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Review article

A comprehensive review of advanced focused ultrasound (FUS) microbubbles-mediated treatment of Alzheimer's disease

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ABSTRACT

Alzheimer's disease (AD) is characterized by progressive neurodegeneration, memory loss, and cognitive impairment leading to dementia and death. The blood-brain barrier (BBB) prevents the delivery of drugs into the brain, which can limit their therapeutic potential in the treatment of AD. Therefore, there is a need to develop new approaches to bypass the BBB for appropriate treatment of AD. Recently, focused ultrasound (FUS) has been shown to disrupt the BBB, allowing therapeutic agents to penetrate the brain. In addition, microbubbles (MBs) as lipophilic carriers can penetrate across the BBB and deliver the active drug into the brain tissue. Therefore, combined with FUS, the drug-encapsulated MBs can pass through the ultrasound-disrupted zone of the BBB and diffuse into the brain tissue. This review provides clear and concise statements on the recent advances of the various FUS-mediated MBs-based carriers developed for delivering AD-related drugs. In addition, the sonogenetics-based FUS/MBs approaches for the treatment of AD are highlighted. The future perspectives and challenges of ultrasound-based MBs drug delivery in AD are then discussed.

1. Introduction

Recently, neurodegenerative diseases have become a major concern, especially because of serious damage to the central nervous

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system (CNS) that is often difficult to treat [1–3]. These disorders encompass a broad spectrum of diseases with deep-seated and severe symptoms, including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and other brain disorders [1, 4–6]. Alzheimer's disease is the most common cause of dementia which affects a person's ability to function *via* a gradual decline in memory, thinking, behavior, and social skills [7–10].

Despite the huge burden and the impact on the patients, there is no definitive cure for AD and thus, developed treatment methods are needed. Significant progress in biotechnology and nanotechnology over the past two decades has yielded some important insights about the basic biology and clinical pathophysiology of AD, leading to more effective therapeutic approaches [11–13]. The novel strategies to be able to improve the permeability of the blood-brain barrier (BBB) have attracted a lot of interest for AD related drug delivery [14,15]. Recently, focused ultrasound (FUS)-induced drug delivery has opened new prospects because it directly delivers the drugs of AD into the brain *via* the penetration of the BBB [16,17]. FUS in combination with the cavity behavior of microbubbles (MBs) can deliver the drug-loaded MBs to the target site in the deep regions of the brain [18–20].

In the present review, a comprehensive approach to understanding the FUS-induced MBs-mediated drug delivery for the treatment of AD and the challenges to be considered in the development of these strategies are discussed.

1.1. Alzheimer's disease and therapeutic approaches

AD refers to one of the major causes of senile dementia with properties including progressive neurodegeneration, cognition impairment, neuronal and axonal loss followed by memory loss [8,21,22]. Progressive cognitive and memory impairment with personality changes in severe AD can lead to dementia and even death [8,23,24]. Memory and cognitive impairment are the main symptoms related to dementia, and it is commonly due to the decrease in acetylcholine (ACh) function in the neurons of the CNS [25]. Aging is among the most important known risk factors for most chronic illnesses, including AD [26]. The increasing population of the elderly in the world is associated with the prevalence of AD [27–29]. The accumulation of amyloid beta (Aβ) plaques and neurofibrillary tangles (NFTs) composed of phosphorylated tau protein in the brain (hippocampus) cells are considered the hallmark lesions of AD [8,27,30,31]. In addition, mitochondrial dysfunction and neuroinflammation (which ultimately leads to neuronal loss) can play a vital role in AD [32,33]. Some drugs (Not approved) have been introduced for AD, but these drugs are often not effective in completely treating the symptoms and their effectiveness decreases over time [34–36]. Therefore, the novel therapy approaches that can provide effective treatment and remarkable advantages for patients who suffer from these devastating disorders are of utmost importance [26, 34].

Challenges in treating AD have partially arisen from difficulties in penetrating the BBB [37,38]. The BBB has a selective permeability and preventing function that tightly controls the entry and exit of exogenous substances especially harmful molecules, which is vital in healthy people [14,37]. This barrier does not allow the required amount of the drug to be delivered into the brain, restricting their potential therapeutics in neurodegenerative diseases [14,38,39]. Recently, micro- and nano-sized carriers have shown great potential as precision medicines that can efficiently penetrate into the brain by crossing, avoiding, or disrupting the BBB and increasing the targeting ability of drugs [14,15]. Regarding, MBs combined with FUS gained special advances as a potential permeable option across BBB [18,40].

1.2. Blood-brain barrier (BBB)

The BBB is a selective semi-permeable membrane between the blood and the interstitium of the brain. BBB regulates the entering of chemicals and drugs into the brain. Physiologically, the BBB is formed by brain microvascular endothelial cells (BMEC), pericytes, and

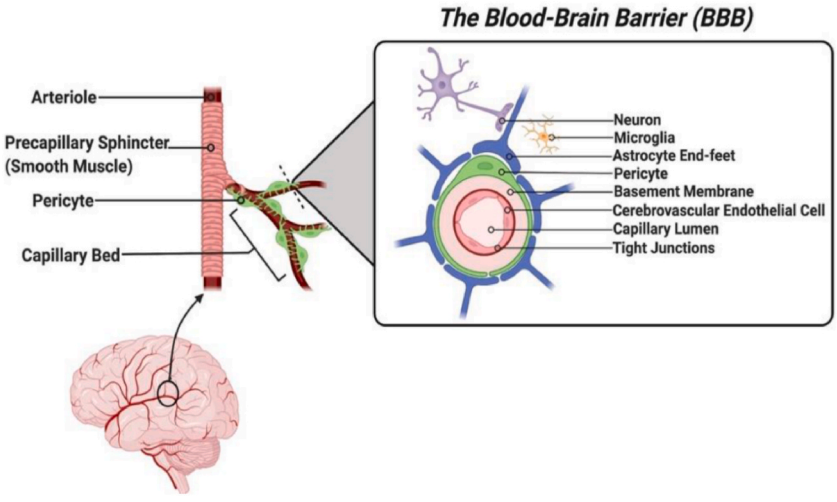

Fig. 1. The structure of Blood-brain barrier (BBB).

astrocytes (Fig. 1). This structure restricts the permeability of delivered drugs from the blood into the brain and makes it difficult to treat many brain disorders effectively. The presence of occludin- and claudin-based tight and adherens junctions, vascular endothelial (VE)-cadherin, and zonula occludens-1 (ZO-1) accessory protein play a role in the transcytosis of molecules through the BBB [41,42].

The pathophysiology of AD is closely related to BBB dysfunction. Changes and dysfunction in the BBB structural components (astrocytes, pericytes) and tight junctions between endothelial cells can cause Aβ accumulation in the brain and increase oxidative stress and beta- and gamma-secretase activity, resulting in Aβ pathology. This condition continues to destroy neurons and glial cells as well as damage the neural network that leads to cognitive decline and the onset of dementia [43–45].

Several approaches have been developed for getting around the BBB, such as intracerebral/intraventricular injections, intranasal delivery, and chemical mediation. However, some drawbacks, such as low delivery, side effects on healthy tissue, and systemic cytotoxicity have been observed [41,45]. Non-invasive methods such as MBs-induced FUS can be alternative options for controlled, reversible, and safe permeability of BBB.

1.3. Microbubbles (MBs)

MBs are a type of microspheres that are structurally contained a nano-sized shell with different compounds and a gas-filled core [46,47]. The shell may be composed of different compounds such as surfactants, proteins, lipids, polymers, which is separated the encapsulated gas from the surrounding aqueous medium (Fig. 2a and b) [20,48,49]. MBs are typically between 0.5 and 10 μm in diameter which allows to circulate in the micro-vessels and capillaries all over the body [46,48]. Changes in core and shell properties determine the strength, acoustic properties, thermal conductivity, and buoyancy of MBs [50].

MBs have widespread application in life science, industry, and medicine especially MBs have been used in drug delivery [52]. MBs can avoid the drugs degradation in the blood circulation and avoid uptake by nonspecific cells or tissues. At the target site, the released drug from MBs can penetrate into cell under ultrasound [53]. The lipophilicity of MBs can facilitate drug penetration into the BBB, but the large size of MBs can be limited the easily cross the barrier [54]. Concomitant use of FUS with MBs can eliminate this problem, which facilitates the penetration of MBs [47]. It has also been proven that this change in BBB permeability is reversible and without serious side effects [18,55].

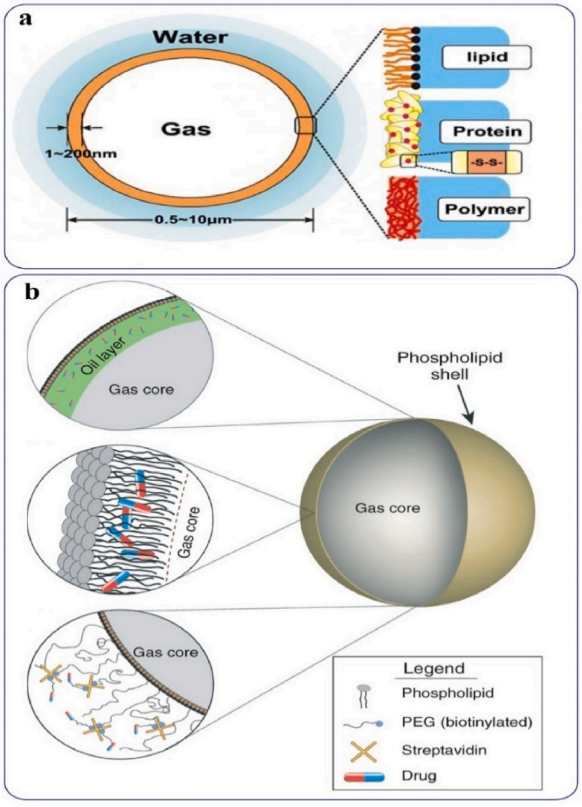

Fig. 2. a) The structure of typical MBs with different shell compositions, b) Drug attachment strategies in MBs mediated drug delivery. Reprinted with permission from Refs. [49,51].

1.4. Focused ultrasound (FUS)

Current strategies to deliver molecules into the brain can be categorized as invasive and non-invasive [38,56]. The invasive manner directly administrated the therapeutic agents into the brain through intracerebral or catheter-guided injection, and opening the tight junctions of endothelial cells that are exposed to a hypertonic solution [38,57]. The invasive methods increase the risk of infection, possible injury of brain tissues, and uncontrolled release of drugs [58]. Therefore, the non-invasive approaches that can deliver the therapeutics to the brain *via* intravenous injection or intranasal administration are commonly preferred. The non-invasive neuromodulation technologies are one of the most promising parts in the development of therapies for neurological diseases [59,60]. However, problems such as limited penetration depth and spatial targeting are seen in common non-invasive approaches, containing transcranial direct current stimulation (tDCS) and transcranial magnetic stimulation (TMS) [59,61].

FUS is a non-invasive strategy for drug/gene delivery to the brain by using ultrasonic energy, which can open the BBB transiently, manipulate the function of cells and proteins, and facilitate the drug's delivery to the target cells with high accuracy [16,17,62]. Studies have shown that FUS can overcome the low penetration of traditional non-invasive methods due to the penetration of sound waves into soft tissue and bone and its high ability to modulate nerve activity in deep areas of the brain with millimeter spatial accuracy. The ultrasound waves used in FUS have a frequency of about 2–18 MHz, which is 100 times more than the human hearing range [63]. The higher frequency (shorter wavelength/higher energy) of the ultrasound waves can penetrate the tissue. A new technology called sonogenetics, allows ultrasound to be directly related to cellular activity [64,65]. The speed and penetration rates of FUS are related to the compressibility of the tissue and its density, which is called different sound impedance [65]. FUS in connection with the cavity behavior of MBs can transport the biomolecules and drugs through various cellular and tissue barriers [66]. In this line, recent reports suggest that the FUS-induced MBs-related technology could be used to treat neurodegenerative diseases [17,67].

2. FUS-induced MBs-based drug delivery mechanism

Cavitation, the mechanical effects of FUS, is the main cause of the disruption of the BBB [68]. The cavitation effects from the

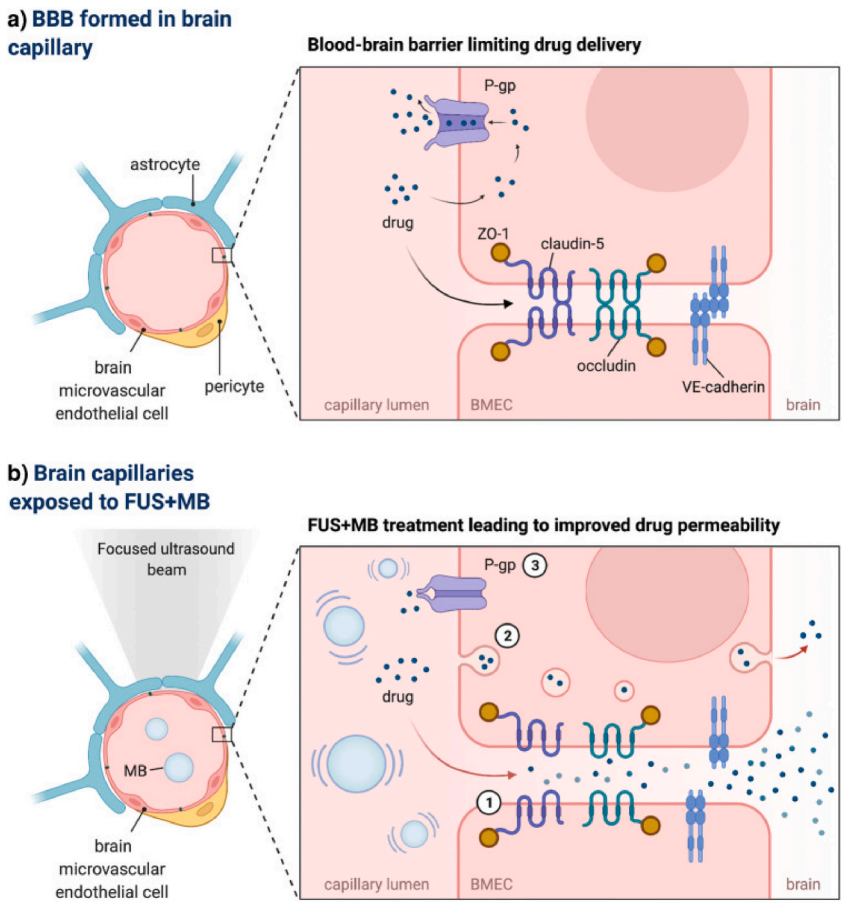

Fig. 3. a) role of BBB barrier in the penetration of drugs into brain, b) FUS-induced MBs-based drug delivery to the brain mechanism. Reprinted with permission from Ref. [75].

circulating MBs during irradiation with FUS sonication are the basis of FUS-induced BBB permeability [47,67]. This phenomenon causes the increase of endocytosis/transcytosis, and paracellular passing *via* reformed tight junctions or through the endothelium cell membrane channels [17,18]. Stable or inertial cavitation are two forms of acoustic cavitation. In stable cavitation, the acoustic microstreaming and pushing/pulling of MBs on the membranes of adjacent cells lead to pores creation and desired BBB permeability [65,69]. Inertial cavitation can potentially disrupt the membrane by the implosion of the MBs and the formation of jets [65].

Selective and regional permeability is a unique advantage of FUS/MBs disruption of the BBB, which allows for improved local delivery of drugs to the brain [70]. In the FUS/MBs technique, low-frequency ultrasound waves are administrated transcranially, ultimately leading to oscillation and concentration of MBs on the capillary walls, which in turn the expansion and contraction of MBs, leading to loosening of the tight junctions between endothelial cells [16].

The intravenous direct administration of the drug or release of the drug locally using drug-loaded MBs provides a high concentration of the drug at the site of BBB disruption, which is needed for the FUS-assisted drug delivery *via* BBB [17,71]. FUS in combination with intravascular MBs can open tight junctions, create endothelial cell openings, and improve endocytosis and transcytosis [72]. The exact mechanisms have still not been fully clarified, but in the presence of FUS, MBs oscillate and apply biomechanical forces on the blood vessel wall, which assist drug delivery through the capillary wall by transcellular or paracellular routes [73]. Based on preclinical observations, three routes were proposed for FUS/MBs-assisted drug delivery at the BBB including paracellular transportation of drug by mechanical forces of MBs, increasing in the number of intracellular vesicles and upregulation of endo- and transcytosis, and limitation of drug efflux at the BBB by decreasing of expression of P-gp at brain microvascular endothelial cells (BMEC) (Fig. 3a and b) [74].

Cavitation of bubbles during irradiation with an ultrasound field can enhance the vascular permeability, facilitating intracellular delivery of small molecules from the blood vessels, a process named as sonoporation. Although the detailed mechanisms of sonoporation are not completely understood, sonoporation, like electroporation, can generate transient pores in cell membranes allowing drug uptake by the cell [76,77]. Sonoporation can also improve the delivery of genes and drugs to the target cells, even in deep tissues [65,76].

Evaluation of the concentration and amount of therapeutic agents that can be delivered to different brain regions is an important aspect of assessing the efficacy of FUS/MBs-released anti-AD drug delivery [78]. Both qualitative and quantitative approaches can be used to analyze the concentration of drugs delivered to the brain. The efficiency of drug delivery and targeting in the brain is usually presented in indirect form. Radioactivity, fluorescence, and UV absorption are used to quantify the drug concentration in the brain. It is difficult to quantitatively estimate the absolute amounts of drug that have reached the brain. The uptake of the drug in the brain is presented as a relative value [79,80]. Basic histologic stains, such as H&E and Nissl, have been used to assess the effects of FUS/MB on tissue health [81]. A quantitative pharmacodynamic analysis can be provided based on the changes in the T1 relaxation after gadolinium injection and quantitative drug concentration maps [78].

3. Role of the MBs properties in the FUS-induced BBB opening

The performance of MBs-based drug delivery is affected by their physicochemical properties such as size, morphology, stability, surface modification, drug loading capacity, and drug release content. When performing the process, some parameters must be considered, such as controlling size, stability, and drug leakage from the MBs. The size of the MBs is usually about 0.5–10 μm, and their size makes them easier to swallow and facilitates the mechanism of drug delivery to the body. The size of the MBs in the range of micrometers may be limited to cross the endothelium, so, a smaller size particle would be preferable [82]. FUS-induced micro-to-nano conversion considerably overcomes the limitation of conventional MBs drug delivery methods [83].

To clarify the role of size of MBs in the FUS-dependent opening BBB, two sets of experiments were carried out on monkeys and mice. Before sonication of the right hippocampus with FUS, 67 mice intravenously received MBs either 1–2, 4–5, or 6–8 μm in diameter. The results showed that when the bubble diameter was similar to the capillary diameter, BBB opened with nonlinear bubble oscillation without inertial cavitation. In monkey model experiments, in all cases with 4–5 μm diameter bubbles, the BBB was opened. This study demonstrated the bubble volume across from BBB was dependent on both the bubble diameter and acoustic pressure [84].

However, the effects of encapsulated MBs on stability and drug delivery efficacy have not been identified, their gaseous core of MBs allows the loading of sufficient drugs and their surrounding shell protect drugs from fast release. Moreover, when drugs are dissolved within the lipid layer of the shell or directly merged into the shell, MBs can protects them from degradation and clearance [85]. To evaluate how loaded drugs alter the stability of MBs, FUS imaging ability, and efficacy of the drug deliver efficacy to the brain, MBs with the same lipid shells can be encapsulated with different FDA-approved gases such as sulfur hexafluoride (SF6), perfluoropropane (C3F8), and perfluorobutane (C4F10) [86]. The interactions between MBs and FUS in the biodistribution of drug within the brain depend on the frequency and power of FUS beams and the dose of MBs [85]. Low-intensity FUS opens the BBB transiently and reversibly, which is followed by the opening of tight junctions of brain endothelial cells and transcellular transportation. These phenomena facilitate the biodistribution of drug within the brain [87]. In a study, the detection of BBB opening following the FUS was assessed by intravenous injection of an NRI agent (gadolinium) as an opening-tracer, which normally does not cross the BBB. The results showed a high amount of the opening volume and gadolinium in the grey matter and an increased FUS-induced permeability and drug concentration with the acoustic pressure [78]. In rodents, physiological properties of the BBB, such as opening volume, permeability, therapeutic agents' concentration, and reversibility timeline should be addressed. So, thoughtful MBs-related pharmacokinetics and physiological variation are vital for emerging safe and stable FUS/MBs treatment protocols [88].

The coating of MBs surface by various polymers, proteins, lipids, or surfactants possesses different properties to MBs consisting of charge, functionality, and hydrophobicity [89]. These are critical hallmarks for their performances and interaction with target

markers. Although the limited surface area and shell thickness of MBs limit their loading capacity, the functionalization of MBs can overcome this shortcoming. Drugs can bind to the MBs shell through specific ligands. Thus, the functional groups are effective criteria for the encapsulation and loading of sufficient content of drugs on the MBs surface. In addition, the MBs that are surface-engineered with functional groups are capable of conjugation with preferred ligands or proteins for site-specific delivery. Lipid MBs are often preferred to polymer MBs due to the permeability of lipid-based material across BBB and their interaction with ultrasound waves. Although the shell of polymeric and lipid-based MBs cracks at high ultrasound pressure, through which the loaded drug can leak the lipid-based MBs become somewhat more resistant to low ultrasound [20].

In addition to the size and type of MBS, the consistent BBB opening is highly related to the dose, and delivery route of MBs [50,54]. In the main protocols of FUS, MBs are intravenously injected with a dynamic dose, permanently rising and falling [90]. Furthermore, the clearance of MBs limits the penetrated drug from opened BBB. Variability in bubble concentrations can pose a potent challenge for MBs-mediated drug delivery [50,91]. MBs at an acceptable serum concentration address these restrictions in the treatment time.

4. MBs as contrast agents in FUS-based imaging of AD

Recently, the serve of MBs as ultrasound contrast enhancement agents has extensively developed in AD imaging. In a study, the left hippocampus of the AD APP/PSI mice was sonicated and the MBs were injected intravenously into the animals. Brain images were obtained before and after injection of Gadolinium (Gd) using MRI. MRI images showed that the BBB was opened on the first day and closed on the second day, although the duration and extent of the opening were different in different regions. The results of this study displayed that FUS can induce the BBB opening and internalization of Gd-loaded MBs in brain cells [92]. MRI technique has been used to characterize AD by diagnosis and checking of amyloid plaques, but invasive interventions are needed to penetrate the contrast agents into the BBB. In a work, unfocused ultrasound in combination with clinically approved MBs was employed for transiently opening the BBB, to determine amyloid plaques in the brain of an APP/PS1 transgenic mouse model of amyloidosis after intravenous injection of a contrast agent. The fabricated method can detect amyloid plaques with a high in-plane resolution at 32 min imaging time. The results showed similar sensitivity to standard brain MRI whenever a contrast agent was injected *via* intra-cerebra-ventricular [93].

In most studies, the researchers have been provided a proper MBs-based theranostic agent for the treatment diagnosis of AD by the

Table 1
FUS-induced delivery of drug-loaded MBs for AD therapy.

Ultrasonic parameters					Microbubbles properties	Animal model	Therapeutic agents	Target	Ref.
Acoustic pressure	Frequency (MHz)	Duration	Duty cycle (%)	PRF (kHz)					
0.4–0.6 MPa	1.14 MHz	2 min	0.5 %	–	1 μm	Mice model of AD	PEG coated, brain-penetrating nanoparticles	Amyloid-beta (Aβ) plaque	[98]
–	–	600 s	–	–	poly-α-cyanoacrylate (pBCA)-based MBs	APP/PS1 Mice model of AD	Quercetin-modified sulfur nanoparticles		[19]
0.6 MPa	1.0 MHz	1 min	10 %	10 Hz	300 nm PLGA-lipid hybrid	APP/PS1 transgenic mice	Nanosized exosome		[99]
0.41–0.5 MPa	400 kHz	60 s	–	1 Hz	SonoVue® SF6-filled MBs	APPswe/PSEN1-dE9 transgenic mice	GSK-3 inhibitor		[100]
0.3 MPa	0.558 MHz	2 min	–	1 Hz	0.16 ml/kg	TgCRND8 mice model of AD	Anti-Aβ antibody BAM10	Amyloid-beta (Aβ) plaque	[101]
0.8 MPa	1 MHz	20 s	–	1 Hz	SonoVue 0.05 ml/kg	New Zealand rabbits	BC-10 anti-Aβ antibody		[102]
	0.55 MHz	120 s		1 Hz	0.04 ml/kg	TgCRND8 mice model of AD	BAM-10 Aβ-antibody and scyllo-inositol		[103]
Constant	1.68 MHz	2 min	–	1 Hz	–	TgCRND8 mouse model of amyloidosis	intravenous immunoglobulin (IVIg)		[104]
0.7 MPa	1 MHz	3 min	10 %	10Hz	1.885 μm Lipid shell	APP23 mouse model of AD	Aducanumab antibody		[105]
–	–	–	–	–	–	APP/PS1dE9 mice	anti-pGlu3 Aβ mAb antibody		[106]
0.33 MPa	2 Hz	100 s	–	–	Optison™ MBs	APP/PS1dE9 mice	anti-pGlu3 Aβ antibody		[107]
0.45 MPa	1.5 MHz	1 min	–	10 Hz	1.4 μm	rTg4510 Mouse Model		Tau protein	[108]
0.42 MPa	1 MHz	60 s	2 %	1 Hz	10 μm	tau transgenic mice			[109]
0.64 MPa	1 MHz	2 min	10 %	1 Hz	4–8 μm lipid shell	Mice model of AD			[52]
25 % of the value	1.68 MHz	120 s	–	1 Hz	0.02 ml/kg	TgCRND8 mice	TrKA agonist D3	TrKA receptor	[110]

combination of MBs conjugate with chemotherapeutic agents. Magnetic resonance-guided focused ultrasound (MRgFUS) coupled with injected MBs is an emerging surgical technology for noninvasive brain treatments that transiently open the BBB with a high degree of spatial and temporal specificity [94]. It was found that the use of FUS in combination with MBs allowed a small fluorescent agent and anti-Aβ antibody as a large molecule to enter the brain in transgenic AD mice. The change in the permeability of the BBB by FUS provided a condition for the delivery of both molecular imaging and therapeutic agents to target the Aβ [95].

5. FUS-induced delivery of drug-loaded MBs for AD therapy

Biomarkers-based diagnosis and treatment of diseases are the most reliable ways to help physicians prevent or limit disease progression. AD-related biomarkers such as Aβ, Tau protein, and apolipoprotein E4 (ApoE 4) have been usually used for the determination of AD in blood and cerebrospinal fluid (CSF) [96,97]. In this line, these specific AD biomarkers are the basis of drug delivery to the brain by FUS/MBs for AD therapy (Table 1).

5.1. Amyloid β (Aβ)

Most studies of FUS/MBs-induced drug delivery to AD treatment have been focused on the permeability of the BBB and subsequently reducing the accumulation of amyloid plaques. Accumulation of Aβ isoforms including insoluble Aβ42 and soluble Aβ40 has a direct effect on the development and progression of AD [97,111,112]. Age-related changes interfere with lymphatic clearance, leading to Aβ accumulation and eventual AD. Based on this, the FUS/MBs combination improved brain-to-cerebrospinal fluid (CSF) Aβ drainage in a mouse model of dementia [113]. Regarding this, FUS/MBs may enhance Aβ entry into the circulation system resulting in increased Aβ clearance by the liver and kidneys. It can be said that MBs treatment may lead to enhanced non-amyloidogenic pathway and suppressed amyloidogenic pathway. It would be great to investigate the effect of MBs on different aspects of Aβ-related pathogenesis including Aβ production pathways and their clearance in future research.

5.1.1. Nanoparticles and therapeutic agent's delivery

Nance et al. investigated a non-invasive approach including MRgFUS, MBs, and polymeric nanoparticles (NPs) as a therapeutic agent with penetrating ability into the brain parenchyma. The coating of brain-penetrating NPs (BP-NPs) with low-molecular-weight poly(ethylene glycol) (PEG) provides long-circulating and stable BP-NPs. However, PEGylation covers NPs and increases their interactions with cells, but limits their cell uptake or passage across intact BBB. MRgFUS can improve the accumulation and spread of BP-

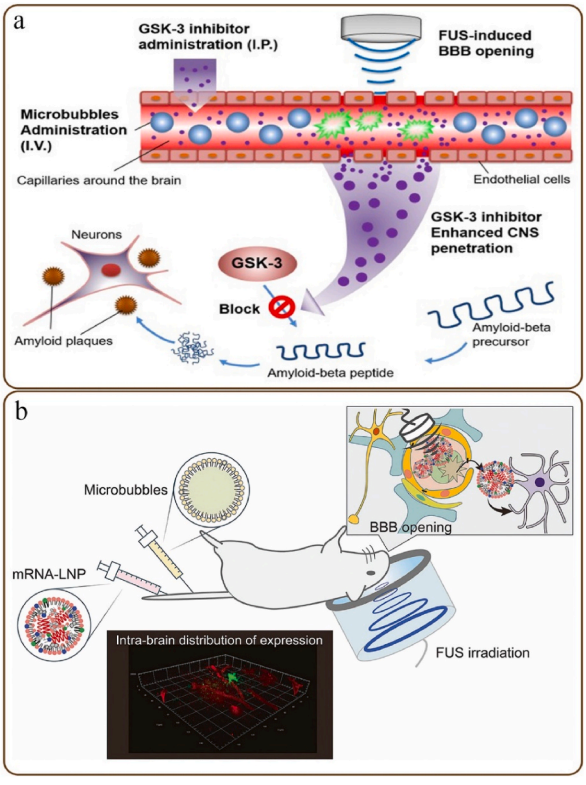

Fig. 4. a) The reduction of Aβ plaque synthesis by FUS-induced BBB opening and the delivery of GSK-3 inhibitor (AR-A014418), b) FUS/MBs-mediated delivery of mRNA encapsulated-LNP (mRNA-LNP) through BBB in an AD model. Reprinted with permission from Refs. [100,114].

NPs in specific areas of the brain. The results showed that the FUS/MBs coupling could deliver 60 nm PEGylated BP-NPs to the brain parenchyma with a 10-fold slower diffuse in normal rat brain tissue. This strategy suggests a potential to improve efficacy, reduce side effects, and provide sustainable drug delivery in the treatment of many CNS-related diseases, especially AD [98].

In a study, the therapeutic potentials of FUS-induced BBB opening were evaluated on the delivery of GSK-3 inhibitor (AR-A014418) and reduction of Aβ plaque synthesis for AD treatment in an AD mice model (Fig. 4a). FUS-mediated BBB opening on APPswe/PSEN1-dE9 transgenic mice was performed unilaterally, with the contralateral hemisphere serving as a control. The immunohistochemistry (IHC) results revealed the reduction of GSK-3 activity up to 61.3 % after FUS-mediated GSK-3 inhibitor delivery. A significant Aβ-plaque reduction up to 31.5 % was also confirmed by autoradiography [100]. Lipid nanoparticles (LNP)-based mRNA delivery has become a novel therapeutic approach. Recently, FUS/MBs-mediated BBB opening can enhance the delivery of mRNA-encapsulated LNP (mRNA-LNP) through BBB to the brain. In a study, it was demonstrated that by applying FUS/MBs, plasmid DNA delivery and exogenous protein (luciferase) expression by mRNA-LNP in the microglia and CD31-positive endothelial cells can be observed, confirming the NPs delivery by BBB opening (Fig. 4b) [114].

A nano-system based on MBs in combination with FUS was constructed to promote the crossing of Quercetin-modified sulfur NPs across the BBB (Fig. 5a). After exposure to ultrasonic pulses, the system is immediately destroyed resulting in improved permeability of the blood vessels and brief opening of the BBB because of the "sonoporation" effect. In addition, after the destruction of the MBs, the nanodrugs embedded in them were released and accumulated in the parenchymal tissue of the brain. Because of instantaneous accumulation in the brain, nanodrug efficiently reduces inflammatory response, apoptosis, oxidative stress, and calcium homeostasis imbalance which is mediated by protecting neuron cells and endoplasmic reticulum stress, thus improving AD. Significant improvement in memory and learning ability was determined without obvious side effects [19]. Exosomes are nano-sized extracellular vesicles that are secreted by different cells of the central nervous system and can be involved in the removal of intracellular material. In AD, increases in Aβ levels can impair the exosome-mediated Aβ clearance pathway. Regarding the role of FUS in the degradation of Aβ, in a study, FUS/MBs were applied for targeted exosome delivery across BBB (Fig. 5b). After FUS, FUS-stimulated HA cells were collected to characterize exosomes. The oligomeric Aβ42 toxicated SH-SY5Y cells were employed for the investigation of the neuroprotective effect of FUS-stimulated HA cells. FUS demonstrated a 5-fold increase in the exosome release from human astrocytes. The decrease of Aβ plaque in APP/PS1 animals following treatment revealed the therapeutic potential of FUS-stimulated HA cells and

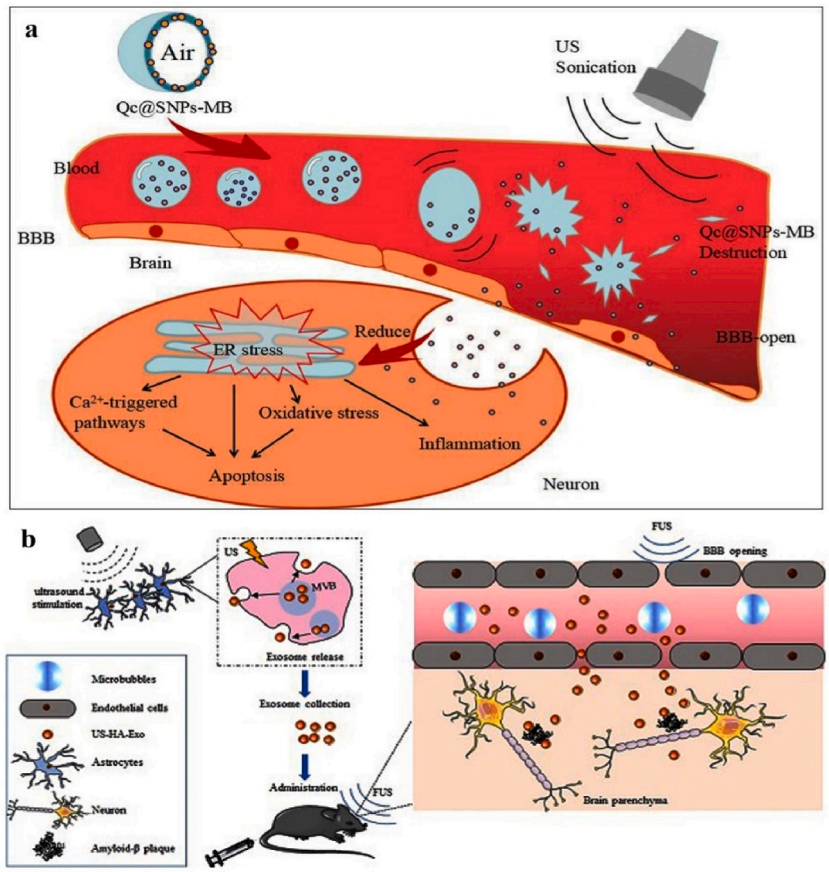

Fig. 5. a) The BBB crossing of quercetin-modified sulfur NPs/MBs in combination with FUS, b) FUS/MBs-mediated exosome delivery across BBB for Aβ clearance. Reprinted with permission from Refs. [19,99].

demonstrated its neuroprotective potential in reversing oligomeric Aβ-induced cytotoxicity [99].

5.1.2. Anti-Aβ antibody delivery

Anti-amyloid mAbs are the first disease-modifying therapies for AD that are directed against the amyloid-β (Aβ) peptide resulting in the slowing of the progression of AD. Over the past years, several mAbs have been engineered to bind and clear Aβ such as bapineuzumab, solanezumab, gantenerumab, crenezumab, and aducanumab [115,116].

In a TgCRND8 mouse model of AD, MRgFUS in combination with MBs was used to deliver BAM-10, an anti-Aβ antibody. In this study, Gd was used as a contrast agent. The antibody was injected into the treated animals at the same time as the ultrasound was applied to a hemisphere of the brain. The results showed that only mice that were exposed to FUS had significant amounts of the antibody in their brains. It was also found that four days after treatment, the level of Aβ in the brains of animals significantly decreased [101]. Alecou et al. reported the treatment of AD in a New Zealand White rabbit model based on MRIgFUS-MB facilitated to entry of the BC-10 anti-Aβ antibody into the brain and its binding to Aβ plaques. Their results revealed a reduction in the number of plaques and elimination of Aβ plaques [102]. In a study, the effect of a combination treatment including initial MRIgFUS/MBs-mediated delivery of BAM-10 Aβ-antibody and scyllo-inositol was investigated on reducing Aβ load through microglial phagocytosis in a TgCRND8 mouse model of AD. After 30 days, a significant reduction of loaded-Aβ and astrocyte activation in the hippocampus and the cortex, and an increase in phagocytic activity of microglia, which relates to Aβ clearance [103].

Intravenous immunoglobulin (IVIg), a centrally and peripherally immunomodulatory, is a human blood product consisting of polyclonal antibodies that can reduce Aβ-related AD. In a study, the potential improvement in the delivery of IVIg to the hippocampus, promotion of neurogenesis, and reduction of amyloid plaque pathology following FUS were investigated. The result demonstrated the FUS can significantly increase the IVIg levels and hippocampal neurogenesis through increased BBB permeability along with a considerable reduction of in amyloid plaque the targeted hippocampus of TgCRN8 mice. The down-regulation of proinflammatory cytokines such as tumor necrosis factor α (TNFα) in the hippocampus confirmed the FUS/MBs-induced inflammation [104]. The results of clinical trials show that Aducanumab as an anti-Aβ can reduce the pathology of amyloid. It has also been shown that this antibody must reach a certain level of accumulation in the brain to improve cognition. In a study, Aducanumab analog efficacy was investigated by FUS merged with intravenously injected MBs in APP23 mice. The results showed that the treatments reduce plaques in the hippocampus, but no significant improvement was seen in combination therapy. However, in the cortex, only combination therapy caused a significant reduction in plaque. It was also found that cognitive improvement was seen only in the combination group and the amount of Aducanumab in this group was 5 times higher than the other groups [105].

A study has shown monoclonal antibody (mAb) reduces the plaques of Aβ and pGlu3 Aβ in APP/PS1dE9 mice by targeting the toxic species of Aβ. In this study, the effect of mAb 07/2a on Aβ clearance and cognition was evaluated through FUS/MBs-induced BBB opening. The result showed that FUS treatment increased mAb levels in the brain, reduced the accumulated hippocampal Aβ, and improved cognition [106]. In another study, the FUS/MBs procedure was employed in the AD model of amyloidogenesis to improve intravenous delivery of mAb 07/2a as an Fc-competent anti-pGlu3 Aβ through the BBB. Pyroglutamate-3 amyloid-β (pGlu3 Aβ) is a form of Aβ with pathogenic specification found in vascular deposits and cerebral amyloid plaques. The results of this study showed that the use of FUS increased the delivery of 07/2a mAb to the brain, and significantly reduced the plaque levels in the hippocampus followed by increased levels of synaptic proteins in synaptosomes, memory, and spatial learning in animals after three weeks. This finding demonstrated that FUS is a helpful tool to increase the efficacy and delivery of an anti-pGlu3 Aβ mAb for immunotherapy through an independent mechanism or an additive effect [107]. Pyroglutamate-3 amyloid-β (pGlu3 Aβ) is an N-terminally modified, toxic form of Aβ that is present in cerebral amyloid plaques and vascular deposits. In a study, FUS/MBs were used to deliver the Fc-competent murine anti-pGlu3 Aβ mAb, 07/2a across the BBB to improve Aβ removal and restore memory in aged AD model APP/PS1 mice. In comparison with control, mice treated with FUS-mediated mAb showed significantly better spatial learning and memory. In addition, the reduction of Aβ42 and pGlu3 Aβ hippocampal plaque and increasing of Iba-1+ microglia and $Ly6G^{+}$ monocytes were observed in the hippocampi of AD mice [117].

In a study, using MRI-guided FUS, the intravenous administration of a much lower dose of anti-Aβ antibodies in transgenic mice resulted in significant plaque reduction 4 days post-treatment, confirming an effective drug for AD treatment [16].

5.2. Tau protein

Tau protein is a microtubules-associated protein (MAP), which is predominantly found in axons to stimulate microtubule polymerization [111,118]. Tau is associated with dementia and neurological disorders [111,119]. Karakastani et al. evaluated the effect of FUS at the early steps of Tau pathology (pre-tangle) in the rTg4510 mice model. The result demonstrated that the FUS reduced phosphorylated Tau in pyramidal CA1 neurons of the hippocampus without an enhancement in the phosphorylated Tau neuronal somas, which is usually associated with disease progression. It was also found that the FUS does not impair nerve integrity [108]. FUS can facilitate the transmission of antibody fragments against pathological Tau in transgenic tau P301L mice. The treatment of transgenic mice with repeated FUS for 15 weeks showed considerable reduction in Tau pathology without obvious histological damage, and improvement of memory and motor function. In addition, FUS promoted the autophagy pathway in neurons revealed through a decrease in the autophagic flux marker p62, reduction of mTOR activity, increase in beclin 1 level, and increase in the autophagosome membrane marker LC3II. The clearance of Tau by autophagy is reflected by a considerable increase in the interaction of p62 and Tau in the treatment group [109]. In a study, triple transgenic AD animals with Tau and Aβ deposits were treated by FUS in combination with MBs twice per week for 6 weeks. Considerable improvement in memory and learning ability, phosphorylated Tau, and Aβ deposits in the sonicated hemisphere were seen in the treated group [52].

6. Sonogenetics-based FUS/MBs approaches on the road to AD treatment

Sonogenetics is a combination of genetics and ultrasound-based methods to noninvasively control cell activity by stimulating the expression of ultrasound-sensitive proteins in the cell [120–122]. For this process, the gene encoding FUS-responsive proteins or other manipulated genes transfers to the preferred cells after the changes caused by ultrasound on the cell. FUS-based sonogenetics technique affects also cell function leading to significant changes in the biological process including differentiation, proliferation, and apoptosis [123]. It is noteworthy that in addition to the mechanical effect of FUS, a thermal effect arises from the motion of target molecules in response to ultrasound radiation, which is due to the increased internal energy of the molecule [120,123].

The genetic tools enable the precise expression of specific FUS-sensing proteins in various cells, providing target cells with enhanced FUS sensitivity compared to other cells [123,124]. sonogenetics targets specific neuronal populations through the use of sound waves activating genetically overexpressed mechanically or thermally sensitive ion channels [125].

Transient receptor potential (TRP) channels are one of the main targets in sonogenetics, whose genes can express at least 20 types of protein ion channels [126,127]. The TRP channels in mammalian tissues are involved in a diverse range of cellular and peripheral signals, allowing them to respond to a variety of chemical and physical stimuli and protect organs against harmful stimuli and acute conditions [128,129]. TRP channels have six transmembrane motifs that lead to the formation of non-selective cationic channels that act as signal transducer by changing membrane potential or intracellular calcium (Ca^{2+}) [128,129].

One of the subfamilies of TRP channels is transient receptor potential vanilloid (TRPV) channels, which are divided into 5 subgroups (TPRV1-5) [130]. Among TPRV subtypes, the TRPV1 channel is commonly used in sonogenetics [123,131]. By the thermal effects of ultrasound waves to TRPV1 channels, the TRPV1 channel temperature reaches 42 °C resulting in calcium ions transportation from the outside of the cell membrane into the cells [120]. It can be surmised that TRP channels contribute to Aβ regulation [132]. Thus, the TRP channels can be a good candidate for sonogenetic FUS/MBs-based treatment of AD.

This technique may also be used as a tool to insert genes into neurons. Three genetic methods are used to insert the ultrasound-sensitive protein genes into the target cell genome, which is transfection, transmission through a suitable viral vector, and the applying transgenic animals. Although the BBB normally prevents the entry of viral vectors carrying manipulated genes to the brain cells, but FUS/MBs-induced approaches can facilitate this process. Gene therapy can make available the long-term accessibility of therapeutic agents in the brain through a single injection. The high transduction efficiency of a non-invasive gene-delivery system to the brain of an amyloidosis model across the BBB was revealed by the FUS combined with intravenous MBs and the recombinant adeno-associated virus (rAAV)-based capsid named rev-PHP.B [133]. In another same study, it was shown that the MRgFUS in combination with MBs could be increased the penetration and delivery of intravenously injected rAAV serotype 1/2 (rAAV1/2) to the hippocampus and cortex of the TgCRND8 mouse AD model hereby enhancing transgene expression in astrocytes surrounding amyloid plaques [134]. Regarding, the rAAV-based vector integrated with specific genes can use in FUS/MBs sonogenetics for the management of AD cells that are genetically regulated with ultrasound-sensitive ion channels (Fig. 6). In a study, the expression of mPrestin, a genetically modified ultrasensitive protein, in the dopaminergic neurons of the substantia nigra in PD mouse by using the 0.5 MHz localized and repeated FUS. The expression of mPrestin in dopaminergic neurons was continued for days after a single administration

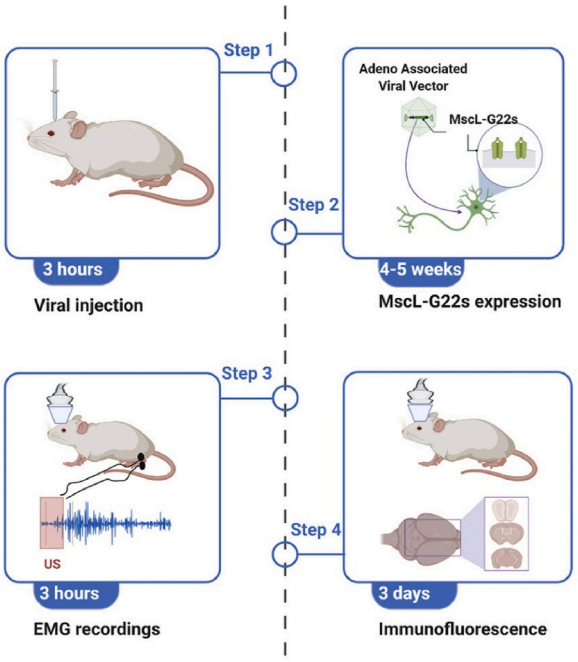

Fig. 6. The sonogenetics neuromodulation based on integrated plasmid into an viral vector and FUS-sensitive ion channels [138].

of the adeno-associated virus. FUS stimulation improved the dopaminergic neurodegeneration and reduced the PD symptoms of the mice [135]. This study suggests that this sonogenetics approach has a therapeutic perspective in other neurodegenerative diseases especially AD due to the changes in the dopaminergic system reported in AD patients with cognitive symptoms [136,137].

The FUS-based sonogenetics have the potential to control cellular signaling and/or the expression of specific genes. Xhima et al. reported that MRIgFUS/MBs effectively delivered TrkA agonist D3 to the basal forebrain, which led to the activation of TrkA-dependent signaling pathways in cholinergic neurons (BFCNs), reducing p75NTR activation, and enhanced cholinergic function, choline acetyltransferase (ChAT) activity and acetycholine (Ach) release in TgCRND8 mice [110].

The BRICHOS domain is a precursor protein related to dementia (Bri2), amyloid lung disease (proSP-C), and cancer. Studies have shown that the recombinant human Bri2 and proSP-C BRICHOS domains reduce Aβ neurotoxicity in animal models of AD through delay in Aβ formation. FUS combined with intravenous MBs were employed to enhance the brain delivery of Rh proSP-C and Bri2 BRICHOS a targeted opening of the BBB. MRI confirmed the BBB opening in the hippocampal region and enhanced delivery of proSP-C and Bri2 domains to the brain parenchyma without any signs of tissue damage [139]. One of the most promising effective targets for AD treatment is the brain-derived nerve growth factor (BDNF), but the crossing of this factor through the BBB is difficult due to its high molecular weight. Wang et al., studied the therapeutic effect of BDNF retrovirus (MpLXSN-BDNF)-loaded MBs in combination with FUS in the animal AD model. In order to open the BBB, low-frequency MRgFUS was used at the same time in the left hippocampus of animals. At the beginning and one month after the application, the effects of overexpression of BDNF on AD rats were investigated. The result exhibited an increase in signal intensity at the BBB disruption zone *via* MR images. The crossing times of the original platform through BBB were considerably also enhanced after FUS-induced treatment. The reduction of contents of ACh and the number of ChAT-positive neurons in the brain confirmed the BDNF delivery to the target site. It can be concluded that FUS combined with viral BDNF-loaded MBs can promote the overexpression of exogenous gene BDNF, and play a therapeutic role in the AD animal model [140].

In a study, the BBB treatments *via* FUS with systematically injected MBs could cause acute inflammatory response through transient upregulation of pro-inflammatory genes. MRgFUS was used to identify the leukocytes and their contribution to reducing Aβ pathology in the TgCRND8 AD model. Intravascular leukocyte activity, trans-endothelial migration, and aggregation of cells as acute inflammation indicators were revealed in this study. The results showed that in the hemisphere exposed to FUS, there were much higher levels of neutrophils than in the control and untreated hemispheres. No considerable neutrophil recruitment and neutrophil phagocytosis of Aβ plaques was seen in TgCRND8 mice in comparison with untreated controls. The results of this study show to some extent the inflammatory cellular aspect of FUS/MBs-based AD treatment [141]. It seems the acoustic cavitation arising from pulsed FUS (pFUS) combined with MBs can initiate an inflammatory response in animals. Accordingly, the probable long-term effects of SIR in the brain after single and six-week sonication were examined by MRI. The animal received bromodeoxyuridine (BrdU) to label the brain cells, before sonication. Ultrasound was used in 9 and 7 focal areas in the right hippocampus and the left cortex, respectively. The result showed pathological changes including cortical atrophy, multiple hypointense areas, astrogliosis, and a persistent BBB in the half of animals. The presence of metallophagocytic in the parenchyma, the high numbers of systemic infiltrating $CD68^{+}$ macrophages along with $BrdU^{+}$ cells, activated astrocytes, increased areas of microglia, and the hyper-phosphorylation of Tau protein were confirmed in the pFUS receiving animals [142].

This technique can be useful in the study and even treatment of neurological diseases such as PD or AD. It is hoped that soon we will see the development of sonogenetics tools to better identify the physiological mechanisms responsible for various behaviors, as well as the development of this method in the treatment of neurological diseases.

7. Safety of FUS-induced MBs-based AD drug delivery

Real-time safety monitoring is another challenge in the clinical usage of FUS-induced MBs drug delivery. The ultrasound levels that exceed MBs thresholds cause inertial cavitation, vascular rupture, permanent tissue damage, and potentially lethal intracranial hemorrhage [69,143]. Headache, numbness and tingling, imbalance, speech, swallowing or memory problems are some possible side effects of FUS/MBs [144]. One of the most common adverse effects of FUS/MBs is the formation of microhemorrhages in the brain, which are raised from the increasing FUS intensity and MB dose, and increased degree of BBB opening [85,145]. It is demonstrated that FUS/MBs-induced brain microhemorrhage often resolves and there are no effects on long-term cognition and neurological function, confirming the repeated FUS treatments without permanently damaging brain tissue [146]. Clinical trials indicate that the repetitive BBB opening by FUS/MBs may be an option for neuromodulation in near-future therapies [147].

A diverse set of FUS parameters influence on BBB disruption efficacy and safety outcomes: MBs, transducer frequency, peak-negative pressure, pulse characteristics, and the dosing of ultrasound applications [148,149]. By employing repeated low frequencies (1 Hz), exposure burst lengths (0.01–10 ms), and pressure amplitudes less than 1 MPa at 20–30 s duration time in FUS/MBs, the chances of permanent tissue damage are minimized [150]. FUS frequencies ranging from 28 kHz to 8 MHz have been used for application in animal models, while a maximum of 1.5 MHz has been suggested for the successful opening of BBB with minimal tissue damage [74]. In clinical trials, one of four FDA-approved clinical-grade ultrasound systems including Exablate model 4000 Type 2, NaviFUS®, NeuroAccess, and Sonocloud9 with customizable parameters are recommended [85]. A variety of experimental models (rodents, rabbits, sheep, pigs, non-human primates (NHPs)) and humans have been employed to monitor safety outcomes following ultrasound-mediated BBB disruption [149].

The rapid restoration of the BBB after treatment is important which is related to the safety of using the delivery method. The time of restoration depends on the size and volume of the therapeutic formulation being delivered. In a study, the effect of opening volume on BBB recovery time after treatment was evaluated. For this purpose, rats received bilateral FUS treatments one hemisphere was exposed to a single sonication and the contralateral received 4 overlapping foci. The contrast-enhanced T1-weighted MRI at 0, 6, and 24 h after

treatment, confirmed the large cross-sectional region of the BBB opening *via* multi-point sonication compared to the single-point case. Six hours after treatment, the opened volumes in 9 of 10 hemispheres were closed and another location was reduced and closed in 24 h. Small morphologic changes were seen by histologic analysis. No signs of hemorrhage and edema were detected at 6 and 24 h by T2-weighted images. The results showed that the opening volume of the BBB was not directly related to the closing time. In addition, the safety of treatment was confirmed *via* MRI [151]. The restoration and safety of FUS combined with intravenously MBs process in BBB opening was also evaluated in Yorkshire pigs as a large animal model of AD. To monitor for tissue damage and BBB opening determination, an MRI was used. The animals underwent neurological tests during treatment (1–4 weeks) and were sacrificed at the end of the study for histopathological studies. No adverse event was seen by neurological testing through treatments. MRI showed restored BBB integrity one week after each session [152].

Neprilysin (NEP) can effectively degrade Aβ, but conventional methods to increase the concentration of this drug, such as the transmission of viral vector, represented some limitations including low gene transfer efficiency, immune responses, and secondary toxicity. Accordingly, a tractable and physical NEP gene-delivery system *via* FUS/MBs was designed for AD treatment. The introduction of human NEP as plasmid into the skeletal muscle of AD mice revealed outstanding reductions of Aβ in the brain after 30 days with improved performance. This approach displayed the safe and effective properties for ameliorating AD-like symptoms in APP/PS1 mice [153].

Lynch et al., appraised the effect of vasculotide (VT) to accelerate the recovery of the disrupted BBB after FUS applying in the TgCRND8 mice AD model. Animals received 250 ng, intraperitoneal VT every 48 h for 90 days. MRI confirmed BBB permeability following FUS with intravenously injected MBs. As predicted, faster restoration of the BBB was seen through VT treatment following FUS in a TgCRND8 animal. This study demonstrated that FUS may induce BBB permeability by affecting the ultra-harmonic pressure of MBs and accelerated BBB restoration in an animal model by VT, which indicates its potential clinical utility to stimulate plasticity, repair, and vascular health in AD [154]. Recently, the safety and efficacy of FUS-targeted MBs destruction in the delivery of MBs-loaded Aβ antibody and neural stem cells (NSCs) in the transgenic mice model of AD was investigated. The treated animals were exposed to diagnostic FUS for 5 min once a week for 4 times and then Aβ plaque deposition, cognitive and memory functions, as well as the expression of synaptophysin (SYN) and BDNF were evaluated. The combined delivery of NSCs and MBs-Aβ antibody by FUS-targeted MBs destruction improved spatial learning and memory function, the clearance of Aβ plaques, and BDNF expression in the treated group in comparison with the control group [155].

Since trials are commonly directed the regulatory approval to the advantages of therapeutics, safety receives limited attention. Despite this, safety has a crucial role in the carrying of therapeutic approaches from *in vitro* to clinic. In addition, most studies of FUS/MBs-induced drug delivery have been performed on rodents, which are differentiated from humans in their many physiological behaviors. Therefore, it is important to consider this treatment in large animals that have more in common with humans to reach a proper idea of the safety of the FUS/MBs process. Although the safety and the noninvasive FUS/MBs-mediated BBB opening in the hippocampus and cortex was indicated in sheep as AD model [156], the safety of FUS/MBs-induced AD drug delivery was recently claimed in the clinical trial studies.

Rezai et al., in an initial clinical trial study, evaluated the feasibility, reversibility, and safety of FUS-based disruption of BBB in the treatment of the entorhinal cortex and hippocampus in 6 patients with early AD. No neurological worsening or side effects and cognitive were observed during treatment. After FUS, a sizable and immediate enhancement in hippocampal parenchymal was confirmed by MRI indicating BBB opening as well as BBB closure within 24 h. The result of the present study exhibited noninvasively, safely, transiently, focally, and reproducibly of FUS/MBs technique in the permeability of BBB in the hippocampus/EC in humans [157]. The primary outcomes of proof-of-concept, prospective, single-arm, feasibility, safety, and non-randomized phase I clinical trial demonstrated that MRgFUS in combination with intravenous MBs administration procedure was reversible and feasible without any serious clinical or radiological side effects. In addition, in 8/10 treatments in five patients opening in the parieto-occipital-temporal was confirmed and in all cases, no side and uneventful effects were seen related to the BBB opening while a cognitive improvement was detected [158]. In an open-label, prospective clinical study, FUS-mediated BBB opening was performed on five patients in Korea, by targeting the bilateral frontal lobe regions twice at three-month intervals. The results confirmed BBB opening at 95 % of the targeted volume in the frontal lobe and a significant decrease in the standardized uptake value ratio 3 months later without adverse effects [159]. In a pilot clinical trial efficacy and safety of implantable FUS device in patients with mild AD was investigated. To target the left supramarginal gyrus, a 1 MHz ultrasound device was extradural implanted in the skull of 10 mild AD patients. Temporary disruption of BBB was carried out using 7 FUS sessions in combination with intravenous administration of MBs for 3.5 months. To monitor cognitive evaluations, amyloid levels, and brain metabolism, the positron emission tomography (PET)/MRI scan was applied 4 and 8 months after sonication. No significant changes in these factors in this study revealed the safety of FUS/MBs-induced therapy for AD patients. However, due to the duration and small sample size of the trial, a larger clinical trial is needed [160].

However, the intravenous injection of MBs has been shown to be safe compared to the use of conventional techniques such as MRI and radiography, but MBs destruction in the circulation of MBs in the bloodstream is also an important challenge. In FUS/MBs, several safety considerations for example the accurate targeting, the risk of tissue heating, and the ability to monitor *in vivo* MBs cavitation may be addressed.

Although stable cavitation of MBs at low-pressure FUS increases vascular permeability and drug penetration, excessive acoustic pressure causes rapid collapse of MB (inertial cavitation) leading to strong mechanical stresses, MB micro-jetting, and thermal effects in the vascular system [70,161]. These phenomena play a key role in the initiation of FUS-induced adverse effects during BBB opening [162].

The parameters of the ultrasound wave are another factors that influence the biological effects of MBs, so a detailed understanding of these can help the safe application of FUS/MBs in humans [70]. It is demonstrated that increasing acoustic pressure below a certain

threshold, modulates the BBB opening, vascular leakage, and adverse effects on the blood vessels. It is now accepted that FUS at pressures at 0.2–1.0 MPa (220 kHz) can safely be applied in small cohort clinical studies [94]. Moreover, burst length, burst repetition frequency and sonication duration affect biological responses to FUS/MBs. The burst length between 0.1 and 10 ms, increasing burst repetition frequency from 0.1 Hz to 1 Hz, and decreasing 10-fold the sonication time at this frequency is able to BBB permeability enhancement [70].

The large temperature changes, generated by FUS, are undesirable due to the risk of causing damage to normal neural tissue. Therefore, the pulsed lower pressure intensity FUS that does not affect MB modulation is recommended. Short-time FUS hyperthermia can remarkably enhance the delivery of drugs to the mouse brain, without affecting uptake in normal, healthy brain tissues [163,164].

8. Experimental to clinical applications of FUS/MBs-assisted AD treatment

To date, the effect of FUS/MBs on the delivery of Aβ and tau therapeutics, behavioral impairments, inflammatory responses, and neuronal health have been investigated. In addition, *In vitro*, *in vivo* FUS/MBs-mediated BBB opening on animal models are well underway and has been used to deliver drugs, antibodies, NPs, and gene therapies for the treatment of AD in preclinical studies [74,124]. In this line, an in-depth understanding of the effect of FUS/MBs physical parameters on the biological performance of the human brain can promise the clinical application of FUS/MBs [70,147]. It is necessary to consider the interactions of MBs and the vasculature in the FUS field, ultrasound parameters (acoustic pressure, sonication duration, frequency, burst repetition frequency, burst length), chemical formulation, size distribution, and half-life of MBs [165]. Modification and standardization of protocols and parameters in future preclinical and clinical studies are required to inform robust clinical translation [74,165].

As FUS technology continues to advance, some challenges in the translatability of preclinical studies to clinical are necessary to be addressed. Treatment manner standardization, the novel therapeutic agent's efficacy, and the development of tools and models reflective of clinical conditions are some instances [85]. In this line, the human induced pluripotent stem cell (hiPSC) has been suggested that can be a novel *in vitro* model for the assessment of FUS/MBs-assisted anti-AD drug delivery, which provides a mimic model for clinical cases responses to FUS/MBs in the future [74].

9. Conclusion and future perspective

The high prevalence of AD and the related progressive challenges are critically evident that new medicine and treatment manner are required. The slow progress in AD therapy arose from BBB. FUS-induced approaches in combination with MBs are an innovative approach due to their advanced ability to penetrate the BBB.

The FUS-induced MBs-based drug delivery is a talented approach to reaching focal delivery of therapeutics including antibodies, NPs, and chemical drugs into the brain in a reversible process [16]. Although it is in the early phase, it has the potential to introduce precision medicine in treating AD patients. Although the above-mentioned FUS-induced MBs drug delivery strategies have shown significant efficacy, the brain delivery performance in some AD-induced animal models has not been quantitatively determined and needs to be further studied. Numerous studies have shown no serious side effects and no chemical or genetic changes in FUS-induced MBs for AD drug delivery. These findings have raised great hopes for the effective treatment of AD. However, despite the great motivation for applying this strategy, the underlying cellular and molecular mechanisms of ultrasonic neuronal modulation remain largely unknown [166,167]. Due to the presence of physical effects such as heating, cavities, and mechanical forces in ultrasound, the study of the mechanism is very important. In MBs-assisted FUS, the FUS frequency influences the penetrability. While, higher frequency FUS provides a limited penetrability, the lower frequency FUS offers excellent penetrability. In addition, low-intensity FUS can induce heating, mechanical forces, and cavitation. Variations in acoustic properties or the expression profiles of endogenous FUS-sensing proteins in different tissues can be affected by the bio-effects of FUS. The overexpression of heterogeneous FUS-sensing proteins is an approach to address this challenge due to the desired neuromodulation *via* FUS stimulation [124].

It is also hypothesized that the FUS/MBs combination can promote hippocampal neurogenesis, which is involved in memory and learning and is effective in neurological illnesses such as AD. In a study in adult mice, FUS/MBs mediated treatment remarkably could increase the proliferated cells number and newborn neurons in the hippocampal dentate gyrus [168]. In a cholinergic degeneration animal model of dementia, the improvement of spatial memory and hippocampal neurogenesis *via* FUS/MBs mechanisms was seen [169]. Despite this, it is not yet clear whether FUS can modulate this process in cholinergic deficiency conditions. FUS/MBs can induce BBB disruption in a wide range of brain regions with varying vasculature and structural properties, such as the hippocampus, striatum, and brainstem [170]. The hippocampus is strongly implicated in the pathology of dementia, offering an attractive drug target region in responses to the FUS/MBs [70]. A significantly increased AChE activity was observed in the hippocampus 18 days after FUS, which implies that the FUS-mediated BBB opening resulted in the recovery of AChE levels [169]. It has demonstrated transcriptional changes in hippocampal microvessels following FUS that are indicative of the initiation of angiogenic processes [171].

The FUS/MBs process involves a microbubble being loaded with a drug and conjugated with targeting moieties such as aptamers, peptides, and antibodies can represent a promising platform for the effective capture of AD biomarkers and specific delivery of therapeutic agents. Using this approach, the drugs can internalize to target cells *via* receptor-mediated delivery [172]. Regarding specific cell targeting for drug delivery, the functionalities can be used as surface functionalization of a biomaterial and target specific cell receptors. Once they bind to the cell receptor, for example receptor mediated endocytosis could occur, and the encapsulated drugs can be released in the lysosome. These functionalized biomaterials could be beneficial for delivering drugs, stimulating or inhibiting the cells, or for imaging and diagnostic purposes [173]. Aptamers could be beneficial in the intra-cellular delivery of therapeutics. They have high affinity and specificity in target recognition which remarkably enhances cellular uptake of aptamers and makes them very

suitable for targeted drug delivery [174].

It should be noticed the modification of the MBs shell with the various component can critically influence their internalization into the target cell [175]. The surface functionalization can improve BBB penetration, target ability, and bioavailability of MBs. However, many concerns still need to be addressed, for instance, the accurate indication of the transition time, degree, location, and diversity of MBs crossing the BBB. Another factor that may influence the functionalized MBs emanates from their interaction with the plasma proteins resulting in the protein corona formation [176,177]. The interaction between nanoparticles with serum biomolecules such as proteins or lipids in biological fluids may form a layer of biomolecules around NPs, which is called the "protein corona" [178]. This phenomenon leads to changes in the physicochemical properties of NPs including size, shape, composition, and surface functionalization of the NPs, and subsequently affects their biomedical functionalities [178,179].

Most FUS-based drug delivery strategies must focus to targeting the pathophysiological factors of AD such as Aβ and tau. However, most studies that have confirmed acceptable results in preclinical studies may have failed in clinical trials, progress in FUS/MBs-based therapy in preclinical models of AD has revealed great potential to go beyond from *in vitro* to the clinic. In a study, the application of the MRgFUS was reported to five patients with AD. However, this study was performed on a small sample size and the efficiency to treat AD was not studied [94]. Further clinical data must be collected to confirm the application of FUS/MBs in humans. Therefore, future research using FUS/MBs-based delivery to the brain should attention to improving safety, precise targeting, and pharmacokinetic properties. In conclusion, it can be determined that FUS/MBs-based sonogenetics approaches have a notable therapeutic potential for AD. The application of nanoformulations will undoubtedly provide talented choices for the diagnosis and treatment of AD in the future. Among various drugs for AD, Aducanumab as an FDA-approved anti-Aβ monoclonal antibody for AD treatment can be an option for FUS/MBs that can effectively cure AD.

Availability of data and materials

Not applicable.

CRediT authorship contribution statement

Nadiyeh Rouhi: Writing – review & editing, Writing – original draft. **Zahra Chakeri:** Writing – original draft, Software. **Behnam Ghorbani Nejad:** Writing – original draft. **Milad Rahimzadegan:** Writing – review & editing. **Mohammad Rafi Khezri:** Writing – review & editing. **Hossein Kamali:** Writing – review & editing, Supervision. **Rahim Nosrati:** Writing – review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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