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REVIEW ARTICLE

Biological effects of blood–brain barrier disruption using a focused ultrasound

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Abstract With focused ultrasound (FUS) and microbubbles, BBB can be transiently disrupted with a localized and non-invasive approach. BBB disruption induced by FUS has made progressions to move forward on delivery of therapeutic agents into a brain in a specific area of brain for better treatment of neurological diseases. In addition to be used as an improvement of drug delivery, BBB disruption has been found to induce biological effects such as a clearance of protein aggregation which cause Alzheimer's disease, regulation of proteins which facilitate drug uptake, and modulation of neuronal function and neurogenesis. In this review, we discuss overview about the principles of BBB opening with FUS and milestones in these biological effects of FUS-induced BBB disruption.

Keywords Focused ultrasound · Blood–brain barrier · Microbubble · Biological effects

1 Introduction

Blood brain barrier (BBB) is a highly selective membrane barrier which separates circulating blood from extracellular fluid in central nervous system. BBB protects the brain from the most of pathogens and toxic materials and maintains the homeostasis. However, endothelial cells in the brain capillaries form tight junctions which interfere therapeutic agents to pass through. Statistically, 98% of small molecule size as less than 400 Da and approximately 100% of large molecule size as bigger than 500 Da drugs cannot pass through the tight junctions [1] which hinders the treatment of brain diseases using neurotherapeutic drugs due to the nature of BBB. Furthermore, in most of brain diseases such as brain cancer and Alzheimer's disease (AD), pathological progression is correlated with the level of BBB degeneration [2–4]. Therefore, it is important to understand the nature of BBB to treat brain disorders.

Various technologies have been attempted to open the BBB (Table 1) such as hyperosmolar therapy with mannitol drug [5], electroporation [6, 7] and ultrashort-pulsed laser [8]. However, when these conventional technologies are applied, there are limitations to be improved. For example, Mannitol opens entire BBB which expose the whole brain to pathogens and toxic materials [9]. Electroporation method is invasive since it requires skull penetration and ultrashort-pulsed laser cannot reach in deep area of the brain. So, these technologies have limitations to go through clinical trials since they may cause side effects or permanent brain damages [7, 8]. Recently, ultrasound energy has been used to open BBB safely by focusing ultrasound energy to targeted regions within brain. [10]. First, microbubbles are injected into blood vessel, then, ultrasound energy interacts with injected microbubbles. Then, the interaction between microbubbles and ultrasound energy stimulates endothelial cells physically and opens up BBB. With this approach, focused ultrasound (FUS) has strong advantages such as non-invasive, localized and safe compared to the conventional technologies [11].

Several studies show progressions on delivery of therapeutic agents into the brain after FUS induced BBB disruption [12–18] (Table 2): the delivery of anticancer drugs [12–16] or nanoparticles [18], reduction of amyloid-βs (Aβs) [17] and so on. Furthermore, these progresses have

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Table 1 Current techniques for BBB disruption

BBB open techniques	Localization	Non-invasiveness	Stability	References
Drug (Mannitol)	Bad	Good	Good	[5]
Electroporation	Good	Bad	Bad	[6, 7]
Ultrashort-pulsed LASER	Good	Good	Bad	[8]
Focused ultrasound	Excellent	Excellent	Excellent	[10]

Table 2 Therapeutic agent delivery after FUS-induced BBB disruption

Drug	Molar mass/size	Findings	References
Doxorubicin	579.98 Da	Estimate the amount of doxorubicin delivered	[12]
Herceptin	148 kDa	Estimate the amount of herceptin delivered	[13]
Methotrexate	545.44 Da	Compare to FUS delivery and ICA injection	[14]
BCNU	214 Da	BCNU delivery, long term survival	[15]
Epirubicin	543.5 Da	Epirubicin delivery, tumor progression slowed	[16]
Aβ antibody	150 kDa	BAM-10 delivery, reduction in Aβ plaque	[17]
MION-47	20 nm	Delivery of nanoparticles (MION-47)	[18]

made researchers to demonstrate safety by experimenting on non-human primates [19]. Studies on the treatment of two representative neurological diseases like brain cancer and AD through FUS-induced BBB disruption proved its potential to be used in clinical setting. First, there have been attempts to study a delivery using anticancer drugs such as doxorubicin [20] and temozolomide [21] to treat brain cancer. In these preclinical studies, effectiveness was investigated in vivo and currently it has reached to the stage of clinical trials (NIH Clinical Trial Identifier: NCT0234391, "Blood–Brain Barrier Disruption Using Transcranial MRI-Guided Ultrasound"). Next, there is a data demonstrating that Aβ is reduced after FUS-induced BBB disruption in AD preclinical trials. Although only few studies on AD have been conducted, promising results are shown. Moreover, there are no reports of significant side effects caused with FUS induced BBB disruption on both representative neurological diseases which can potentially open new era of treatment.

The purpose of this review is to arrange results of possible biological effects of BBB disruption induced by FUS because the mechanical stimulation caused by interactions between ultrasound and microbubbles can affect the function of brain endothelial cells where the changes may induce biological effects on mechanism of BBB opening in the brain. We also suggest to conduct further studies and analysis on neurological diseases treatments.

2 Focused ultrasound-induced blood–brain barrier disruption

The first report that FUS could increase permeability of the BBB was in the 1950's. This study showed that ultrasound increased BBB permeability and its mechanism was based on thermal effects generated by FUS [22, 23]. However, BBB disruption using thermal effects caused tissue damage in most cases. So, to reduce magnitude of acoustic pressure required for effective BBB disruption without brain tissue damage, researchers injected ultrasound contrast agents (i.e microbubbles) intravenously [20, 21]. With a short pulse duration, gas bubble oscillation corresponds to the rapid changes in pressure within the brain vasculature which lead to the enhancement of permeability of BBB and minimize side effects with reduced FUS pressure. In addition to that, BBB disruption with combination of FUS and microbubbles has advantages of being consistent, reproducible and transient.

2.1 FUS conditions for BBB disruption

Over the past decade, various parameters of FUS and microbubble were evaluated to open the BBB. To decide appropriate FUS frequency, considerations on ultrasound wave to penetrate skull and to be focused at small spot in brain had been made. Along with the idea, the FUS frequency mainly used in preclinical test was at range of 0.2–1.5 MHz FUS frequency [24]. The acoustic pressure was also one of the important factors to safely disrupt the BBB so to deliver FUS energy, acoustic pressure was used in the range of 0.3–0.8 MPa peak rarefactional pressure with 10 ms burst for 1–2 min. The degree of BBB disruption were also dependent on concentration and size of microbubbles [25, 26]. Microbubble was injected through a tail vein catheter since higher concentrations of microbubbles may cause brain tissue damage [26].

2.2 Safety of BBB disruption

Severe damages at undesired region during FUS treatment can cause permanent brain damage or death. Minimizing

variables and improving safety during BBB disruption induced by FUS are in demands to be developed for clinical translation.

Standing waves in the brain increase in situ pressures unexpectedly, especially near interfaces of the bone where high energy absorption are occurred already. The standing waves can lead to unpredictable disturbance in the brain. Irregular shapes and differences in the thickness of the skull bone influences a reflection angle of the sound, where unwanted high pressures aside from targeted area can be present. With short burst (<3 μs in length) technique, safety concerned with standing waves can be improved. In addition to that, standing waves can be regulated with the large aperture arrays consists of 1024 elements which independently perform arrays to transmit beam at desired depth and location.

There were several studies to improve safety of FUS-induced BBB disruption with microbubbles. In these studies, optimization not only on ultrasound parameters but also on concentration and amount of microbubbles were investigated in rat [10, 27]. Although these studies optimized ultrasound parameters, the other parameters like differences of skull thickness according to age of animals and state of cerebral vessels in brain diseases could affect BBB disruption. Several groups had developed methods to detect the acoustic cavitation of microbubbles for real-time monitoring of FUS treatment [28, 29]. Since inertial cavitation and high pressure can cause hemorrhages, monitoring acoustic emissions during BBB disruption by FUS is important key to regulate safety. In order to secure BBB disruption safely, there are two experiments on a development of acoustic cavitation algorithms. First, when threshold where setting in subharmonics is exceed, 50% of ultrasound power is reduced and increased the power gradually until the subharmonic appears again [29]. Another algorithm is a detection of the substances on ultraharmonics. Ultrasound energy is regulated in between the set-parameter of the ultraharnomic threshold for the safety. In addition to that, when broad band noise is presented which cause brain vasculature damage, power of ultrasound is set to be shut down to minimize an emergency situation [28]. These developments provide a guide to adjust FUS acoustic pressure to avoid any side effects in the brain. With this real-time acoustic feedback technology, a subset of the macaques underwent behavioral testing, in which they completed tasks on a touch screen, to evaluate their visual acuity and higher-order cognitive abilities after BBB disruption. Results revealed that repeated BBB opening in the visual cortex caused no impairments in complex visual acuity tasks. These findings on application of BBB disruption induced by FUS from small animal like mice to large animal like non-human primates demonstrated that prolonged and repeated opening of the BBB were safe [19].

2.3 Benefits of MRI guidance

The use of magnetic resonance imaging (MRI) was beneficial for guidance of targeting ultrasound wave into brain and evaluation on BBB permeability and brain damage. There was a report that quantifies BBB permeability using dynamic contrast enhanced-MRI (DCE-MRI) [12]. In the study, the BBB was opened with different FUS parameters and the degree of BBB disruption was quantified. So, advanced MRI imaging techniques along with FUS could be useful tool to evaluate BBB permeability and biological effects.

3 Biological effects

Many studies on biological effects, especially on therapeutic agent delivery with FUS-induced BBB disruption, have been actively carried out recently. In this review, we describe biological effects mainly on protein regulation, neuromodulation and neurogenesis of BBB disruption by FUS.

3.1 Protein regulation

3.1.1 Tight junction (TJ) proteins

Reduction of TJ proteins such as occludin, claudin-5, claudin1 and zonula occluden-1 after FUS-induced BBB disruption had been detected using immunoelectron microscopy [30]. Especially, significant reductions in occludin, claudin-5 and zonula occludens were observed at 1 and 2 h post-FUS treatment compared to control. 69% of occludin proteins were decreased at 1 h post-FUS and at 2 h, 62% was reduced compare to control ($p < 0.01$). Although claudin-1 showed level of reduction but it was not statistically significant. In claudin-5, 75% of reduction at 1 h and 62% of reduction at 2 h-post FUS were observed ($p < 0.01$). Lastly in zonula occluden-1, 66% of protein level decreased at 1 h after FUS given, and 62% reduction at 2 h ($p < 0.01$). The levels of these proteins were recovered to pre-sonication levels within 4 h confirming that the effects of FUS on the BBB disruption were transient. Disintegration of TJ proteins lasts up to approximately 4 h after sonication indicating a promising time window for the delivery of targeted drug or antibodies to brain.

Both ultrasound interaction with microbubbles in the microvasculature and its relation to biology of the BBB are

complex processes. The interaction can induce microbubble dynamics such as acoustic radiation force, bubble oscillation, acoustic streaming or inertia cavitation. Ultrasound driven microbubbles can generate changes in concentration of Ca^{2+} [31]. Similar range of elevation of calcium concentration is observed with bradykinin, histamine, mannitol and ATP known to increase the permeability of the BBB. Although correlation between calcium and tight junction protein changes in brain endothelial cells remains elusive, this can be an important aspect in FUS-induced BBB disruption in the molecular mechanisms of BBB disruption. Further studies to reveal the mechanism in relation to tight junction proteins are needed.

3.1.2 P-glycoprotein (P-gp)

Cho et al. [10] reported that P-gp was down-regulated by FUS-induced BBB disruption in rat brain [10]. To confirm BBB disruption, MR T1-weighted image with contrast agent and Evans blue dye intensity were used and P-gp expression was analyzed with immunofluorescence. Intensity of P-gp in BBB disrupted region of brain was decreased by $63.2 \pm 18.4\%$ compared to non-sonicated brain region. Overall results demonstrated the inhibition of P-gp induced by FUS and microbubbles. Furthermore, they could draw a strong correlation between the down-regulated P-gp expression and the level of the BBB disruption magnitude (MR and Evans Blue intensities, $R = -0.687$, $p < 0.001$, $n = 31$; $R = -0.731$, $p < 0.001$, $n = 31$, respectively).

P-gp substrates are used as anticancer drugs which are doxorubicine, daunorubicine, vinblastine, vincristine, etoposide and teniposide. Inhibition of P-gp by FUS and microbubbles may retain these drugs temporarily in brain tissue after across BBB. By P-gp modulation through FUS with microbubbles, drug retention is increased. So, dose and frequency necessary to treat brain disease could be possibly reduced as well as toxicity and side effects on surrounding tissues. Moreover, this can be applied to other multi-drug efflux transporters such as breast cancer resistance protein (BCRP) and members of the multi-drug resistance protein (MRP) family. With the potential provided, BBB disruption induced by FUS with anti-cancer drug may efficiently induce therapeutic outcomes.

3.1.3 Amyloid beta (Aβ) plaque

A few studies showed that FUS-induced BBB disruption could be used for the reduction of Aβ plaques in AD animal models [32, 33]. First study was conducted on TgCRND8 mice. Plaque size and total surface area in cortex targeted with FUS region were reduced by 20% and 13%, respectively with a single treatment compared to untreated region ($n = 9$) [32]. Also, the number of Aβ plaques was reduced to 9% ($n = 9$). With APP23 transgenic mice model of AD, researchers opened entire BBB giving weekly treatment for 6 to 9 weeks to remove Aβ [33]. Plaque burden was reduced in ultrasound treated AD mice and clearance of plaque was observed in 75% of them. In these two previous studies, FUS improved bioavailability of endogenous antibodies and temporal activation of glial cells, providing plaque reduction mediated with BBB disruption induced by FUS. Application to treat AD and neurological disorders can get benefit from this.

Currently, treatment on AD is based on antibodies, however, many clinical trials have failed. First, with administration of any exogenous antibodies cause inflammation and cerebrovascular side effects. Next, not only side effects, antibodies used are not successful enough since low amount are deliver across BBB. Solely, BBB disruption with FUS itself can be used as AD treatment since it reduces amyloid beta plaque. Moreover, FUS can be used as companion method with current antibodies that cannot pass through BBB to enhance AD treatment.

3.2 Neuromodulation

There was a study on BBB disruption induced by FUS accompanied by neuromodulation [34]. This study showed that functional effects of FUS-induced BBB disruption with microbubble by measuring changes in somatosensory evoked potentials (SSEP) and blood-oxygen-level dependent responses (BOLD). To examine neuronal activity under various parameters, FUS was sonicated at 0.2, 0.35 or 0.5 MPa and neuromodulation was identified by changes in SSEP and BOLD. FUS reduced the SSEP amplitude for the 0.35 and 0.5 MPa groups, but not in control or 0.2 MPa groups, suggesting that the reduction in SSEPs only occurred when BBB was opened. Similarly, BOLD responses were reduced in BBB disruption group (0.35 and 0.5 MPa group) while the BOLD did not respond at all in control or 0.2 MPa groups. Furthermore, 0.5 MPa group induced a higher magnitude of reduction in BOLD responses than in 0.35 MPa group.

There were many studies that FUS without BBB opening were enough to induce neuromodulation, depending on the FUS parameters of the energy into neurons [35, 36]. However, to the best of our knowledge, only one study showed neuromodulation accompanying FUS-induced BBB disruption.

Still, the mechanism whereby FUS-induced BBB disruption affects neuronal activity remains unclear, but this technology can be applied and used for brain mapping or treatment of neurologic disorders such as chronic pain, obesity, Parkinson disease, epilepsy, obsessive compulsive disorder, and mental or movement disorders.

3.3 Neurogenesis

FUS-induced BBB disruption could increase cell proliferation in dentate gyrus of the sonicated hemisphere [37]. This study was examined to find out whether specific pressure of FUS and intravenous administration of microbubbles promoted neurogenesis or not. FUS was applied to unilateral hippocampus at 0.39 and 0.78 MPa with microbubbles and 1.56 and 3.0 MPa without microbubbles. Only at 0.78 MPa, significant BBB disruption was observed through MR guided-image where BBB was not disrupted in the other parameters. So, 0.78 MPa was set as the standard pressure and 50% reduction value to standard as 0.39 MPa, twice increased value as 1.56 MPa and four times to standards as 3.00 MPa were used. Additionally, since ultra-harmonic substances were observed at 0.7 MPa, microbubbles were not used at higher power. Immediately prior to sonication start, 0.39 and 0.78 MPa groups were administered with microbubble contrast agent intravenously at a dose of 0.02 ml/kg. Only 0.78 MPa pressure amplitude with microbubbles promoted hippocampal neurogenesis when BBB permeability was increased.

FUS-induced BBB disruption can be potentially used to treat damaged brain. Increasing neurogenesis to recover damaged neurons has been proposed as a treatment for various neurodegenerative disorders.

4 Discussion and conclusion

This paper reviewed representative biological effects by FUS-induced BBB disruption. FUS induced BBB disruption may trigger other biological effects in brain, but discussed results in this research focus mainly on the protein regulation, neuromodulation and neurogenesis. Mechanisms on how FUS stimulates these biological effects are unknown but it seems to be evoked only when BBB is transiently opened. It could be caused by mechanical stimulation on brain endothelial cells or immunological response in the brain parenchyma after BBB disruption.

BBB is a main hurdle to overcome to treat neurological diseases but with FUS, it can be a breakthrough technology to enhance treatments of the suffering patients. Although limited information on BBB disruption with FUS are known and further investigation on the mechanisms underlying BBB opening to induce other biological effects and safety are in demands to be examined, promising outcomes suggest its potentials to be used in clinical translations.

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References

1. Pardridge WM. Blood–brain barrier drug targeting: the future of brain drug development. Mol Interv. 2003;3(90–105):51.
2. Kalaria RN. Vascular basis for brain degeneration: faltering controls and risk factors for dementia. Nutr Rev. 2010;68(Suppl 2):S74–87.
3. Zlokovic BV. Neurovascular pathways to neurodegeneration in Alzheimer's disease and other disorders. Nat Rev Neurosci. 2011;12:723–38.
4. Carvey PM, Hendey B, Monahan AJ. The blood-brain barrier in neurodegenerative disease: a rhetorical perspective. J Neurochem. 2009;111(2):291–314
5. Brown RC, Egleton RD, Davis TP. Mannitol opening of the blood–brain barrier: regional variation in the permeability of sucrose, but not 86Rb+ or albumin. Brain Res. 2004;1014:221–7.
6. Garcia PA, Rossmeisl JH, Robertson JL, Olson JD, Johnson AJ, Ellis TL, et al. 7.0-T magnetic resonance imaging characterization of acute blood–brain-barrier disruption achieved with intracranial irreversible electroporation. PLoS One. 2012;7(11):e50482.
7. Hjouj M, Last D, Guez D, Daniels D, Sharabi S, Lavee J, et al. MRI study on reversible and irreversible electroporation induced blood brain barrier disruption. PLoS One. 2012;7(8):e42817.
8. Madsen SJ, Hirschberg H. Site-specific opening of the blood–brain barrier. J Biophotonics. 2010;3(5–6):356–67.
9. Ikeda M, Bhattacharjee AK, Kondoh T, Nagashima T, Tamaki N. Synergistic effect of cold mannitol and Na(+)/Ca(2+) exchange blocker on blood–brain barrier opening. Biochem Biophys Res Commun. 2002;291:669–74.
10. Cho H, Lee H-Y, Han M, Choi J-R, Ahn S, Lee T, et al. Localized down-regulation of P-glycoprotein by focused ultrasound and microbubbles induced blood–brain barrier disruption in rat brain. Sci Rep. 2016;6:31201.
11. Etame AB, Diaz RJ, Smith CA, Mainprize TG, Hynynen K, Rutka JT. Focused ultrasound disruption of the blood–brain barrier: a new frontier for therapeutic delivery in molecular neurooncology. Neurosurg Focus. 2012;32:E3.
12. Park J, Zhang Y, Vykhodtseva N, Jolesz FA, McDannold NJ. The kinetics of blood brain barrier permeability and targeted doxorubicin delivery into brain induced by focused ultrasound. J Control Release. 2012;162:134–42.
13. Kinoshita M, McDannold N, Jolesz FA, Hynynen K. Noninvasive localized delivery of Herceptin to the mouse brain by MRI-guided focused ultrasound-induced blood–brain barrier disruption. Proc Natl Acad Sci USA. 2006;103:11719–23.
14. Mei J, Cheng Y, Song Y, Yang Y, Wang F, Liu Y, et al. Experimental study on targeted methotrexate delivery to the rabbit brain via magnetic resonance imaging-guided focused ultrasound. J Ultrasound Med. 2009;28:871–80.
15. Liu H-L, Hua M-Y, Chen P-Y, Chu P-C, Pan C-H, Yang H-W, et al. Blood–brain barrier disruption with focused ultrasound enhances delivery of chemotherapeutic drugs for glioblastoma treatment. Radiology. 2010;255:415–25.
16. Liu H-L, Hua M-Y, Yang H-W, Huang C-Y, Chu P-C, Wu J-S, et al. Magnetic resonance monitoring of focused ultrasound/magnetic nanoparticle targeting delivery of therapeutic agents to the brain. Proc Natl Acad Sci USA. 2010;107:15205–10.
17. Jordão JF, Ayala-Grosso CA, Markham K, Huang Y, Chopra R, McLaurin J, et al. Antibodies targeted to the brain with image-guided focused ultrasound reduces amyloid-β plaque load in the TgCRND8 mouse model of Alzheimer's disease. PLoS One. 2010;5(5):e10549.
18. Hynynen K, McDannold N, Vykhodtseva N, Raymond S, Weissleder R, Jolesz FA, et al. Focal disruption of the blood–

brain barrier due to 260-kHz ultrasound bursts: a method for molecular imaging and targeted drug delivery. J Neurosurg. 2006;105:445–54.
19. McDannold N, Arvanitis CD, Vykhodtseva N, Livingstone MS. Temporary disruption of the blood–brain barrier by use of ultrasound and microbubbles: safety and efficacy evaluation in rhesus macaques. Cancer Res. 2012;72:3652–63.
20. Aryal M, Park J, Vykhodtseva N, Zhang Y-Z, McDannold N. Enhancement in blood-tumor barrier permeability and delivery of liposomal doxorubicin using focused ultrasound and microbubbles: evaluation during tumor progression in a rat glioma model. Phys Med Biol. 2015;60:2511–27.
21. Wei KC, Chu PC, Wang HYJ, Huang CY, Chen PY, Tsai HC, et al. Focused ultrasound-induced blood–brain barrier opening to enhance temozolomide delivery for glioblastoma treatment: a preclinical study. PLoS One. 2013;8(3):e58995.
22. Bakay L, Ballantine HT Jr, Hueter TFSD. Ultrasonically produced changes in the blood–brain barrier. AMA Arch Neurol Psychiatry. 1956;76:457–67.
23. Shealy CN, Crafts D. Selective alteration of the blood–brain barrier. J Neurosurg. 1965;23:484–7.
24. McDannold N, Vykhodtseva N, Hynynen K. Blood–brain barrier disruption induced by focused ultrasound and circulating preformed microbubbles appears to be characterized by the mechanical index. Ultrasound Med Biol. 2008;34:834–40.
25. Yang FY, Fu WM, Chen WS, Yeh WL, Lin WL. Quantitative evaluation of the use of microbubbles with transcranial focused ultrasound on blood–brain-barrier disruption. Ultrason Sonochem. 2008;15:636–43.
26. Samiotaki G, Vlachos F, Tung YS, Feshitan J, Borden M, Konofagou EE. Pressure and microbubble size dependence study of focused ultrasound-induced blood–brain barrier opening reversibility in vivo. AIP Conf Proc 2012;1481:300–6.
27. Choi JJ, Small SA, Konofagou EE. Optimization of blood–brain barrier opening in mice using focused ultrasound. IEEE Int Ultrason Symp. 2006;540–3.
28. Arvanitis CD, Livingstone MS, Vykhodtseva N, McDannold N. Controlled ultrasound-induced blood–brain barrier disruption using passive acoustic emissions monitoring. PLoS One. 2012;7(9):e45783.
29. O'Reilly MA, Hynynen K. Blood–brain barrier: real-time feedback-controlled focused ultrasound disruption by using an acoustic emissions-based controller. Radiology. 2012;263:96–106.
30. Vykhodtseva N, McDannold N, Hynynen K. Progress and problems in the application of focused ultrasound for the blood–brain barrier disruption. Ultrasonics. 2008;48:279–96.
31. Park J, Fan Z, Kumon RE, El-Sayed MEH, Deng CX. Modulation of intracellular Ca^{2+} concentration in brain microvascular endothelial cells in vitro by acoustic cavitation. Ultrasound Med Biol. 2010;36:1176–87.
32. Jordão JF, Thévenot E, Markham-Coultes K, Scarcelli T, Weng YQ, Xhima K, et al. Amyloid-β plaque reduction, endogenous antibody delivery and glial activation by brain-targeted, transcranial focused ultrasound. Exp Neurol. 2013;248:16–29.
33. Leinenga G, Gotz J. Scanning ultrasound removes amyloid- and restores memory in an Alzheimer's disease mouse model. Sci Transl Med. 2015;7:278ra33.
34. Chu P-C, Liu H-L, Lai H-Y, Lin C-Y, Tsai H-C, Pei Y-C. Neuromodulation accompanying focused ultrasound-induced blood–brain barrier opening. Sci Rep. 2015;5:15477.
35. Younan Y, Deffieux T, Larrat B, Fink M, Tanter M, Aubry J-F. Influence of the pressure field distribution in transcranial ultrasonic neurostimulation. Med Phys. 2013;40:82902.
36. Tyler WJ, Tufail Y, Finsterwald M, Tauchmann ML, Olson EJ, Majestic C. Remote excitation of neuronal circuits using low-intensity, low-frequency ultrasound. PLoS One. 2008;3(10):e3511.
37. Mooney SJ, Shah K, Yeung S, Burgess A, Aubert I, Hynynen K. Focused ultrasound-induced neurogenesis requires an increase in blood–brain barrier permeability. PLoS ONE. 2016;11:1–11.