A phantom for visualization of three-dimensional drug release by ultrasound-induced mild hyperthermia

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Purpose: Ultrasound-induced mild hyperthermia has advantages for noninvasive, localized and controlled drug delivery. In this study, a tissue-mimicking agarose-based phantom with a thermally sensitive indicator was developed for studying the spatial drug delivery profile using ultrasound-induced mild hyperthermia.

Methods: Agarose powder, regular evaporated milk, Dulbecco's phosphate-buffered saline (DPBS), n-propanol, and silicon carbide powder were homogeneously mixed with low temperature sensitive liposomes (LTSLs) loaded with a self-quenched near-infrared (NIR) fluorescent dye. A dual-mode linear array ultrasound transducer was used for insonation at 1.54 MHz with a total acoustic power and acoustic pressure of 2.0 W and 1.5 MPa, respectively. After insonation, the dye release pattern in the phantom was quantified based on optical images, and the three-dimensional release profile was reconstructed and analyzed. A finite-difference time-domain-based algorithm was developed to simulate both the temperature distribution and spatial dye diffusion as a function of time. Finally, the simulated dye diffusion patterns were compared to experimental measurements.

Results: Self-quenching of the fluorescent dye in DPBS was substantial at a concentration of 6.25×10^{-2} mM or greater. The transition temperature of LTSLs in the phantom was 35 °C, and the release reached 90% at 37 °C. The simulated temperature for hyperthermia correlated with the thermocouple measurements with a mean error between 0.03 ± 0.01 and 0.06 ± 0.02 °C. The R^2 value between the experimental and simulated spatial extent of the dye diffusion, defined by the half-peak level in the elevation, lateral and depth directions, was 0.99 (slope = 1.08), 0.95 (slope = 0.99), and 0.80 (slope = 1.04), respectively, indicating the experimental and simulated dye release profiles were similar.

Conclusions: The combination of LTSLs encapsulating a fluorescent dye and an optically transparent phantom is useful for visualizing and modeling drug release *in vitro* following ultrasound-induced mild hyperthermia. The coupled temperature simulation and dye-diffusion simulation tools were validated with the experimental system and can be used to optimize the thermal dose and spatial and temporal dye release pattern. © 2013 American Association of Physicists in Medicine. [<http://dx.doi.org/10.1118/1.4813299>]

Key words: ultrasound, mild hyperthermia, tissue-mimicking phantom, low temperature sensitive liposome, FDTD

1. INTRODUCTION

The development of techniques to enhance drug delivery in cancer, while minimizing systemic toxicity, is highly desirable. Ultrasound is attractive for inducing hyperthermia and controlling delivery as it is noninvasive, localized, and free of ionizing radiation.^{1,2} Techniques employing ultrasound in cancer therapy include high-intensity focused ultrasound (HIFU) ablation,² magnetic resonance-guided focused ultrasound surgery (MRgFUS),³ gene transfer,⁴ and hyperthermia.^{5–7} In mild hyperthermia, the temperature is maintained between 37 and 42 °C to enhance vascular permeability⁸ and drug delivery efficiency.^{7,9} Closed-loop control of ultrasound-induced mild hyperthermia has been demonstrated both *in vitro* and *in vivo* with an error in temperature of less than ± 0.1 °C.¹⁰

Drug delivery systems, including polymers, liposomes, peptides, and protein-based materials, have been developed to decrease system toxicity and increase treatment efficacy.⁹ Liposomes are phospholipid-based drug delivery vehicles that can entrap both hydrophobic and hydrophilic drugs.¹¹ Traditional temperature sensitive liposomes (TTSLs) undergo a phase transition between 41.5 and 42.5 °C, above which the cargo can cross the bilayer membrane; dipalmitoylphosphatidylcholine (DPPC) is a major component of TTSLs, and therefore, dominates the phase transition temperature.^{12,13} However, the delivery efficiency of TTSLs is low because of the slow speed and limited volume of release. Therefore, modified formulations incorporating lysolipids in the lipid shell have been developed and are referred to as low temperature sensitive liposomes (LTSLs).^{13–15} Monopalmitoylphosphatidylcholine (MPPC) is a commonly used lysolipid that

is incorporated to facilitate rapid release in LTSLs.^{12–16} An-yarambhatla and Needham demonstrated that the presence of MPPC at a molar ratio of 6%–20% significantly increased the rate and volume of release in PBS compared with the release from the liposomes without MPPC. Further, the phase transition temperature decreased by $\sim 1^\circ\text{C}$ as the MPPC concentration increased from 0% to 20%.^{12,13,17} In mouse serum at 42°C , 100% cargo release was detected from 10% MPPC-containing LTSLs in less than 10 s, whereas 25% release was observed from TTSLs after 10 min of incubation.¹⁶ Local drug delivery resulting from the combination of MPPC-containing LTSLs and mild hyperthermia has been shown to be feasible and can improve the treatment of local lesions.^{13,15,18,19}

Fluorescence and bioluminescence are useful imaging modalities for examining drug release from carrier vehicles.^{15,20} In the work reported here, the near-infrared (NIR) fluorescent dye bis-1,1'-(4-sulfobutyl)indotricarbocyanine-5,5'-dicarboxylic acid (SIDA, molecular weight = 741) was employed as a model drug molecule.²¹ The structure of SIDA is similar to indocyanine green (ICG), but SIDA is highly hydrophilic (partition coefficient: SIDA: <0.005 ; ICG: >200), which reduces plasma protein binding and clearance from the liver.^{21,22} The self-quenching effect of SIDA at high concentrations reduces the total emitted fluorescence, and therefore, SIDA is a useful probe for drug release studies.

Although ultrasound-induced drug delivery systems are becoming more common, methods to determine the three-dimensional (3D) drug release profile following insonation, thus optimizing both the vehicle and the ultrasound exposure, have not been developed. A suitable phantom material has an acoustic absorption, attenuation, and speed of sound which are similar to soft tissue, in addition to being optically transparent at NIR wavelengths. Water-based gels using gelatin have been investigated as a material for mimicking the properties of soft tissues. The addition of n-propanol adjusts the speed of sound, and graphite is used to alter the attenuation coefficient and ultrasonic scattering properties.²³ However, the low melting point of gelatin (e.g., 32.5°C for calfskin-derived gelatin) makes the phantom less stable with temperature fluctuations in the environment, and the introduction of a small amount of formaldehyde is required for stability.²⁴ Another solution is to substitute agarose for gelatin in the phantom construction. The high melting point of agarose keeps the phantom stable to a temperature of $\sim 80^\circ\text{C}$.²⁴ Madsen *et al.* developed agarose-based tissue-mimicking phantoms in which evaporated milk was added to enhance absorption. Since evaporated milk has a high attenuation coefficient ($\sim 0.8\text{ dB/cm/MHz}$) with a speed of sound that is typical of biological fluids (1547 m/s), the milk solution is diluted to achieve an attenuation coefficient ranging from 0.3 to 0.7 dB/cm/MHz, and the speed of sound is increased by adding n-propanol. The final speed of sound in the work of Madsen was near 1540 m/s.²⁵

The objective of this paper is to present a new method for visualizing drug release resulting from ultrasound-induced mild hyperthermia. LTSLs incorporating MPPC in the shell

and encapsulating SIDA dye in the core were embedded in an agarose-based tissue mimicking material and exposed to ultrasound-induced mild hyperthermia. NIR optical imaging was used to quantify the release patterns. Thermal diffusion and dye diffusion were coupled in a finite-difference time-domain (FDTD) algorithm for simultaneously predicting temperature distribution, drug release, and drug diffusion. Simulations were then compared to experimental measurements.

2. MATERIALS AND METHODS

2.A. Synthesis and calibration of SIDA fluorescence dye

The synthesis of SIDA followed previously reported methods.^{21,26} In brief, 5-carboxy-1-(4-sulfobutyl)-2,3,3-trimethyl-3*H*-indolenine was synthesized from hydrazinobenzoic acid in two steps. Further reaction with *N*-[5-(phenylamino)-2,4-pentadienyldiene]aniline monohydrochloride (Sigma-Aldrich Co. LLC., St. Louis, MO) yielded SIDA as a dark blue crystal after recrystallization. The isolated SIDA was characterized by ^1H NMR and electron spray ionization mass spectrometry (ESI). The peak absorbance and emission wavelengths of SIDA were measured in a Dulbecco's phosphate-buffered saline (DPBS) solution by scanning over a wavelength range of 600–850 nm (1-nm step) using a plate reader (Infinite M1000 Pro, Tecan Group Ltd., Männedorf, Schweiz). The bandwidth of the plate reader (full-width half-maximum, FWHM) was 5 nm. Calibration of SIDA fluorescence at the peak wavelengths was determined by serial dilution in DPBS from a 0.5 mM solution, resulting in 13 concentrations ranging from 0.5 mM to 1.22×10^{-4} mM. Before being encapsulated into LTSLs, SIDA was dissolved in DPBS at a concentration of 25 mM, and the pH value was adjusted to 7.4.

2.B. LTSLs

DPPC (1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine), DSPE-PEG2000 (1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxy(polyethylene glycol)-2000] (ammonium salt)), and MPPC (1-palmitoyl-2-hydroxy-*sn*-glycero-3-phosphocholine) were purchased from Avanti Polar Lipids (Alabaster, AL). The molar ratio of the LTSL formulation in this study was DPPC:DSPE-PEG2000:MPPC = 86:4:10.¹⁵ LTSLs were synthesized at a size of 100 nm and with the encapsulation of SIDA at a concentration of 25 mM (see the supplementary material³⁶).

2.C. Phantom production

The phantom production procedure was modified from a protocol developed by Madsen *et al.*²⁵ The volume ratio of regular evaporated milk (Sunny Select, Super Store Industries, Lathrop, CA), DPBS, n-propanol (Sigma-Aldrich Co. LLC.), and LTSLs was 50:46:4:1, respectively. Agarose powder (Agarose Low Gel Temperature, MP Biomedicals LLC., Solon, OH) and silicon carbide powder (HSC1200, d50 = 6 μm , Superior Graphite, Chicago, IL) were combined

in a weight/total-volume ratio of 2% and 1.5%, respectively. First, DPBS and agarose powder were mixed and microwaved until completely dissolved (90 °C), followed by mixing with evaporated milk preheated to 90 °C. The agarose-milk solution was then placed in a vacuum for 10 min for degassing. After degassing, the solution was microwaved again to boiling, and silicon carbide powder and n-propanol were added. The solution was stirred until cooled to 30 °C, when the gel-filtered LTSL solution was added. The LTSLs were gently mixed into the cooled agarose-milk solution for 5 min, and then the phantom solution was poured into a 25.4-mm cubic mold and allowed to solidify for at least 3 h at room temperature (23 °C).

In order to determine the components responsible for the reduced transition temperature of the LTSLs when suspended in the solid milk phantom, as compared with LTSLs in DPBS (Sec. 2.D.1), additional studies in DPBS were conducted. First, the release efficiency of LTSLs was examined in the 4% n-propanol solution. Second, the effect of milk fat on liposomal release was tested by comparing fat-free evaporated milk (Carnation, Nestle USA, Inc., Solon, OH) with regular evaporated milk. The fat residue in the evaporated milk was 0.08 ± 0.01 g/100 ml for the fat-free milk and 6.71 ± 0.01 g/100 ml for the regular milk (LactoScope FTIR Advanced, Delta Instruments BV, AJ Drachten, The Netherlands). Third, the fat in the regular evaporated milk was extracted by centrifugation and mixed with the fat-free evaporated milk, creating a fat-added fat-free evaporated milk as compared with the fat-free evaporated milk. Fourth, fetal bovine serum (FBS, Omega Scientific, Inc., Tarzana, CA) was used as an alternative protein-rich environment to examine the release of LTSLs. In addition, a milk filtrate without caseins, the primary proteins in milk, was tested. The concentrations of the fat-free milk, regular milk, FBS, and milk filtrate were 50%, 10%–50%, 20%–99%, and 50%–99%, respectively. Lastly, in order to understand the performance of the LTSLs in the agarose matrix, a phantom consisting of only DPBS, 2% agarose powder, and LTSLs was synthesized (see the supplementary material³⁶).

2.D. LTSL release tests

2.D.1. LTSL release in DPBS

All of the LTSL release tests described in Secs 2.D.1 and 2.D.2 were conducted in a microprocessor-controlled water bath (Precision Microprocessor Controlled 280 Series, Thermo Fisher Scientific Inc., Rockford, IL) at a fixed temperatures of 23 °C and 30–42 °C (in steps of 1 °C). Samples for each temperature point consisted of 495 μ l DPBS placed in a 500- μ l Eppendorf tube. The tube was immersed in the water bath at a fixed temperature for 12 min. After reaching thermal equilibrium, 5 μ l of gel-filtered LTSL solution was added into the preheated DPBS. The sample was heated for 3 min before being placed in a 0 °C water bath to stop release. A 100%-release reference sample was produced by mixing 485 μ l DPBS, 5 μ l LTSL solution, and 10 μ l of 10% Triton X-100 (Sigma-Aldrich Co. LLC). Fluorescence intensity at

789 nm was measured by the plate reader. The temperature at which 10% of the cargo is released is defined as the transition temperature for the results reported in this paper.

2.D.2. LTSL release in the phantom

The phantom was cut into thin slices with a thickness of 2.0 mm. Slices were sealed in water-proof plastic bags and immersed into the water bath for 15 min. Care was taken to eliminate air bubbles between the phantom slices and the bag wall in order to achieve uniform heat conduction. Due to the thin slices, thermal equilibrium was assumed to occur instantaneously after the bag was immersed in the water bath; however, an incubation time of 15 min was used to insure stable measurements. After incubation, slices were cooled in 0 °C water to stop release, and a fluorescence imaging system (Maestro 2, Cambridge Research & Instrumentation, Inc., Woburn, MA) was used to image the slices. The bandwidth of the Maestro system was 40 nm and a near-infrared filter set was used in imaging, in which a broad range of excitation light was applied with the images acquired at the 790 nm emission wavelength (nearest to 789 nm). In this test, the fluorescence response at 42 °C was used as the 100%-release reference. The fluorescence intensity as a function of treatment temperature was fit with a cumulative Gaussian curve stepped by 0.1 °C intervals using a least squares method coded in MATLAB (The MathWorks, Inc., Natick, MA). All of the exposure times and wavelength settings of the Maestro system were consistent for all of the measurements.

2.E. Calibration of SIDA fluorescence as a free dye in the phantom

In order to create the calibration curves between the fluorescence intensity and varied SIDA concentration as a free dye in the phantom, phantoms with serial SIDA dilutions ranging from 0.5 mM to 1.22×10^{-4} mM were cured, sliced, and imaged by the Maestro system. A linear fit was performed on the measured fluorescence intensity versus SIDA concentration.

2.F. Ultrasound-induced dye release

2.F.1. Ultrasound system design and experimental setup

The ultrasound system used in this study was reported previously (see the supplementary material³⁶).¹⁰ Briefly, the ultrasound system consisted of a Siemens Antares ultrasound scanner (Sonoline Antares, Siemens Medical Systems, Inc., Issaquah, WA), a custom dual-mode linear array transducer, and a PC equipped with temperature control system (Fig. 1). The dual-mode linear array transducer has three parallel arrays, including two outer arrays operating at 1.54 MHz used in this study.^{10,27} During insonation, a proportional-integral-derivative (PID) controller with temperature feedback from a needle-type thermocouple (HYP-1, Omega Engineering, Inc., Stamford, CT) controlled the duty cycle of the acoustic pulses by varying the pulse repetition frequency (PRF).

FIG. 1. Experimental system. A custom dual-linear array transducer (1.54 MHz center frequency) insonified a 25.4-mm cubic tissue-mimicking agarose phantom. Temperature feedback from a needle thermocouple was sent to a PC for closed-loop control of an Antares ultrasound scanner using LabVIEW. The phantom was immersed in a 3-L 32 °C warm water bath, and a 15 °C transducer cooling chamber was placed between the transducer and the warm water bath. The dimensions in this figure are not to scale.

All of the insonation experiments in this paper were conducted in a 3-L water tank in which degassed water was filled and maintained at 32 °C using a stirrer and a heater (Model 71, PolyScience, Niles, IL) (Fig. 1). The cubic phantom created in Sec. 2.C was immersed in the water bath, and the beam focus was located at the center of the phantom. The needle thermocouple was placed 6 mm distal to the insonation focus along the center axis of the beam. Heating of the phantom was accomplished using 100-cycle acoustic pulses at 1.54 MHz, with a varied PRF up to a maximum of 5 kHz. At the maximum PRF, the total acoustic power (TAP) was 2.0 W. The TAP was measured using a radiation force balance (UPM-DT-1AV, Ohmic Instruments Co., Easton, MD), and the peak-negative acoustic pressure was 1.5 MPa measured using a needle hydrophone (Müller-Platte needle probe, Onda Inc., Sunnyvale, CA), which corresponds to a mechanical index of 1.2. Between the transducer and the warm water tank, a cooling chamber was filled with 15 °C water to keep the transducer surface cool. The acoustic windows between the transducer, cool water chamber, and warm water tank were made from polyethylene film with a thickness of $\sim 70 \mu\text{m}$. Insonation was started when the core phantom temperature reached an equilibrium temperature of 32 °C. Single-beam insonation with a single focal location was applied for this study. Insonation continued until the temperature at the location of the needle thermocouple reached 35, 37, or 39 °C.

2.F.2. Optical imaging and reconstruction

Immediately upon reaching the desired temperatures, the phantom was cooled in 0 °C water. The phantom was then sliced every 2.0 mm along the depth direction and imaged in the Maestro system (Fig. 2). The first phantom slice (furthest from the insonation focus) was treated in a 41 °C water bath for 15 min for a 100%-release reference. The spatial resolution of the optical imaging system was $0.07 \times 0.07 \text{ mm}$.

Quantification of fluorescence and reconstruction of the release profile was accomplished in the following steps. First, the normalized fluorescence intensity for each image pixel

FIG. 2. Flow chart for experiments and simulations in this study. LTSLs were synthesized and a fluorescent dye was encapsulated. A tissue-mimicking agarose phantom was created with homogeneously mixed LTSLs. Insonation was applied until thermocouple readings reached desired temperatures of 35, 37, or 39 °C. Fluorescence was quantified by an optical system. FOCUS was then used to simulate the 3D acoustic pressure field, the 3D intensity field was calculated, and an FDTD algorithm was used for simulation of temperature and dye diffusion.

was first calculated according to the following equation:

$$FI_{\text{norm}} = \frac{FI_{\text{post}} - FI_0}{FI_{100} - FI_0}, \quad (1)$$

where FI_{norm} is normalized fluorescence intensity, FI_{post} is measured fluorescence intensity after heating, FI_{100} is average intensity for 100% release, and FI_0 is the average fluorescence intensity of the sample without heating, i.e., the autofluorescence of the phantom.

Second, the fluorescence intensity map obtained from each slice was smoothed using a $0.4 \times 0.4 \text{ mm}$ 2D smoothing filter (smooth2a, Greg Reeves, MATLAB Central open exchange community, The MathWorks, Inc.). Finally, slices were stacked into a 3D array and spline interpolation was applied over depth to generate isometric voxels (e.g., converting $0.07 \times 0.07 \times 2.0 \text{ mm}$ voxels to $0.07 \times 0.07 \times 0.07 \text{ mm}$ voxels). Because the range of measured fluorescence intensity in this study was a linear function of dye concentration with a zero intercept, the normalized fluorescence intensity map was equivalent to a normalized dye concentration map. From the filtered and interpolated 3D normalized fluorescence intensity maps, the 50%-release spans in the elevation, lateral, and depth directions were measured.

2.G. Ultrasound beam simulation

The Fast Object-oriented C++ Ultrasound Simulator (FOCUS) was used in this study to simulate single-beam insonation from the dual-mode linear array. The acoustic simulation was divided into two parts: generation of 2D acoustic pressure fields near the dual-mode linear array using the Fast Near-field Method (FNM),²⁸ and the formation of 3D acoustic pressure volumes by propagating the predicted 2D

field along the depth direction using the Angular Spectrum Approach (ASA).²⁹ Beam simulation parameters were as follows: number of rows of the transducer: 2; spacing between the two rows: 12.9 mm; element numbers in each row: 64; spacing between two adjacent elements: 0.6 mm; length of an element: 6 mm; width of an element: 0.56 mm; focal length of the row: 35 mm; operating frequency: 1.54 MHz; speed of sound: 1552 m/s. The normalized 3D intensity field was calculated by squaring the magnitude of the normalized 3D acoustic pressure field.

2.H. Simulation of temperature and dye diffusion

For temperature simulation, the FDTD method was applied to solve Pennes' bioheat transfer equation:³⁰

$$\rho C_p \frac{\partial T}{\partial t} = \nabla \cdot (K \nabla T) + q - b(T - T_b), \quad (2)$$

where ρ is density, C_p is the specific heat for the tissue, T is temperature ($^{\circ}\text{C}$), t is time (s), K is the thermal conductivity, q is the heat deposition source given by $2\alpha I$, α is the acoustic absorption, I is the acoustic intensity, b is the blood perfusion constant, and T_b is the blood temperature. A second-order FDTD approximation to Eq. (2) was used in the simulation,^{10,20,31} and the parameters used were: $\rho = 1059 \text{ kg/m}^3$ (measured); $C_p = 4000 \text{ J/kg}^{\circ}\text{C}$ (approximated); $K = 0.25 \text{ W/m}^{\circ}\text{C}$ (approximated); $\alpha = 0.6 \text{ dB/cm/MHz}$ (measured); $I = 20.8 \text{ W/cm}^2$ (measured); $b = 0 \text{ W/m}^3/^{\circ}\text{C}$ (known); $T_b = 32^{\circ}\text{C}$ (measured); spatial resolution = 10^{-4} m .

The simulated temperature profile in the elevation direction has a main lobe and two prominent side lobes due to the overlapping beams. To separate the main lobe and side lobe heating, three Gaussians were fit to the temperature profile in the elevation direction at the depth corresponding to the highest temperature in the main lobe (Curve Fitting Toolbox, MATLAB). The peak amplitudes of the decomposed Gaussians were used as the temperature increases contributed by the main lobe and the two side lobes.

Dye diffusion in 3D was simulated simultaneously with the temperature simulation process based on the diffusion equation:

$$\frac{\partial \phi(r, t)}{\partial t} = D \nabla^2 \phi(r, t), \quad (3)$$

where $\phi(r, t)$ is concentration of dye at location r and time t . D is the diffusion coefficient which is estimated to be $5 \times 10^{-6} \text{ cm}^2/\text{s}$ in the simulation. As in the temperature simulation, a second-order FDTD approximation to Eq. (3) was used to simulate dye diffusion.

The procedure for simulating temperature and dye diffusion is illustrated in Fig. 3. The temperature profile (T) was calculated using the second-order FDTD approximation involving the simulated 3D acoustic intensity field and thermal parameters detailed above. Indexes N and n represent total iteration steps and an individual step, respectively. FI and d are fluorescence intensity from the dye release and the corresponding dye concentration at each step, respectively. Conversion between temperature and normalized fluorescence intensity (C1), and conversion between fluorescence intensity and dye concentration (C2), were used to calculate the dye concentration. $d_{\text{diff}}(n-1, n)$ and $d_{\text{diff}}(n, n+1)$ represent the difference in dye concentration between steps n and $n+1$, respectively. At step n , when the accumulated dye concentration profile after diffusion was calculated ($d_{\text{accu}}(n)$), the difference in dye concentration between steps n and $n+1$, $d_{\text{diff}}(n, n+1)$, was added to $d_{\text{accu}}(n)$ and the result was $d_{\text{accu}}(n+1)$. The FDTD algorithm was applied on $d_{\text{accu}}(n+1)$ to calculate the diffused dye profile, $D_{\text{accu}}(n+1)$, and so on. After N iterations, the final accumulated dye concentration profile ($D_{\text{accu}}(N)$) was normalized by the 100%-release dye concentration and compared with experimental results.

FIG. 3. Detailed procedure for calculating dye concentration profile from simulation of temperature and dye diffusion. The temperature profile (T) was calculated using the intensity field of the beam in the second-order FDTD approximation of the bioheat equation. Indexes N and n represent total iteration steps and an individual step, respectively. FI is fluorescence intensity from the dye release and d is the corresponding dye concentration. Conversion between temperature and normalized fluorescence intensity (C1), and conversion between fluorescence intensity and dye concentration (C2), were used to calculate the dye concentration. $d_{\text{accu}}(n)$ and $D_{\text{accu}}(n)$ represent the accumulated dye concentration profiles at step n before and after the diffusion calculation, respectively. At step n , when the accumulated dye concentration profile after diffusion was calculated ($d_{\text{accu}}(n)$), the difference in dye concentration between steps n and $n+1$, $d_{\text{diff}}(n, n+1)$, was added to $d_{\text{accu}}(n)$ and the result was $d_{\text{accu}}(n+1)$. The FDTD algorithm was applied on $d_{\text{accu}}(n+1)$ to calculate the diffused dye profile, $D_{\text{accu}}(n+1)$, and so on. After N iterations, the final accumulated dye concentration profile ($D_{\text{accu}}(N)$) was normalized by the 100%-release dye concentration and compared with experimental results.

sity (C1), and conversion between fluorescence intensity and dye concentration (C2), were used to calculate the dye concentration at every iteration step. Here, the experimentally obtained calibration curves relating fluorescence intensity from released dye to temperature and fluorescence intensity to dye concentration were combined to give dye concentration as a function of temperature. The difference in dye concentration in contiguous steps was then calculated (d_{diff}) and added to the accumulated dye concentration profile at the previous step (d_{accu}). The new accumulated dye concentration profile (d_{accu}) was then passed into the second-order FDTD approximation of the diffusion equation to obtain an updated profile. Thus, the notations $d_{\text{accu}}(n)$ and $D_{\text{accu}}(n)$ represent the accumulated dye concentration profiles at step n before and after the diffusion calculation performed by the FDTD algorithm, respectively. At step n , when the accumulated dye concentration profile after diffusion was calculated ($d_{\text{accu}}(n)$), the difference in dye concentration between steps n and $n+1$, $d_{\text{diff}}(n, n+1)$, was added to $d_{\text{accu}}(n)$ and the result was $d_{\text{accu}}(n+1)$. Again, the FDTD algorithm was applied on $d_{\text{accu}}(n+1)$ to calculate the diffused dye profile, $D_{\text{accu}}(n+1)$, and so on. After N iterations, the final accumulated dye concentration profile ($D_{\text{accu}}(N)$) was normalized by the 100%-release reference, and then the 50%-release spans in the elevation, lateral, and depth directions were measured and compared with experimental results.

FIG. 4. Calibration of SIDA fluorescence intensity as a free dye in DPBS. Thirteen serial dilutions from a 0.5 mM solution resulted in a concentration ranging from 0.5 mM (the leftmost point) to 1.22×10^{-4} mM (the rightmost point). The highest fluorescence intensity occurred at a concentration of 6.25×10^{-2} mM ($8 \times$ dilution of 0.5 mM), indicating a substantial self-quenching effect at the SIDA concentration at or above 6.25×10^{-2} mM. ($n = 3$; error bars show $\pm$ one standard deviation)

3. RESULTS

3.A. Characterization of SIDA in DPBS after synthesis

The overall yield of SIDA synthesis was 47%. The peak absorbance and emission wavelengths of SIDA in DPBS were measured as 756 and 789 nm, respectively. Fluorescence intensity in DPBS buffer showed that the self-quenching effect was substantial for a concentration at or above

6.25×10^{-2} mM ($8 \times$ dilution of 0.5 mM) (Fig. 4), indicating that the encapsulated SIDA (at 25 mM) was self-quenched in the LTSLs prior to release.

3.B. Characterization of LTSLs

The diameter of LTSLs was 106.2 ± 15.6 and 105.3 ± 15.4 nm ($n = 5$, error shows $\pm$ one standard deviation) based on intensity weighting and volume weighting, respectively. The LTSL yield was 14.1 ± 1.3 mg lipid from 15 mg of starting lipid, or $94 \pm 8.7\%$ ($n = 3$, error shows $\pm$ one standard deviation). Assuming our LTSL membrane was a single (unilamellar) bilayer and the area of one DPPC molecule was $\sim 65 \times 10^{-2}$ nm²,^{32,33} for a liposome diameter of ~ 105 nm, the number of total LTSLs created from 1 mg total lipid with the composition in this study (DPPC:DSPE-PEG2000:MPPC = 86:4:10) was estimated to be 7.1×10^{12} .

In the solid milk phantom, release was minimal ($<3\%$) below 33°C . A dramatic increase in release occurred at 35°C and reached 90% at 37°C [Fig. 5(a), solid line]. Based on the least-squares method, the cumulative Gaussian function was fit to the experimental results with an average absolute error of $2.6 \pm 2.5\%$ of the normalized fluorescence intensity [Fig. 5(a), dash line]. Based on the fitting, the release approached $\sim 100\%$ when the temperature was above 40°C [Fig. 5(a), inset]. The same transition temperature was observed for both DPPC and LTSL liposomes in the solid milk phantom (Table I). In DPBS, less than 3% of the contents were released when the temperature was below 39°C , and rapid release occurred above 39°C and reached 93% at 42°C [Fig. 5(a), dotted line]. Calibration between the fluorescence intensity

TABLE I. Summary of the diluents tested and resulting release temperatures. In each case MPPC or DPPC-containing liposomes (1% of the final volume) were suspended in a phantom or solution with the constituents varied between groups. The groups are ordered from low to high release temperature. The temperature of 10% release is defined as the transition temperature in the remainder of the paper.

Lowest melting point lipid species		Diluent groups	Temperature of 10% and (80%) release (°C)	Temperature of full cumulative release ^c (°C)	Form	Addition of n-propanol
MPPC	50% fat-free milk + 49% DPBS		32 (36)	40	Solution	No
MPPC	DPBS phantom ^a		34 (38)	41	Solid	No
MPPC	50% (fat-free milk + fat) ^b + 49% DPBS		35 (39)	42	Solution	No
DPPC	50% regular-milk + 4% n-propanol + 45% DPBS, phantom form		35 (37)	40	Solid	Yes
MPPC	50% regular-milk + 4% n-propanol + 45% DPBS, phantom form		35 (37)	40	Solid	Yes
MPPC	10% regular milk + 89% DPBS		37 (40)	40	Solution	No
MPPC	20% regular milk + 79% DPBS		37 (40)	40	Solution	No
MPPC	50% regular milk + 49% DPBS		37 (40)	40	Solution	No
MPPC	99% DPBS		39 (41)	42	Solution	No
MPPC	4% n-propanol + 95% DPBS		39 (41)	43	Solution	Yes
MPPC	20% FBS + 79% DPBS		39 (41)	41	Solution	No
MPPC	50% FBS + 49% DPBS		39 (41)	41	Solution	No
MPPC	99% FBS		39 (41)	42	Solution	No
MPPC	50% milk filtrate (without casein) + 49% DPBS		39 (41)	42	Solution	No
MPPC	99% milk filtrate (without casein)		39 (41)	42	Solution	No

Note: The formulation of the MPPC liposomes was DPPC:DSPE-PEG2000:MPPC = 86:4:10 (mol.%). The formulation of the DPPC liposomes was DPPC:DSPE-PEG2000 = 96:4 (mol.%). All of the milk described here was evaporated milk. "Phantom" indicates that the experiment was conducted in a solid phantom, otherwise the experiment was conducted in solution.

^aThe phantom composition was 2% agarose (weight/volume%) in DPBS.

^bThe fat was extracted from regular evaporated milk.

^cMaximum cumulative release in the temperature range between 23 and 44°C .

FIG. 5. Calibration of fluorescence intensity from LTSL release in phantom (a) and from free dye in phantom (b); release efficiency of LTSLs in different solutions (c) and SDS-PAGE analysis of fat-free evaporated milk and milk filtrate (d). (a) LTSL release in the phantom was demonstrated from 23 to 42 °C (solid line, this is also the *conversion C1* in Fig. 3). The release intensity with 42 °C-treatment was used as 100% release efficiency. There was little release below 33 °C, and the intensity increased dramatically from 35 °C and reached 90% at 37 °C. LTSL release in DPBS showed a rapid increase from 39 °C (dotted line), indicating that the transition temperature of LTSLs in the phantom is lower than that in DPBS. *Inset*, Zoom-in of the curve between 36 and 42 °C. The release of LTSLs approached ~100% when the temperature was above 40 °C. A cumulative Gaussian function (dash line) was used to fit the curve by a least-squares method. (b) Correlation between fluorescence intensity of free dye and dye concentration (*conversion C2* in Fig. 3) in phantom showed high linearity. (c) Release efficiency of LTSLs in different solutions for a comparison of transition temperature. LTSLs in DPBS (dash line), 99% FBS (dotted line), and 99% milk filtrate (black solid line) showed a transition temperature of 39 °C, while LTSLs in the regular evaporated milk (gray solid line) and fat-free evaporated milk (dashed-dotted line) showed a transition temperature of 37 and 32 °C, respectively. (d) MW: Molecular weight ladder; M: fat-free evaporated milk; F: milk filtrate from the fat-free evaporated milk. Bands of the caseins, β -lactoglobulin (β -Lg) and α -lactalbumin (α -La) appeared in the lane M at molecular weight of ~30, 18, and 14 kDa, respectively. In the lane F, bands of the β -Lg and α -La were distinguishable, and the band corresponding with the caseins was weak. Both lanes M and F were loaded with 30 μ g of total protein [In (a)–(c): $n = 3$; error bars show $\pm$ one standard deviation].

and SIDA concentration showed high linearity [Fig. 5(b), $R^2 = 0.99$] when the dye concentration was under 1.5×10^{-2} mM (corresponding to fluorescence intensity of 0.15 scaled counts/s). In this study, the fluorescence intensity obtained from the phantom slices was in the linear region of the calibration curve. Therefore, the conversion in this study between fluorescence intensity and SIDA concentration was linear.

3.C. LTSL performance with alternative phantom constituents

The release of LTSLs in the 4% n-propanol solution showed a transition temperature of 39 °C (Table I). Selected

release curves as a function of temperature in the regular evaporated milk, fat-free evaporated milk, FBS, and milk filtrate showed that FBS did not change the transition temperature of the LTSLs, while the regular evaporated milk and the fat-free evaporated milk resulted in transition temperatures of 37 and 32 °C, respectively [Fig. 5(c)]. The 50% fat-added fat-free evaporated milk demonstrated a higher transition temperature (35 °C, Table I) than the 50% fat-free evaporated milk (32 °C). When caseins were removed from the fat-free evaporated milk, the transition temperature returned to 39 °C [Fig. 5(c), black solid line]. Comparing the liposomal release in the 50% regular evaporated milk environment in the solid [Fig. 5(a), solid line] and solution forms

FIG. 6. Beam profile of single-beam insonation. (a) 3D profile. 3D dimensions of the main lobe were 1.3, 0.9, and 7.0 mm in the elevation, lateral, and depth directions, respectively. The 50%-intensity side lobe had a volume of 0.5% of the main lobe, and the highest intensity of side lobes was 51% of the maximum main lobe intensity. (b) Beam pattern in the depth-elevation plane shows the relative positions of the three lobes, where the contours were the boundaries of 50% intensity. The transducer surface was located at zero on the depth axis.

[Fig. 5(c), gray solid line], the transition temperature was 2°C lower in the solid form (resulting in a transition temperature of 35°C). The liposomal release in the DPBS phantom (DPBS + 2% agarose) showed a 5°C reduction in the solid phantom form (34°C) compared to the transition tempera-

ture in the DPBS solution (39°C). A full comparison of all of the transition temperatures examined in this study is listed in Table I.

The major milk proteins, including caseins, β -lactoglobulin (β -Lg) and α -lactalbumin (α -La), show protein bands in the SDS-PAGE at molecular weights of ~ 30 , 18, and 14 kDa, respectively.³⁴ The SDS-PAGE analysis in our study showed obvious bands of caseins, β -Lg, and α -La in the lane of fat-free evaporated milk. In the lane of milk filtrate, β -Lg and α -La were visible and the casein signal was greatly reduced, indicating our filtration procedure successfully removed the caseins from the fat-free evaporated milk [Fig. 5(d)].

3.D. Simulation of the acoustic beam

The simulated beam profile showed a main lobe and two side lobes in the depth-elevation plane (Fig. 6). The dimensions of the 50%-intensity main lobe were 1.3, 0.9, and 7.0 mm in the elevation, lateral, and depth directions, respectively. Side lobes due to aligning the two linear arrays in parallel had 50%-intensity dimensions of 0.2, 0.1, and 0.9 mm in the elevation, lateral, and depth directions, respectively. The center of the side lobe was 2.7 mm from the center of the main lobe in the elevation direction [Fig. 6(b)]. The 50%-intensity side lobe had a volume of 0.5% of the main lobe, and the highest intensity in the side lobes was 51% of the maximum main lobe intensity. The contours in Fig. 6(b) denote boundaries of 50% intensity.

FIG. 7. Optical images and 3D reconstruction of an example case in the 37°C controlled heating group. (a)–(j) Optical images of phantom slices showed 790 nm fluorescence intensity of SIDA resulting from the release of LTSs. (a) and (j) were first and last phantom slice with detectable fluorescence, respectively. Each slice had a thickness of 2.0 mm, and spacing between the slices was 2.0 mm. The bar in (a) represents 10 mm in length. (k) and (l) 3D reconstruction of the 50%-release profile. Contribution of the side lobes was observed in the depth-elevation plane (k, dash arrows).

FIG. 8. Analysis of 3D release profile in the experiments. Distinct 50%-release spans were shown in the elevation, lateral, and depth directions. Summary of the 50%-release spans were in Table II ($n = 5$; error bars show $\pm$ one standard deviation).

3.E. Optical imaging, reconstruction, and analysis

Optical intensity images of phantom slices in the lateral-elevation plane obtained at 790 nm validate the release of SIDA dye from LTSs following ultrasound heating [Figs. 7(a)–7(j)]. Figures 7(a) and 7(j) correspond to the first

and last phantom slice with detectable fluorescence intensity, respectively. The zero-coordinate location in the depth direction corresponded to the phantom boundary nearest to the transducer surface. The dye release morphology in each slice was elliptical in contour with a major axis along the elevation direction, consistent with the elevation side lobes. In an example case in the 37°C controlled heating group, the 3D reconstruction of the 50%-release span yielded dimensions of 7.6, 6.2, and 16.5 mm in the elevation, lateral, and depth directions, respectively [Figs. 7(k) and 7(l)]. The contribution of side lobes was clearer in the 3D reconstruction profile [Fig. 7(k)]. In the depth-lateral plane, the release profile was widest in the near-field.

The average dimensions of the 50%-release spans for all of the controlled heating groups are summarized in Fig. 8 and Table II. The 50%-release spans in all groups were distinct in the three axes (elevation, lateral, and depth directions).

3.F. Temperature simulation

The temperature simulations showed good agreement with the thermocouple measurements. In an example case in the 37°C controlled heating group, the simulated temperature at the location of the thermocouple tip [Fig. 9(a), dash line]

FIG. 9. Simulation results and effects of the main lobe and the side lobes. (a) In an example case in the 37°C controlled heating group, the simulated temperature in the thermocouple tip (dash line) closely matched the thermocouple measurements (solid line) with an average error of 0.04°C. (b) Gaussian decomposition of the simulated temperature profile (black solid line) in the elevation direction in a case of 37°C controlled heating. Three Gaussian distributions were initialized in the curve fitting tool, and the results for the main lobe (dash line) and the two side lobes (gray solid lines) were demonstrated. The amplitude of the decomposed Gaussian curves was used as the temperature increase contributed by the main lobe and the side lobes. (c) Temperature increase induced by mainlobe and sidelobes. In (c) $n = 5$; error bars show $\pm$ one standard deviation.

TABLE II. Summary of experimental and simulated dye-release spans. ($n = 5$; error bars show $\pm$ one standard deviation).

Controlled heating group ($^{\circ}\text{C}$)	Experimental span (mm)			Simulation span (mm)		
	Elevation	Lateral	Depth	Elevation	Lateral	Depth
35	5.4 ± 0.1	3.9 ± 0.3	13.5 ± 1.2	5.8 ± 0.1	3.1 ± 0.1	11.6 ± 0.1
37	7.5 ± 0.4	6.2 ± 0.4	16.0 ± 0.5	7.9 ± 0.2	6.1 ± 0.1	17.6 ± 0.1
39	9.5 ± 0.4	8.3 ± 0.5	18.6 ± 0.4	10.5 ± 0.3	8.7 ± 0.2	20.4 ± 0.1

closely matched the thermocouple measurements [Fig. 9(a), solid line]. The error obtained by averaging the absolute error point-to-point from both curves was 0.04°C . The mean of the average errors (MAE) in the 35, 37, and 39°C controlled heating groups was 0.03 ± 0.01 , 0.04 ± 0.01 , and $0.06 \pm 0.02^{\circ}\text{C}$, respectively ($n = 5$, error shows $\pm$ one standard deviation), and the maximum average error in this study was 0.6°C .

Evaluation of the temperature profile in the elevation direction demonstrated a distance of 2.6 ± 0.1 mm between the main lobe and the side lobes [Fig. 9(b)]. All of the controlled heating groups resulted in a similar distance ($n = 15$, error shows $\pm$ one standard deviation), which is approximately equal to the distance of 2.7 mm obtained in the simulated beam profile [Fig. 6(b)]. The temperature increase contributed by the side lobes was approximately 60–70% of the temperature elevation from the main lobe [Fig. 9(c)]. The temperature increase induced by the main lobe and the side lobes for the 35, 37, and 39°C control heating groups were 6.3 ± 0.2 , 8.9 ± 0.2 , $10.8 \pm 0.6^{\circ}\text{C}$, and 3.9 ± 0.0 , 5.7 ± 0.2 , $7.9 \pm 0.3^{\circ}\text{C}$, respectively.

The 3D temperature simulation in the phantom is shown in Fig. 10 for the depth-elevation plane [Fig. 10(a)] and depth-

lateral plane [Fig. 10(b)]. Here, the contribution from the side lobes is apparent in the depth-elevation plane [Fig. 10(a)].

3.G. Dye diffusion simulation

The 3D dye concentration profiles were similar to the 3D temperature simulations. In an example case in the 37°C controlled heating group, the 50% normalized dye concentration profile was measured as 7.7, 6.1, and 17.5 mm in the elevation, lateral, and depth directions, respectively [Figs. 10(c) and 10(d)], compared to the experimental results of 7.6, 6.2, and 16.5 mm in the elevation, lateral, and depth directions, respectively (Sec. 3.E). The dimensions of the 50% normalized dye concentration profile for all of the simulations are summarized in Table II.

3.H. Relationship between experiments and simulation

The overlaid contours of the 50% dye-release profile in experiments and the predicted 50% dye concentration profile from simulation show similarities in the depth-elevation

FIG. 10. Simulated temperature profiles for 37°C controlled heating at the thermocouple tip (a) and (b) and dye concentration profiles (c) and (d) in the depth-elevation (a) and (c) and depth-lateral (b) and (d) planes. (a) and (b) In an example case, contours of temperature distribution were marked on both depth-elevation and depth-lateral planes and the highest temperature was 41.8°C . (c) and (d) In the same case, simulation of normalized dye concentration profile showed a 50% dye release span of 7.7, 6.1, and 17.5 mm in elevation, lateral, and depth directions, respectively.

FIG. 11. Overlaid simulated and experimental contours. (a)–(c) The 50% dye-release contours from experiments (black) and 50% normalized dye-concentration contours from simulation (gray) in the depth-elevation plane in the 35, 37, and 39 °C controlled heating groups, respectively. (d)–(f) The 50% contours in the depth-lateral plane in the 35, 37, and 39 °C controlled heating groups, respectively.

and depth-lateral planes (Fig. 11). Comparisons between the experimental and simulated data in the elevation, lateral, and depth directions resulted in R^2 values of 0.99, 0.95, and 0.80, respectively, showing a high correlation (Fig. 12). The lower correlation in the depth direction likely resulted from insufficient resolution in the phantom slicing (Fig. 11). The slopes of the experimental and simulated data were 1.08, 0.99, and 1.04 in the elevation, lateral, and depth directions, respectively, indicating that the simulation and experimental results were similar.

4. DISCUSSION AND CONCLUSIONS

A direct 2D mapping between the release of magnetic resonance (MR) contrast agents from TTSLs and a temperature map was demonstrated by de Smet *et al.* using MR-guided high intensity focused ultrasound-induced hyperthermia.³⁵ However, the spatial drug distribution resulting from liposomal release depends upon several factors, including the target temperature, treatment duration (i.e., thermal dose), and the diffusion properties of drugs in the target. In this work, we proposed a simulation model that considers the dynamics of liposomal release with changes in temperature, as well as the drug diffusion spatially and temporally, and thus a more

accurate prediction of drug release can be accomplished. In addition, a new and stable tissue phantom model for examining ultrasound-stimulated release of drug molecules from LTSLs was developed. The stability and thermal activity of LTSLs were preserved for at least three weeks, and those properties were also maintained within the phantom. The phantom is optically transparent at NIR wavelengths, and the self-quenching effect of the dye enables visualization of release. Using a therapeutic ultrasound array, we demonstrated controlled heating within the phantom, and we quantified dye release from LTSLs and diffusion within the phantom material. The high correlation between dye release profiles in experiment and simulation indicate that the phantom and the simulation tool may be applied to validate hyperthermia from complex array geometries or different insonation protocols.

The LTSLs are unique in their rapid release and tunable phase transition temperatures that can be used to achieve release over a wide range of physiologically relevant temperatures. Below the phase transition, all lipids are in gel form and the compact alignment between lipids provides a low bilayer permeability.¹² During the phase transition, lipids transform to the liquid crystalline phase, and the packing defects resulting from the coexistence of gel and liquid crystalline phases enhance permeability.¹² In this study, very low levels of release from LTSLs were observed when the environmental temperature was below the phase transition temperature [Figs. 5(a), dotted line]. At the phase transition temperature, rapid release occurred; when the temperature exceeded the phase transition (42–44 °C), release decreased as expected (not shown) due to the re-packing of lipids in the liquid crystalline phase, resulting in decreased membrane permeability.

4.A. Self-quenching effect

The self-quenching effect of the hydrophilic SIDA dye combined with encapsulation facilitates rapid and sensitive optical detection in this study. LTSLs with encapsulated SIDA yield a fluorescence density too low to be detected. A calculation of the encapsulated volume of SIDA results in a percentage of $\sim 0.04\%$ within the phantom. According to the serial dilution test in Fig. 4, the fluorescence intensity of SIDA at this concentration is $\sim 0.03\%$ compared to the 100% normalized fluorescence intensity. When all dye is released, a normalized fluorescence intensity of approximately 78% is achieved (1.01×10^{-2} mM), which is a ~ 2600 -fold increase in fluorescence intensity compared with 0.03%. The difference in fluorescence intensity before and after release shows that the self-quenching effect plays an important role.

4.B. Properties and effect of the phantom composition

Based on Figs. 5(c) and 5(d), the removal of caseins restored the transition temperature of LTSLs in the 50% fat-free evaporated milk to 39 °C, demonstrating that the caseins

FIG. 12. Comparison of 50% dye-release spans between experiments and simulation. R^2 value of 0.99, 0.95, and 0.80 were calculated in the elevation, lateral, and depth directions, respectively. Solid lines are linear trend lines with intercept set at zero, and dashed lines represent the $x = y$ ideal trend.

contributed to the reduction in the transition temperature of LTSLs. The analysis of evaporated milk using the FTIR spectrometer showed that the casein concentration in the regular and fat-free evaporated milk were 5.06 ± 0.01 and 6.03 ± 0.02 g/100 ml, respectively. The higher concentration of casein in the fat-free evaporated milk could lower the transition temperature of LTSLs as compared to the regular evaporated milk.

Although the higher transition temperature in regular evaporated milk, as compared to fat-free evaporated milk, shows the effect of fat on liposomal stability, additional evidence was obtained by directly adding fat into the fat-free evaporated milk. This addition resulted in a higher transition temperature, indicating that milk fat stabilizes the LTSLs (Table I).

The solid phantom environment reduced the transition temperature of LTSLs [Figs. 5(a) and 5(c), Table I] whether the phantom contained milk or 99% DPBS, indicating that the interaction between the agarose polymer structure and the LTSLs could be a cause of the decreased stability. Within the solid phantom, DPPC liposomes and LTSLs showed the same transition temperatures (35°C), and therefore, incorporation of lysolipid did not impact the transition temperature in a solid phantom. Also, *n*-propanol had no effect on the transition temperature. Further investigation is required to under-

stand the mechanisms for the increased or reduced permeability of the liposomal shells.

4.C. Low-temperature agarose gel

The use of a low-temperature agarose gel in this study makes it possible to preserve intact encapsulated LTSLs in the phantom. During congealing, the phantom does not start to solidify until the temperature drops below 26°C , thus the phantom is an appropriate material to embed LTSLs with a transition temperature of $35\text{--}40^\circ\text{C}$ without inducing accidental release. In this study, we mixed LTSLs with the phantom solution when the temperature was 30°C . Optical imaging shows that unexpected release due to the phantom production process is negligible, and a uniform distribution of LTSL particles was accomplished. After the phantom is solidified, the melting point is 65°C (data from the manufacturer), which provides stability in the temperature range of mild hyperthermia.

4.D. Simulation of the temperature and resulting release

Our simulation of the beam indicated that the majority of the heat was delivered through the main lobe, as expected. In addition, the side lobes resulting from the crossing of parallel

beams in the dual-mode linear array geometry led to a smaller temperature increase [Figs. 9(b) and 9(c)]. The temperature elevation contributed from the side lobes extended effective hyperthermia region in the elevation direction. Experimental and simulated results for the extent of dye release were similar in the elevation, lateral, and depth directions. Improvement in the limited resolution in depth due to slicing, including a thinner slicing technique or slicing at different orthogonal planes, will increase the accuracy of estimating the drug-release profile.

The dye-diffusion simulation tool was added to temperature simulations for the dual-mode linear array presented previously.¹⁰ The coupled nature of the temperature-dependent dye diffusion provides an approximate model of drug release in tissue. Information is then extracted at each stage, e.g., thermal dose may be extracted from the time-dependent temperature, and time-dependent drug concentration is extracted from the diffusion estimate. Whereas our study primarily focused on developing a tool for studying ultrasound-hyperthermia mediated drug diffusion *in vitro*, it represents an important step towards developing a more accurate model for drug release *in vivo*.

4.E. Summary

We have shown that identical liposomal formulations present different transition temperatures with changes in the environment (e.g., phantom versus solution). However, once the release kinetics are determined and calibrated, the experiments and simulations developed here can be applied to evaluate release with a given set of conditions. A solid phantom, as described here, is required for the evaluation of the spatial profile of drug concentration and changes in the profile resulting from ultrasound. Liposomal formulations can be evaluated in solution to preserve the transition temperature and to compare the effect of formulation on release. Further, the methodology shown in this paper is translatable to other drug-release indicators. Encapsulating radio-isotopes or MR-compatible contrast agents into LTSLs would facilitate 3D validation for drug release distribution with PET or MR imaging systems, respectively.

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