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Observation and modulation of the dissolution of histotripsy-induced bubble clouds with high-frame rate plane wave imaging

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Abstract

Focused ultrasound therapies are a noninvasive means to ablate tissue. Histotripsy utilizes short ultrasound pulses with sufficient tension to nucleate bubble clouds that impart lethal strain to the surrounding tissues. Tracking bubble cloud dissolution between the application of histotripsy pulses is critical to ensure treatment efficacy. In this study, plane wave B-mode imaging was employed to monitor bubble cloud motion and grayscale at frame rates up to 11.25 kHz. Minimal changes in the area or position of the bubble clouds were observed 50 ms post excitation. The bubble cloud grayscale was observed to decrease with the square root of time, indicating a diffusion-driven process. These results were qualitatively consistent with an analytic model of gas diffusion during the histotripsy process. Finally, the rate of bubble cloud dissolution was found to be dependent on the output of the imaging pulse, indicating an interaction between the bubble cloud and imaging parameters. Overall, these results highlight the utility of plane wave B-mode imaging for monitoring histotripsy bubble clouds.

Keywords

histotripsy; bubbles; plane wave imaging

Introduction

Histotripsy is an ablative form of therapeutic ultrasound under development for the treatment of several pathological conditions (Khokhlova et al 2015). A clinical trial was recently completed to assess the safety of histotripsy technology to alleviate the symptoms of benign prostatic hyperplasia (Schuster et al 2018). Tissue is not damaged directly by the acoustic field, but through the formation and mechanical activity of bubble clouds generated within the focal region (Maxwell et al 2012, Bader et al 2019). A critical dose of bubble activity is required to liquefy the target tissue, and diagnostic ultrasound is employed for image guidance (Hall et al 2007, Bader et al 2016b, 2018a).

Tracking bubble cloud dissolution between the application of pulses is critical to ensure uniform tissue liquefaction throughout the focal zone. Standard B-mode ultrasound utilizes a

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sequence of transmitted pulses for image acquisition (Szabo 2004). Several milliseconds are required to execute the imaging sequence, over which time the bubble cloud can undergo significant changes and multiple histotripsy pulses may be applied (Xu et al 2007, Maxwell et al 2011b). Plane wave B-mode transmits and receives with all element in parallel, shortening the imaging sequence to less than 1 ms. The frame rates for plane wave B-mode imaging cannot track volumetric oscillations of individual bubbles at frequencies utilized for therapeutic ultrasound (Khokhlova et al 2011, Maxwell et al 2011b, Vlaisavljevich et al 2015, Raymond et al 2016), but is sufficient to assess the dynamics of the bubble cloud as a whole. In addition to monitoring the presence of bubbles, imaging pulses can also be used to instantiate particular bubble behaviors (Radhakrishnan et al 2013). High output pulses destroy microbubble contrast agents (Couture et al 2012), and may also be useful for mitigating residual histotripsy-induced bubble clouds (Wang et al 2012).

The objective of this study was to monitor histotripsy bubble cloud dissolution in a tissue-mimicking phantom with plane wave B-mode imaging. The potential to modulate the bubble cloud behavior with high acoustic output from the plane wave B-mode imaging pulse was also explored. To predict the bubble cloud behavior, an analytic model based on histotripsy-induced bubble expansion (Bader and Holland 2016) and a zero-order diffusion equation (Eller 1965) was developed to compute the time for bubble dissolution. Predictions from the analytic model were compared to numerical computations (Bader and Bollen 2018) and observations in this study.

Methods

Tissue phantom production

Tissue phantoms composed of agarose (3.7 g), deionized water (147.2 ml), n-propanol (12.8 ml), and evaporated milk (240 ml) were manufactured utilizing an established protocol (Bader et al 2016a, 2018a). Commercially available evaporated milk was gently stirred on a hot plate to reach a final temperature of 55 °C. Agarose powder (A9539 Sigma-Aldrich Co. St. Louis, MO, USA) was dissolved into a 0.2 μ m filtered, deionized water (NANOPure Diamond, Barnstead International, Dubuque, IA, USA) and n-propanol solution by heating in 30 s increments in a microwave (700 W power) until clear. The heated agarose/n-propanol solution was degassed in a heated (55 °C) ultrasonic cleaning bath for 30 min while continuously evacuating at 50 kPa. The degassed agarose/n-propanol solution was combined with the heated evaporated milk, poured into a mold, and allowed to solidify at 5 °C overnight. This formation has previously been shown to replicate the density, sound speed, elastic modulus, and frequency-dependent acoustic attenuation spectra of ex vivo prostate tissue (Bader et al 2016a).

Histotripsy insonation

A 1 MHz, 8-element annular array source that generated bubble activity via shock scattering (Maxwell et al 2011b) with a 10 cm aperture and 9 cm focal length (Imasonic, Voray sur l'Ognon, France) was utilized in this study. All elements of the transducer were simultaneously driven in parallel by a custom class D amplifier and matching network (Hall and Cain 2006). The acoustic field and focal pressure of the transducer were recorded with a

fiber optic hydrophone (FOPH 2000, RP Acoustics, e.K., Leutenbach, Germany) (Bader et al 2016b). Under linear conditions (~ 500 kPa peak positive/negative pressure), the -6 dB width of the main lobe was $10 \text{ mm} \times 1.8 \text{ mm} \times 1.8 \text{ mm}$. As the waveform steepened due to the combined effects of nonlinear propagation and diffraction, the -6 dB width of the main lobe was $5.4 \text{ mm} \times 0.6 \text{ mm} \times 0.6 \text{ mm}$ for the positive pressure, and $13.4 \text{ mm} \times 2.4 \text{ mm} \times 2.4 \text{ mm}$ for the negative pressure (70 MPa/11 MPa for the peak positive/negative pressure). Peak negative pressures up to 18.3 MPa could be measured in the focal zone (figure 1). Direct calibration of the histotripsy transducer was not possible for peak negative pressures greater than 18.3 MPa due to cavitation, and the focal pressures were estimated following Maxwell et al (2013). Pulses of 5, 10, or 20 μs duration were delivered with peak negative pressures of 12, 18, or 23 MPa, derated based on the acoustic attenuation coefficient of the phantom assuming a 1 MHz fundamental frequency (0.46 dB cm^{-1}) (Bader et al 2016a). The derated peak positive pressures were estimated following Canney et al (2010) to be 77, 105, and 123 MPa. The largest pressure level, beyond the transducer calibration, was estimated based on numerical simulation (Roznitskiy et al 2017). The insonation conditions employed in this study span those previously employed for histotripsy (Maxwell et al 2012, Khokhlova et al 2015).

Experimental protocol

Phantoms were degassed for two hours in deionized water at a partial pressure of 50 kPa, after which they were affixed to a three-axis positioning system (TDC001, Thorlabs Inc, Newton, NJ, USA) immersed in a tank of degassed (20% dissolved oxygen), filtered (10 μm pore size) water. Bubble clouds generated by 5 μs histotripsy pulses in the water tank were visualized with plane wave B-mode images acquired with an L11-4v imaging array (Verasonics, Inc., Kirkland, WA, USA) driven by a research ultrasound scanner (Vantage 128, Verasonics, Inc., Kirkland, WA, USA). The azimuthal axis of the imaging plane coincided with the acoustic axis of the histotripsy source (figure 2(A)). The bubble cloud location in the image was denoted as the free-field focus of the histotripsy transducer (Vlaisavljevich et al 2013b). The phantom was then positioned such that the histotripsy transducer focus was at a depth of 2 cm into the phantom (figure 2(A)), and histotripsy pulses were applied. The exposure conditions were randomized for each insonation location within the phantom. The distance between the imaging array and the histotripsy focus was fixed at 3 cm (figure 2(A)). During the excitation, a plane wave B-mode imaging sequence was triggered by the histotripsy electronics (figure 2(B)). The peak negative pressure of the plane wave B-mode pulse was assessed at a 3 cm image depth with a calibrated needle hydrophone (HNP-0400, Onda Corporation, Sunnyvale, CA, USA). Transmitted plane wave B-mode pulses with peak negative pressures between 420 kPa and 6.7 MPa were used to visualize the bubble cloud. Image frames were acquired 179 μs to 10 ms after the histotripsy excitation at a rate of 11.5 kHz, and from 10 ms to 50 ms at a rate of 1 kHz (142 frames total per insonation). Strong hyperechoic interference patterns prevented acquisition of frames prior to 179 μs post focal insonation. The 50 ms bubble cloud dissolution observation window in this study was longer than the pulse repetition periods employed commonly for *in vivo* (Maxwell et al 2011a, Darnell et al 2015, Vlaisavljevich et al 2016, 2017, Zhang et al 2017) and *in vitro* (Zhang et al 2015, Bader et al 2018, Shi et al 2018a) histotripsy studies (1–50 ms). The imaging sequence was acquired every 200th histotripsy pulse due to data

transfer limitations, and to ensure observation of a new bubble cloud when the imaging sequence was triggered. A total of 50 data sets were analyzed for each combination of histotripsy and imaging array insonation conditions ($N = 50$ for each arm, 1200 total data sets in 24 different phantoms).

Image processing

Plane wave B-mode images were downloaded and analyzed offline. The threshold grayscale value separating the bubble cloud and phantom background was determined via Otsu's method, minimizing the intraclass variance of the black and white pixels via the 'imbinarize' function in MATLAB® (The Mathworks, Natick, MA, USA) (figure 3). For each frame, the bubble cloud area and mean grayscale value were recorded. The azimuth position of the bubble cloud, reported in terms of the bubble cloud centroid, was also assessed (Haworth et al 2015). The range of the histotripsy focus was center on 30 mm in the imaging array plane (figure 2(A)). Trends of the bubble cloud area, azimuthal position, or grayscale with time were computed via the Pearson correlation coefficient.

Computations of gas diffusion during histotripsy excitation

The time-dependent bubble diameter was computed numerically using a modified version of the Gilmore equation, as described previously (Church 1989, Bader and Bollen 2018). Briefly, an adaptive fourth-order Runge-Kutta algorithm was implemented in MATLAB® (The Mathworks, Natick, MA, USA) to solve a modified version of the Gilmore model:

$$\left(1 - \frac{\dot{R}}{C}\right)R\ddot{R} + \frac{3}{2}\left(1 - \frac{\dot{R}}{3C}\right)\dot{R}^2 = \left(1 + \frac{\dot{R}}{C}\right)H + \frac{\dot{R}}{C}\left(1 - \frac{\dot{R}}{C}\right)R\frac{dH}{dR} \quad (1)$$

where R is the time dependent bubble radius, the diacritic dot denotes the temporal derivative, and C is the sound speed in the medium at the bubble wall. The enthalpy, H , is defined in terms of the medium equation of state:

$$H = \int_{P_{\infty}}^{P(R)} \left(\frac{P' + B}{A}\right)^{1/m} dP' \quad (2)$$

where A , B , and m are defined following Lastman and Wentzell (1981), and P_{∞} is the pressure far from the bubble wall. The pressure at the bubble wall, $P(R)$, is defined in terms of the surface tension σ , viscosity μ , shear modulus G , gas pressure P_g and initial bubble radius R_0 :

$$P(R) = P_g - \frac{2\sigma}{R} - \frac{4\mu\dot{R}}{R} - \frac{4G}{3}\left[1 - \left(\frac{R_0}{R}\right)^3\right]. \quad (3)$$

The gas pressure is dependent on the time-varying number of moles of gas in the bubble n , and was computed following Church (1989).

Analytic estimation of diffusion was computed based on the high-frequency zero-order solution of Fick's equation (Eller 1965):

$$n = n_0 + 8\sqrt{\pi Dt \left(\frac{R}{R_0}\right)^4} R_0^2 C_0 \left[\frac{C_\infty}{C_0} - \frac{\left(\frac{R}{R_0}\right)}{\left(\frac{R}{R_0}\right)^4} \right] \quad (4)$$

where n_0 is the initial number of moles of gas within the bubble, D is the diffusion constant, t is time, C_0 is the saturated gas concentration, and C_∞ is the gas concentration in the medium. For histotripsy-induced bubble expansion, the second term in the curly brackets is on the order of 10^{-12} , and can be neglected for even well-degassed media.

The angled brackets denote a time-averaged quantity, and can be computed analytically following Bader and Holland (2016). Over the first acoustic cycle, the time-dependent bubble radius can be estimated as:

$$R = R_0 + Vt \quad (5)$$

where the bubble wall velocity V is as described by Apfel (1981). Over the compressional phase of the histotripsy pulse, the time-averaged bubble radius is approximately equal to $R_1 \sim R_0 + V\tau_r$, where τ_r is the duration of the tensile phase of the histotripsy pulse, and the time-averaged quantity in equation (4) can be approximated as:

$$\left\langle \left(\frac{R}{R_0}\right)^4 \right\rangle \sim \left(\frac{R_1}{R_0}\right)^4 \left(1 - \frac{f_0}{2f_{eff}}\right) + \frac{1}{160} \frac{f_0}{f_{eff}} \left(\frac{V}{R_0 f_{eff}}\right)^4 \quad (6)$$

where f_0 is the fundamental frequency of the histotripsy pulse, and $f_{eff} = 1/\tau_r$ (see equation (7) in Bader and Holland (2016)). Beyond the first cycle, the time-dependent bubble radius can be approximated as $R_1 + \sqrt{\frac{2|p_{AC}|}{3\rho}} t \sim R_1 + dRt$, where p_{AC} is the time-dependent acoustic pressure waveform. The time-averaged bubble radius to the fourth power in equation (4) can thus be computed to be:

$$\left\langle \left(\frac{R}{R_0}\right)^4 \right\rangle \sim \left\langle \left(\frac{R}{R_0}\right)^4 \right\rangle_1 / \tau + \frac{f_0}{5\tau R_0^4 dR} \left[\left(R_1 + \frac{dR(\tau-1)}{f_0}\right)^5 - R_1^5 \right] \quad (7)$$

where τ is the number of cycles of the histotripsy pulse, and $\left\langle \left(\frac{R}{R_0}\right)^4 \right\rangle_1$ is the time-averaged bubble radius to the fourth power over the first cycle as evaluated by equation (6).

Utilizing equation (7) in (4), the gas content of the bubble for histotripsy-induced bubble expansion can be computed analytically. Once the number of moles n in the bubble is determined analytically via equation (4) or numerically via equation (1), the molar-dependent equilibrium radius of the bubble, R_{0n} is computed as:

$$R_{0n} = \left[\frac{3k_B T n}{4\pi \left(P_0 + \frac{2\sigma}{R_0} \right)} \right]^{1/3} \quad (8)$$

where k_B is Boltzmann's constant and T is the medium temperature. The time for passive dissolution of the gas-filled bubble assessed via equation (8) was computed following Neppiras (1980).

The following values were used in the calculation: surface tension $\sigma = 0.056 \text{ N m}^{-1}$ (Holland and Apfel 1989, Church et al 2015), dynamic viscosity $\mu = 0.005 \text{ kg(m} \cdot \text{s)}^{-1}$ (Holland and Apfel 1989, Church et al 2015), shear modulus $G = 44 \text{ kPa}$ (Bader et al 2018), and temperature $T = 293 \text{ K}$. The diffusion constant $D = 1.94 \times 10^{-9} \text{ m}^2 \text{ s}^{-1}$ was based on the diffusion of gases in agarose gels (Muhr and Blanshard 1982), and the saturated gas concentration $C_0 = 0.822 \text{ mol m}^{-3}$ was based on air dissolved in water. The gas concentration in the medium was set to 50% based on the measured dissolved oxygen concentration of the solidifying agarose/evaporated milk mixture. The diameter of the bubble nucleus, $2R_0$, was set to 20 nm which corresponds to $n_0 = 1.84 \times 10^{-17} \text{ m}^3$ of gas. The calculated maximum bubble diameter due to a histotripsy pressure waveform is weakly dependent on the bubble nucleus size, provided the nucleus diameter is greater than 20 nm (Bader and Holland 2016). Therefore, calculations in this study are an upper estimate for the maximum diameter of the bubble (Bader and Holland 2016). Validation of the analytic model was confirmed by comparing predictions of the equilibrium bubble diameter for the analytic and numerical models. The histotripsy insonation conditions for the validation computations had peak negative pressures of 14.5, 16.1, or 18.3 MPa with durations of 1, 2, 3, 4, or 5 μs . The histotripsy excitation term imbedded in P_∞ was implemented with measured pressure waveforms of the 1 MHz source (Bader and Holland 2016). The Gilmore equation is particularly apt for predicting bubble response to highly nonlinear waveforms similar to the shocked histotripsy pulses used in this study (Church 1989, Bader and Holland 2016). Additional analytic predictions were considered for conditions representative of the experimental conditions utilized in this study (5–20 μs pulse duration, peak negative pressures of 12, 18, or 23 MPa).

Results

Bubble cloud generation

Typical observations of the bubble cloud dynamics are displayed in figure 4 for plane wave pulses with a peak negative pressure of 420 kPa. For all insonation conditions, bubble clouds were generated. The azimuthal centroid of the bubble cloud moved towards the therapy source by $3.8\% \pm 3.1\%$ ($0.18 \pm 0.15 \text{ mm}$, figure 5), and the area of the bubble cloud

decreased by $13.0\% \pm 7.8\%$ ($12.6 \pm 10.1 \text{ mm}^2$, figure 6) on average over the 50 ms observation period. The changes in position and area of the bubble cloud with time were statistically significant for most insonation conditions ($p < 0.05$ except bubble cloud area for the 20 μs pulse duration with a 23 MPa peak negative pressure pulse).

Bubble cloud grayscale

The time-dependent bubble cloud grayscale is shown for all insonation conditions in figure 7. The bubble cloud grayscale changed by less than 1% over the first millisecond after application of the histotripsy pulse. Over the 50 ms observation period, the bubble cloud grayscale decreased on average by $30\% \pm 10.1\%$ for all insonation conditions. For a diffusion-driven process, the bubble size will decrease with the square root of time (Neppiras 1980). The averaged time-dependent grayscale GSV was fit in the least-squares manner to a power-law function of the form:

$$\frac{\text{GSV}}{G_0} = 1 - \alpha t^\beta \quad (9)$$

where G_0 is the grayscale value at time $t = 0$. The fitting parameters α and β , along with the goodness of fit metrics coefficient of determination and root mean squared error, are shown in table 1. Over all insonation conditions, β was found to be 0.54 ± 0.09 , and α was 0.04 ± 0.01 normalized GSV/s.

The predicted time for dissolution of the bubble cloud was computed as $1/\alpha^{1/\beta}$ using the fitting parameters of equation (9), and is shown in figure 8. At 12 MPa peak negative pressure, the bubble cloud dissolution time was similar for the 5 and 10 μs duration histotripsy pulses, but was increase by a factor of 2 for the 20 μs pulse. For peak negative pressures 18 and 23 MPa, the bubble cloud dissolution time increased with the duration of the histotripsy pulse. The bubble cloud dissolution time increased with the peak negative pressure for the 5 and 10 μs duration histotripsy pulses. For the 20 μs histotripsy pulse, the bubble cloud dissolution time was similar for the peak negative pressures 18 and 23 MPa.

Modulation of bubble cloud grayscale with imaging pulse amplitude

The time-dependent bubble cloud grayscale is shown in figure 9 parametrically with the peak negative pressure of the plane wave B-mode pulse. The histotripsy pulse utilized to generate the bubble cloud had a fixed peak negative pressure of 23 MPa. The bubble cloud grayscale decreased more rapidly with increasing output from the imaging array. For a given peak negative pressure of the plane wave imaging pulse, the averaged time-dependent bubble cloud grayscale was fit in the least squares sense to a power-law equation, equation (9). The parameter β did not decrease significantly with increasing output from the imaging array ($\beta = 0.46 \pm 0.06$). However, the term α increased with increasing peak negative pressure of the plane wave B-mode pulse (figure 10(A)). The predicted time for bubble cloud dissolution, computed as $1/\alpha^{1/\beta}$ using the fitting parameters of equation (9), is shown in figure 10(B). Over the range of acoustic outputs from the imaging array considered in this study, the predicted time for the bubble cloud dissolution was reduced by 87.4% (255.6 ms), 91.7%

(573.2 ms), and 88.3% (749.0 ms) for the 5, 10, and 20 μ s pulse durations, respectively. The area of the bubble cloud decreased by $4.6 \pm 4.9\%$ ($12.8 \pm 16.6 \text{ mm}^2$) for all pressure levels of the imaging array over the 50 ms data acquisition period, similar to that observed for the low output fields (figure 6). The azimuthal position of the bubble cloud shifted by approximately 3% (less than 1 mm) over the course of the 50 ms observation period.

Analytic calculation of bubble dynamics

A comparison of numerical and analytic prediction of the equilibrium bubble diameter is shown in figure 11 as a function of the histotripsy pulse duration. Predictions from the analytic and numerical models agreed within $6.8\% \pm 6.5\%$ ($0.10 \pm 0.09 \mu\text{m}$) for the 16.1 and 18.3 MPa peak negative pressure pulses. The analytic model overpredicts the bubble size by $29.0\% \pm 21.7\%$ ($0.29 \pm 0.23 \mu\text{m}$) compared to the numerical model for the 14.5 MPa pulse.

The dependence of the diffusion-dependent bubble equilibrium diameter was computed analytically for the insonation conditions considered in this study (figure 12(A)). For the largest two peak negative pressures, the equilibrium bubble diameter increased linearly with the duration of the histotripsy pulse. For the lowest peak negative pressure (12 MPa), the tension was not sufficient to induce strong bubble growth throughout the duration of the pulse (Bader and Holland 2016). The corresponding predicted time for passive bubble dissolution is shown in figure 12(B). The bubble dissolution time increases with peak negative pressure for a given pulse duration, and with the pulse duration for a given peak negative pressure. The bubble dissolution time increased with the peak negative pressure of the histotripsy pulse (fixed pulse duration) or the pulse duration (fixed peak negative pressure), consistent with experimental observations (figure 10(A)).

Discussion

Bubble cloud dissolution profile

In this study, histotripsy-induced bubble clouds were visualized following the histotripsy excitation with plane wave B-mode imaging. Previous studies have relied on high speed videography to monitor bubble activity (Khokhlova et al 2011, Maxwell et al 2011b, Vlaisavljevich et al 2015). While useful for investigating bubble dynamics in a transparent medium, such observations would not be feasible for the optically opaque targets of histotripsy therapy (Khokhlova et al 2015). Here, studies focused on the dissolution profile of a cloud of bubbles with a bulk medium after a single histotripsy pulse. Plane wave B-mode imaging has previously been utilized to monitor the changes in bubble cloud activity from pulse-to-pulse along a fluid/tissue interface (Hu et al 2015), or the activity of a single bubble (Gateau et al 2011, Arnal et al 2017).

In contrast to the mercurial behavior typically associated with cavitation (Willard 1953), the primary interesting feature of the bubble clouds observed in this study is the lack of interesting behavior. The bubble cloud position appears stationary within 3% over the observation period, with an approximate 13% reduction in the cloud area. Observations in this study indicate that the change in position and area of the bubble cloud is slow over the millisecond time scale (figure 7), which may be in part due to the timing of image

acquisition. Constructive interference between the imaging and therapy pulses prevented assessment of the bubble cloud echogenicity prior to 179 μs post insonation. Previous studies indicate the bubble cloud collapses 50 to 300 μs following the histotripsy exposure (Xu et al 2007, Maxwell et al 2011b). Thus the hyperechoic spots observed here are likely remnants of the bubble cloud following the inertial collapse (Xu et al 2007, Prieur et al 2015, Bader et al 2018). Persistent residual bubbles can limit the treatment efficacy by causing preferential mechanical activity at discrete locations within the focal zone (Wang et al 2012, Shi et al 2018a, 2018b). Plane wave imaging appears to be ideally suited to track the dissolution of these treatment-limiting residual bubble clouds.

High-frame rate data indicate a trend of bubble cloud translation away from the therapy source with increasing pulse duration for a given peak negative pressure (figure 5). Observations with high speed videography note that a shock-induced bubble cloud grows towards the transducer, and the bubble cloud axial length is proportional to the pulse duration (Maxwell et al 2011b). It should be noted that the high-speed videography data reflect a change in the relative length of the bubble cloud with pulse duration, not the absolute position of the bubble cloud within the acoustic field. The relationship between the bubble cloud position and the histotripsy pulse duration may be in part due to nonlinear effects associated with the incident pressure wave. The degree of acoustic nonlinearity is influenced by the pulse duration (Karzova et al 2012), which may alter the diffraction field and therefore the location of bubble activity (Maxwell et al 2011b). Alternatively, primary radiation force from the incident pulse, which increases with pulse duration, may push the bubble cloud distal to the focal zone (Hamilton and Blackstock 1998).

The slow change in grayscale tracked with plane wave imaging may indicate that dissolution of the bubble cloud is dictated by diffusion of gas from the bubble into the surrounding medium (Bader and Bollen 2018), which operates over time scales of several seconds (Neppiras 1980). Indeed, the grayscale decreases approximately as $t^{1/2}$ (table 1), consistent with a diffusion process (Landau and Lifshitz 1987). It should be noted that the peak negative pressure for the lowest amplitude plane wave pulse in this study was 420 kPa. The imaging pulse at this pressure level may influence the diffusion profile of the bubble cloud, though to a lesser extent than the largest imaging pulses employed in this study (figure 9).

Studies here indicate that the bubble cloud dissolution time increases with the histotripsy pulse duration (figure 8). Observations with high speed videography note that bubbles within the cloud grow over the duration of the pulse (Maxwell et al 2011b). The bubble persistence will increase with size (Bader and Bollen 2018), indicating the dissolution should increase with the pulse duration. The bubble cloud dissolution time increases with the peak negative pressure of the pulse for the 5 and 10 μs duration pulses, consistent with the observations that larger peak negative pressures generate larger bubbles (Vlaisavljevich et al 2015, Bader and Holland 2016). For the 20 μs duration pulses, bubble cloud dissolution times are similar for the 18 and 23 MPa peak negative pressure pulses. This may indicate a saturation effect of bubble cloud growth, or extension of the bubble cloud beyond the focal zone (Maxwell et al 2011b). Such bubble behavior could be corroborated with high speed videography. There was some indication that the bubble cloud azimuthal position shifted with the peak negative pressure of the histotripsy pulse (figure 5). The location of bubble cloud formation has been

shown concatenate with regions of high intensity within the focal zone (Maxwell et al 2011b), which will shift spatially with increasing nonlinear distortion of the pressure waveform (Rosnitskiy et al 2017).

Observations of bubble cloud dissolution with increased output of plane wave imaging pulse

Residual bubbles cause mechanical activity in discrete locations within the histotripsy focal zone, resulting in partial ablation of the target tissue (Wang et al 2012). Insonation schemes are being developed to remove residual bubbles and improve the treatment efficacy of histotripsy (Duryea et al 2015b, Shi et al 2018a). The deleterious effects of persistent bubble cloud can be mitigated by combining ablative histotripsy pulses with low-amplitude pulses to force bubble coalescence (Duryea et al 2015a), significantly improving the speed and efficacy of ablation (Duryea et al 2015b, Shi et al 2018a). Bubble coalescing pulses are generated via specialized transducer fixtures integrated within the histotripsy source (Shi et al 2018b).

The destruction of microbubbles has been demonstrated with diagnostic imaging pulses (Radhakrishnan et al 2013). Following a microbubble destructive pulse, plane wave B-mode imaging provides visualization of contrast reperfusion within the vessel (Couture et al 2012). In this study, the computed dissolution time of histotripsy-induced bubble clouds was reduced by 87%–91% (255–749 ms) when the peak negative pressure of the plane wave imaging pulse was increased from 0.5 to 6.7 MPa (figure 10(A)). The bubble cloud grayscale decreased as $t^{1/2}$ for all outputs of the plane wave B-mode sequence, indicating diffusion was still a dominant mechanism for the reduction in bubble cloud echogenicity for the imaging pulses employed here (Landau and Lifshitz 1987). The fitting parameter α increased with the peak negative pressure of the plane wave B-mode pulse (figure 10(B)). For a diffusion-driven process, α is proportional to the square of the diffusion constant D (Landau and Lifshitz 1987). Thus, higher outputs from the plane wave B-mode pulses may increase the effective diffusion of gases from the bubble cloud.

The choice of the plane wave B-mode sequence may therefore have an impact on the size of individual bubbles within the cloud. Acoustically-driven diffusive effects are most prominent for bubbles driven off resonance (Church 1988). Histotripsy pulses activate nanometer-sized nuclei to become bubbles that are tens to hundreds of micrometers in diameter (Maxwell et al 2011b, Vlaisavljevich et al 2015), much larger than a resonant-sized bubble of the 6.25 MHz plane wave B-mode pulse. While gas would initially be expelled via the bubble oscillations under the plane wave B-mode pulse, diffusion would reduce as the bubbles approach resonance size (Church 1988), approximately 1.33 μm diameter (Bader and Holland 2012). Further assessment is necessary to determine if such a reduction in the bubble size is sufficient to provide uniform mechanical activity throughout the focal zone, thereby improving the histotripsy treatment efficacy.

Beyond forcing bubble dissolution, plane wave B-mode imaging provides a high temporal-resolution assessment of the residual bubble cloud burden. Information of the bubble cloud grayscale could be utilized to automate the timing between the application of histotripsy pulses for efficient tissue liquefaction. A limitation of plane wave B-mode imaging to

modulate bubble behavior is the width of the acoustic field. For the L11-4v imaging array employed in these studies, the -3 dB elevational width is approximately 2 mm. Any bubbles nucleated out of plane from the imaging array are unlikely to be modulated by the imaging pulse. Another limitation is acoustic attenuation. The distance between the imaging array and bubble cloud was set at the natural focus of the L11-4v for this *in vitro* study. For a confocal arrangement of the imaging array and histotripsy source, the distance between the array and bubble cloud would be dictated by the focal distance (typically greater than 60 mm). Propagation of the imaging pulse along the tissue path would significantly attenuate the 6.25 MHz imaging pulse (Bader et al 2016a), reducing the degree of acoustically-driven diffusion (Crum and Hansen 1982). Other bubble deleting schemes operate a peak negative pressures less than 3 MPa (Shi et al 2018a), indicating that there may be means to optimize the imaging sequence to still substantiate bubble dissolution at depth.

Analytic predictions of bubble diffusion

Results from the analytic model for predicting diffusion during histotripsy-induced bubble expansion were in good agreement with numerical predictions for peak negative pressures 16.1 and 18.3 MPa. There is a divergence of the analytic and numerical predictions as the pulse duration increased, likely due to additive errors in the analytic model. The analytic model over estimates bubble growth for peak negative pressures less than 15 MPa (Bader and Holland 2016), causing an overestimate for the degree of diffusion for bubble expansion for the 14.5 MPa comparison of the numeric and analytic calculations (figure 11). For the peak negative pressures where the analytic model is accurate, the analytic and numerical predictions were within $6.8\% \pm 6.5\%$ ($0.10 \pm 0.09 \mu\text{m}$, figure 11). The inclusion of diffusive effects in bubble oscillation numerical theory can be computationally expensive, particularly with the nanoscale nuclei used here (Bader and Bollen 2018). Beyond ease and accessibility compared to numerical calculations, analytic models provide a physical intuition as to the important parameters that effect the bubble dynamics. The identification of such parameters can help guide the development of regulatory standards for regimes where the efficacy of histotripsy may be mitigated due to bubble persistence.

The dependence of bubble dissolution time on the insonation parameters predicted by the analytic model in figure 12 are consistent with trends observed for the reduction in grayscale (figure 8). However, the computed bubble dissolution time is much shorter than that observed with plane wave imaging (figure 8). This may be in part due to the nature of the calculations performed here, which models the growth of a single bubble, whereas a dense bubble cloud is formed during the shock scattering process (Maxwell et al 2011b). Any bubble-bubble interactions such as coalescence were not considered in the computational or analytic bubble oscillation models. The analytic calculation models bubble growth due to the incident pressure wave (Bader and Holland 2016), whereas most bubbles are nucleated via scattering of the incident shock wave (Maxwell et al 2011b). The tension from the scattered shock wave may be much greater than the incident pulse (Maxwell et al 2010), producing larger bubbles (Bader and Holland 2016), and therefore longer dissolution times (Bader and Bollen 2018). Nevertheless, the shock scattering process is initiated by a microbubble within the focal zone that grows under the action of the incident pulse (Maxwell et al 2011b). Based on the assumed 20 nm diameter of the bubble nucleus, calculations in this study

provide an upper estimate to the maximum bubble size for shocked histotripsy pulses (Bader and Holland 2016). Therefore, the reported bubble dissolution time would also correspond to an upper estimate for a single bubble. However, the exact nature and size distribution of nuclei activated by histotripsy pulses is still unknown (Bader et al 2019).

Limitations

There are a number of limitations that prevent generalization of the findings here. The free-field focus of the histotripsy transducer was located with hyperechoic bubble clouds generated from 5 μ s histotripsy pulses (Maxwell et al 2011a, Vlasisavljevich et al 2013a). The bubble cloud grows azimuthally into the nearfield compared to the geometric focus of the histotripsy transducer (Bader and Holland 2016). For a 5 μ s histotripsy pulse, the bubble cloud would be approximately 1 mm in diameter (Maxwell et al 2011b). Thus, the reported azimuthal positions reported in figure 5 are shifted by 1 mm relative to the geometric focus of the histotripsy transducer.

A tissue phantom was used in this study instead of viable tissue. The use of a phantom allowed specified, consistent medium properties (Bader et al 2016a). However, this *in vitro* approach may not be representative of bubble cloud behavior *in vivo*. The population of bubble nuclei may not replicate that found in real tissue, altering the threshold for individual bubble formation. However, the bubble dynamics initiated by highly shocked histotripsy excitations are largely independent of the initial bubble diameter and the viscoelastic properties of the medium (Maxwell et al 2013, Bader and Holland 2016) for elastic moduli less 1 MPa (Bader 2018). A lack of scatterers used in the phantom minimized the presence of cavitation nuclei, but also resulted in a medium that was more hypoechoic than tissue (Szabo 2004). Ultrasound images were also only acquired in a fixed 2D plane, whereas a volume of bubble cloud was generated.

The dynamics of the bubble cloud were observed over the millisecond time scale with plane wave imaging, which prevented obtaining insight into the microsecond bubble oscillations. High speed videography may elucidate the interaction of the histotripsy bubble cloud with the plane wave imaging pulses (Khokhlova et al 2011, Maxwell et al 2011b, Vlasisavljevich et al 2015, Raymond et al 2016). Such information may discern the mechanism by which the bubble cloud grayscale is mitigated by high output plane wave imaging pulses.

Plane-wave B-mode images in this study were analyzed 179 μ s post insonation due to constructive interference between the therapy and imaging pulses. Thus, the hyperechoic bubble clouds observed in this study with plane wave B-mode imaging may be due to residual bubbles and not the original bubble cloud responsible for inducing mechanical liquefaction. Further, the temporal resolution of the plane wave imaging sequence would not be sufficient to capture the inertial collapse of the bubble cloud (Maxwell et al 2011b). The bubble behavior observed here may vary for images acquired at alternate times, or using alternate B-mode image acquisition methods (e.g. plane wave versus conventional or pulse inversion (Hall et al 2007)). Plane wave imaging may capture the bubble behavior at a particular instant in time, though generally has poorer resolution than conventional scanline B-mode (Szabo 2004). The short image acquisition time may be necessary if the histotripsy pulses are applied at a high rate. Bubble cloud dissolution was tracked in this study for one

in every 200 applied histotripsy pulses, in part due to hardware limitations. Parallelized image processing could provide a means to increase the plane wave sequence application rate (Haworth et al 2007). Alternatively, a subset of the received waveforms could be processed to increase the frequency of the sequence execution (Gateau et al 2011). The time for bubble cloud dissolution was longer than the 50 ms observation period of this study. Tracking the bubble cloud over the entirety of its lifetime may alter the fitting parameters for equation (9) shown in table 1, and the estimated times to dissolution presented in figures 8 and 10.

Summary

Histotripsy is a promising ultrasound ablative therapy under development for numerous disease pathologies. A primary limitation in the efficacy of histotripsy are bubble clouds that persist between the application of consecutive pulses. Here, plane wave B-mode imaging was employed to monitor bubble cloud dissolution after the application of the histotripsy pulse. The area and location of the bubble cloud exhibited little change over the 50 ms observation period, and the grayscale decreased as with the square root of time ($t^{1/2}$). The trends in the decrease in grayscale were consistent with an analytic model developed here to predict the time for bubble dissolution. Finally, the rate of bubble cloud grayscale decrease was found to be susceptible to the output of the imaging array. Overall, these results indicate that high-frame rate imaging can be used to monitor and modulate the behavior of histotripsy bubble clouds.

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Figure 1.
Representative histotripsy waveforms with (A) 12/76 MPa peak negative/peak positive pressure and (B) 18/107 MPa peak negative/peak positive pressure excitation.

Figure 2.

(A) Side view of experimental set up for histotripsy bubble cloud generation in the tissue mimicking phantom. An L11-4v imaging array was oriented to monitor bubble clouds with plane wave B-mode imaging along the central axis (azimuth/range plane of the imaging array) of the histotripsy source. (B) Timing diagram for the acquisition of plane wave B-mode images following the histotripsy focal insonation.

Figure 3.

(A) Representative bubble cloud visualization with the plane wave B-mode imaging sequence. The histotripsy pulse ($10\ \mu\text{s}$ pulse duration, 18 MPa peak negative pressure) propagated left to right in the image. The pixel values are reported in terms of dB relative to the maximum grayscale value, as indicated in the colorbar. (B) Binarized plane wave B-mode image using Otsu's method.

Figure 4.

Representative examples of high-frame rate plane wave image observations of histotripsy-induced bubble clouds. (Left column) 5 μs duration histotripsy pulse. (Middle column) 10 μs duration histotripsy pulse. (Right column) 20 μs duration histotripsy pulse. For each row, the time at which the image was acquired after the conclusion of the histotripsy pulse is noted along the left. The grayscale colormap is reported in terms of decibels, normalized to the maximum grayscale of the image acquired at 500 μs for each respective pulse duration. The peak negative/peak positive pressure of the histotripsy pulse was 18/107 MPa, and was propagating from left to right in each image. The white line in the lower right portion of the image corresponds to 5 mm (top left most panel only).

Figure 5.

Azimuthal position of the bubble cloud centroid as a function of time for (A) $5\ \mu\text{s}$, (B) $10\ \mu\text{s}$, or (C) $20\ \mu\text{s}$ duration histotripsy pulse. The peak negative pressure of the histotripsy pulse is noted in the legend. Azimuthal positions are relative to the focus of the histotripsy source under linear conditions, with negative values indicating movement towards the transducer. The peak negative pressure of the plane wave imaging pulse was 420 kPa. The mean bubble cloud centroid for all data sets is plotted here ($N=50$ for each arm). The error bars represent the standard deviation over all acquired data sets for each respective arm.

Figure 6.

Bubble cloud area as a function of time for (A) 5 μ s, (B) 10 μ s, or (C) 20 μ s duration histotripsy pulse. The peak negative pressure of the histotripsy pulse is noted in the legend. The peak negative pressure of the plane wave imaging pulse was 420 kPa. The mean bubble cloud area for all data sets is plotted here ($N=50$ for each arm). The error bars represent the standard deviation over all acquired data sets for each respective arm.

Figure 7.

Bubble cloud grayscale as a function of time for a (A) 12 MPa, (B) 18 MPa, or (C) 23 MPa histotripsy pulse peak negative pressure. The histotripsy pulse duration is noted in the legend. Error bars are representative of data throughout the duration of image acquisition. The peak negative pressure of the plane wave imaging pulse was 420 kPa. The mean bubble cloud grayscale for all data sets is plotted here ($N=50$ for each arm). The error bars represent the standard deviation over all acquired data sets for each respective arm.

Figure 8.

The predicted time for dissolution of the bubble cloud as a function of the histotripsy insonation parameters. The dissolution time was computed as $1/\alpha^{1/\beta}$, where α and β are the fitting parameters to equation (9). The fitting parameters are listed in table 1. Error bars represent the 95% confidence interval of the least-square fits. The peak negative pressure of the plane wave imaging pulse was 0.5 MPa.

Figure 9.

Normalized bubble cloud grayscale as a function of time for a (A) 5 μ s, (B) 10 μ s, or (C) 20 μ s duration histotripsy pulse of 23 MPa peak negative pressure. The peak negative pressure of the plane wave imaging pulse is noted in the legend. Error bars are representative of data throughout the duration of image acquisition.

Figure 10.

(A) Fitting parameter α of equation (9) as a function of the peak negative pressure of the plane wave imaging pulse. Error bars represent the 95% confidence interval of the least-squares fit. (B) The predicted time for dissolution of the bubble cloud as a function of the plane wave imaging pulse peak negative pressure. The dissolution time was computed as $1/\alpha^{1/\beta}$, where α and β are the fitting parameters to equation (9). Error bars represent the 95% confidence interval of the least-square fits.

Figure 11.

Comparison of numerical (solid bar) and analytic (striped bar) prediction of diffusion-dependent equilibrium bubble diameter as a function of the histotripsy pulse duration. The peak negative pressure of the histotripsy pulse is noted in the legend. The initial bubble diameter was 20 nm.

Figure 12.

(A) Analytic prediction of diffusion-dependent equilibrium diameter for insonation conditions considered in this study. (B) Prediction of passive bubble dissolution time based on insonation condition-dependent bubble equilibrium bubble diameter.

Table 1.

Parameters for power law fit of the normalized bubble cloud grayscale value (nGSV) as a function of time, equation (9). Goodness-of-fit parameters coefficient of determination (r^2) and the root mean square error (RMSE) are also reported.

$5 \mu s$	α [nGSV/s β]	β [A.U.]	r^2	RMSE [nGSV]
12 MPa	0.02 (0.002)	0.72 (0.02)	0.996	0.005
18 MPa	0.05 (0.002)	0.52 (0.01)	0.989	0.011
23 MPa	0.04 (0.003)	0.54 (0.02)	0.997	0.005
$10 \mu s$				
12 MPa	0.04 (0.001)	0.60 (0.010)	0.994	0.008
18 MPa	0.04 (0.001)	0.51 (0.007)	0.995	0.005
23 MPa	0.04 (0.001)	0.50 (0.024)	0.994	0.006
$20 \mu s$				
12 MPa	0.05 (0.001)	0.50 (0.007)	0.994	0.006
18 MPa	0.06 (0.007)	0.41 (0.025)	0.989	0.007
23 MPa	0.03 (0.001)	0.52 (0.015)	0.979	0.010