Ultrafast Three-Dimensional Microbubble Imaging Predicts Tissue Damage Following Nonthermal Brain Ablation

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Introduction

Nonthermal ablation via focused ultrasound and contrast agent microbubbles is under pre-clinical investigation for non-invasive brain surgery [1-7]. Existing sources of variability during the procedures can lead to inconsistent treatment outcomes and bioeffect generation outside of the intended target volume(s) [1,6,7], warranting the development of systems and methods for online monitoring and control prior to clinical translation. Our group has shown that three-dimensional (3D) microbubble imaging can be used to calibrate ultrasound exposure levels for inducing transient blood-brain barrier permeabilization without causing overt tissue damage [8]. In separate work, megahertz-rate processing of the acquired volumetric imaging data uncovered details regarding the spatiotemporal evolution of microbubble activity in vivo that were missed when temporal averaging was carried out over the duration of ultrasound on-time [9]. Here we investigate ultrafast 3D microbubble imaging for predicting the spatial distribution of tissue necrosis induced following nonthermal brain ablation.

Methods

Experiments were performed on craniotomized rabbits (≈ 2 cm x 2 cm window, 3-4 kg) using a multi-frequency transmit/receive ultrasound phased array consisting of 256 transducer modules (3 concentric cylindrical PZT4 elements, \( f_0 = 306/612/1224 \) kHz, inner/outer diameter = 1.4\( \lambda \)/2.0\( \lambda \), \( \lambda \) = wavelength) sparsely distributed over a 31.8 cm diameter hemispherical shell [8]. Pulsed ultrasound (\( f_0 = 612 \) kHz, pulse length = 10 ms, pulse repetition frequency = 1 Hz, duration = 120 s) was electronically steered over a 2 x 2 point square grid (axial plane, side length = 6 mm) starting simultaneously with an intravenous microbubble infusion (200 \( \mu \)l/kg Definity™ over 90 s, 10 x maximum allowable clinical imaging dose) using a 3D subharmonic imaging-based feedback approach [8] that was modified to enable multi-point exposure level calibration. For all grid points, channel data were acquired throughout each transmit pulse (capture length = 14 ms, sampling rate = 10 MS/s) using the array elements tuned to the subharmonic frequency (\( f_0/2 = 306 \) kHz). Exposures were carried out at 0/50/100/150% of the peak negative pressure required to detect spatially-coherent subharmonic activity in vivo \( (p_{sub}) \) via multi-channel 3D beamforming [8]. The locations of the different exposure levels were randomized between animals. Short-time analysis of the acoustic emissions data (moving, non-overlapping rectangular beamforming windows spanning ultrasound on-time, integration time = 1 \( \mu \)s) was performed offline. MRI was carried out at 3T to assess the induced tissue effects. Animals were sacrificed 48 hr post-treatment for histological examination.

Results

Multi-point exposure level calibration via 3D subharmonic imaging was feasible in vivo \( (p_{sub} = 0.67 \pm 0.19 \) MPa, intra-grid \( p_{sub} \) range = 0.14 ± 0.05 MPa, in-situ estimates). No statistical differences were found in the peak negative pressure subharmonic threshold between subgroups when the grid points were stratified based on either the exposure level or the target location within the brain \( (p > 0.60, \) one-way ANOVA). \( T_2^* \)-weighted \( (T_2^*w) \) MR images acquired immediately post-sonication displayed regions of signal hypointensity induced by the exposures at 100% \( p_{sub} \) and 150% \( p_{sub} \), but not at lower target levels (Fig. 1). Hematoxylin-eosin (H&E) stained tissue sections associated the \( T_2^*w \) MRI signal hypointensities with the presence of red blood cell (RBC) extravasations and regions of tissue necrosis, the spatial extent
of which were both found to increase with increasing exposure level (Fig. 1). H&E histology also revealed small zones of RBC extravasations and tissue necrosis resulting from exposures at 50% $p_{sub}$ that were not evident on T2*w MRI (Fig. 1). Ultrafast 3D subharmonic imaging data correlated well with the spatial distribution of T2*w MRI signal hypointensities (Fig. 1).

**Conclusions**

Volumetric imaging of ultrasound contrast agents *in vivo* over microsecond timescales shows promise as a method for predicting the spatial morphology of tissue damage induced following nonthermal brain ablation. The information provided by ultrafast 3D microbubble imaging is expected to aid in the development of active exposure control strategies for bubble-mediated ultrasound treatments both in the brain and in other parts of the body.

*Figure 1: Spatial correlation of ultrafast 3D microbubble imaging data with ultrasound-induced tissue damage in rabbit brain *in vivo*. Axial (left) and coronal (middle) T2*w MR images acquired immediately post-sonication demonstrate regions of signal hypointensity induced by the exposures at 0.8 MPa (100% $p_{sub}$) and 1.2 MPa (150% $p_{sub}$). Yellow contours: -8 dB source field intensity distributions from the corresponding anatomical plane (1 MHz imaging volume rate, spatial-peak source field intensity location/magnitude integrated over each pulse for the full treatment duration). Axial H&E stained tissue section from the same animal 48 hours post-sonication (right) shows regions of RBC extravasations and lightly stained necrotic areas (arrows).*

**References**


