab-made US device. The AAV9 viral vectors were administered intravenously immediately after US exposure. Analysis of GFP expression in brain tissue confirmed the feasibility and efficiency of MB-assisted US for delivering AAV9 viral vector in both HD and wild-type mice at ages 2, 6, and 12 months. However, Owusu-Yaw *et al.* did not evaluate the i.c. production of miR10150 or its therapeutic benefit (Table 10).

Altogether, these findings demonstrate that MB-assisted US is a promising modality for the delivery of therapeutics in the treatment of HD.

9. Discussion

Accessing the brain for drug delivery remains a significant challenge in the treatment of neurodegenerative diseases. BBB opening is a rapidly expanding field, as evidenced by the growing number of publications on this topic. This method first emerged in the early 2000s with Hynynen's publications on BBB disruption in a rabbit model,[26] and a new momentum has been achieved thanks to advances in the understanding of the mechanisms and composition of MB/ND, the development of new transducer models, and the emergence of clinically approved devices in the 2010s. Many pre-clinical studies have demonstrated the safety, feasibility, and reversibility of opening the BBB. Among these, two studies have focused on the delivery of gadolinium in animal models of neurodegenerative diseases,[63,87] while others have investigated the delivery of therapeutic molecules to assess their effects on behavior.[112,115,132] A few

Table 10. Drug delivery with MB-assisted US for pre-clinical studies in Huntington's disease

References	Drug, dye, particle	Animal model	US devices/parameters	Targeted area	MBs	Therapy duration
Burgess *et al.*, 2012[134]	cc-siRNA-Htt	Rat	Lab-made MRgFUS device; center frequency of 558 kHz, burst length of 10 ms, 1 Hz PRF, 0.3 MPa	Striatum	Definity® MBs (0.02 mL/kg)	2 sonications at 1-h interval
Lin *et al.*, 2019[115]	GDNF	R6/2 mouse	Lab-made US device; 1Hz PRF, 10 ms burst length, DC 1%, 0.33 MPa for 30s at the contralateral hemisphere and for 60s	Striatum	SonoVue® MBs (0.1 mg/kg)	1 sonication
Owusu-Yaw *et al.*, 2024[135]	AAV9 viral vector encoding the microRNA, miR10150	zQ175 mouse	Lab-made US device; 1 Hz, 10 ms, 0.34 MPa for 120 s	Striatum	Optison® MBs (100 µL/kg)	1 sonication, sacrifice 3 weeks later

Abbreviations: DC: Duty cycle; MB: Microbubble; MRgFUS: Magnetic resonance imaging-guided focused ultrasound; PRF: Pulse repetition frequency; US: Ultrasound.

studies achieved both objectives, with most demonstrating enhanced i.c. delivery of therapeutics and improvements in cognitive functions. There is a notable preference for using MRgFUS in pre-clinical studies; however, the majority has been conducted on small animals, primarily rodents such as mice and rats. Only one study to date has utilized a large animal model, specifically a non-human primate, within the context of PD.[111] Expanding pre-clinical research to include large animals could significantly enhance the understanding of BBB opening and lend greater weight to the results. In addition, rodent models, whether transgenic or induced through neurotoxic lesions such as MPTP or 6-OHDA, have inherent limitations, posing challenges in interpreting results and extrapolating findings to humans.

Compared to pre-clinical trials, most clinical studies involving BBB disruption do not focus on drug delivery. Instead, they primarily assess the feasibility, safety, and reproducibility of BBB opening in patients with neurodegenerative disease.[74,76,119] Notably, one study in PD investigated glucocerebrosidase (GCase),[120,121] while another in AD evaluated the delivery of aducanumab.[96] These recent studies are promising for the advancement of BBB opening techniques in neurodegenerative diseases. However, all clinical trials conducted thus far have involved a very small number of participants, typically ranging from 3 to 9, which is surprising given the high prevalence of these conditions. To reliably evaluate the efficacy of therapeutics in humans, future studies need to include larger patient cohorts to determine whether BBB opening significantly enhances therapeutic effects compared to treatment alone. It is worth noting that no standardized treatment exists for neurodegenerative diseases, and therapeutic responses vary widely among patients. If a significant effect is not observed with BBB opening, it may not necessarily reflect a limitation of the technique but rather resistance to the treatment itself. Future studies should therefore not only focus on the feasibility of the technology but also prioritize clinical outcomes such as preserving memory, cognition, or motor functions in patients with neurodegenerative diseases.

One of the key challenges in slowing the progression of these diseases lies in their neurobiological characteristics. Diseases such as PD and ALS are more localized, whereas conditions, such as AD are diffusive. Targeted diseases are seemingly easier to treat, as neurodegeneration is confined to a specific or limited region in the brain, allowing the US to be focused on these areas. In contrast, treating diffuse diseases is more complex, as all affected regions would require treatment with FUS.

There is also a noticeable preference for clinically approved devices in this field. For instance, the NaviFUS® system has not been used in clinical trials for neurodegenerative diseases and has only been used for brain tumors, such as glioblastoma.[72,142] Similarly, the SonoCloud® system has only been tested in one clinical trial for AD as of 2022.[69] ExAblate Neuro® remains the most widely used system, including in a pre-clinical trial involving a non-human primate.[111] The non-invasive nature of MRgFUS and its relatively short intervention times (2 – 4 h) make it an attractive option for research. However, its use entails significant logistical challenges, particularly due to the reliance on MRI. An MRI system must be on-site, available for the procedure, and operated by experienced personnel, all of which contribute to the high costs associated with this approach.

A major limitation across these studies is the variation in protocols. Both in the pre-clinical and clinical phases, the use of MBs/NDs, US devices, and parameters lack consistency, resulting in limited reproducibility between studies. Differences are evident in the type of MBs used (i.e., lab-made, SonoVue®, Definity®, Optison®, and Sonazoid®) as well as in their method of administration. MBs are sometimes injected as a bolus and at other times as an infusion. O'Reilly *et al.*[143] compared BBB disruption *in vivo* using a long infusion (2 min) versus a bolus injection (15 s) of Definity® MBs at the same dose. Their findings revealed better BBB disruption with bolus injection, attributed to the higher peak concentration of MBs in the circulation during bolus administration. The method of administering therapeutics alongside MBs also varies. In clinical practice, sequential administration – infusion of the therapeutic treatment followed by bolus injection and sonication – is often preferred for the convenience of both healthcare staff and patients.[96,121] This method is similarly adopted in pre-clinic phases, with MBs typically administered as a bolus rather than an infusion. Additional protocol variations include the use of loaded or unloaded MBs, as well as targeted versus non-targeted MBs. At present, there is insufficient data to compare the therapeutic efficacy of drug-loaded versus unloaded MBs in neurodegenerative diseases. Few pre-clinical studies explore this comparison, and no drug-loaded MBs have been clinically approved, making such comparisons impossible in clinical trials.

Another challenge is the small sample size in clinical trials, which limits the ability to draw rigorous or generalized conclusions about the therapeutic efficacy of molecules delivered through sonoporation. In pre-clinical studies, researchers often use ab-made MBs, allowing greater flexibility in loading or tagging MBs. In contrast, clinical trials must use clinically approved MBs, which restricts this flexibility. Beyond MB characteristics, US parameters also vary widely between studies. Sonication duration may range from 30 s to 2 min, with acoustic

pressure varying between 0.4 MPa and 0.5 MPa. Parameters such as time, acoustic pressure, and frequency are tailored to each study. Pre-clinical protocols further differ across research laboratories, often due to the use of lab-made US devices. The number of sonication session also varies, with some studies employing a single treatment while others span 3 weeks with 2 sessions per week, for example. Despite these variations, all methods demonstrate success in BBB opening, suggesting the need for standardized protocols. It would be beneficial to establish defined parameters tailored to specific species (e.g., mice, rats, non-human primates, and humans) and target areas (e.g., striatum, motor cortex, hippocampus).

Regarding NDs, only one pre-clinical study has employed them for BBB opening in neurodegenerative diseases.[62] One reason for this limited use is the relative novelty of NDs, which are still being optimized despite the known advantages of ADV since 1998.[44] In addition, no NDs are clinically approved, making their use in clinical settings impossible. The greater familiarity and clinical approval of MBs make them a more accessible option. Nonetheless, studies using NDs have validated BBB opening in wild-type animals, such as with dextran in mice[57] and Evan's blue in rats.[108] Chen *et al.*[57] demonstrated that NDs are more effective than MBs in delivering drugs. While only one study has used NDs for neurodegenerative diseases (specifically AD[62]), other studies have focused on cancer therapy. These include *in vivo* studies on ovarian cancer,[144] hepatocellular carcinoma,[145] and glaucoma,[146] as well as anticancer drug delivery (lung and breast cancer cells)[147] and *in vitro* studies on breast and lung cancer.[148] These studies have shown significant tumor progression slowdown.

10. Conclusion

MB/ND-assisted US offers a promising approach for improving the i.c. delivery of therapeutic molecules by enabling BBB opening in both animal models and humans. While the studies reviewed here focus primarily on neurodegenerative diseases, this approach could also be applied to other conditions requiring barrier-crossing treatments. This approach holds great promise for advancing patient care by providing less invasive alternatives in the future.

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Conflict of interest

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