Towards Subject-Specific Therapy Planning for Non-Invasive Blood Brain Barrier Opening in Mice by Focused Ultrasound

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***Abstract*—Focused ultrasound (FUS) is a promising method to open the blood brain barrier (BBB) for treatment of neurodegenerative diseases. Accurate targeting is essential for a successful BBB opening (BBBo). We aim to develop a robust therapy planning for BBBo in mice, which is challenging due to the size of the brain and the influence of the skull on the ultrasound pressure distribution. For enabling mouse individual therapy planning, a simulation tool is proposed, developed and validated. We used the k-Wave toolbox to enable 3D acoustic simulations of the commercial FUS system from Image Guided Therapy (IGT). Micro-CT scans were used to model the geometry of skulls. Simulations using a mouse skull showed an attenuation of approx. 20-24% depending on the position of penetration, which was validated by hydrophone measurements in the same range. Based on these validations we planned BBBo in mice by placing the transducer at different positions over the mouse brain and varying the excitation amplitude. With different transducer positions, the peak pressure in the brain varied between $0.54\,MPa$ and $0.62\,MPa$ at 11% output level, which is expected to enable safe BBBo. Subsequently, *in vivo* experiments were conducted using the aforementioned simulation parameters. BBBo was confirmed by contrast enhanced T1 weighted magnetic resonance images immediately after sonication.**

***Index Terms*—Transcranial focused ultrasound, Blood brain barrier opening, Therapy planning, Acoustic simulations**

I. Introduction

Neurodegenerative diseases such as Parkinson's or Alzheimer's disease affect millions of people every year, with an enormous impact on the daily life of the patient [1]. A direct medication into the affected regions is a complex issue of modern medicine. Focused ultrasound (FUS) could be a non-invasive method to enable targeted placement of medication in the human brain [2]. In this type of therapy, the blood brain barrier (BBB) is opened to enable particle flow into the cranial tissue. In recent years the injection of microbubbles directly before FUS therapy has been established as a promising method in order to achieve the opening. This process uses cavitation created by US waves, which leads to an oscillation of the bubbles and a sudden pressure change in the vessels [3]. When the BBB was opened successfully, particles are able to pass the BBB and can accumulate in the brain tissue.

For monitoring and control of the treatment magnetic resonance guidance (MRgFUS) is performed. The BBB opening can be seen in magnetic resonance MR images by applying the contrast agent gadolinium to the animal directly following the BBB opening (BBBo).

Though several studies have been performed in animals, the parametrization of the FUS therapy still poses many uncertainties to the pressure field, such as attenuation, reflection and distortion of the wave field by the skull. In particular, for small animals like mice a therapy planning is believed to be beneficial due to the small size of the brain compared to the size of the focal spot. This work presents a simulation tool, which enables systematic animal specific sonication planning to derive settings for more robust BBBo in mice.

II. Methods

In order to create a robust and adaptable simulation tool, the development and testing process was split into three parts. First, we identified all necessary parameters for the simulation and determined their values, and if possible, evaluated them experimentally (II-A). Then, we developed a simulation tool based on the k-wave toolbox [5] in order to conduct multiple studies to analyze the influence of several parameters on FUS application (II-B). Eventually, we applied these settings to *in vivo* experiments with a MRgFUS system (II-C).

A. Reference Measurement

To determine the impact of the mouse skull on the pressure field a dissected mouse skull was placed in front of a custom built US transducer [4], in an experimental water tank setup. The output signal, a chirp with a center frequency of $2.5\,\mathrm{MHz}$ and a bandwidth of $3\,MHz$, was recorded by a hydrophone, which was moved in a 2D semi-spherical pattern, with a radius of $15\,cm$ to the transducer. The top of the skull was placed at $7.5\,cm$ in front of the transducer. The measurement was performed with and without the skull to estimate the impact of it on the resulting pressure field and to determine an average

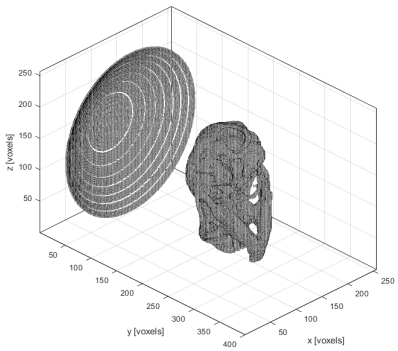

Fig. 1. 3D model of the simulation framework including the FUS transducer and a mouse skull converted from CT data. The placement of the skull is optimized to position the focal area in one hemisphere of the mouse brain.

value for the attenuation caused by the skullcap. The recorded data from the skull measurements were matched filtered to minimize the impact of the transducer characteristics and the measurement setup.

B. Simulation Framework

The simulation tool models an experimental MRgFUS system manufactured by Image Guided Therapy (IGT), France. It uses a bowl-shaped transducer manufactured by *IMASONIC* with a diameter of $25\,mm$, which consists of 8 elements arranged in concentric rings. Each element can be individually excited by the IGT software. The transducer is integrated into a plastic casing with a mount and fits in a controllable fixture. The maximum acoustic output power is $11\,W$, which leads to a peak pressure of approximately $7\,MPa$ at a distance of $20\,mm$ using a center frequency of $1.5\,MHz$. In front of the transducer a latex membrane which can be filled with water achieves coupling with the mouse scalp.

The developed three-dimensional simulation is designed to propagate the ultrasound wave in the y-direction and the transducer is placed in the x-z plane. The resolution is set to $0.1\,mm$ in each spatial direction. This takes into account the required discretization for the used frequencies and allows to represent the model of a skull in enough details while keeping the computation time manageable. The pixel size of the modeled domain is chosen as a power of two to reduce computation time.

The simulated volume is $30 \times 20 \times 40 mm^3$. The time resolution *dt* was set to a fixed value of $10\,ns$, because automated selection by k-wave did not lead to a stable simulation. This allows a high resolution of the $1.5\,MHz$ source signal, which has a duration of one pulse of $66.6\,ns$.

To model the propagation of the wave through the water filled membrane, the background medium in the simulation was modeled with a speed of sound of $1500\,m/s$, a density of $1000\,kg/m^3$ and is considered as lossless in this simulation.

The geometry of the anatomical model of a mouse skull was derived from a micro-CT scan. It was resampled to the resolution of the computation grid ($0.1\,mm$) of the simulation and positioned centrally in the medium at a realistic distance of approx. $16\,mm$ from the transducer, such that the natural focal spot was located approximately $3-4\,mm$ below the skull. The skull was filled with brain mimicking material. Acoustic material parameters for the bone were chosen from literature [6]–[9]. The setup is shown in figure 1.

C. In vivo Experiments

Subsequently, we performed *in vivo* experiments with mice using the FUS parameters which we had simulated before and which were considered robust to reach a peak pressure in the desired window between 0.4 and $0.6\,MPa$, which leads to stable BBBo according to the literature [10], [11].

Prior to each experiment, the mice were anaesthetised and placed on a heating pad to maintain its body temperature. For optimal coupling, the hair was removed and ultrasound gel was applied between scalp and transducer membrane for coupling. Finally, an access was placed in the caudal tail vein and the mouse was placed on an MRI coil.

For monitoring the BBBo, MR images are acquired after application of FUS. A gadolinium-based contrast agent was applied to the mouse after the FUS treatment. It diffused through the open BBB and results in a change of the MR signal. The FUS setup is controlled by the ThermoGuide software provided by IGT. It was used to estimate the focus point in the brain by calibrating the transducer position with the MR image.

Considering an estimated attenuation of the mouse skull of approximately $20\,\%$ (section III-A), and a desired peak pressure for successful BBB opening of $0.6\,MPa$ (section II-C), a peak pressure of $0.75\,MPa$ must be achieved in the focus point without a skull. This results in the initial setting of $11\,\%$ of the maximum pressure in the natural focus that can be generated by the FUS system ($7\,MPa$). As shown in our simulations, the peak pressure varies depending on the position of sonication, however the setting of $11\,\%$ amplitude resulted in a predicted peak pressure well in the pressure window considered safe for BBBo.

A total sonication duration of one minute was defined consisting of 600 shots with $3\,ms$ time per shot and $97\,ms$ recovery period between each shot to avoid overstressing the tissue. These parameters follow the established protocol by Magnin et al. [12]. Directly before the execution, microbubbles were administered to the mouse via the tail vein access. Immediately after, the FUS treatment was executed. After sonication, the contrast agent was administered to the mouse. Multiple MR images were acquired to document the uptake of gadolinium in the brain tissue.

III. Results

A. Reference Measurements

The matched filtered and normalized result of the reference measurement of the attenuation caused by the mouse skull is shown in figure 2. The position of the skull in the water bath can be well recognized though the projected size of the skull

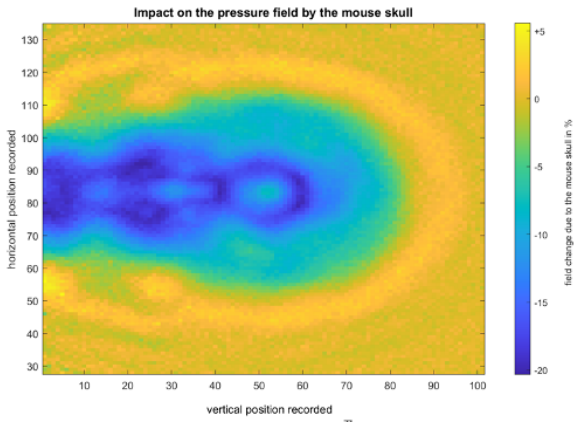

Fig. 2. Results of the 2D hydrophone measurement with a custom-built US transducer. The outline of the skull can be recognized. Around the skull there is an increase in the pressure values due to reflections at flat incident angles. The actual size of the skull is slightly smaller than the contours in the image.

is smaller than the outlines in the figure. Ultrasound waves hitting the skull in a flat angle lead to an increase in the pressure field, which can be recognized as a halo around the center of the skull with positive pressure change. In the central part of the skull, i.e. behind the skull cap, the bone dampens the signal by approximately $20\,\%$. For the *in vivo* application we expect the attenuation to be slightly higher, caused by skin and brain tissue.

B. Simulation and Optimization of FUS Settings

In the first step of the development, the transducer output pressure in the simulation was calibrated according to the specification of the FUS system, reaching a maximum peak pressure of $7\,MPa$ in the focus point in water at $100\,\%$ signal amplitude. Simulation settings have subsequently been adjusted to meet a target peak pressure of approximately $0.6\,MPa$ in the focal spot, i.e. $11\,\%$ amplitude setting according to initial estimates, see section II-C. A slice of the resulting pressure field including the elliptical focal area is shown in figure 3.

In order to optimize sonication settings, parameters from multiple simulations with the skull model have been extracted and analyzed. The position of the skull was moved in x and z direction up to $8\,mm$ in steps of $0.2\,mm$.

In figure 4 the resulting peak pressure in the focus point at each simulated sonication position is given. The resulting pressure in the hemispheres (center top and center bottom of the image) ranges from $0.5\,MPa$ to $0.6\,MPa$. The highest pressure can be observed when the US focus misses the skull (top left, bottom left and bottom right).

Furthermore, the skull was rotated to analyze the influence of the incident angle. In this work two different ways of rotation have been examined in detail. In the first case, the desired focal point is located in the middle axis of the brain. The skull is rotated around the z-axis to simulate pressure fields in the left and right hemisphere of the skull, with an angle other than normal to the skull surface. The simulation was performed

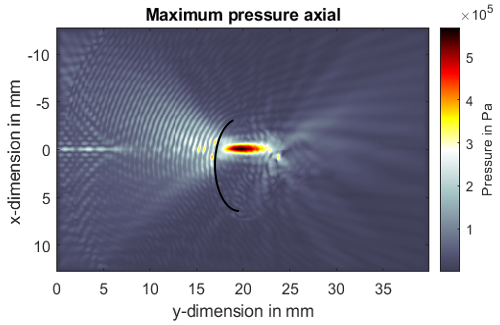

Fig. 3. Pressure distribution from the 3D simulation in the mouse skull, showing the slice of the maximum pressure with an elliptical focal area. The target was located in one hemisphere.

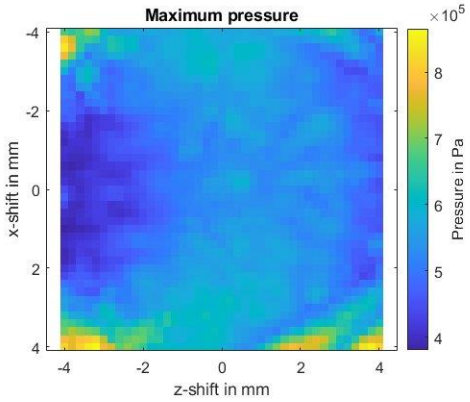

Fig. 4. Resulting maximum of the pressure by simulation of placing the FUS transducer in a 8x8 mm^2 area above the skullcap. The resulting attenuation in the center is in the same range as the reference measurement at approximately $24\,\%$.

from -45° to +45°, with 0° being the skullcap facing directly to the transducer. Figure 5 shows the attenuation of the signal compared to a simulation through water only. The values range from $22\,\%$ to $32\,\%$. In the hemispheres, the thickness of the skull is slightly reduced, resulting in less attenuation while at even higher rotation angles the influence of the incident angle increases resulting in a high attenuation at the boarders of figure 5, left.

In the second case, the desired focal position is in one hemisphere, which corresponds to the setting of our *in vivo* experiments. Figure 5, right shows the attenuation as a function of the rotation angle. For negative angles, the right side of the skull is rotated step by step towards a normal incident angle to the skull surface resulting in attenuation in the range between $24\,\%$ and $25\,\%$. Rotation angles between $-45°$ and $-25°$ show an increased attenuation. All positive rotation angles show an increased attenuation, peaking at over $40\,\%$ due to the considerably higher incident angle.

Based on these results we decided for a sonication of one hemisphere with the transducer approximately normal to the skull surface. Before *in vivo* application we simulated this setting. The results of the simulation are shown in table I. The

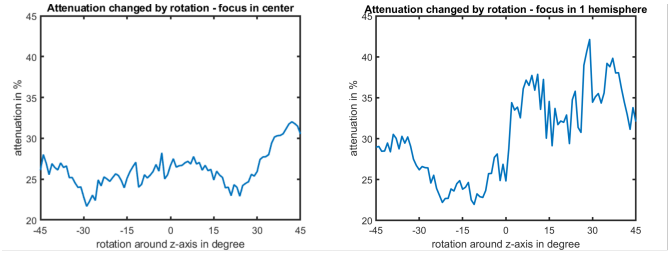

Fig. 5. Attenuation caused by the skull as a function of the rotation angle. Rotation around the z-axis from -45° to +45°. Left: skull placed centrally in front of the FUS Transducer. Right: FUS transducer placed over one hemisphere of the skull.

TABLE I
RESULTS OF THE SIMULATION OF THE FOCUSED ULTRA TRANSDUCER IN ON HEMISPHERE OF THE MOUSE BRAIN WITH 11 % AMPLITUDE.

Parameter	Simulation result
Focus axial in mm	7.4
Focus lateral in mm	1.5
Maximum pressure in MPa	0.57
Peak location in mm	20
Peak volume in mm^3	18.8
Attenuation	24 %

simulation shows an estimated attenuation of approximately 24 %. The position of the maximum pressure is perfectly in the geometrical focus of the FUS transducer. The maximum pressure of $0.56\,MPa$ is considered sufficient and safe for a BBBo.

C. In vivo Experiments with MRgFUS

After predicting a reasonable set of parameters (approximate position and amplitude setting) we conducted *in vivo* experiments in four mice using MRgFUS. If the opening was successful, injected gadolinium could pass the BBB and was visible in a T1-weighted MR image. In figure 6 the uptake of contrast agent in the sonicated hemisphere of an exemplary mouse is displayed. This indicates that the BBB has been opened successfully. Subsequentially performed histological analysis - which will be covered in future analysis - showed no major bleeding. Using this procedure, we were able to successfully open the BBB in four mice, thereby validating the aforementioned sonication planning based on simulations and reference measurements.

IV. DISCUSSION AND CONCLUSION

We consider subject specific planning of FUS applications as a key component for BBBo in mice due to the narrow peak pressure window which enables successful BBBo while at the same time minimizing hemorrhages. We developed a simulation framework to address this challenge, which is based on the acoustic wave equation. The resulting pressure field is reasonable considering earlier experiments with the IGT FUS system and our reference measurement with a dissected mouse skull. Shear wave losses which are expected to occur in solids, will be considered in an extended version of our simulation in the future.

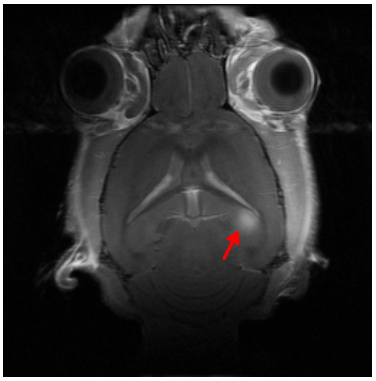

Fig. 6. MR image after sonication. Contrast enhancement is visible in the right hemisphere, indicating that the BBB was opened successfully.

An analysis of parameters as the transducer positioning and inclination showed the considerable changes in the peak pressure in the focus point. Our findings are in line with earlier experiments in rats [13].

Using our estimates from the simulation framework we successfully performed BBBo in four mice. We therefore consider this method to be a reliable simulation tool, helping therapy planning for *in vivo* sonication procedures. In the future, individual mouse anatomy is directly modeled based on segmentation of the mouse head in e.g. separate *in vivo* CT scans or even directly from MR images.

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