Spring 5-19-2022

# Effect of Static Magnetic Field on Focused Ultrasound Induced Blood-brain Barrier Opening

Leqi Yang

Washington University in St. Louis

McKelvey School of Engineering

Department of Biomedical Engineering

# Effect of Static Magnetic Field on Focused Ultrasound Induced Blood-brain Barrier Opening

By

Leqi Yang

A thesis presented to the McKelvey School of Engineering of Washington University in St. Louis in partial fulfillment of the requirements for the degree of Master of Science

May 2022

St. Louis, Missouri

**Acknowledgements**

I would like to thank Dr. Hong Chen for the guidance and generous research resources she provided. I sincerely appreciate Christopher Pacia, Yaoheng Yang, and Yan Gong for their guidance and help. I also want to thank Dr. Dezhuang Ye, Dr. Jinyun Yuan, Yimei Yue, Chih-Yen Chien, Dr. Zhongtao Hu, Lu Xu, Kevin Xu, Yajie Liu and Dr. Si Chen for their selfless help.

I would like to thank Dr. James D. Quirk for his support on MRI and Dr. Erik Dumont for his help on optimizing imaging guided therapy procedure.

I would like to thank Dr. Hong Chen, Dr. Chao Zhou and Dr. Song Hu for agreeing to become members in the committee of my thesis defense and providing constructive suggestion on this thesis.

Most importantly, I want to thank my family members, especially my parents, for supporting my dream and giving me this extraordinary opportunity to learn at Washington University in St. Louis.

# Table of Contents

List of Figures ..... ii

Abstract ..... iii

Chapter 1: Introduction ..... 1

Background ..... …1

Focused ultrasound-induced blood-brain barrier opening ..... 2

Microbubbles cavitation ..... 3

Objective……..3

Chapter 2: Materials and Methods ..... 4

Animal preparation ..... 4

Experimental Setup and Timeline ..... 4

Focused Ultrasound Treatment ..... 5

Mircrobubbles Cavitation Detection and Quantification ..... 6

Evans Blue Extravasation Quantification ..... 7

Chapter 3: Results ..... 9

Static Magnetic Filed Decreased Microbubble Cavitation ..... 9

Static Magnetic Filed Decreased Evans Blue Delivery ..... 13

Chapter 4: Discussion ..... 16

References……..18

## List of Figures

Figure 1 Structure of the blood-brain barrier……...…….....2

Figure 2.1 Experimental Setup and timeline. (A) Setup (B) Timeline…….5

Figure 2.2 Microbubbles cavitation analysis procedure……...…7

Figure 2.3 Representative Evans blue intensity quantification procedure……...…….8

Figure 3.1 Microbubbles cavitation activities in mice during sonication at different magnetic field……12

Figure 3.2 Evans blue delivery efficiency via MRI-guided focused ultrasound-induced blood-brain barrier opening with microbubbles…….15

Abstract

Effect of Static Magnetic Field on Focused Ultrasound-induced

Blood-brain Barrier Opening

By

Leqi Yang

Master of Science in Biomedical Engineering

Washington University in St. Louis, 2022

Research Advisor: Professor Hong Chen

Magnetic resonance-guided focused ultrasound (MRgFUS) with microbubble has been widely used to achieve blood-brain barrier (BBB) opening, followed by delivering drugs into the brain or obtaining biomarkers of brain diseases. Recently, there is one paper pointing out static magnetic field generated by the 4.7 T MR scanner dampens BBB opening volume. This thesis aims to study the effect of a higher static magnetic field (9.4 T) on focused ultrasound-induced BBB opening with microbubbles (MBs). Eight mice were randomly assigned to two groups (n = 4 in each group), followed by the same procedure under different magnetic fields (approximately 0 T or 9.4 T). After treatment, Evans Blue (EB) was injected into mice. Passive cavitation dose and Evans blue intensity were analyzed afterward. As a result, the MBs cavitation dose decreased by an average of 1.9 dB at 9.4 T (P = 0.01), compared with that outside the magnetic field (approximately 0 T). Evans blue intensity reduced by 2 fold at 9.4 T (P = 0.04), compared with that at approximately 0 T. This study indicts high static magnetic field (9.4 T) dampens focused ultrasound-induced BBB opening, consistent with previously published results. Findings in these

two studies suggest static magnetic field generated by the MR scanner should be considered as one factor in terms of delivering drugs into the brain with MRgFUS.

# Chapter 1: Introduction

## Background

The brain is one of the most important organs in humans, which controls thought, memory, motor skills, breathing, and other critical processes. In humans, the brain and spinal cord together are named the central nervous system, or CNS. The brain contains the cerebrum, the brainstem, and the cerebellum. As for cellular structure, the brain is composed mainly of two broad classes of cells: neurons and glial cells. Glial has several types and performs plenty of critical functions, including structural support, metabolic support, insulation, and guidance of development. Neurons are usually considered the most important cells in the brain and they are in charge of sending signals to specific target cells over long distances.[1] Over the years, brain diseases, such as Alzheimer's disease, brain cancers, and Parkinson's disease, are not only hard to treat, but also super challengeable to diagnose. One of the major reasons is the existence of the blood-brain barrier (BBB). BBB consists of a specialized vascular endothelium that interacts directly with astrocytes, neurons, and pericytes (Figure 1).[2, 4] BBB prevents ∼100% of large molecules and more than 98% of small molecules from coming into the brain, which helps to prohibit serious brain disease to some extent.[3] However, BBB also limits the delivery of therapeutic agents into the brain to treat brain disease and block the way of liquid biopsy of brain diseases. Therefore, there is a need to investigate a technology to achieve reversible BBB opening. Focused ultrasound-induced blood-brain barrier opening (FUS-BBBO) is one of the most promising technologies to reach this goal.

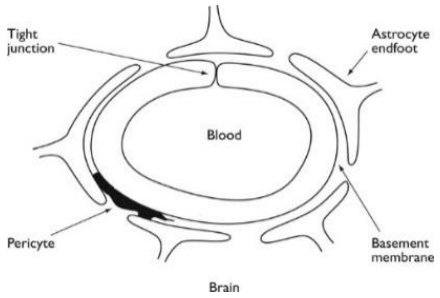



Figure 1 Structure of the blood-brain barrier

## Focused Ultrasound-induced Blood-brain Barrier Opening

In 2001, the first paper introduced the feasibility of combining focused ultrasound with microbubbles to achieve blood-brain barrier opening (FUS-BBBO) in rabbits.[3] Over the years, this technology has been widely used and developed because it can noninvasively, locally, and reversibly enhance BBB permeability. Using FUS-BBBO, different kinds of therapeutic agents, including small molecules, proteins, and cells, have been delivered across the BBB to treat brain diseases and brain tumors.[4, 12] Based on the idea of FUS-BBBO, another innovative technology, Sonobiopsy, has been established to improve the sensitivity of biomarker detection released from brain tumors.[5] Plenty of experiments had been performed with different FUS parameters but there is no standard one. The well-accepted FUS parameters are peak negative pressure (PNP) and mechanical index (MI).[4] Different parameters values are introduced based on the purpose of the experiments. Despite the FUS parameters values are not consistent in different experiments, FUS-BBBO has already been shown success in clinical. In 2018, the first clinical trial was performed to examine the feasibility and safety of FUS-BBBO in patients with Alzheimer's disease.[6] The first-in-human experiment of Sonobiopsy was conducted in 2021 to detect cell-free DNA in patients with WHO grade IV glioblastoma (GBM).[13]

Among all these preclinical or clinical experiments, magnetic resonance imaging (MRI) is commonly combined with FUS-BBBO to localize, target and monitor the treatment procedure in

real time[7]. MRI is a mature, widely-used medical imaging modality in radiology to image the anatomy and the physiological processes of the body. Different MRI scanners generate various strong static magnetic fields. Among those, 1.5T, 3T, 4.7T, and 9.4T MRI scanners have been used to treat patients in clinical.[7-9] Preclinical studies usually focused on optimizing FUS parameters, microbubbles parameters, and other treatment protocols to optimize the outcome of magnetic resonance-guided focused ultrasound (MRgFUS) treatments. Though the effect of magnetic field on microbubble cavitation activity has been widely investigated using numerical modeling or phantom experiment, its effect on microbubble cavitation in vivo and the consequent FUS-BBBO treatment outcome was only recently reported in magnetic field of 1.5 T, 3.0 T and 4.7 T.[9]

## Microbubbles cavitation

MBs are micron-sized gas-filled vesicles stabilized by phospholipids, proteins or polymers.[15] One of the FDA-approved microbubbles is Definity®, which is an injectable ultrasound enhancement agent comprised of lipid-coated echogenic microbubbles filled with octafluoropropane gas.[11] Microbubbles cavitation can range from stable cavitation to inertial cavitation based on the acoustic pressure. Stable cavitation occurs under low pressure, which can cause the increase of vascular permeability without damaging the vascular. High pressure can induce inertial cavitation, leading to MBs collapse suddenly and possible damage to the vascular.[14] Stable cavitation and inertial cavitation are often detected by passive cavitation detection to monitor MBs behavior and improve the safety of BBBO.

## Objective

The objective of the present study is to investigate the effect of strong static magnetic field (9.4 T) on microbubbles cavitation and its consequent FUS-BBBO outcome *in vivo* mice brain.

# Chapter 2: Materials and Methods

## Animal Preparation

Animal protocols used in this study were reviewed and approved by the Institutional Animal Care and Use Committee in accordance with the National Institutes of Health guidelines for animal research (approval no. 20180185). Eight Cr. NIH Swiss mice (7–9 weeks, ~20 g body weight, female) were ordered from Charles River Laboratory (Wilmington, MA, USA). Mice were anesthetized with vaporized isoflurane (approximately 1.7%) mixed with oxygen throughout the experiment. The body temperature was maintained at approximately 37°C by a water-circulating heating pad.

## Experimental Setup and Timeline

A schematic of the experiment is shown in Figure 2.1A. An MR-compatible focused ultrasound system (Imasonics) was used. This system contained a seven-element transducer with a center frequency of 1.5 MHz, an aperture of 25 mm, and a radius of curvature of 20 mm. The full width at half maximums of the focused ultrasound transducer was 5.5 mm in axial direction and 1.2 mm in lateral direction. The transducer was connected to an MR-compatible motor, allowing it to move along the x-axis and y-axis. The passive cavitation detection (PCD) sensor at the center of the transducer had a center frequency of 1.6 MHz. The signal detected with the PCD sensor was obtained by PicoScope. The transducer elements were connected to a water balloon filled with degassed and deionized water and coupled to the mouse head with degassed ultrasound gel. The generator, PicoScope, and computers were well away from the MRI scanner (outside 5 Gauss line) to avoid their influence on the magnetic field intensity. The experimental timeline is shown in Figure 2.1B.

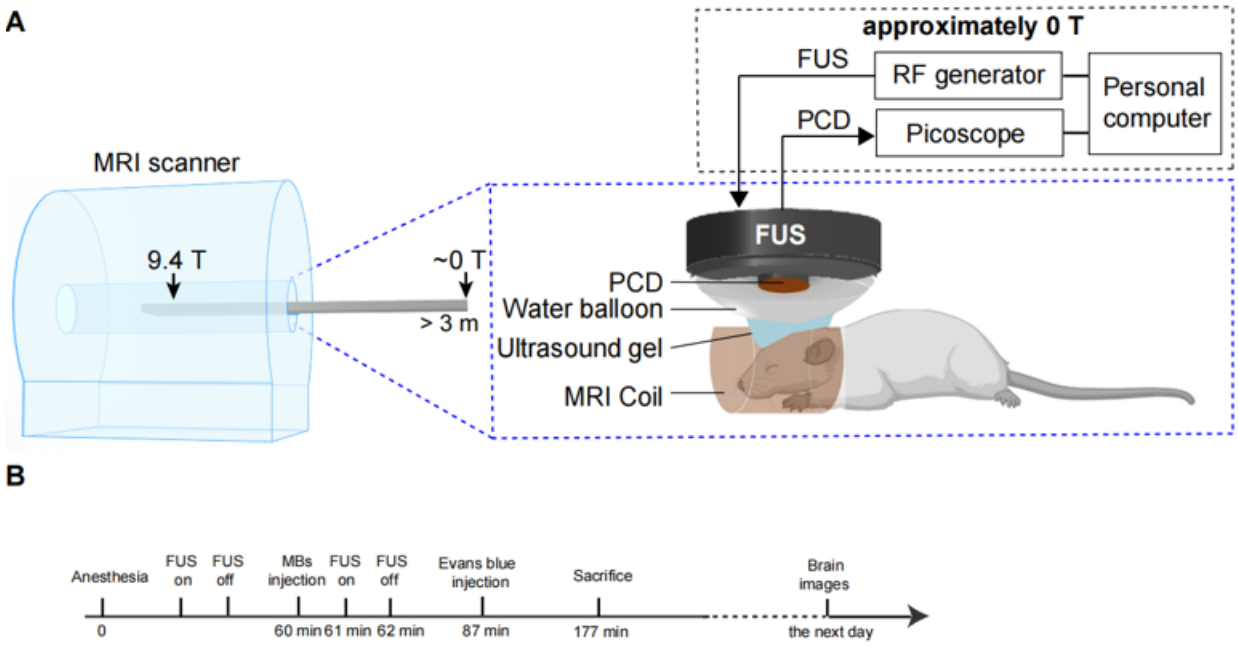



Figure 2.1 Experimental Setup and timeline. (A) Setup (B) Timeline

## Focused Ultrasound Treatment

Eight mice were randomly assigned to two groups (n = 4 in each group). Each group was treated under 2 static magnetic field intensity (approximately 0T or 9.4T) by placing the mouse at different distances from the center of the MR scanner. The magnetic field intensity was measured by a gaussmeter (RoHS; FW Bell). Except for under different magnetic fields, all two groups of mice were treated by the same experimental procedure with the same microbubble concentration and focused ultrasound parameters. Microbubbles (Definity; $8\times10^8$ #/mL, 30 µL) with 0.9% sodium chloride were injected intravenously. After 1 minute, mice were treated by focused ultrasound with the target at the brainstem, which was located by MR-guided focused ultrasound software (ThermoGuide). The focused ultrasound parameters (center frequency, 1.5 MHz; peak negative pressure, 0.6 MPa; duty cycle, 3.33%; burst length, 6.66 msec; pulse repetition frequency, 5 Hz; and sonication duration, 1 minute) were kept consistent for all subjects.

## Microbubbles Cavitation Detection and Quantification

Before MBs injection, the PCD signal was recorded for 1 minute to define the baseline of cavitation for each subject. 1-minute after MBs injection, the subject was treated with focused ultrasound and the PCD signal was recorded continuously during this 1-minute treatment. A fast-Fourier transform was performed for each signal acquired during the sonication of each focused ultrasound pulse. The stable cavitation level was defined as the root mean squared amplitude of the subharmonic ($1/2f_0$; $f_0$: center frequency of the transducer), second ($2f_0$) and third ($3f_0$) harmonic signals within 3 kHz bandwidths. The inertial cavitation level was defined as the root mean squared amplitude of the frequency spectrum after excluding 300-kHz bandwidths around the center frequency and harmonic signals (used to calculate stable cavitation). The stable cavitation level after treatment was then normalized to the stable cavitation level before treatment and was named as normalized stable cavitation level. Likewise, the normalized inertial cavitation level was calculated in the similar way. The stable cavitation dose was defined by the cumulative sum of the stable cavitation levels during the whole sonication and then normalized to the corresponding cavitation dose of the baseline. The inertial cavitation dose followed the same guideline. The microbubbles cavitation analysis procedure is shown in Figure 2.2.

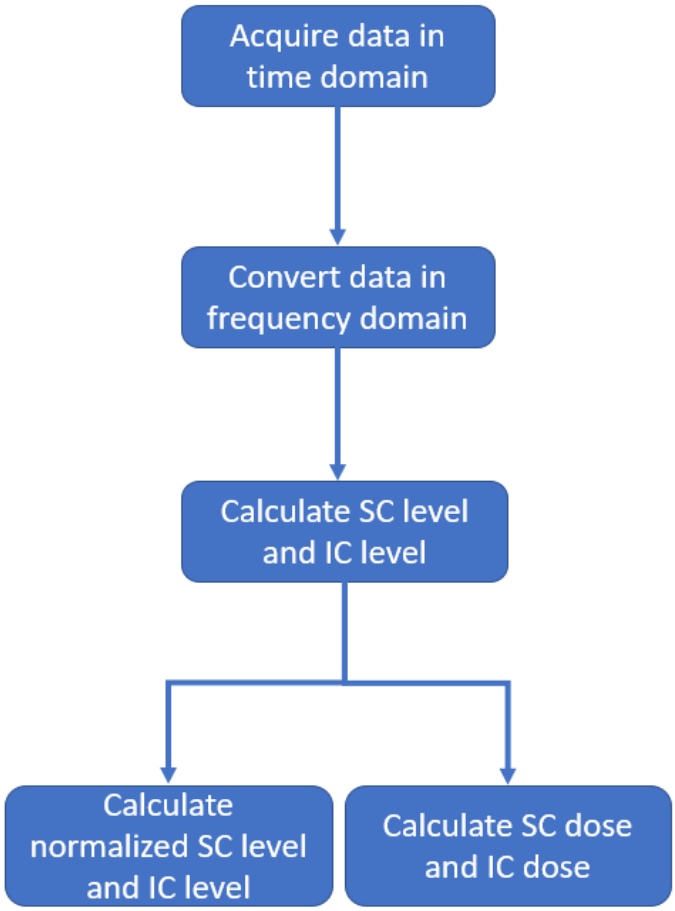



Figure 2.2 Microbubbles cavitation analysis procedure. SC means stable cavitation. IC means inertial cavitation.

## Evans Blue Extravasation Quantification

Evans blue was used to evaluate whether the static magnetic field affects the efficiency of drug delivery by FUS-BBBO. Evans blue (4%, 50 µL) was injected intravenously into mice 25 minutes after sonication. 90 minutes after Evans blue injection, mice were sacrificed by injecting phosphate-buffered saline (0.01 mol/L, 40 mL) transcardially. The brains were stored in 4% paraformaldehyde in a 4°C refrigerator. The next day, brains were cut into slices and then imaged with the Pearl system (LICOR Biosciences) using the 700 nm channel with the same exposure settings for all mice. For each mouse, ROI (a 2mm-diameter circle) centered at the location with

the maximum fluorescence signal were drawn and calculated by using one untreated brain slice as the baseline. A representative Evans blue quantification procedure is shown in Fig 2.3.

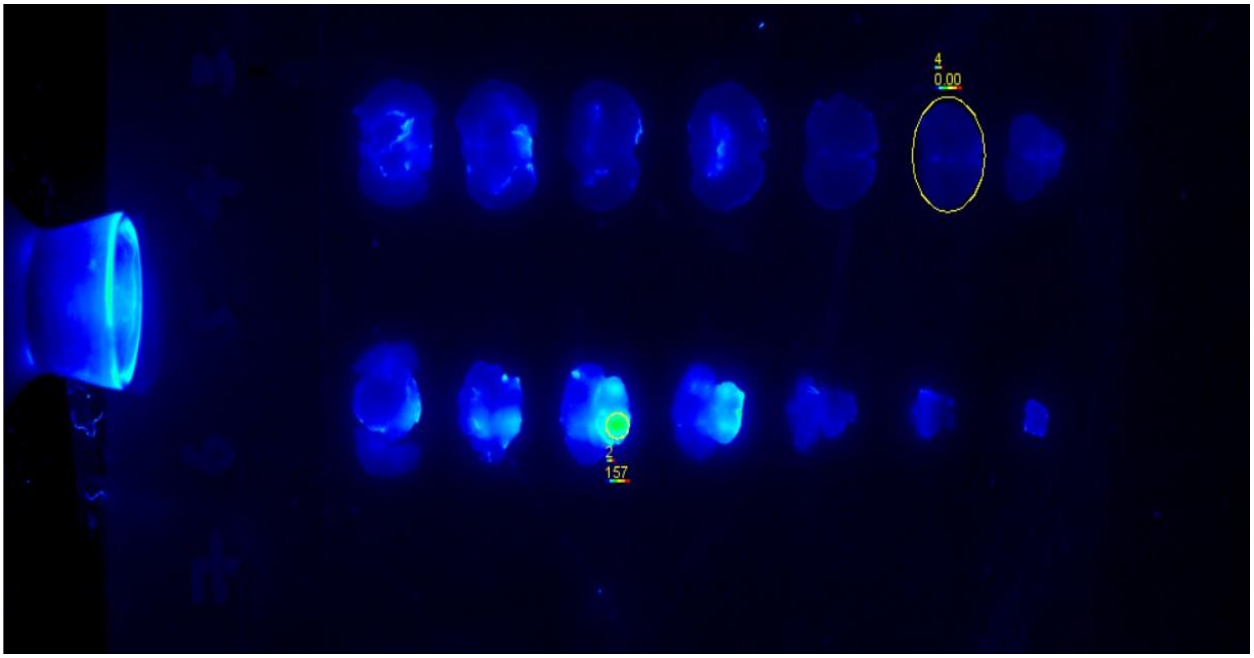


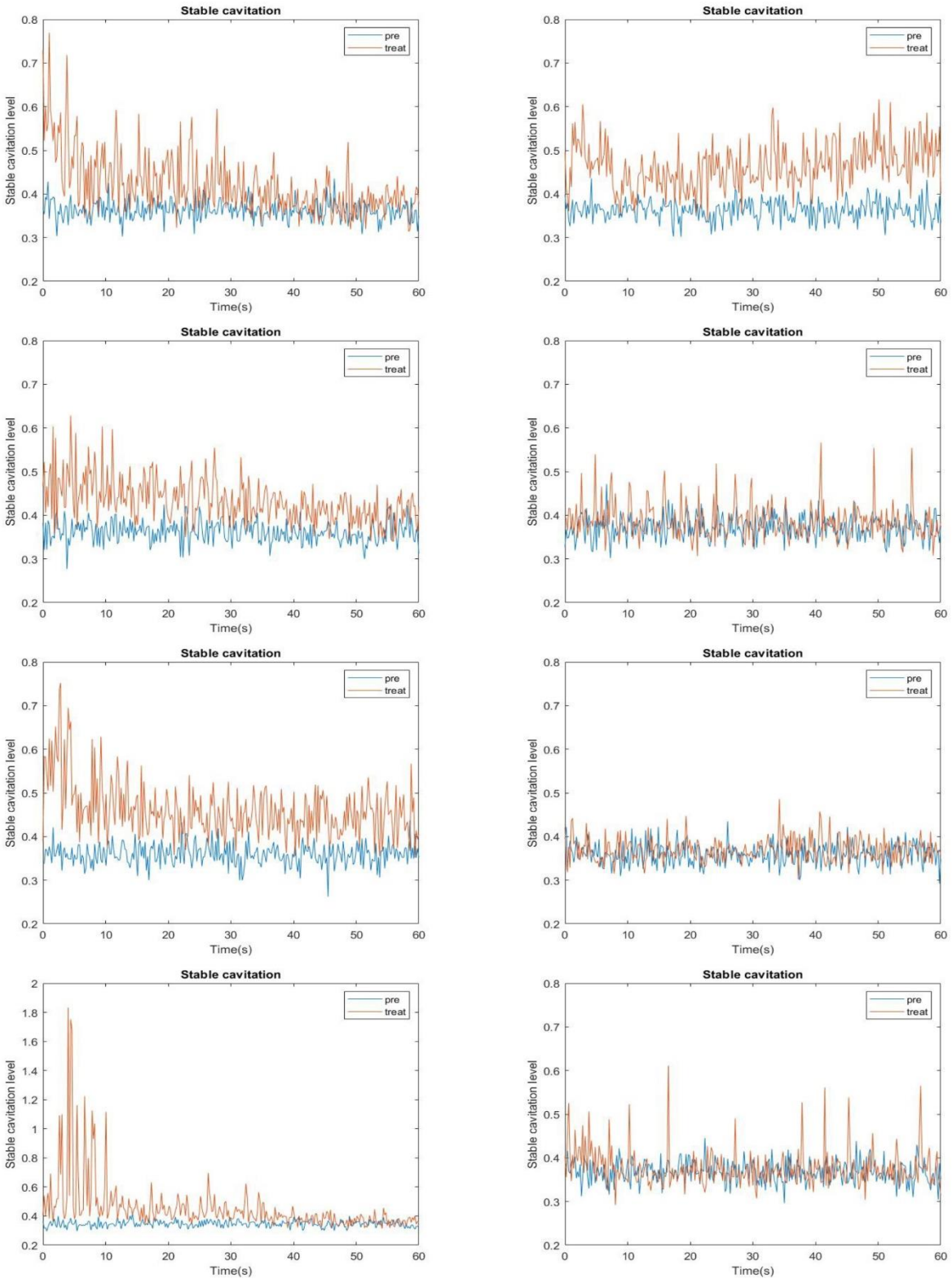

Figure 2.3 Representative Evans blue intensity quantification procedure. The yellow circles indicate the selected ROI (region of interest).

# Chapter 3: Results

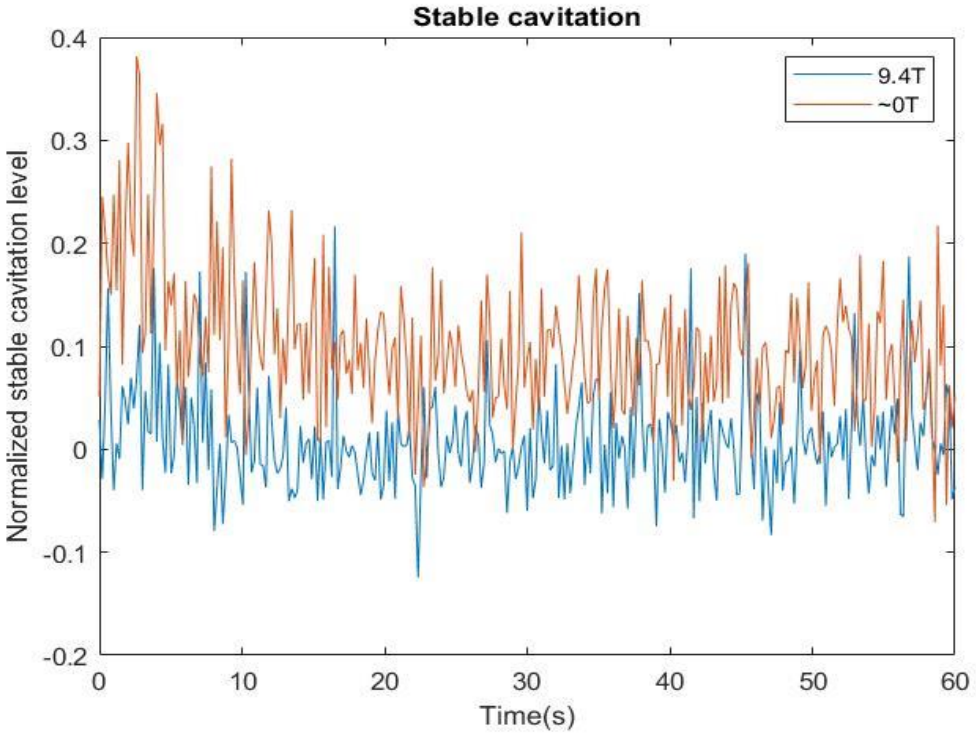
## Static Magnetic Field Decreased Microbubble Cavitation

All the stable cavitation levels at approximately 0 T or 9.4 T are plotted in Fig 3.1A. There is a clear dampening effect on stable cavitation amplitude at 9.4 T compared to the one outside the MRI scanner (~0 T) (Figure 3.2B). The mean stable cavitation dose at approximately 0 T was 2.79 dB ± 0.47 (standard error of the mean) while, it significantly dropped to 0.85 dB ± 0.34 (standard error of the mean) in 9.4 T magnetic field (Figure 3.1D). Since the acoustic pressure was relatively low, the inertial cavitations was undetectable both at approximately 0 T or 9.4 T (Figure 3.1 C and E).

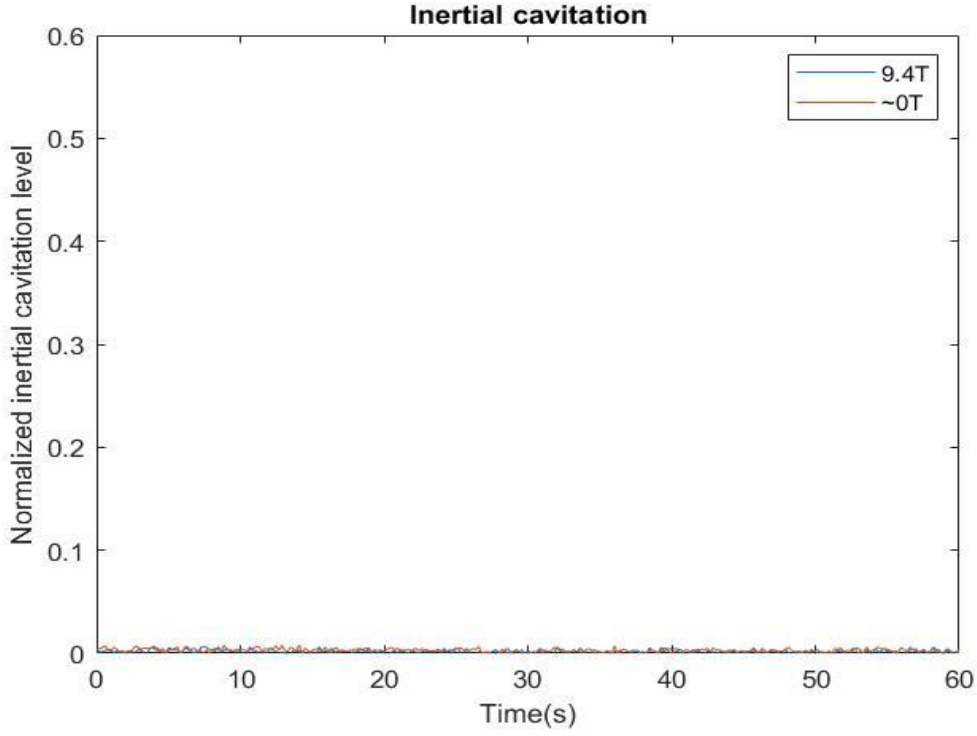
A ~0 T 9.4 T


Stable cavitation
pre
treat
Stable cavitation level
Time(s)

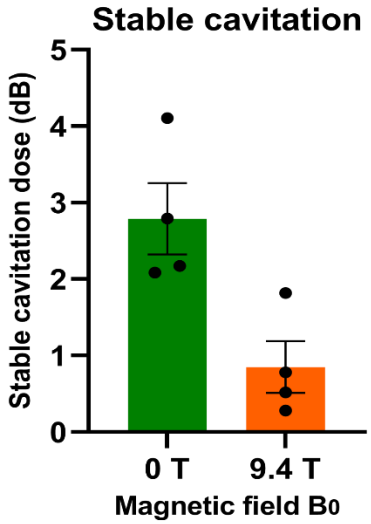
B


Stable cavitation
9.4T
~0T
Normalized stable cavitation level
Time(s)
0.4
0.3
0.2
0.1
0
-0.1
-0.2
0
10
20
30
40
50
60

C


Inertial cavitation
9.4T
~0T
Normalized inertial cavitation level
Time(s)
0.6
0.5
0.4
0.3
0.2
0.1
0
0
10
20
30
40
50
60

D




E

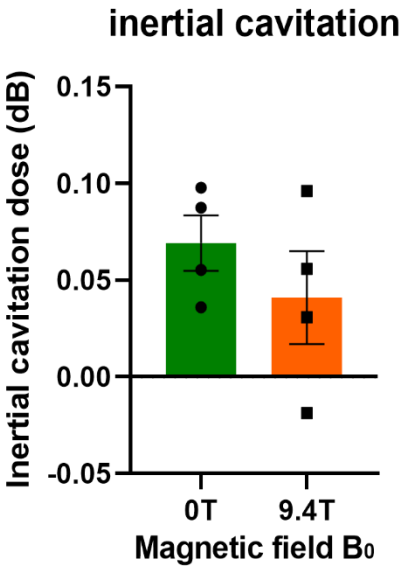


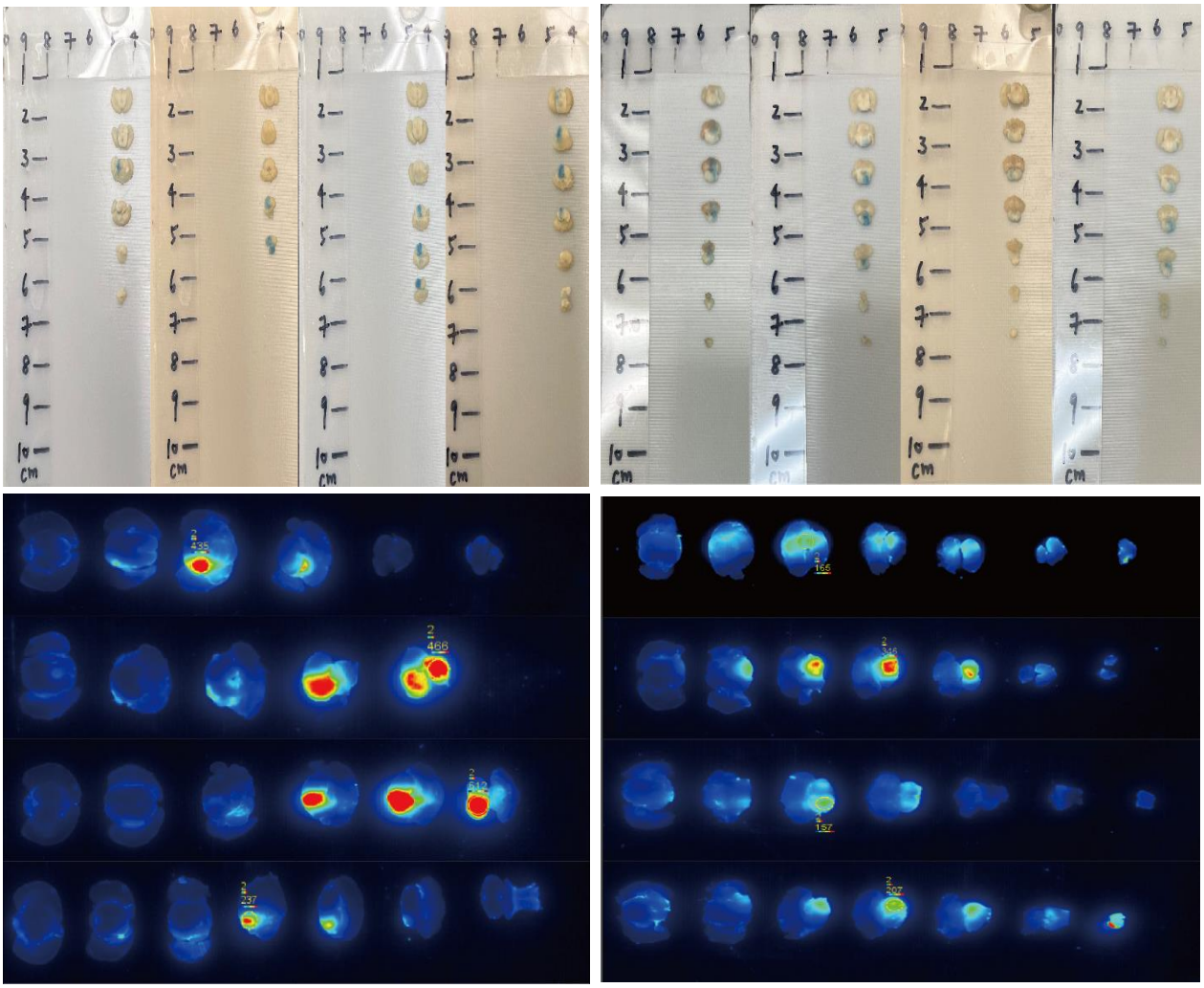

Figure 3.1 Microbubbles cavitation activities in mice during sonication at different magnetic fields. (**A**) All subjects' spectrums of stable cavitation levels during sonication in approximately 0 T or 9.4 T. (**B**) Representative plots of normalized stable cavitation level as a function of time for mice

treated in different magnetic fields. (**C**) Representative plots of normalized inertial cavitation level as a function of time for mice treated in different magnetic fields. (**D**) Stable cavitation doses of mice sonicated in different magnetic fields. Each dot represents an outcome from one mouse. (**E**) Inertial cavitation doses of mice sonicated in different magnetic fields. Each dot represents an outcome from one mouse. Error bars represent standard error of the mean.

## Static Magnetic Field Decreased Evans Blue Delivery

Two representative florescence images are shown in Fig 3.2A (9.4 T) and Fig 3.2B (~ 0 T). The fluorescence images of mice brain slices indict that the Evans blue intensity in 9.4 T was much lower than that in ~ 0 T. The GraphPad analysis shows that Evans blue intensity was 445.50 ± 77.04 (mean ± standard error of the mean) in approximately 0 T while 217.30 ± 44.06 in 9.4 T. Therefore, the Evans blue intensity was decreased by 2-fold at 9.4 T than that in approximately 0 T ($P$ = 0.04, Figure 3.2C).

A


~0 T
9.4 T

B

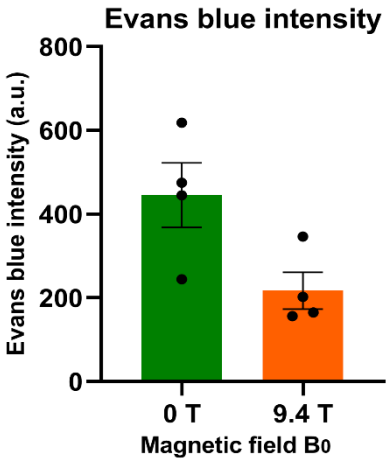

Evans blue intensity
800
600
400
200
0
Evans blue intensity (a.u.)
0 T
9.4 T
Magnetic field B0

Figure 3.2: Evans blue delivery efficiency via MRI-guided FUS-BBBO (A) Photographs (top row) and corresponding fluorescence images (bottom row) of mouse brain slices treated at approximately 0 T (left column) and 9.4 T (right column). (B) Evans blue intensity quantification of mice treated at approximately 0 T and 9.4 T. Error bars indicate standard error of the mean.

## Chapter 4: Discussion

Ever since FUS-BBBO was introduced, MRI plays a critical role in targeting and monitoring the treatment procedure. Almost all the studies aim to find the optimized parameters of MRI-guided FUS-BBBO in terms of FUS parameters (pressure, duty cycle et. al), MRI parameters (echo time, contrast agent concentration et. al) and experiment procedures. However, the effect of the static magnetic field generated by the MR scanner on MRg-FUS-BBBO was recently discovered. Here, we examine the strong magnetic field (9.4 T) impact on MBs cavitation and BBB opening efficiency indicted by the Evans blue delivery efficiency. Consistent with previous results, static magnetic field not only dampens microbubbles cavitation but also decreases Evans blue delivery. Previous studies have shown the magnetic field can change bubbles dynamics because moving water molecules around a cavitating bubble react with the magnetic field, which causes the transformation of kinetic energy into heat.[10] Moreover, the microbubbles used in this experiment, Definity microbubbles, have negative charges on their lipid shell,[11] which also contributes to the dampening effect of the magnetic field on microbubble cavitation.[9] The dampening effect of microbubble causes the decrease of mechanical force on the blood vessel wall, which decreases the BBBO volume and Evans blue delivery efficiency.

This study had a few limitations. Most importantly, due to equipment damage and time limitation, the perfusion time after Evans blue injection under different magnetic fields was not consistent. For mice under 0 T, the perfusion time was 1 hour and 30 minutes after Evans blue injection while, for mice under 9.4 T, the perfusion time was 32 hours, 6 hours and 30 minutes, 24 hours and 30 minutes or 25 hours, respectively. For future study, the perfusion time should be kept consistent to evaluate the real impact of the static magnetic field on Evans blue delivery efficiency. Second, this study didn't use MR images to quantify BBB opening volume since the signal-to-

ratio of the MR images was too low and the images often contained lots of artifacts. This is because the coil used in this study had been damaged and it was no longer compatible with the MR scanner perfectly. Another limitation of this study was that it missed other common static magnetic fields, including 1.5 T, 3 T and 4.7 T. In clinical, the most commonly used magnetic field for MRgFUS is 3 T. Future studies should also examine the impact of more different static magnetic fields on FUS-BBBO with a different MR scanner from the previous study.

This study, for the first time, shows strong static magnetic field (9.4 T) has an obvious dampening effect on microbubbles cavitation, which also leads to the decrease of BBB opening volume and the decrease of Evans blue delivery efficiency. Specifically, the MBs cavitation dose decreased by an average of 1.9 dB at 9.4 T ($P = 0.01$), compared with that outside the magnetic field (approximately 0 T). Evans blue intensity was reduced by 2 fold at 9.4 T ($P = 0.04$), compared with that at approximately 0 T. This study supports the idea of the previous study that the static magnetic field generated by the MR scanner should be considered as a parameter by using MRgFUS. Combining this study and the previous one, it is recommended to apply FUS far away from the MR scanner to achieve a higher BBB opening volume. Future study is needed to examine the impact of the static magnetic field on focused ultrasound-induced liquid biopsy (Sonobiopsy)

## References


1. Kandel, E. R., Schwartz, J. H., Jessell, T. M., Siegelbaum, S., Hudspeth, A. J., & Mack, S. (Eds.). (2000). Principles of neural science (Vol. 4, pp. 1227-1246) without doi. New York: McGraw-hill.
2. Pardridge, W. M. (2005). The blood-brain barrier: bottleneck in brain drug development. NeuroRx, 2(1), 3-14. https://doi.org/10.1602/neurorx.2.1.3
3. Hynynen K, McDannold N, Vykhodtseva N, et al. Noninvasive MR imaging-guided focal opening of the blood-brain barrier in rabbits. Radiology 2001; 220: 640–646 https://doi.org/10.1093/bjaceaccp/mkr018
4. Chen, S., Nazeri, A., Baek, H., Ye, D., Yang, Y., Yuan, J., Rubin, J. B., & Chen, H. (2022). A review of bioeffects induced by focused ultrasound combined with microbubbles on the neurovascular unit. Journal of cerebral blood flow and metabolism : official journal of the International Society of Cerebral Blood Flow and Metabolism, 42(1), 3–26. https://doi.org/10.1177/0271678X211046129
5. Zhu, L., Cheng, G., Ye, D., Nazeri, A., Yue, Y., Liu, W., ... & Chen, H. (2018). Focused ultrasound-enabled brain tumor liquid biopsy. Scientific reports, 8(1), 1-9. https://doi.org/10.1038/s41598-018-24516-7
6. Lipsman, N., Meng, Y., Bethune, A. J., Huang, Y., Lam, B., Masellis, M., ... & Black, S. E. (2018). Blood–brain barrier opening in Alzheimer's disease using MR-guided focused ultrasound. Nature communications, 9(1), 1-8.MR-guided focused ultrasound liquid biopsy enriches circulating biomarkers in patients with brain tumors

https://doi.org/10.1038/s41467-018-04529-6

7. Jolesz, F. A. (2009). MRI-guided focused ultrasound surgery. Annual review of medicine, 60, 417-430.Clinical advantages of 3.0 T MRI over 1.5 T https://doi.org/10.1146/annurev.med.60.041707.170303
8. Thomas, D. L., De Vita, E., Roberts, S., Turner, R., Yousry, T. A., & Ordidge, R. J. (2004). High-resolution fast spin echo imaging of the human brain at 4.7 T: implementation and sequence characteristics. Magnetic Resonance in Medicine: An Official Journal of the International Society for Magnetic Resonance in Medicine, 51(6), 1254-1264. https://doi.org/10.1002/mrm.20106
9. Yang, Y., Pacia, C. P., Ye, D., Yue, Y., Chien, C. Y., & Chen, H. (2021). Static Magnetic Fields Dampen Focused Ultrasound–mediated Blood-Brain Barrier Opening. Radiology, 300(3), 681-689. https://doi.org/10.1148/radiol.2021204441
10. Yasui, K. (1999). Effect of a magnetic field on sonoluminescence. Physical Review E, 60(2), 1759. https://doi.org/10.1103/PhysRevE.60.1759
11. Ja'afar, F., Leow, C. H., Garbin, V., Sennoga, C. A., Tang, M. X., & Seddon, J. M. (2015). Surface charge measurement of SonoVue, definity and optison: a comparison of laser doppler electrophoresis and micro-electrophoresis. Ultrasound in Medicine & Biology, 41(11), 2990-3000. https://doi.org/10.1016/j.ultrasmedbio.2015.07.001

12. Dasgupta, A., Liu, M., Ojha, T., Storm, G., Kiessling, F., & Lammers, T. (2016). Ultrasound-mediated drug delivery to the brain: principles, progress and prospects. Drug Discovery Today: Technologies, 20, 41-48. https://doi.org/10.1016/j.ddtec.2016.07.007
13. Meng, Y., Pople, C. B., Suppiah, S., Llinas, M., Huang, Y., Sahgal, A & Lipsman, N. (2021). MR-guided focused ultrasound liquid biopsy enriches circulating biomarkers in patients with brain tumors. Neuro-oncology, 23(10), 1789-1797. https://doi.org/10.1093/neuonc/noab057
14. Chen, H., & Konofagou, E. E. (2014). The size of blood–brain barrier opening induced by focused ultrasound is dictated by the acoustic pressure. Journal of Cerebral Blood Flow & Metabolism, 34(7), 1197-1204. https://doi.org/10.1038/jcbfm.2014.71