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Simultaneous passive acoustic mapping and MR thermometry for monitoring of cavitation-enhanced tumor ablation in rabbits using focused ultrasound and phase-shift nanoemulsions

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Abstract

Thermal ablation of solid tumors via focused ultrasound (FUS) is a noninvasive image-guided alternative to conventional surgical resection. However, usefulness of the technique is limited in vascularized organs due to convection of heat, resulting in long sonication times and unpredictable thermal lesion formation. Acoustic cavitation has been shown to enhance heating but requires use of exogenous nuclei and sufficient acoustic monitoring. In this study, we employed phase-shift nanoemulsions (PSNE) to promote cavitation, and incorporated passive acoustic mapping (PAM) alongside conventional MRI thermometry within the bore of a clinical MRI scanner. Simultaneous PAM and MRI thermometry were demonstrated in an *in vivo* rabbit tumor model, with and without PSNE to promote cavitation. Vaporization and cavitation of the nanoemulsion could be detected using PAM, which led to accelerated heating, monitored using MRI thermometry. The maximum heating assessed from MRI was well correlated with the integrated acoustic emissions, illustrating cavitation-enhanced heating. Examination of tissue showed thermal lesions which were larger in presence of PSNE, in agreement with the thermometry data. Using fixed exposure conditions over 94 sonications in multiple animals showed an increase in the mean amplitude of acoustic emissions and resulting temperature rise, but with significant variability between sonications, further illustrating the need for real-time monitoring. The results demonstrate the utility of combined PAM and MRI for monitoring of tumor ablation, and provide further evidence for the ability of PSNE to promote cavitation-enhanced lesioning.

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Keywords

passive acoustic mapping; magnetic resonance imaging; thermal ablation; tumor ablation; phase-shift nanoemulsion; cavitation; focused ultrasound; rabbit; VX2

Introduction

Focused ultrasound (FUS) is a promising therapeutic technology which enables delivery of energy to tissues within the body for applications such as noninvasive surgery for treatment of cancer (Illing et al. 2005; Kennedy 2005; Rowland et al. 1997). Current clinical implementations of the technique are primarily based on thermal effects, in which absorption of ultrasound at high acoustic intensity leads to focal heating that can be used to ablate tissue and create lesions in place of conventional surgery (ter Haar and Coussios 2007). Such thermally-dominated procedures may be monitored using MRI thermometry, which facilitates temperature mapping during sonication (Cline et al. 1994; McDannold et al. 2000) as well as conventional MR imaging of tissue which may be used for targeting and assessment of results. Unfortunately, it can take several hours to thermally ablate a solid tumor with FUS, especially in well vascularized organs where blood circulation can carry away heat downstream (Illing et al. 2005). Various studies have shown that acoustic cavitation can enhance ultrasound absorption in tissue, accelerating FUS-mediated heating and reducing the power required for thermal ablation (Farny et al. 2010; Holt and Roy 2001; Hynynen 1991). However, MRI is unable to detect non-thermal effects of FUS such as acoustic cavitation. Current devices - particularly those targeting the brain - thus generally seek to avoid cavitation (InSightec 2016), as they lack the capability to use it in a controlled fashion. These issues ultimately limit the areas of the body in which FUS may be used. The introduction of improved acoustic monitoring techniques could thus greatly expand the performance and scope of FUS to include more challenging targets by allowing cavitation-enhanced therapy.

Cavitation activity may be monitored using acoustic methods and technology (Leighton 1994). By employing an array of ultrasound sensors for passive detection it is possible to use beamforming methods to map the spatial distribution of acoustic activity (Gyongy and Coussios 2010a; Salgaonkar et al. 2009), referred to in this paper as passive acoustic mapping (PAM). The technique is particularly applicable in the presence of acoustic cavitation, which results in characteristic acoustic emissions (Leighton 1994). PAM has previously been employed for applications such as investigation of microbubble behavior (Choi et al. 2014; Deng et al. 2016), transcranial imaging in the brain (Arvanitis et al. 2013; Jones et al. 2013; O'Reilly et al. 2014) and monitoring *ex vivo* thermal ablation (Haworth et al. 2015; Jensen et al. 2012). Integration of acoustic monitoring techniques such as PAM with FUS could thus provide additional information which could facilitate controlled cavitation-enhanced therapy.

The implementation of cavitation-enhanced FUS-mediated thermal ablation *in vivo* presents several additional challenges. In particular, the pressure amplitudes required in order to induce cavitation in tissue can be several megapascals (Hynynen 1991; Maxwell et al. 2013)

and vary in an unpredictable manner due to tissue heterogeneity. Introduction of exogenous nuclei such as microbubbles can significantly reduce the pressure threshold for cavitation (Crum 1982). However, typical microbubble suspensions have circulation times of only a few minutes (Morel et al. 2000) and are confined to the vasculature due to their micro-scale size, limiting their utility for cavitation-enhanced FUS. Alternatively, nano-scale agents such as liquid emulsions (Dayton et al. 2006) may be employed, which may offer improved circulation time compared to gas bubbles and allow extravasation out of blood vessels and into tissue due to their small size. By combining a volatile liquid core with a suitable stabilizing shell such emulsions may be vaporized into microbubbles upon ultrasound exposure or elevated temperature (Rapoport et al. 2010; Zhang and Porter 2010), termed phase-shift nanoemulsions (PSNE). PSNE could thus allow cavitation-enhanced FUS at reasonable acoustic pressures while avoiding shortcomings of conventional microbubble agents.

Previous work has shown that PSNE can accumulate in tumors (Kopechek et al. 2013) and can enhance cavitation and hence temperature rise in MR-guided focused ultrasound experiments (Kopechek et al. 2014). However, acoustic monitoring in past *in vivo* studies was limited to single-channel detectors which cannot provide any information on the spatial extent of cavitation activity. The purpose of this small-scale pilot study was to incorporate passive acoustic mapping alongside MR thermometry (Arvanitis and McDannold 2013; Crake et al. 2017) for monitoring thermal ablation of tumors using focused ultrasound and PSNE. The use of PAM and MRI together allows simultaneous mapping of cavitation and the resulting temperature rise. By combining the speed and sensitivity to cavitation of PAM with the anatomical imaging and thermometry capabilities of MRI, the combined system could facilitate control of cavitation-enhanced thermal ablation. Clinically, this could greatly expand the scope of FUS therapy to include targets which cannot be treated with current technology. The aim of this work was to demonstrate the use of combined PAM and MRI thermometry for monitoring thermal ablation of tumors with focused ultrasound.

Materials and methods

Production of phase-shift nanoemulsions

PSNE were produced using a published protocol (Kopechek et al. 2014). The lipids 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-snglycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG2000) were obtained from Avanti (Alabaster, AL, USA) and dodecafluoropentane (DDFP) was purchased from Fluoromed (Round Rock, TX, USA). Lipid powder (164mg DPPC, 26mg DSPE-PEG200) was weighed into a glass vial and dissolved to mix in chloroform. The chloroform was evaporated leaving a film which was rehydrated with 10ml phosphate-buffered saline (PBS). The lipid was dispersed by heating the vial to 50°C in a water bath followed by sonication (2min, 20% power) with a probe sonicator (VC505; Sonics & Materials, Newtown, CT, USA). The vial was then placed in an ice bath and 0.4ml DDFP added, followed by sonication (25% power) using a pulsed mode (10s on, 50s off, 60s total on time) to prevent heating. To produce PSNE the mixture was then passed 16 times through a 200nm polycarbonate filter (Whatman, Piscataway, NJ, USA) using an extruder (Lipex,

Northern Lipids, Burnaby, BC, Canada). Samples of the resulting suspension were analyzed using dynamic light scattering (90Plus, Brookhaven Instruments, Holtsville, NY, USA) which showed mean size 175.2 ± 1.4 nm and polydispersity index 0.058 ± 0.013 . The solution was stored at 4°C until use.

Animals

Experiments were performed in accordance with protocols approved by the Brigham and Women's Hospital Institutional Animal Care and Use Committee. Rabbits ($n=6$, male, 3kg; Millbrook Breeding Labs, Amherst, MA, USA) were injected subcutaneously in the thigh muscle with 4×10^7 VX2 cells and allowed to form palpable tumors before commencing experiments.

The mean duration from implant to treatment was 10.3 ± 4.9 days for control rabbits ($n=3$) and 11.0 ± 4.4 days for PSNE rabbits ($n=3$). During experiments rabbits were anesthetized with a mixture of ketamine (35mg/kg; Aveco, Fort Dodge, IA, USA) and xylazine (7mg/kg; Lloyd Laboratories, Shenandoah, IA, USA) injected as an intramuscular bolus each hour. Hair was removed from the skin surrounding tumors using clippers and depilatory cream. For experiments using PSNE a catheter was placed in the ear vein and 0.5ml/kg PSNE solution injected 1–2h before the first sonication and flushed with 0.5ml saline. Previous work with gadolinium-labelled PSNE (Kopechek et al. 2013) showed rapid accumulation in tumors over the first 1–2h from injection, which was maintained for at least 6h, so this schedule allowed time for PSNE to accumulate in tumors. Tumors were imaged using MRI before commencing sonications and volumes estimated using the ellipsoid formula $V = \pi/6 \times L \times W \times H$ (Tomayko and Reynolds, 1989). The mean tumor volumes for control ($n=3$) and PSNE ($n=3$) animals were $4.1 \pm 2.3 \text{ cm}^3$ and $4.9 \pm 2.0 \text{ cm}^3$ respectively, which was not significantly different between the two groups ($p > 0.05$, unpaired two-tailed t-test).

Experimental protocol

The results in this study were obtained from a total of 106 sonications conducted in 6 animals (3 animals treated with PSNE, 3 controls without PSNE). A summary of the sonications conducted in each animal is provided in Table 1. In the first two animals, the exposure conditions were varied over three different power levels (23, 32, 42W) to find appropriate conditions for PSNE-enhanced ablation. Three sonications were conducted at each of the power levels in each animal. The peak focal heating was then compared at each power level between the PSNE-treated and control animals in order to select the conditions which best demonstrated PSNE-enhanced ablation (i.e. the power level at which the increase in heating with PSNE was greatest). The selected exposure conditions were then used to conduct further sonications in the same animals and 4 additional animals. In order to avoid overlapping treated areas the number of sonications conducted in each animal varied depending on the size of the tumors. During sonications the temperature rise was monitored with MRI and acoustic emissions with PAM as described below. The mean durations of each experiment from first to last sonication were 75 ± 35 and 65 ± 33 minutes in the control and PSNE-treated animals respectively. After experiments the animals were sacrificed and tumors extracted.

Experimental setup

A schematic of the experimental setup is given in Figure 1. A 1.5 MHz MR-compatible focused ultrasound transducer (model BW2, 10cm diameter, 8cm focal length; GE Healthcare, Milwaukee, WI, USA) and ultrasound linear array (model L558, 5MHz center frequency, 128 elements, 58mm aperture; Acuson, Siemens Inc., Malvern, PA, USA) were clamped to an acrylic holder such that FUS transducer focus was within the array imaging plane (Fig. 1a). The assembly was placed in a water tank containing degassed water and attached to a 3-axis manual positioning stage *via* an aluminum rod such that the FUS transducer and array could be moved together within the bore of the MRI scanner. Animals were placed on an acrylic board with a cutout for ultrasound propagation. The board was mounted above the water tank and acoustically coupled to the transducers using a plastic bag filled with degassed water. A receive-only MRI surface coil (constructed in house) was mounted to the underside of the board such that the imaging plane of the coil was as close as possible to the FUS transducer focus. The whole tank, transducer and positioning system assembly was clamped to the patient table of a 3T clinical MRI scanner (Signa HDxt 3.0T; GE Healthcare, Milwaukee, WI, USA).

The imaging array was connected to a research ultrasound platform (model V1-128, Verasonics Inc., Kirkland, WA, USA) *via* an extension cable and penetration panel (constructed in house) through the wall of the MRI suite (Fig 1b). The FUS transducer was driven using a two-step 1.5MHz sinusoidal waveform as per previous studies with PSNE (Kopechek et al. 2014; Zhang and Porter 2010) produced using two function generators (Model 396, Fluke, Everett, WA, USA) that were summed together using a power combiner (ZSC-2-2, Mini Circuits, Brooklyn, NY, USA). The first function generator produced a 100-cycle high-amplitude burst to promote PSNE vaporization (65W) and subsequently triggered a 1 million cycle burst at a lower amplitude (23–42W) from the second function generator to promote cavitation and temperature rise, giving a total burst length of 667ms. The pulsing scheme was repeated every 741ms (90% duty cycle) for 40s. The output from the power combiner was amplified using a 50dB power amplifier (2100L, ENI, Rochester, NY, USA) and delivered *via* an impedance matching network supplied by the transducer manufacturer. The electrical power delivered to the transducer was monitored using a power meter (E4419B, Agilent, Palo Alto, CA, USA) which was calibrated to the effective acoustic power using a radiation force balance (XS205, Mettler Toledo, Switzerland) with a brush target (constructed in-house) (Hynynen 1991). The electrical to acoustic conversion efficiency of the transducer measured using this method was $70.1 \pm 0.5\%$.

During sonications the MRI scanner was used to perform temperature mapping to monitor the thermal effects of treatment, while the ultrasound platform was used to monitor the acoustic emissions due to effects such as cavitation. In order to reduce interference between the two modalities, the ultrasound platform was triggered using the RF exciter unblank output from the MRI scanner to occur at a time when MRI RF transmission was inactive (Fig. 1b-d). To provide consistent timing between the modalities and set the sonication duration, the ultrasound platform was also used to trigger the function generators driving the FUS transducer. For the temperature mapping sequence used in this study (see below), the MRI scanner produced a trigger out every $TR = 19.5\text{ms}$, and produced a new image every

2.6s ($\approx 128 \times \text{TR}$). To achieve a 90% duty cycle the sonication sequence was configured to repeat every 38 triggers (741ms). Within the routine the ultrasound platform was configured (see below) to take a B-mode image for the first trigger, followed by initiating FUS and passive acquisition on the second. Within each FUS burst a total of seven passive acquisitions were conducted (every 5 triggers). The routine was repeated for the 40s duration of each sonication.

MR imaging and thermometry

MRI was used before and during treatment for planning and thermometry. Imaging parameters are shown in Table 2 and are similar to those reported in previous work (Arvanitis et al. 2013; Arvanitis and McDannold 2013). T2-weighted imaging was conducted in each of the 3 planes to identify tumors. During sonications a fast-spoiled gradient echo sequence was used to acquire magnitude and phase images in a single slice every 2.6s. The phase images were processed immediately after acquisition to produce temperature maps using the proton resonance frequency shift method (Ishihara et al. 1995) which relates phase shift to temperature change as

$$\Delta T = \frac{\Delta \phi}{2\pi TE \cdot B_0 \gamma \alpha} \quad (1)$$

where TE is the echo time of the MR sequence (i.e. time over which the phase shift developed), B_0 the flux density of the main magnetic field (3.0 T), γ the gyromagnetic ratio for protons (42.58 MHz/T) and α the temperature coefficient for water (-0.01 ppm/ $^{\circ}\text{C}$). Representative example images for both sequences are shown in Fig 1c (left, mid).

Prior to commencing animal experiments the position of the FUS transducer focus was identified by sonicating a gel phantom (DQA phantom, InSightec, Israel). The phantom was initially roughly aligned relative to the MR imaging space using the laser landmark built in to the MR scanner. The focal coordinates were then identified by sonicating the phantom (30s, 5–20W power) while performing temperature mapping in each of the 3 principal planes in turn. Once the location of the focus was identified the phantom was replaced with the rabbit, and tumor aligned with the focus using T2-weighted imaging. Sonications were monitored using the temperature mapping sequence as described above. To capture baseline data for MRI thermometry, the sequence was initiated 5s before sonication. Between each sonication the transducer assembly was moved using the positioning stage to put the focus in a new area of the tumor.

Passive acoustic mapping

B-mode and passive imaging were interspersed using the linear array in a similar manner to our previous studies (Crake et al. 2015; Crake et al. 2016). B-mode imaging was conducted once every 38 MRI triggers (1.3Hz frame rate) utilizing 7 angled full aperture flash transmit events covering a steering range $\pm 6^{\circ}$ per frame, and reconstructed utilizing the routines supplied by the system manufacturer. Due to the angle of incidence at the skin surface and

lack of contrast between tumor and thigh muscle B-mode images provided little anatomical information (Fig 1c right), but were beneficial for alignment purposes and *in vitro* testing.

Passive imaging was conducted by acquiring 8,192 samples×128 channels at 20MHz sample rate (i.e. 409.6μs duration) per acquisition. To improve the frame rate, the receive events, DMA transfer, processing and saving of data were parallelized. To maintain timing relative to the MR scanner passive acquisition was set to occur every 5 trigger cycles, resulting in ~10Hz frame rate. For real-time visualization data were processed over a large region of interest (40 mm transverse × 100 mm axial, 60 × 100 pixels) relative to the imaging array and encompassing the acoustic focus. To optimize speed this was conducted using a frequency-domain beamformer (Arvanitis et al. 2016; Salgaonkar et al. 2009) over a small number of frequency bins. To provide baseline measurements recording of acoustic data was initiated 5s before FUS. After experiments the data were reprocessed using a robust time-domain beamformer (Coviello et al. 2015) utilizing the full bandwidth after a high pass digital filter to remove the 1.5MHz FUS excitation frequency.

Histology

Immediately after experiments rabbits were sacrificed and tumors and surrounding tissue extracted and placed in formalin. The tissue samples were stored in a 4°C cold room for at least 1 week to allow fixation. After this time, the samples were manually cut in the plane perpendicular to FUS propagation into approximately 4mm thick slices. The slices were then dehydrated with ethanol, embedded in paraffin, sectioned into 100μm slices and stained using hematoxylin and eosin (H&E) stain. The stained sections were scanned with a 20× objective using a slide scanning microscope (Axio Imager M1, Zeiss, Germany) for histological evaluation.

Data analysis

The PAM and MRI data were analyzed after experiments as a function of the exposure conditions. To examine the effect of the acoustic power on cavitation and heating enhancement the data were summarized from each exposure by summing the acoustic maps over time, and by extracting the temperature map at the time of maximum heating. We also examined individual acoustic and temperature maps and extracted metrics related to the amplitude and spatial characteristics of cavitation-enhanced heating at each time point. From the acoustic data, we defined the ‘peak amplitude’ as the maximum pixel value from each map (or sum of maps over time), which is proportional to the power (or energy when summed over time) of the acoustic emissions. To produce a spatial metric, we first amplitude normalized the maps, i.e.

$$M_{norm} = \frac{M - \min(M)}{\max(M) - \min(M)} \quad (2)$$

where M is an acoustic map, and the min and max operators represent the minimum and maximum values of all the pixels in that map, so the values of M_{norm} are in the range 0–1. This expression is undefined for a map where all the pixels are equal, but in practice this non-meaningful situation never occurs due to noise. We used the normalized maps to define

a ‘focal index’ metric, as a measure of focal-like activity in the mapping region. The focal index was calculated by comparing the area under the map to the size of the mapping region, i.e.

$$Focal\ Index = \frac{A}{\iint_{x,y} M_{norm}} \quad (3)$$

where the double integral represents numerical integration over the two spatial dimensions of the map, and A the area of the mapping region. This metric gives an indication of ‘focal-like’ activity, where randomly distributed noise has low index value, and a single high pixel has high index value. As a numerical example, for random noise normalized to the range 0–1 the expected area under the map is $\sim 0.5 \times A$ (so focal index = 2). At the opposite extreme, a map with a single high pixel has an area under the map equal to the area of one pixel; for example, a 0.5mm^2 pixel in a 500mm^2 mapping region gives a focal index value of 1000. A similar metric has been described before in the beamforming literature, using a ratio of areas of a beam pattern (Kelly et al. 2014). Similarly, from the temperature maps we extracted the maximum pixel value as an indication of heating amplitude. As an indication of the size of the heated region we extracted the full-width half-maximum of the region in which the temperature exceeded 50°C , at which point proteins begin to denature. Data were analyzed in MATLAB (The MathWorks, Inc., Natick, MA, USA). Metrics were compared between two groups (PSNE vs. control) using an unpaired two-sample t-test (MATLAB function *ttest2*), or between three groups (Fig. 9) using 1-way ANOVA followed by Tukey-Kramer multiple comparison testing (MATLAB functions *anova1*, *multcompare*).

Results

Effect of PSNE on cavitation

Acoustic data from tumors sonicated with and without PSNE at three different power levels are shown in Fig. 2. Maps summed over the duration of exposures are shown in Fig. 2a. In absence of PSNE, focal activity was just visible above the background noise at the highest power level. This may represent nonlinear absorption or low amplitude cavitation activity seeded by endogenous nuclei in the tissue. In presence of PSNE, focal cavitation activity was clearly evident at all three power levels. This suggests that PSNE were vaporized and resulted in increased cavitation in the tumors. The peak amplitude of activity increased between 23 and 32W as expected. At 42W several regions of cavitation activity were visible, with similar peak value to that at 32W.

These results are summarized for 3 trials of each of the 6 conditions in Fig. 2b. The peak amplitude of cavitation with PSNE increased significantly ($p < 0.05$) above the control sonications at 32W (548.2 ± 298.2 AU with PSNE vs. 193.5 ± 13.9 AU control) and 42W (644.1 ± 382.7 AU with PSNE vs. 247.2 ± 47.3 AU control). There was no significant difference in peak cavitation amplitude between the two groups ($p > 0.05$) at 23W (328.8 ± 134.6 AU with PSNE vs. 165.9 ± 13.2 AU control). As a comparison to the PAM data, averaged spectra of the raw RF data from the array are shown in Fig. 2c. An increase in

harmonics (multiples of $f_0=1.5\text{MHz}$), second- and third-order ultraharmonics (multiples of $f_0/2$ and $f_0/3$), and a small increase in broadband noise (between $1-2\times f_0$) are visible.

Acoustic data showing the variation in emissions over time for tumors sonicated with and without PSNE are shown in Fig. 3. For display purposes the maps in Fig. 3a were summed over subsets of the frames of data; each subfigure shows a summation over 9s duration. Fig. 3b shows amplitude and focal index metrics (defined above) for 3 trials of each condition, as well as the correlation between the two metrics themselves.

In the absence of PSNE a ‘focus’ of activity was just visible in the real-time acoustic maps above the noise floor when the FUS transducer was activated (Fig. 3a, bottom two rows), however this appeared steady over time so is likely due to non-cavitation effects of FUS such as generation of harmonics due to nonlinear propagation in tissue. The amplitude of emissions increased in amplitude on the order of $5-10\times$ in presence of PSNE (Fig. 3a, top row). The amplitude of emissions initially rose rapidly over the first 2.5 seconds to a peak value, before falling to a lower level which was maintained over the duration of sonication (Fig. 3b, top), suggesting vaporization and cavitation of PSNE occurred in the tumors. In one trial the amplitude of emissions began to increase again towards the end of sonication, which may indicate thermally-induced vaporization of PSNE occurred towards the end of sonication when temperature was highest (see thermometry results below). The focal index metric (defined above) shows similar trends to the amplitude data (Fig. 3b, mid), suggesting that most of the maps were dominated by a small number of sources. Comparison of the two metrics (Fig. 3b, bottom) suggests that they are strongly correlated (Pearson’s correlation coefficient $\rho=0.96$) due to focal cavitation.

Effect of PSNE on temperature rise

MRI thermometry data from tumors sonicated with and without PSNE at three different power levels are shown in Fig. 4. Temperature maps from the time point of maximum heating in each experiment are shown in Fig. 4a. The maximum temperature rise and width of the heated region are shown for 3 trials per condition in Fig. 4b. In the absence of PSNE, the temperature rise and size of the region heated above 50°C both scaled approximately linearly with power as expected, from $14.0\pm 3.4^\circ\text{C}$ at 23W to $20.3\pm 3.7^\circ\text{C}$ at 42W. In presence of PSNE the peak heating approximately doubled, to a mean of $32.4\pm 9.6^\circ\text{C}$ over all 9 trials. The peak temperature achieved using PSNE appeared approximately independent of the electrical power delivered, suggesting that heating in presence of PSNE was cavitation-dominated. The peak temperature with PSNE increased significantly ($p < 0.05$) above the control sonications at 32W ($35.8\pm 11.6^\circ\text{C}$ with PSNE vs. $16.5\pm 1.8^\circ\text{C}$ control). The peak temperature was not significantly different ($p > 0.05$) at 23W ($30.5\pm 10.8^\circ\text{C}$ with PSNE vs. $14.0\pm 3.4^\circ\text{C}$ control) and 42W ($31.0\pm 9.4^\circ\text{C}$ with PSNE vs. $20.3\pm 3.7^\circ\text{C}$ control). The results at 23W and 32W are consistent with the acoustic data (Fig. 2). At 42W a significant increase in cavitation was observed, but this did not translate to a significant increase in temperature, as the increase in power resulted in greater temperature rise in the control animal. The size of the heated region scaled approximately linearly with power both with and without PSNE, and increased in size by approximately a factor of 3 using PSNE ($4.2\pm 1.4\text{mm}$ with PSNE vs. $1.4\pm 0.8\text{mm}$ without).

MRI thermometry data showing the evolution of focal heating over time is shown in Fig. 5. Illustrative temperature maps every 15s from sonications with and without PSNE are shown in Fig. 5a, and the maximum temperature for all time points over 3 trials per condition is shown in Fig. 5b. Both with and without PSNE the temperature rise showed a typical logarithmic heating curve, rapidly increasing over the first 15–20s of sonication before reaching a plateau. The rate of heating during the first 15 seconds of sonication was increased from $0.5 \pm 0.2^\circ\text{C}$ without PSNE to $1.8 \pm 0.5^\circ\text{C}$ with PSNE. After sonication ceased the temperature began to fall as expected.

Correlation between PAM and MRI

The correlation between the PAM and MRI data was investigated by comparing the maximum heating in each temperature map with the maximum cavitation amplitude and focal index metrics from the PAM data at the same time points (Fig. 6). As the frame rate of the PAM data was higher than that of the MRI, the PAM data were averaged over the duration between adjacent MRI frames to make this comparison. This data includes a total of 266 time points from the 18 experiments ($3 \text{ trials} \times 3 \text{ powers} \times 2 \text{ with/without PSNE}$), i.e. approximately 15 MR time points were acquired during the FUS on time during each sonication as shown in Fig 5b.

Comparing the peak amplitude of the PAM data to the maximum temperature rise at each time point (Fig. 6a) shows a positive correlation (Pearson's correlation coefficient $\rho=0.67$) suggesting cavitation-enhanced heating. The effect of PSNE was readily apparent, particularly with respect to the amplitude of acoustic emissions (x axis), which was generally higher using PSNE.

Alternatively, the PAM data may be summed cumulatively from the beginning of each exposure to the current time points (Fig. 6b). Comparing the maximum value from these cumulative maps to the temperature shows an improved correlation ($\rho=0.74$), suggesting that the build-up of cavitation-enhanced heating 'dose' is cumulative. Finally, comparing the focal index of the cavitation maps (Fig. 6c) to the maximum heating achieved shows a similar correlation ($\rho=0.70$) but with the advantage that this can be calculated from individual time points.

While this comparison is rudimentary, it illustrates that the PAM data could potentially be used as an indication of the likelihood of cavitation-enhanced heating. For applications in which cavitation is not desired (i.e. for safety) the PAM data alone may be sufficient without MRI: for example, in Fig. 6a, below approximately 1 AU on the horizontal axis no significant heating enhancement occurred. Alternatively, for applications in which cavitation is desired, PAM could be used as a monitoring tool for optimization of the acoustic parameters and to confirm that cavitation occurred. The effects of PSNE were readily apparent in both modalities.

Effect of PSNE on thermal lesioning

Representative histological images of lesions produced without and with PSNE are shown in Figs. 7 and 8 respectively. In both control and PSNE-treated animals, we identified large

tumor nodules with typical invasion of VX2 tumor cells into muscle layers. The adjacent muscle (not shown) presented almost normal characteristic with no signs of necrosis.

In tumors from animals treated without PSNE we observed typical thermal lesions (Fig. 7) with three zones of tissue injury: a thermally coagulated central core (labeled a), surrounding ring of edematous tissue (b), and a region presenting almost normal characteristics, with some signs suggestive of thermal damage and small tumor cell foci adjacent to the central necrotic areas (c). Intact areas of the tumor away from lesions (d) exhibits a relatively solid pattern: the tumor cells are round or oval, are rich in cytoplasm and show many mitotic figures (labeled M).

In tumors from animals treated with PSNE (Fig. 8), we observed larger thermal lesions, exhibiting a central core with large areas of coagulative necrosis (labeled a), cavities which may have been produced by vaporization of the nanoemulsion (b), multiple hemorrhages resulting from inertial cavitation (c), hemorrhagic necrotic areas (d) and some small tumor cell foci adjacent to the necrotic areas (e).

These results confirm that thermal lesions were produced, and that the lesions were larger and more destructive to tumor tissue in the PSNE-treated tumors, as expected from the PAM and MRI data.

Variability between animals

To assess the variability in response between animals the conditions from Fig. 4, which resulted in maximum heating enhancement, were tested in additional animals. These results are summarized in Fig. 9, which shows the peak value from the cumulative PAMs summed over the duration of each experiment, as well as the peak temperature rise at the time of maximum heating. Due to differences in the tumor properties the fixed exposure conditions nonetheless resulted in a substantial variation in cavitation and heating response between sonications and animals, reinforcing the need for feedback and real-time monitoring. In particular, cavitation occurred in some control sonications, and did not occur in every one of the PSNE sonications. Nonetheless, over 94 sonications conducted in 6 animals (3 animals with PSNE, 3 control), the PAM data showed that the amplitude of the acoustic emissions was significantly increased ($p < 0.01$) in presence of PSNE (mean 811.0 ± 1063.4 AU with PSNE vs. 276.8 ± 100.9 AU without). To attempt to decouple variability in endogenous cavitation from PSNE-enhancement, separately analyzing the subset of sonications in which the maximum cavitation amplitude in PSNE-treated animals exceeded that of the control animal treated on the same day showed a further significant ($p < 0.001$) increase in acoustic emissions (mean 1766.8 ± 1292.1 AU). MRI data for the same set of sonications showed the maximum temperature rise was significantly increased ($p < 0.01$) in the PSNE-treated animals (mean $29.6 \pm 9.3^\circ\text{C}$ with PSNE vs. $24.3 \pm 6.6^\circ\text{C}$ without). Similarly, the average temperature rise in the subset of PSNE sonications where cavitation exceeded the respective control was significantly higher ($p < 0.001$) than the control animals (mean $32.4 \pm 10.1^\circ\text{C}$). These results suggest that cavitation-enhanced heating was achieved using PSNE and further illustrate the need for robust monitoring to account for *in vivo* variability.

Discussion

In this study the use of simultaneous PAM and MRI was investigated for monitoring of PSNE-enhanced tumor ablation.

Passive acoustic mapping of the acoustic emissions recorded during sonication showed that the amplitude of emissions was increased in the presence of PSNE and that this could be resolved spatially to an area close to the focus of the FUS transducer. As the electrical power was varied the amplitude of emissions in the control animals began to rise slightly, suggesting that the higher power levels tested were approaching the endogenous cavitation threshold in the tumors and thus reducing the scope for benefit of PSNE. Using PSNE the mean amplitude of acoustic emissions increased only slightly when the electrical power was increased, suggesting that once vaporization of the nanoemulsion is achieved there is little additional benefit to further increasing the power. By contrast, the spatial extent over which the emissions appeared in the maps did continue to increase with electrical power, since the portion of the FUS beam that exceeded the vaporization threshold increased. Previous *in vivo* work without spatial mapping also found little variation in the amplitude of acoustic emissions above the cavitation threshold of PSNE (Kopechek et al. 2014), as well as our recent *in vitro* PAM study (Crake et al. 2017).

Over time, the PAM data showed that the amplitude of acoustic emissions rapidly increased in the presence of PSNE, particularly over the first ~2.5 seconds of sonication. This suggests that PSNE were quickly vaporized into microbubbles, which subsequently resulted in increased cavitation. The rapid increase in emissions suggests that PSNE were primarily vaporized acoustically, i.e. acoustic droplet vaporization (Shpak et al. 2014). After the initial peak the amplitude of acoustic emissions fell but was maintained above control sonications for the 40s duration of treatment, suggesting sustained vaporization and cavitation. Previous work has shown sustained response of PSNE on similar timescales *in vitro* (Crake et al. 2017) and *in vivo* (Rapoport et al. 2011). These results may be contrasted to previous work with PAM using microbubbles in a tumor model which showed a sharp peak in acoustic emissions of a few seconds duration followed by rapid clearance (Choi et al. 2014; Crake et al. 2016). Due to their nanoscale size PSNE are able to extravasate and accumulate in tumors (Kopechek et al. 2013). As a result, a combination of liquid droplets, vaporized microbubbles and so-called ‘daughter bubbles’ (Borden et al. 2005) produced following cavitation may persist in the focal region for longer than would occur using agents such as conventional microbubbles which are confined to the vasculature and thus rapidly cleared by blood flow. It should be noted that previous *in vitro* studies have demonstrated that a continued ‘cascade’ of cavitation is possible using conventional microbubbles if the time between pulses is too short for bubble dissolution (Chen et al. 2003), and that microbubble cavitation can be optimized by matching the pulse repetition rate to the flow conditions (Choi and Coussios 2012); however, translation of such results remains a challenge due to poor *in vivo* stability and limited knowledge of vessel geometry and flow rate. In one trial the amplitude of acoustic emissions was observed to rise towards the end of sonication: this is likely due to thermally-triggered vaporization, as the maximum temperature and size of the heated region (and hence likelihood of this exceeding the vaporization temperature for PSNE) would be greatest at this time.

The focal index metric considered in this paper as a normalized indication of the spatial extent of acoustic emissions showed broadly similar trends to the amplitude data. For PSNE-enhanced sonications the focal index initially rose rapidly to a peak value, indicating a sharp peak in activity in the acoustic maps, before falling to a lower level that was maintained for the sonication duration. Generally speaking the focal index for PSNE sonications exceeded that in the control treatments; however, the variability (e.g. standard deviation relative to the mean) between trials appeared larger than that from the amplitude of the maps. Comparison of the amplitude and focal index showed that the two were closely correlated, with clear separation between the PSNE and control cases. Although it was based on normalized maps the focal index was nonetheless correlated with the peak amplitude of the maps within the narrow range of parameters considered in these experiments, so can at best be considered pseudo-independent. It is unsurprising that the amplitude and spatial metrics are linked, as individual maps produced during sonication with a focused transducer would be expected to be clustered around the transducer focus. In addition, it should be noted that the maps produced using PAM represent a convolution of the true distribution of acoustic sources with a point spread function (Gyongy and Coussios 2010b). It should also be stressed that we employed metrics extracted from the PAM (and MRI) data in this study primarily for the purpose of providing a form of pseudo-quantitative analysis over multiple sonications. In practice, even without any such analysis, the difference in amplitude and/or ‘focal-like’ behavior was visually obvious in real-time between the PSNE-enhanced and control experiments, which itself demonstrates that the technique could be useful for detection of cavitation-enhanced heating. While the analysis considered here was relatively basic, it demonstrates that PAM was able to detect changes in the amplitude and spatial distribution of the acoustic emissions as a result of PSNE vaporization and cavitation behavior *in vivo*, which suggests that it could be useful for monitoring of cavitation-enhanced therapy.

MRI thermometry showed that both the maximum temperature and size of the heated region increased using PSNE compared to the control experiments without PSNE. Coupled with the acoustic data above, this finding suggests that cavitation-enhanced heating occurred. The size of the heated region increased with electrical power using PSNE but maximum temperature achieved did not significantly increase. This observation is consistent with threshold effects, as observed in previous work (Kopechek et al., 2014) - i.e. provided the power is sufficient to result in vaporization/cavitation of PSNE, increasing the power further may not result in significantly greater amplitude of cavitation enhanced heating. However, as the power is increased the size of the focal region that exceeds the pressure threshold for vaporization and/or cavitation to occur will increase, such that cavitation-enhanced heating could occur over a larger volume. These effects were also consistent with the PAM data, which showed little effect on the peak amplitude of acoustic emissions but greater spatial extent in the maps as the electrical power was increased.

Over time, the MR thermometry data showed that the greatest difference in heating between PSNE and control sonications occurred during the first few seconds of each treatment. The rate of heating over the first 10–15s of each exposure was approximately 3–4 times greater using PSNE than the control experiments. While the temporal resolution of the MRI data is limited compared to the acoustic data, the rapid jump in temperature during sonication did occur around the same time as the maximum amplitude of acoustic emissions in the PAM

data, suggesting that the increase in heating rate was as a result of vaporization and cavitation of the nanoemulsion. In both PSNE and control sonications the rise in temperature followed an approximately logarithmic relationship in which the rate of heating slowed over time, as the temperature at the focus approached thermal equilibrium. After sonication ceased the temperature in both control and PSNE-treated animals fell at a similar rate as expected. We did not monitor the full duration of cooling in this study as our primary interest was in heating enhancement, however each sonication was carried out in a new area of tumor which would be less affected by previous heating, after a delay of several minutes to allow for manual movement of the transducer.

A basic comparison of the PAM and MRI data was conducted in this paper utilizing the maximum temperature at each MRI time point for each of the sonications and amplitude of the acoustic emissions at the equivalent time points in the PAM data. This initial comparison of the real-time data showed that the PSNE-enhanced sonications could be clearly separated from the control experiments based on either the amplitude of heating or acoustic emissions. Repeating the analysis after performing a cumulative sum on the PAM data up to each of the time points showed an improved correlation with temperature, suggesting a 'dose' effect. Previous work has shown that cavitation dose metrics can be related to temperature rise (Farny et al. 2010), as well as various other bioeffects such as ultrasound-driven hemolysis (Chen et al. 2003), thrombolysis (Datta et al. 2008) or blood-brain barrier disruption (Tung et al. 2010). Equivalently, inspection of the heating (Fig. 5) and acoustic emissions (Fig. 3) over time suggests that the *rate* of heating is correlated with the amplitude of acoustic emissions, as the maximum heating rate occurred towards the beginning of sonication when the acoustic emissions were at their strongest. However, in practice integration of the PAM data over time may be preferred as this averages out incoherent noise in the data, while taking the derivative of the thermometry data would serve to amplify the (already noisy) thermal data.

To confirm that thermal lesions were produced, tissue samples were examined histologically. The results confirmed that thermal lesions were produced in this study, in agreement with previous work (Kopechek et al. 2014). Repetition of the optimal conditions from the parametric part of the study in additional animals showed substantial variability in results primarily due to variation in tumor growth. Variable heating response in this tumor model have been reported previously (McDannold et al. 1998). Nonetheless, the average maximum heating achieved in the 94 sonications at 32W in this paper was significantly increased using PSNE than without. The average magnitude of the effect of PSNE on heating was similar to previous work (Kopechek et al. 2014) which observed ~5°C difference between PSNE and control sonications. The power level used in this study was higher than that quoted in previous work (~30W vs. ~5W); as a result, the maximum absolute temperature elevations observed here were also higher (up to 50°C with PSNE vs. ~20°C control). Our intention was to use higher power to increase the size of lesions with a view to later developing the technique for ablation of whole tumors, however this also had the effect of increasing the 'baseline' effect in the control animals. It should be noted that this observation is largely a consequence of the tumor model used here and is not in itself detrimental to the potential utility of PSNE: due to their accessibility surface tumors are arguably among the easiest of targets in which to achieve high rarefactional pressures and thus produce cavitation, such

that this could be achieved in some cases even without injection of nuclei. It stands to reason that if PSNE can result in an increased likelihood of cavitation in surface tumors then they could also do so in more challenging (e.g. deeper) targets in which endogenous cavitation is less likely.

The study as presented has several limitations, which could be improved upon in future work. First, passive imaging made use of a linear array, so was only able to capture the acoustic emissions in a single plane. Due to space constraints within the scanner this plane was positioned at an angle with respect to the FUS propagation direction. The acoustic maps thus represent a projection of the distribution of acoustic emissions onto this plane (which has finite elevational slice thickness) of the field of view of the array. The use of a larger aperture 3D array (O'Reilly et al. 2014) would allow reconstruction of the emissions in an arbitrary plane, which could then be more readily compared with the MRI data. Improvements in array design and signal conditioning (e.g. hardware filtering, amplification) and in the extension cable used to enable operation within the MRI could also improve the mapping. Second, the VX2 tumor model showed substantial variability in response (i.e. propensity of cavitation, and resulting temperature rise) between the multiple sonications in each animal, and particularly between multiple animals. Coupled with the study design using largely fixed exposure conditions (discussed below), this showed that while PSNE certainly appeared to increase the likelihood of cavitation-enhanced heating, the exact degree to which this occurred could not be simply predicted from the exposure conditions. Such variability is not without precedent - for example (McDannold et al. 1998) observed 'untypically low temperature rise' in some sonications for a given set of exposure conditions using a similar tumor model. Such variability is not especially surprising in an *in vivo* study, as the properties of the tumors, which grow following cell injection, cannot be precisely controlled as they depend upon the host animal. In particular, tumor-specific factors which were not measured in this study such as the depth, perfusion, degree of necrosis, and position relative to other structures such as fat, bone, or boundaries within the muscle could all impact wave propagation, absorption, and heating within tumors. As this affects the likelihood of cavitation, the effects of small changes in tissue properties are further magnified in the acoustic emissions and heating. Finally, a significant limitation of this study is the small number of animals which were used. As this was a small-scale pilot study, our goal was to extract the maximum amount of information from the smallest number of animals, the results of which could then be used to inform a future larger study. Given the variability in tumor response observed between animals in this study, such a larger study would be more appropriate to account for inter-animal variability. The design of this study also relied upon an initial parametric stage in which the exposure conditions were varied, followed by repetition of the 'optimum' conditions in subsequent animals. The number of sonications that could be conducted in each tumor was additionally limited, as we wished to place each in a new non-overlapping area to avoid retreating areas which could be depleted of PSNE from a previous sonication. Clearly for practical use the exposure conditions should be adjusted for each individual sonication either manually by the operator (for example by gradually ramping the power) or by a suitable automated controller based on feedback from the acoustic emissions and MRI data. In essence this is precisely the point of this study, as if the effects on tissue could be accurately predicted then we would have no

need for real-time monitoring with either PAM or MRI. Nonetheless, despite these limitations this study demonstrates the utility of combined PAM and MRI for monitoring of ultrasound-mediated tumor ablation, and provides further evidence for the ability of PSNE to promote cavitation-enhanced heating.

Conclusions

Simultaneous passive acoustic mapping and MRI thermometry were demonstrated in an *in vivo* rabbit tumor model, with and without injection of phase-shift nanoemulsions to promote cavitation. Vaporization and cavitation of the nanoemulsions could be detected using PAM, which led to enhanced temperature rise assessed using MRI thermometry. The maximum heating assessed from MRI was well correlated with the integrated acoustic emissions over time from PAM, illustrating cavitation-enhanced heating. Examination of tissue showed thermal lesions which were larger in presence of PSNE, in agreement with the thermometry data. Repetition of fixed exposure conditions over multiple animals showed substantial variation in cavitation and heating enhancement, illustrating the need for real-time monitoring. The results demonstrate the utility of combined PAM and MRI for monitoring of tumor ablation and provide further evidence for the ability of PSNE to promote cavitation-enhanced lesioning.

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Figure 1:

Setup for dual mode imaging of focused ultrasound (FUS) ablation. (a) Experimental setup (not to scale). A 1.5MHz single element FUS transducer and ultrasound (US) imaging array were aligned and attached to a 3-axis positioning system. A tumor-bearing rabbit was coupled to the transducer using water. A receive-only surface coil was used for MR imaging. (b) Electrical setup. A two-step waveform generated by two arbitrary waveform generators (AWG) was combined, amplified and delivered to the FUS transducer. The US array was connected to a research ultrasound platform (Verasonics) via an extension cable and MR penetration panel. (c) Example images showing (left) T2-weighted MRI for treatment planning: the FUS transducer, tumor and approximate focus position are shown; (mid) MRI phase image showing focal heating; (right) B-mode US image: the outline of the thigh and approximate focus position are shown. Scale bar = 1cm. (d) Timing diagram. A trigger signal from the MR scanner was used to synchronize B-mode, passive acoustic mapping (PAM) and FUS pulses. A new MR image was obtained every 2.6s. The routine shown was repeated for the duration of each treatment.

Figure 2:

Effect of phase-shift nanoemulsion (PSNE) on passive acoustic mapping as a function of electrical power. (a) Acoustic maps showing the summation of acoustic emissions with and without PSNE. The imaging array is located at the top of the images and focused ultrasound propagation is out of the page. (b) Peak amplitude from summed acoustic maps for 3 trials of each condition ($n=3$ repeats $\times$ 3 powers $\times$ 2 animals). (c) Averaged spectra from raw ultrasound data with and without PSNE at 32W. The frequency axis is normalized with respect to the FUS transmission frequency of 1.5MHz.

Figure 3:

Effect of phase-shift nanoemulsion (PSNE) on passive acoustic mapping over time (32W electrical power). (a) Acoustic maps showing the summation of acoustic emissions for subsets of the data with and without PSNE. The imaging array is located at the top of the images and focused ultrasound propagation is out of the page. The control maps are shown with two different color scales for comparison. (b) Top: peak amplitude and Mid: focal index over time for 3 repeats of each condition ($n=3$ repeats $\times$ 2 animals); Bottom: comparison of the two metrics over all of the time points.

Figure 4:

Effect of phase-shift nanoemulsion (PSNE) on MRI-observed temperature rise as a function of electrical power. (a) Temperature maps about the focus from sonications with and without PSNE at three power levels. Maps at the time point of maximum heating are shown. Focused ultrasound propagation is from bottom to top. Scale bar = 1cm. (b) Top: Maximum temperature rise; Bottom: width (FWHM = full-width half-maximum) of the heated region exceeding 50°C for 3 trials of each condition (n=3 repeats $\times$ 3 powers $\times$ 2 animals).

Figure 5:

Effect of phase-shift nanoemulsion (PSNE) on MRI-observed temperature rise over time (32W electrical power). (a) Temperature maps about the focus at selected time points for sonications with/without PSNE. Focused ultrasound propagation is from bottom to top. Scale bar = 1cm. (b) Maximum temperature rise in all temperature maps for 3 trials of each condition (n=3 repeats $\times$ 2 animals).

Figure 6:

Correlation between MRI and passive acoustic mapping (PAM) metrics. Maximum temperature rise from MRI vs. (a) peak amplitude from PAM at each time point, (b) cumulative peak amplitude from PAM up to each time point, (c) focal index from PAM at each time point. Data from 266 time points are included ($n=3$ repeats $\times$ 3 powers $\times$ 2 animals $\times$ c. 15 time points per treatment). The PAM and MRI data were matched based upon the time stamps of each recording; PAM data were averaged over the time between adjacent MR images. ρ =Pearson's correlation coefficient.

Figure 7:

No phase-shift nanoemulsion (control): histological section of the thigh muscle of a rabbit with a VX2 tumor after treatment. The lesion has an appearance typical for thermal lesions. A well-demarcated area of tumor (green arrows) has three zones of tissue injury: a thermally coagulated central core (a), surrounding ring of edematous tissue (b), and adjacent tumor region (c) with some signs of thermal damage, and small tumor cell foci adjacent to the necrotic area. Tumor cells in the central core are shrunken with condensed nuclei. Beyond the thermal lesion (d), the intact tumor exhibits a relatively solid pattern. The tumor cells are round or oval, are rich in cytoplasm and show many mitotic figures (M). Dotted areas (a-c) are enlarged on right to show detail; detail image (d) of normal tumor was taken from a region away from any thermal lesions. Scale bar = 1mm for main image, 100 μ m for detail images.

Figure 8:

With phase-shift nanoemulsion (PSNE): histological section of the thigh muscle of a rabbit with a VX2 tumor after treatment. The lesion has an appearance typical for thermal lesions. Note the large areas of coagulative necrosis (a) in the central lesion core, the cavities produced by vaporization of the nanoemulsion (b), multiple hemorrhages resulted from inertial cavitation (c), hemorrhagic necrotic areas (d), and small tumor foci adjacent to the central lesion core (e). Dotted areas (a-e) are enlarged on right to show detail. Scale bar = 1mm for main image, 100 μ m for detail images.

Figure 9:

Cumulative amplitude of acoustic emissions and maximum heating at 32W electrical power (n=3 animals with PSNE, 3 control). (a) Peak amplitude from cumulative passive acoustic maps (PAM) over duration of each sonication. (b) Peak temperature rise at time of maximum heating from each sonication. 'Control' indicates sonications without phase-shift nanoemulsion (PSNE) (n=54 sonications), 'PSNE' indicates sonications with PSNE (n=52 sonications), 'PSNE + Cav' indicates the subset of PSNE sonications in which acoustic emissions exceeded those in the corresponding control animal (n=19 sonications). Stars indicate statistical significance assessed using 1-way ANOVA followed by Tukey-Kramer multiple comparison testing (***=p<0.001, **=p<0.01, n.s.=not significant).

Table 1:

Summary of experimental protocol.

Animal #	Group	Power	# Sonications	Purpose
1	Control	23, 32, 42W	9	Power ramp to select conditions
2	PSNE	23, 32, 42W	9	
1 (cont'd)	Control	32W	12	Additional sonications at 'best' conditions (to limit of tumor size)
2 (cont'd)	PSNE	32W	3	
3	Control	32W	13	Repeat sonications to account for variation in response within each tumor, and between animals
4	PSNE	32W	14	
5	Control	32W	20	
6	PSNE	32W	26	

Table 2:

MR imaging parameters. TR = repetition time, TE = echo time, FOV = field of view, NEX = number of exposures, ETL = echo train length, BW = bandwidth.

Sequence	Use	TR (ms)	TE (ms)	Flip angle	FOV (cm)	Matrix size	Thickness (mm)	NEX	ETL	BW (kHz)
T2-weighted fast spin echo	Treatment planning	4500	84.9	90°	12–20	256 × 256	3	1	8	15.6
Fast spoiled gradient echo	Temperature maps	19.5	13.0	30°	16–20	256 × 128	3	1	1	15.6