Published in final edited form as:

IEEE Trans Ultrason Ferroelectr Freq Control. 2013 January ; 60(1): 53–64. doi:10.1109/TUFFC.2013.2537

Influence of Shell Properties on High-Frequency Ultrasound Imaging and Drug Delivery Using Polymer-Shelled Microbubbles

Parag V. Chitnis [Member, IEEE]

F. L. Lizzi Center for Biomedical Engineering, Riverside Research, New York, NY
(pchitnis@riversideresearch.org)

Sujeethraj Koppolu [Student Member, IEEE]

F. L. Lizzi Center for Biomedical Engineering, Riverside Research, New York, NY

Jonathan Mamou [Senior Member, IEEE]

F. L. Lizzi Center for Biomedical Engineering, Riverside Research, New York, NY

Ceciel Chlon

Department of Biomolecular Engineering, Philips Research Europe, Eindhoven, The Netherlands

Jeffrey A. Ketterling [Senior Member, IEEE]

F. L. Lizzi Center for Biomedical Engineering, Riverside Research, New York, NY

Abstract

This two-part study investigated shell rupture of ultrasound contrast agents (UCAs) under static overpressure conditions and the subharmonic component from UCAs subjected to 20-MHz tonebursts. Five different polylactide-shelled UCAs with shell-thickness-to-radius ratios (STRRs) of 7.5, 30, 40, 65, and 100 nm/ μm were subjected to static overpressure in a glycerol-filled test chamber. A video microscope imaged the UCAs as pressure varied from 2 to 330 kPa over 90 min. Images were postprocessed to obtain the pressure threshold for rupture and the diameter of individual microbubbles. Backscatter from individual UCAs was investigated by flowing a dilute UCA solution through a wall-less flow phantom placed at the geometric focus of a 20-MHz transducer. UCAs were subjected to 10- and 20-cycle tonebursts of acoustic pressures ranging from 0.3 to 2.3 MPa. A method based on singular-value decomposition (SVD) was employed to obtain a cumulative subharmonic score (SHS). Different UCA types exhibited distinctly different rupture thresholds that were linearly related to their STRR, but uncorrelated with UCA size. The rupture threshold for the UCAs with an STRR = 100 nm/ μm was more than 4 times greater than the UCAs with an STRR = 7.5 nm/ μm . The polymer-shelled UCAs produced substantial subharmonic response but the subharmonic response to 20-MHz excitation did not correlate with STRRs or UCA-rupture pressures. The 20-cycle excitation resulted in an SHS that was 2 to 3 times that of UCAs excited with 10-cycle tonebursts.

I. Introduction

STABILIZED micrometer-scale spheres composed of a thin biocompatible shell that encapsulates an inert gas are known as ultrasound contrast agents (UCAs) [1]. UCAs provide increased sensitivity and specificity because of their high echogenicity relative to surrounding tissue devoid of UCAs [2]. Furthermore, their nonlinear oscillations generate harmonics of the incident ultrasound that can be specifically detected to further enhance the contrast-to-noise ratio [3], [4]. Subharmonic ultrasound contrast imaging is a particularly attractive method because it reduces the loss in signal-to-noise ratio resulting from frequency-dependent attenuation and the subharmonic signals originate specifically from UCAs [3], [5]. A free bubble (no encapsulation) optimally generates a subharmonic response when it is driven at a frequency twice its resonance frequency. When a bubble is encapsulated, the thickness and

elasticity of the shell increase the resonance frequency [6]. Consequently, the propensity of an UCA to generate a subharmonic response depends on the combination of its size, shell parameters, the driving frequency, and incident acoustic pressure.

UCAs also can be driven to destruction by acoustic forcing. Ultrasound-mediated destruction of UCAs can be employed for therapeutic applications such as localized drug delivery and gene transfection [7], [8]. UCA-based delivery of a therapeutic payload can be targeted to specific pathologies by bioconjugating UCAs with appropriate ligands or antibodies [9]–[11]. Ultrasound can noninvasively rupture UCAs bound to the disease site and trigger the local release of therapeutics. Furthermore, ultrasound–UCA interactions can produce mechanical effects such as cavitation that enhance local uptake [12]. Accumulation of UCAs at the target site and their ultrasonically induced destruction can be detected by using ultrasound to guide and monitor the treatment in real time.

Small animals, such as mice, offer convenient *in vivo* models for preclinical studies of the efficacy of ultrasound-mediated targeted drug delivery and gene transfection [13], [14]. However, such studies necessitate ultrasound imaging at high frequencies (>20 MHz), which produces fine-resolution images of the microcirculation and anatomical features for monitoring progression of diseases, such as cancers [15], [16], and response to therapeutics [17], [18]. Consequently, small-animal high-frequency ultrasound (HFU) imaging is an area of growing interest. In fact, commercial ultrasound machines have been developed specifically for this purpose [19]. Therefore, the development of UCAs that can be employed in the HFU regime for subharmonic imaging and ultrasound-mediated drug delivery is desirable.

Currently, contrast-enhanced HFU imaging studies predominantly employ lipid-shelled UCAs. Although lipid-shelled UCAs were originally developed for ultrasound-contrast enhancement in conventional diagnostic frequencies, their ability to produce subharmonic signals in response to HFU excitation also has been demonstrated [20]–[22]. The mechanism of subharmonic generation from lipid-shelled UCAs also has been studied extensively [23], and generally is attributed to nonlinear radial oscillations of the lipid shells. However, optimization of UCAs for these emerging HFU applications continues to be a challenge. Outstanding issues exist with respect to the optimal agent construction and the associated acoustic forcing. The size of the UCA and the material properties and thickness of the UCA shell influence the resonance behavior of UCAs when responding to ultrasound [23]–[25]. Polymer-shelled agents have been developed to address the need for a high level of manufacturing control in terms of the UCA size and shell parameters [26], [27]. The manufacturing process involved in producing polymer-shelled UCAs is amenable to customizing the shell-thickness-to-radius ratio (STRR) of a UCA population with a polydisperse size distribution. Because the destruction threshold of polymer-shelled UCAs directly relates to STRR [28]–[30], polymer-shelled UCAs potentially can be designed to rupture at a prescribed acoustic pressure by choosing an appropriate STRR, irrespective of their size distribution. The UCAs designed in this manner tend to remain intact below the threshold for acoustically induced rupture, which facilitates imaging; when subjected to ultrasound that exceeds this threshold, the UCAs would immediately rupture without leaving a substantial amount of intact, residual UCAs. Upon rupture of the shell, the interior gas may escape as free gas bubbles, which have been hypothesized to produce clinically relevant ultrasound backscatter, including a subharmonic response [25], [31] that can facilitate imaging and treatment monitoring. Recent studies have observed generation of subharmonic responses from polymer-shelled UCAs when excited at high frequencies (40 MHz) [32].

Polymer-shelled agents are studied less than lipid-shelled agents, and a better understanding of their response as a function of shell parameters is needed so that they can be optimized

for targeted drug and gene delivery applications. The shell material properties and the thickness are necessary parameters for developing realistic theoretical models that describe the dynamic response of polymer-shelled agents [6], [30]; the robustness and predictive value of these models are highly dependent on accurate values of the shell parameters.

High-speed video microscopy of UCAs [25], [33] and studies that measured acoustic backscatter from UCAs [32], [34] have been preliminary steps in characterizing polymer-shelled UCAs. Although these techniques provide sizes of single agents and insight into the dynamic response of UCAs to ultrasonic excitation, they do not provide information on the shell's material parameters. Leong-Poi *et al.* measured the volumetric change resulting from a change in static pressure and estimated the bulk modulus of polymeric UCAs using a novel technique to microscopically monitor multiple UCAs subjected to overpressure [35]. Recently, an atomic force microscope (AFM) was used to estimate the shell material properties based on measurements of UCA deformation caused by an external force [36]. Although the AFM method provided novel quantitative insight into the elasticity of UCA shells, this method cannot be implemented efficiently to characterize a large number of UCAs.

The present study examined polymer-shelled UCAs of different STRRs to investigate their potential utility in ultrasound-mediated drug delivery applications involving small animals. Specifically, the influence of shell parameters on the propensity of UCA rupture and the subharmonic response produced by UCAs when excited at 20 MHz was studied.

II. Theory

Polymer-shelled contrast agent shells have been considered as homogeneous, curved, solid, thin-walled structures. For such thin-walled structures, the stability of their shape is of primary importance to their function. We conducted theoretical studies to gain insights into the compression-induced rupture of UCA shells and the influence of shell properties on their resonance behavior when excited with HFU.

A. Shell Buckling and Rupture

When subjected to compression, spherical shells may buckle and undergo catastrophic failure. The linear elastic solution for compression-induced stress on a perfectly spherical thin shell can be expressed as

$$\sigma_{\text{crit}} = \frac{E}{\sqrt{3(1-\nu^2)}} \left(\frac{2h}{d} \right), \quad (1)$$

and the corresponding critical buckling pressure ($P_{\text{crit}} = 4h\sigma_{\text{crit}}/d$) is given as

$$P_{\text{crit}} = \frac{2E}{\sqrt{3(1-\nu^2)}} \left(\frac{2h}{d} \right)^2 = A\chi^2, \quad (2)$$

where E is the Young's modulus, ν is the Poisson's ratio, h is the shell thickness, d is the diameter, A is a lumped constant describing the material properties, and χ is the STRR [37]. A Poisson's ratio of 0.3 was considered an appropriate approximation for most common materials, resulting in $A = 1.21E$ [38] (a positive coefficient of A implies compression of the sphere). Early experimental investigations indicated that the critical buckling pressure measured for spherical structures was approximately one-fourth the theoretically predicted value, and this discrepancy was attributed to imperfections or inhomogeneities in the shell [38].

This classical expression for the critical buckling pressure assumes that the shell has uniform thickness and that the transverse shear components are negligible. However, variations in shell thickness can result in an effective bending thickness and transverse shear. A more complete formulation, introduced by Ru [39], accounts for shear components that may be significant in biological spherical shells. The critical buckling pressure derived using the Ru model can be expressed as

$$P_{\text{crit}} = \left(\frac{h_0}{h}\right)^{3/2} \left(\frac{2Eh^2}{R^2 \sqrt{3(1-\nu^2)}} \right) - \frac{EGh_0^3}{3(1-\nu)R^3 k_s G_t}, \quad (3)$$

where h_0 is the effective bending thickness (arising as a result of nonuniform shell thickness), R is the median radius of the shell, G is the in-plane shear modulus, G_t is the transverse shear modulus, and k_s is the shear coefficient. The corresponding critical pressure values were found to be significantly less than those predicted using the classical model for a perfect shell. Eq. (3) reduces to the classical linear elastic equation shown in (2) when the shell is perfectly homogeneous ($h_0 = h$) and the transverse shear is negligible ($G_t \gg G$). Structural heterogeneities and imperfections, such as those considered in the Ru model, have been suggested as important considerations in previous studies involving polymeric agents [35], [40], [41].

A recent theoretical study by Marmottant *et al.* investigated the compression-induced rupture and buckling of polymer-shelled UCAs [30]. Critical rupture pressure was defined as the Laplace pressure that results in critical elongation (maximum strain e_{max}) of the shell, which was expressed as $P = 3E\chi e_{\text{max}}$. They concluded that thin-shelled ($\chi < 10 \text{ nm}/\mu\text{m}$) UCAs exhibited distinct buckled and ruptured states with corresponding critical pressures that were quadratic and linear functions of UCA STRR, respectively. In comparison, they predicted that UCAs with thicker shells do not exhibit distinct buckling of the shell, i.e., the critical pressures for buckling and rupture are essentially the same. Because four out of five UCAs investigated in the present study had a nominal STRR greater than $10 \text{ nm}/\mu\text{m}$, the UCA-rupture data (details in the following section) was fitted to Ru's expression for critical buckling pressure (3) to gain insight into the material properties of the UCA shell and its propensity for ultrasound-mediated shell rupture.

B. Microbubble Resonance

The theoretical model for ultrasonically driven polymer-shelled microbubbles developed by Hoff *et al.* [6] was used to calculate the nominal frequency-dependent scattering cross-section of each UCA population employed in this study; the nominal resonance frequency of the UCAs was inferred from the frequency peak of the scattering cross-section. The model assumes that the polymer shell is thin compared with the radius and depends on the viscous and elastic properties of the shell. The scattering cross-section (σ_s) of an encapsulated bubble predicted using the Hoff model can be expressed as

$$\sigma_s = \frac{\Omega^4}{(\Omega^2 - 1)^2 + \Omega^2 \delta^2} = 4\pi a_e^2 \frac{|p_s|^2}{|p_A|^2}, \quad (4)$$

$$\omega_0 = \frac{1}{2\pi a_e} \sqrt{\frac{1}{\rho_L} \left(3\kappa p_0 + 12G_s \frac{ds_e}{a_e} \right)}, \quad (5)$$

$$\delta = \frac{1}{\omega_0 \rho_L a_e^2} \left(4\mu_L + 12\mu_s \frac{ds_e}{a_e} \right), \quad (6)$$

where a_e is the equilibrium radius of the bubble, $\omega_0 = 2\pi f_0$, f_0 is the UCA resonance frequency, $\Omega = 2\pi f/\omega_0$, f is the excitation frequency, p_A is the incident acoustic pressure, p_s is the scattered pressure, ρ_L is the liquid density, κ is the polytropic constant, p_0 is the ambient pressure, G_s is the shear modulus, ds_e is the equilibrium shell thickness, μ_L is the liquid viscosity, μ_s is the shear viscosity, and δ is the viscous dampening term.

The shell material properties used to theoretically calculate the scattering cross-section of polymer-shelled UCAs were estimated from independent measurement of responses of the UCAs to static overpressure [28] and values inferred in previous works [6], [35], [42]. Specifically, the following values for shell properties were employed: $E = 1.35$ GPa, $G = E/(2(1 + \nu))$, $G_t = 0.4G$, $\mu = 0.4$, and $\nu = 0.4$. For each UCA population, the mean of measured radii of the UCAs employed in the overpressure study was used to determine a_e . Hoff simulations were performed for three scenarios that described the shell thickness ds_e : ruptured UCA, i.e., $ds_e = 0$; a perfectly homogenous, intact shell, i.e., $ds_e = \chi \times a_e$ (nominal); and an imperfect shell, i.e., $ds_e = h_0$, where h_0 is the effective bending thickness of the shell described in the Ru model (3) and is obtained by fitting the UCA-rupture data from the overpressure study to the Ru model; the effective bending thickness is less than the nominal shell thickness.

III. Materials and Methods

Two experimental studies were conducted in the course of this work: an overpressure study and a HFU-backscatter study. Five varieties of polylactide-shelled UCAs were employed in this study. The first variety, PB127, was manufactured by Point Biomedical (San Carlos, CA; intellectual property now transferred to University of Pittsburgh, Pittsburgh, PA); the other four varieties were manufactured by Philips Research (Eindhoven, The Netherlands). Each UCA population had a distinct STRR. The PB127 UCAs had a STRR of 7.5 nm/ μ m and a nominal mean diameter of 3.4 μ m; the four Philips UCA populations had STRRs of 30, 40, 65, and 100 nm/ μ m and each population had a nominal mean diameter of 2 μ m. The UCA parameters are presented in Table I. The manufacturing methods for the Point UCAs are proprietary. The steps involved in manufacturing and estimating the STRR of the Philips UCAs are presented in the following section.

A. Philips UCA Preparation

Poly-L-Lactide with a molecular weight of 2400 and a fluorinated end group was synthesized as described previously [27], [40]. Hollow microcapsules were obtained by emulsifying a mixture of 3% (w/w) polyvinyl alcohol (PVA; molecular weight = 13 000 to 23 000 Da; Aldrich, Zwijndrecht, The Netherlands) in water and 0.79 g of a stock solution containing poly-L-lactide and cyclodecane (Aldrich) in dichloromethane (DCM, Merck, Haarlem, The Netherlands). The emulsion was then added drop-wise to 16 g of 0.3% (w/w) PVA (molecular weight = 13 000 to 23 000 Da) in water by pressing it through an Acrodisk 1- μ m glass filter (Pall Corp., Port Washington, NY). The emulsion was stirred for 3 h at 500 rpm to remove the DCM by dissolution in the aqueous phase and subsequent evaporation. After DCM evaporation, the sample was centrifuged at 3000 rpm (g -force was 968 g) for 30 min. The top fraction was retrieved and washed with 5% poly(ethyleneglycol) (PEG, molecular weight of 3015 to 3685 Da, Aldrich) in water. The centrifugation step was repeated once. PEG was added to the retrieved sample, after which the sample was rapidly frozen at -80°C in a precooled glass vial. The ice and the cyclodecane fraction were

removed by freeze-drying for 20 h (Epsilon 2–6 freeze dryer, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) at 0.2 kPa followed by 20 h at 0.03 Mbar while maintaining the shelf temperature at -10°C . After freeze-drying, the system was filled with nitrogen to obtain nitrogen-filled polymer-shelled microbubbles. Finally, the samples were stored at 4°C and capsules were re-dispersed in water or Dulbecco's phosphate-buffered saline (DPBS) to yield 10^9 microbubbles/mL. After re-dispersion, the microbubbles were placed on a rotating plate for at least 10 min before use.

The STRR of the UCA populations obtained in this manner was adjusted through the weight ratio of the polymer in the polymer-cyclodecane stock solution; Polymer ratios (% w/w) of 2.85, 1.9, 1.27, and 1.03 yielded microbubbles that had volumetric shell-to-core ratios of 1:3, 1:5, 1:8, and 1:10, respectively. These shell-to-core ratios and the median UCA radii were then used to estimate the STRR of each UCA population. Lensen *et al.* [29] employed cryoscanning electron microscopy to confirm that the STRRs of polymer-shelled UCAs calculated using the methods described above correlated well with the measured values; however, these calculations underestimated the STRR by up to 30%, particularly for UCA populations with large nominal STRRs.

B. Overpressure Study

1) Experimental Setup and Protocol—A cylindrical test chamber was custom designed for subjecting the UCAs to static overpressure [Fig. 1(a)]. The chamber had a permanent glass window at the bottom and a removable microscope reticule (secured using a lid and a rubber o-ring) at the top. The chamber was mounted on top of a right-angle light collimator which mated with a 6.35-mm light guide. This arrangement allowed uniform white-light illumination within the chamber from below. Two flow ports were installed on the chamber to facilitate the flow of fluid in and out of the chamber.

The test chamber was washed with distilled water and dried using compressed air before each experimental run. The outlet port was connected to a pressure sensor (PM100D, World Precision Instruments, Sarasota, FL) for monitoring the static pressure within the chamber. A syringe pump (BS-8000, Braintree Scientific, Inc., Braintree, MA) was used to slowly pump glycerol (to avoid introducing air bubbles) through the inlet of the chamber, which initially was open at the top, until the meniscus of glycerol exceeded the top of the chamber. Approximately 0.2 mL of distilled water was then added on top of the glycerol surface using a dropper. A small amount of the dry-form UCA was added to the water and reconstituted; care was taken to obtain a uniform layer of UCA solution on top of the glycerol without mixing glycerol and water. The microscope reticule was gently placed on top of the chamber to prevent the unwanted intrusion of air bubbles. Thus, a relatively uniform layer of UCAs was placed directly under the markings on the microscope reticule.

The UCAs were subjected to overpressure that slowly increased from 2 to 330 kPa over a 90 min duration through the continuous injection of glycerol at a rate of 5 mL/hour. Pressure-induced changes in the UCAs were visually monitored (through the microscope reticule) using a video microscope consisting of a 1-Mpixel CCD camera (Qcam, Qimaging, Surrey, BC, Canada), and a long distance, $100\times$ microscope objective (EO Infinity-Corrected LWDO, Edmund Optics, Barrington, NJ), which provided a field of view (FOV) of $64\text{ }\mu\text{m}$. A customized dynamic-focusing algorithm was implemented to automatically reposition the camera to maximize the gradient at the edges of the reticule markings in the FOV. This algorithm ensured that the UCAs in contact with the reticule remained in the optimal focal plane of the imaging system. The dynamic focusing resulted in a variable image-capture rate, which remained between 1 and 4 frames/s. The static pressure corresponding to each captured frame was recorded; an illustrative frame is shown in Fig. 1(b).

2) Image Processing—All acquired images were filtered using a $0.5 \times 0.5 \mu\text{m}$ median filter to reduce noise. Individual UCAs in each frame were sized using semi-automated post-processing [illustrated in Fig. 1(c)–(e)] involving the following three steps: 1) a region of interest (ROI; $30 \times 30 \mu\text{m}$) centered around a user-specified UCA in the image was binarized; 2) the edges of the UCAs in the ROI were detected by thresholding the gradient calculated using a Sobel operator; and 3) the position and the diameter of the UCAs within the ROI were determined by applying the generalized Hough transform for a circle [43]. Thus, the algorithm detected and sized the UCAs, including those partially obscured, as shown in Fig. 1(e). UCAs that migrated outside of the FOV or that clumped together were discarded. For each UCA population, overpressure data from a minimum of 300 individual UCAs were acquired. Rupture pressure of each individual UCA was recorded upon visual identification of a rupture event from the sequence of images. The dependence of rupture pressure on UCA diameter and STRR was investigated. The resulting data were analyzed using the statistics tool-box in Matlab (The MathWorks Inc., Natick, MA) and fitted to the theoretical model for spherical-shell rupture (Section II-A).

C. HFU-Backscatter Study

1) Experimental Setup and Protocol—Individual UCAs were streamed through the focus of a 20-MHz, PVDF transducer (PI 20–2 Panametrics, Waltham, MA) that had a focal length of 12 mm, an aperture diameter of 6 mm, and bandwidth of 50% (Fig. 2). The transducer had a -6-dB depth-of-field of approximately 1 mm and a -6-dB lateral beamwidth of $200 \mu\text{m}$. The transducer was excited by the output of a power amplifier (1040L, ENI, Rochester, NY) driven by an arbitrary-waveform generator (Tabor 1281, Tel Hanan, Israel). The receive echo was digitized at 400 MHz with an 8-bit digitizer (DC271A, Acqiris, Monroe, NY) after undergoing 50 dB of amplification (AU-1313, Miteq, Hauppauge, NY). The transducer was calibrated using a fiber-optic hydrophone (Precision Acoustics Ltd., Dorset, UK).

A dual-channel tubing pump (REGLO Digital MS-2/8, Ismatec, Glattbrugg, Switzerland) with 2.06-mm inner diameter ID tubing (CP-95608–42, Cole-Parmer, Vernon Hills, IL) was employed to provide UCA flow. The pump-flow rate was 0.625 mL/min. One line of the pump drew the diluted mixture of contrast agents from a 200-mL reservoir into a test tank through a $200\text{-}\mu\text{m}$ needle mounted on the wall, and a second line of the pump drew liquid out of the tank into a waste reservoir. The transducer was mounted to a motorized positioning system to accurately align its geometric focus with the needle tip. After alignment, the transducer was displaced downstream by 2 mm. The UCAs were subjected to 10- or 20-cycle tonebursts at peak-negative pressures of 0.3 to 2.3 MPa to facilitate UCA examination under unconstrained conditions in open fluid.

The data-acquisition system was controlled using a custom software package (Labview, National Instruments, Austin, TX) and was configured to screen consecutively acquired 20-line, M-mode data sets for valid backscatter events, which were then logged. The pulse repetition frequency of M-mode acquisition was 3 kHz. With this data-acquisition approach, each UCA was exposed to about six to eight pulses. Multiple exposures per agent guaranteed capturing a backscatter event as the UCA moved through a local pressure maximum.

2) Signal Processing—A postprocessing scheme based on singular-value decomposition (SVD) was employed to compute the subharmonic score (SHS) that represented the overall subharmonic activity detected from each UCA population. A detailed description of this processing scheme is available in [32].

SVD-based processing is a particularly robust method of analyzing large sets of data that were obtained with the same experimental conditions. For a given acoustic pressure and pulse duration, single pulse–echo events recorded from individual UCAs can be interpreted as the realizations of a random process (i.e., UCA response to ultrasound). The decomposition of UCA echoes onto an SVD-derived base is statistically optimal in the sense that the greatest variance by any projection of the data are on the first analyzing vector (AV), the second greatest variance on the second AV, etc. [44]–[46].

For each exposure condition, 500 UCA-backscatter events were recorded into a matrix, M . The SVD of M yielded AVs that were sorted in decreasing order of singular value, and the first AV whose Fourier-transform (FT) magnitude peaked at 10 MHz (AV10) was selected; SHS was computed by multiplying the average of the decomposition coefficient of M on AV10 by the FT magnitude of AV10 at 10 MHz. In other words, SHS represented the mean energy of the entire 500-echo data set in the subharmonic band defined only by the subharmonic response. The SVD-derived SHS provides a sensitive metric for quantifying subharmonic activity that represented the frequency of subharmonic events as well as the strength of the subharmonic component in the backscattered signal. Previously, the performance of the SVD approach was compared with the so-called conventional method, which detected a peak above the noise level at half the excitation frequency [32]. Results of that study demonstrated that the conventional and SVD methods performed well, but that the SVD approach led to more robust quantification of the subharmonic activity. The SVD-based processing is less susceptible to errors associated with echo signals of low signal-to-noise ratios.

IV. Results

A. Overpressure Study

A histogram (12 bins) of UCA diameters is shown in Fig. 3(a). The Point UCA had a fairly broad size distribution that was approximately centered around its nominal mean diameter of 3.4 μm . The four Philips UCAs had relatively narrower size distributions, which significantly overlapped each other and were centered around the nominal mean diameter of 2 μm .

A histogram (12 bins) of rupture pressures for each of the UCA populations is shown in Fig. 3(b). Each UCA population exhibited a distinct rupture-pressure range. The majority of UCAs that had STRRs of 7.5, 30, 40, or 65 $\text{nm}/\mu\text{m}$ ruptured when subjected to overpressure in the range of 2 to 330 kPa; however, only 50% of the UCAs that had a STRR of 100 $\text{nm}/\mu\text{m}$ ruptured in this pressure range. Rupture pressure did not exhibit a correlation with UCA size. However, the rupture pressure was strongly correlated with the nominal STRRs of each UCA population. Fig. 4 shows the results of one-way analysis of variance (ANOVA) performed on rupture-pressure measurements obtained from each UCA population. Mean rupture pressure obtained from each population was statistically different from the mean rupture pressures of other UCA populations ($p < 0.05$) and the mean rupture pressure exhibited a linear relationship to STRR ($R^2 = 0.9998$).

B. Subharmonic Study

When excited at 20 MHz, individual polymer-shelled UCAs produced substantial backscatter, and in some cases, a subharmonic component. Representative subharmonic waveforms obtained for 10- and 20-cycle excitations are shown in Figs. 5(a) and 5(b), respectively. The envelopes of the echo signals (shown in red) highlight the harmonic components present in the signal. Corresponding power spectra are shown in Figs. 5(c) and 5(d). UCA responses to both pulse durations exhibited a subharmonic peak that was within

20 dB of the fundamental peak. In addition to the subharmonic peak, an ultraharmonic peak at 30 MHz and a second-harmonic peak at 40 MHz also were observed.

Fig. 6 shows the SVD-derived SHS for each UCA population as a function of peak-negative pressure (P_-) ranging from 0.4 to 2.3 MPa. Each data point in the SHS curve represents a mean of SHS obtained from 6 data sets, each containing 500 backscatter events from individual UCAs. The mean SHS value provided a quantitative, cumulative indication of the frequency and strength of subharmonic events detected in a 3000-UCA data set for each incident pressure and pulse duration. The UCA-SHS data are presented in an increasing order of STRR in Figs. 6(a)–(e). Acoustic threshold (p) for subharmonic onset for each SHS curve was obtained by fitting the SHS data to an exponential function, $\text{SHS}(P_-) = \text{SHS}(P_- - p) = 0.4 \text{ MPa}) (10^{P_-/p})$; p determined in this manner provided the value of peak-negative pressure at which the SHS was 10 times the value at 0.4 MPa (indicated by vertical black lines in Fig. 6).

For all UCA populations, the UCAs subjected to 20-cycle excitation produced 2 to 3 times greater subharmonic activity in comparison to UCAs subjected to 10-cycle excitation. UCA-SHS data indicated that Point UCAs produced substantially less subharmonic activity than any of the Philips UCAs; the maximum SHS values for the Philips UCAs were between 12 and 28 times the peak SHS for the Point UCAs. However, the subharmonic-onset threshold determined by fitting the Point data to an exponential function was approximately half that for the Philips-UCA populations (1.2 to 1.5 MPa).

The UCA-SHS data did not exhibit a correlation with STRR. All four Philips UCAs exhibited similar SHS- P_- profiles with nearly the same onset threshold; SHS values increased rapidly as P_- increased beyond the onset threshold. Unlike the Point UCAs, none of the Philips UCAs exhibited a distinct SHS peak in the range of P_- investigated in this study. The Philips UCAs with the highest STRR (100 nm/ μm) produced the largest SHS values. The Philips UCAs with a STRR of 40 nm/ μm produced a SHS that was approximately 50% of the SHS obtained from the other Philips UCAs. This disparity might be attributed to the fact that the 40-nm STRR UCAs were manufactured approximately six months before the other Philips UCAs; although relatively long, the shelf life of polymer-shelled UCAs is not well documented.

V. Discussion

A. UCA-Shell Rupture

The overpressure results (Fig. 4) indicated that the UCA-rupture threshold is linearly related to STRR. This experimental observation is in agreement with recent theoretical predictions of compressional response of a polymer shell by Marmottant *et al.* [30]. This result also is consistent with another recent experimental study [29], which subjected polymer-shelled UCAs of STRRs ranging from 22 to 100 nm/ μm to 1-MHz ultrasound (incident pressures ranging from 0.1 to 1.6 MPa) and reported a linear relationship between STRR and incident acoustic pressure necessary for inducing rupture. These prior studies also indicated that UCAs can be divided into two categories: one that exhibits a distinct buckling phase before shell rupture (critical buckling pressure < rupture pressure), and the other that exhibits abrupt rupture, i.e., no distinct buckling (critical buckling pressure $\approx$ rupture pressure). The UCAs investigated in the presented study likely corresponded to the latter category.

The linear relationship between STRR and rupture pressures implies that the fragility of polymer-shelled UCAs can be customized by appropriately prescribing the STRR of UCAs during the production phase. The ability to control this attribute makes polymer-shelled UCAs ideally suited for ultrasound-mediated, localized drug delivery. A previous study by

Böhmer *et al.* [47] employed three of the same agents used in the present study (STRRs of 40, 65, and 100 nm/ μ m) to demonstrate that the ability of an agent to disperse therapeutics triggered by shell rupture was influenced by its STRR. UCAs were embedded in gel phantoms and subjected to 1-MHz ultrasound at different incident pressures. The area of the UCA-destruction zone was assessed by measuring the area of the echogenic region in ultrasound images (Fig. 7). The diameter of the UCA-destruction zone increased with an increase in incident acoustic pressure, but decreased with an increase in STRR for each incident pressure. Böhmer *et al.* also showed a similar trend *in vivo* using dye-loaded UCAs with the same three STRRs [47].

In the context of prior results reported by Böhmer *et al.* [47] and Lensen *et al.* [29], the overpressure-rupture results in the present study indicated that the fragility of UCAs was directly related to their dynamic response to ultrasound and subsequent ultrasonically induced rupture. This conjecture is further supported by prior observations made by Bouakaz *et al.* [25] in which polymer-shelled UCAs were shown to undergo a compression-only behavior analogous to stable cavitation when subjected to ultrasonic excitation in the range of 1 to 5 MHz; although occurring in a faster time scale than rupture dynamics involved in the current overpressure study, the compression-only response to ultrasound is likely to subject the UCA shell to stresses similar to those induced by overpressure and could result in buckling and rupture. Therefore, the overpressure methods potentially offer a convenient means of selecting or optimizing UCAs and their shell properties for different drug-delivery applications.

In addition to characterizing rupture behavior, the overpressure results can be used to estimate specific shell properties such as thickness and the Young's modulus, or gain an insight into the inhomogeneities and imperfections in the UCA shell. For instance, when fitted to (2), the measured rupture pressure for the Philips UCAs with a STRR of 40 nm/ μ m results in a predicted Young's modulus of 65 MPa, which is inconsistent with the Young's modulus value obtained from an AFM-based estimate of approximately 1500 MPa [36]. The rupture-pressure data also could be fitted to theoretical models such as the spherical capsule model (3) proposed by Ru [39], which describes the critical buckling threshold while accounting for an effective shell thickness and non-negligible transverse shear resulting from a nonuniform shell. A fit between experimentally inferred UCA-rupture pressure and the theoretically predicted rupture pressure obtained from the Ru model indicated that the inhomogeneities and imperfections in the shell of the same UCA (STRR = 40 nm/ μ m) might result in structural integrity equivalent to a perfectly intact and homogenous UCA of STRR $\sim$ 5 nm/ μ m. Similar analysis based on fitting the rupture data to the Ru model was performed for all five UCA populations, and the corresponding equivalent bending thicknesses are presented in Table I. This equivalent bending thickness might play an important role in the dynamic response of UCAs and will be discussed in the following section.

B. UCA-Resonance Behavior

Although the majority of UCAs, including those employed in this study, were originally designed for applications involving conventional diagnostic frequencies (1 to 10 MHz), the polymer-shelled UCAs, particularly the Philips UCA populations, produced substantial subharmonic activity when excited at 20 MHz, as evidenced by the SHS inferred from backscatter measurements. The presence of subharmonic activity from these UCAs at high frequencies could provide a means for ultrasound-based vascular imaging in small animals, and can potentially facilitate monitoring of UCA-based drug delivery.

1) Rupture-Based Hypothesis—One of hypothesized mechanisms of subharmonic generation involves UCA rupture and subsequent release and oscillation of the free gas

bubble. Observations made in a previous study involving low-frequency (<10 MHz) excitation of polymer-shelled UCAs are consistent with this hypothesis [25]. However, the results of the present study (20-MHz excitation frequency) appear inconsistent with the rupture-based mechanism for subharmonic generation. UCAs with the highest STRRs produced the greatest SHS values, but our overpressure study indicated that these UCAs were the most resistant to shell rupture. Furthermore, the SVD-derived SHS was uncorrelated to STRRs or the mean rupture pressures for the UCA populations examined in the present study. Finally, if the subharmonic activity observed in our HFU-backscatter study did result from shell rupture and oscillation of gas bubbles, the resonance frequency of these gas bubbles is expected to be in the range of 1 to 6 MHz (based on a bubble diameter ranging from 1 to 4 μm); the 20-MHz excitation frequency employed in this study is more than three times the bubble resonance frequency and, therefore, is unlikely to result in nonlinear oscillations of free gas bubbles necessary to produce subharmonic activity [48]. Other alternatives to the rupture hypothesis include higher-order shell waves [49] or radial oscillations similar to a free gas or lipid microbubble [6], [50].

2) Influence of Shell Thickness on Radial Oscillations—The shell parameters such as elasticity and thickness strongly influence the resonance frequency of encapsulated microbubbles [6]. An examination of the UCA scattering cross-section, which depends on UCA size, shell properties, and the excitation frequency, can provide an insight into the propensity of a particular polymer-shelled UCA for producing subharmonic activity. The UCA-shell properties listed in Table I were employed to calculate the scattering cross-section of each UCA population as a function of excitation frequency for two cases: a shell of nominal thickness and a shell corresponding to the effective bending thickness obtained from the Ru model (Fig. 8). The peak of the scattering cross-section corresponds to the resonance frequency of the UCA. Because the compliance of the UCA shell is critically dependent on shell properties, imperfections and heterogeneities in the shell were conjectured to increase the compliance of UCAs to ultrasonic excitation, thereby lowering the expected resonance frequency. For instance, an UCA that has a 40-nm shell with imperfections might exhibit a resonance behavior of an UCA with a perfectly intact, homogenous, 5-nm shell.

When using the shell properties corresponding to an intact, homogenous shell, the Hoff model predicted a resonance frequency of the Philips UCAs that was in excess of 60 MHz [Fig. 8(a)]. However, the resonance frequency of the Point UCAs was predicted to be 24.1 MHz, which is relatively close to the acoustic driving frequency of 20 MHz. The disparity in predicted resonance behavior between the Point and Philips UCAs indicated that the Philips UCAs might require stronger acoustic forcing to induce nonlinear oscillations and subsequent subharmonics in comparison to the Point UCAs. This is consistent with our experimental observations based on UCA SHS (Fig. 6): the subharmonic-onset threshold (i.e., the minimum acoustic pressure that results in SHS equivalent to 10 times the baseline) for Philips UCAs was approximately twice that for the Point UCAs.

Although the Point UCAs had a lower subharmonic-onset threshold and a predicted resonance frequency that was in the vicinity of the 20-MHz excitation frequency, they produced substantially weaker subharmonic activity in comparison to the Philips UCAs. Furthermore, the peak subharmonic activity from the Point UCAs was observed for an excitation pressure of approximately 0.8 MPa; no subharmonic activity was observed from Point UCAs beyond an acoustic pressure of 1 MPa. This reduction in SHS beyond 0.8 MPa excitation pressure might be a result of ultrasound-induced destruction of Point UCAs; overpressure studies had indicated that the Point UCAs, which have a STRR of 7.5 $\text{nm}/\mu\text{m}$, have a rupture threshold that is between 20% and 50% of the rupture pressures measured from the Philips UCAs. However, broadband noise emissions similar to those previously

reported as the hallmark of ultrasonic destruction of lipid-shelled UCAs [51], were not detected in measurements acquired by the 20-MHz transducer.

As indicated by the Ru model, the imperfections in the UCA shell can weaken the integrity of the shell structure, thereby resulting in an effective shell thickness that is less than the nominal shell thickness corresponding to a perfectly homogenous microstructure. When using the effective shell thickness values (determined by fitting the rupture-pressure data to the Ru model), the Hoff model predicted a significant decrease in UCA resonance frequencies [Fig. 8(b)]. This observation was particularly dramatic for thick-shelled Philips UCAs. In the context of these theoretical predictions, the strong subharmonic response experimentally observed from thick-shelled Philips UCAs indicates that the shell parameters, particularly the imperfections and heterogeneities, might have a substantial influence on the response. However, the Hoff model only accounts for radial oscillations of the bubble. The analysis presented in this study cannot exclude other methods of subharmonic generation such as shell waves or higher-order oscillation modes.

VI. Conclusion

Polymer-shelled microbubbles can serve as highly engineered agents for enhancing ultrasound contrast and delivering therapeutic payloads. The manufacturing process facilitates customization of STRR, which directly influences the threshold for acoustically induced rupture of a UCA population despite the differences in the size of individual UCAs. We investigated polymer-shelled UCAs made using the same shell material, but with different STRRs to determine the influence of shell properties on UCA rupture. A convenient method for characterizing the mechanical response of UCA populations to overpressure was presented. The mechanical response was shown to be directly related to the dynamic response of UCAs when excited with ultrasound. Therefore, the overpressure method provides a suitable means for characterizing UCA-shell properties for predicting and comparing the propensity of ultrasound-induced rupture of different UCA populations before costly *in vivo* experiments. The HFU-backscatter study demonstrated that the polymer-shelled UCAs can produce substantial subharmonic activity when excited at 20 MHz, which can facilitate vascular imaging. This attribute further enhances practical utility of these UCAs in image-guided, ultrasound-mediated, localized drug delivery.

Acknowledgments

This research was supported by National Institutes of Health research grant EB006372. The authors thank Dr. X. Chen and Dr. M. Böhmer for providing access to the Point and Philips UCAs, respectively. The authors also thank Dr. J. S. Allen for helpful discussions.

Biographies

Parag V. Chitnis (M'08) was born in Bhopal, India, in 1979. He received a B.S. degree in engineering physics and mathematics from the West Virginia Wesleyan College,

Buckhannon, WV, in 2000. He received M.S. and Ph.D. degrees in mechanical engineering from Boston University in 2002 and 2006, respectively. His dissertation focused on experimental studies of acoustic shock waves for therapeutic applications. After a two-year postdoctoral fellowship at Boston University involving a study of bubble dynamics, Dr. Chitnis joined Riverside Research as a Member of the Research Staff in 2008. His fields of interest include therapeutic ultrasound, photoacoustic imaging, high-frequency ultrasound, and ultrasound contrast agents.

Sujeethraj Koppolu (S'11) was born in Nellore, India, in 1987. He received his B.E. degree in biomedical engineering from Osmania University, India, in 2005 and his M.S. degree in biomedical engineering from the Polytechnic Institute of New York University (NYU), in 2012. He is currently working as a research intern at the Frederic L. Lizzi Center for Biomedical Engineering, Riverside Research, New York. His research interests lie in the areas of contrast agents and drug delivery systems for ultrasound and MR imaging.

Jonathan Mamou (SM'11) was born in Saint-Germain-En-Laye, France, in 1978. In July 2000, he graduated from the Ecole Nationale Supérieure des Télécommunications in Paris, France. In January 2001, he began his graduate studies in electrical and computer engineering at the University of Illinois at Urbana-Champaign, Urbana, IL. He received his M.S. and Ph.D. degrees in May 2002 and 2005, respectively. He is now a Principal Member of the Research Staff at Riverside Research in New York, NY. His fields of interest include theoretical aspects of ultrasonic scattering, ultrasonic medical imaging, ultrasound contrast agents, and biomedical image processing.

Jonathan Mamou is a Senior Member of IEEE, a Senior Member of the American Institute of Ultrasound in Medicine, and a Member of the Acoustical Society of America.

Ceciel Chlon was born in Horst, The Netherlands, in 1979. She received her B.Sc. degree in chemistry in 2001 from the Fontys Hogeschool in Eindhoven, The Netherlands. After working for 5 years in the field of coatings and polymers at a Dutch research institute, she joined Philips Research in Eindhoven, The Netherlands, in 2006. At Philips, she started her career in the field of ultrasound contrast agent preparation and process optimization. In the last few years, her research activities focused on ultrasound-mediated delivery of drugs.

Jeffrey A. Ketterling (SM'11) was born in Seattle, WA, in 1970. He received the B.S. degree in electrical engineering from the University of Washington, Seattle, WA, in 1994. He received the Ph.D. degree in mechanical engineering from Yale University, New Haven, CT, in 1999. His thesis focused on experimental studies of phase-space stability in single bubble sonoluminescence.

Dr. Ketterling joined the Lizzi Center for Biomedical Engineering at Riverside Research in 1999 as a Member of the Research Staff, served as Research Manager from 2008 to 2012, and currently acts as the Associate Director for the group. He serves as the principal investigator for programs supported by the National Institutes of Health that deal with high-frequency annular arrays for small animal imaging and ophthalmic imaging, high-frequency acoustic contrast agents for microcirculation imaging in small animals and eyes, and hydrophone arrays for characterizing the instantaneous acoustic fields of lithotripters.

Dr. Ketterling was the Technical Chair for the Biomedical Acoustics Committee of the Acoustical Society of America from 2008 through 2011 and he is currently a member of the IEEE International Ultrasonics Symposium's Medical Ultrasonics Technical Program Committee.

References

- [1]. Ophir J, Parker KJ. Contrast agents in diagnostic ultrasound. *Ultrasound Med. Biol.* 1989; 15(4): 319–333. [PubMed: 2669297]
- [2]. Goldberg BB, Liu J, Forsberg F. Ultrasound contrast agents: A review. *Ultrasound Med. Biol.* 1994; 20(4):319–333. [PubMed: 8085289]
- [3]. Goertz DE, Frijlink ME, de Jong N, van der Steen A. Nonlinear intravascular ultrasound contrast imaging. *Ultrasound Med. Biol.* 2006; 32(4):491–502. [PubMed: 16616596]

- [4]. Bouakaz A, Frigstad S, Ten Cate FJ, de Jong N. Super harmonic imaging: A new imaging technique for improved contrast detection. *Ultrasound Med. Biol.* 2002; 28(1):59–68. [PubMed: 11879953]
- [5]. Shi W, Forsberg F, Hall A, Chiao R, Liu J, Miller S, Thomenius K, Wheatley M, Goldberg B. Subharmonic imaging with microbubble contrast agents: Initial results. *Ultrason. Imaging.* 1999; 21(2):79–94. [PubMed: 10485563]
- [6]. Hoff L, Sontum P, Hovem JM. Oscillations of polymeric microbubbles: Effect of the encapsulating shell. *J. Acoust. Soc. Am.* 2000; 107(4):2272–2280. [PubMed: 10790053]
- [7]. Unger EC, McCreery TP, Sweitzer RH. Ultrasound enhances gene expression of liposomal transfection. *Invest. Radiol.* 1997; 32(12):723–727. [PubMed: 9406011]
- [8]. Price RJ, Skyba DM, Kaul S, Skalak TC. Delivery of colloidal particles and red blood cells to tissue through microvessel ruptures created by targeted microbubble destruction with ultrasound. *Circulation.* 1998; 98(13):1264–1267. [PubMed: 9751673]
- [9]. Unger EC, Matsunaga TO, McCreery T, Schumann P, Sweitzer R, Quigley R. Therapeutic applications of microbubbles. *Eur. J. Radiol.* 2002; 42(2):160–168. [PubMed: 11976013]
- [10]. Klivanov AL. Microbubble contrast agents: Targeted ultrasound imaging and ultrasound-assisted drug-delivery applications. *Invest. Radiol.* 2006; 41(3):354–362. [PubMed: 16481920]
- [11]. Böhmer MR, Klivanov AL, Tiemann K, Hall CS, Gruell H, Steinbach OC. Ultrasound triggered image-guided drug delivery. *Eur. J. Radiol.* 2009; 70(2):242–253. [PubMed: 19272727]
- [12]. Ferrara K, Pollard R, Borden M. Ultrasound microbubble contrast agents: Fundamentals and application to gene and drug delivery. *Annu. Rev. Biomed. Eng.* 2007; 9(1):415–447. [PubMed: 17651012]
- [13]. Suzuki R, Takizawa T, Negishi Y, Utoguchi N, Maruyama K. Effective gene delivery with liposomal bubbles and ultrasound as novel non-viral system. *J. Drug Target.* 2007; 15(7–8):531–537. [PubMed: 17671899]
- [14]. Haag P, Frauscher F, Gradl J, Seitz A, Schfer G, Lindner JR, Klivanov AL, Bartsch G, Klocker H, Eder IE. Microbubble-enhanced ultrasound to deliver an antisense oligodeoxynucleotide targeting the human androgen receptor into prostate tumours. *J. Steroid Biochem. Mol. Biol.* 2006; 102(1–5):103–113. [PubMed: 17055720]
- [15]. Foster FS, Burns PN, Simpson DH, Wilson SR, Christopher DA, Goertz DE. Ultrasound for the visualization and quantification of tumor microcirculation. *Cancer Metastasis Rev.* 2000; 19(1–2):131–138. [PubMed: 11191052]
- [16]. Wirtzfeld LA, Wu G, Bygrave M, Yamasaki Y, Sakai H, Moussa M, Izawa JI, Downey DB, Greenberg NM, Fenster A, Xuan JW, Lacefield JC. A new three-dimensional ultrasound microimaging technology for preclinical studies using a transgenic prostate cancer mouse model. *Cancer Res.* 2005; 65(14):6337–6345. [PubMed: 16024636]
- [17]. Banihashemi B, Vlad R, Debeljevic B, Giles A, Kolios MC, Czarnota GJ. Ultrasound imaging of apoptosis in tumor response: Novel preclinical monitoring of photodynamic therapy effects. *Cancer Res.* 2008; 68(20):8590–8596. [PubMed: 18922935]
- [18]. Goertz DE, Yu JL, Kerbel RS, Burns PN, Foster FS. High-frequency Doppler ultrasound monitors the effects of antivascular therapy on tumor blood flow. *Cancer Res.* 2002; 62(22):6371–6375. [PubMed: 12438217]
- [19]. Foster FS, Zhang MY, Zhou YQ, Liu G, Mehi J, Cherin E, Harasiewicz KA, Starkoski BG, Zan L, Knapik DA, Adamson SA. A new ultrasound instrument for in vivo microimaging of mice. *Ultrasound Med. Biol.* 2002; 28(9):1165–1172. [PubMed: 12401387]
- [20]. Goertz D, Cherin E, Needles A, Karshafian R, Brown A, Burns P, Foster F. High frequency nonlinear B-scan imaging of microbubble contrast agents. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control.* Jan.2005 52(1):65–79. [PubMed: 15742563]
- [21]. Needles A, Goertz D, Karshafian R, Cherin E, Brown A, Burns P, Foster F. High-frequency subharmonic pulsed-wave Doppler and color flow imaging of microbubble contrast agents. *Ultrasound Med. Biol.* 2008; 34(7):1139–1151. [PubMed: 18328617]
- [22]. Sprague MR, Chrin E, Goertz DE, Foster FS. Nonlinear emission from individual bound microbubbles at high frequencies. *Ultrasound Med. Biol.* 2010; 36(2):313–324. [PubMed: 20018429]

- [23]. Helfield BL, Cherin E, Foster FS, Goertz DE. Investigating the subharmonic response of individual phospholipid encapsulated microbubbles at high frequencies: A comparative study of five agents. *Ultrasound Med. Biol.* 2012; 38(5):846–863. [PubMed: 22402024]
- [24]. Shankar PM, Krishna PD, Newhouse VL. Subharmonic backscattering from ultrasound contrast agents. *J. Acoust. Soc. Am.* 1999; 106(4):2104–2110. pt. 1. [PubMed: 10530033]
- [25]. Bouakaz A, Versluis M, de Jong N. High-speed optical observations of contrast agent destruction. *Ultrasound Med. Biol.* 2005; 31(3):391–399. [PubMed: 15749563]
- [26]. Bjerknes K, Sontum PC, Smistad G, Agerkvist I. Preparation of polymeric microbubbles: Formulation studies and product characterisation. *Int. J. Pharm.* 1997; 158(2):129–136.
- [27]. Chlon C, Guedon C, Verhaagen B, Shi WT, Hall CS, Lub J, Bohmer MR. Effect of molecular weight, crystallinity, and hydrophobicity on the acoustic activation of polymer-shelled ultrasound contrast agents. *Biomacromolecules.* 2009; 10(5):1025–1031. [PubMed: 19351154]
- [28]. Chitnis PV, Lee P, Mamou J, Allen JS, Böhmer M, Ketterling JA. Rupture threshold characterization of polymer-shelled ultrasound contrast agents subjected to static overpressure. *J. Appl. Phys.* 2011; 109(8) art. no. 084906.
- [29]. Lensen D, Gelderblom EC, Vriezema DM, Marmottant P, Verdonchot N, Versluis M, de Jong N, van Hest JCM. Biodegradable polymeric microcapsules for selective ultrasound-triggered drug release. *Soft Matter.* 2011; 7(11):5417–5422.
- [30]. Marmottant P, Bouakaz A, Jong N, Quilliet C. Buckling resistance of solid shell bubbles under ultrasound. *J. Acoust. Soc. Am.* 2011; 129(3):1231–1239. [PubMed: 21428486]
- [31]. Shi W, Böhmer M, de Winter S, Steenbakkers J, Emmer M, van Wamel A, de Jong N, Hall C. Ultrasonic characterization of novel monodispersed contrast agents. *IEEE Ultrasonics Symp.* 2006:301–304.
- [32]. Mamou J, Ketterling JA. Subharmonic analysis using singular-value decomposition of ultrasound contrast agents. *J. Acoust. Soc. Am.* 2009; 125(6):4078–4091. [PubMed: 19507989]
- [33]. De Jong N, Frinkning PJ, Bouakaz A, Goorden M, Schourmans T, Xu J, Mastik F. Optical imaging contrast agent microbubbles in an ultrasound field with a 100-MHz camera. *Ultrasound Med. Biol.* 2000; 26(3):487–492. [PubMed: 10773381]
- [34]. Sboros V, Moran CM, Pye SD, McDicken WN. The behaviour of individual contrast agent microbubbles. *Ultrasound Med. Biol.* 2003; 29(5):687–694. [PubMed: 12754068]
- [35]. Leong-Poi H, Song J, Rim S, Christiansen J, Kaul S, Lindner JR. Influence of microbubble shell properties on ultrasound signal: Implications for low-power perfusion imaging. *J. Am. Soc. Echocardiogr.* 2002; 15(10):1269–1276. pt. 2. [PubMed: 12411916]
- [36]. Sboros V, Glynos E, Pye SD, Moran CM, Butler M, Ross JA, McDicken WN, Koutsos V. Nanomechanical probing of microbubbles using the atomic force microscope. *Ultrasonics.* 2007; 46(4):349–354. [PubMed: 17720211]
- [37]. Fok SL, Allwright DJ. Buckling of a spherical shell embedded in an elastic medium loaded by a far-field hydrostatic pressure. *J. Strain Anal.* 2001; 36(6):535–544.
- [38]. Kaplan, A. Buckling of spherical shells. In: Fung, YC.; Sechler, EE., editors. *Thin-Shell Structures; Theory, Experiment and Design.* Prentice Hall; Upper Saddle River, NJ: 1974.
- [39]. Ru CQ. Buckling of empty spherical viruses under external pressure. *J. Appl. Phys.* 2009; 105(12) art. no. 124701.
- [40]. Kooiman K, Böhmer MR, Emmer M, Vos HJ, Chlon C, Shi WT, Hall CS, de Winter S, Schron K, Versluis M, de Jong N, van Wamel A. Oil-filled polymer microcapsules for ultrasound-mediated delivery of lipophilic drugs. *J. Control. Release.* 2009; 133(2):109–118. [PubMed: 18951931]
- [41]. Ottoboni, TB.; Tickner, EG.; Short, RE.; Yamamoto, RK. Hollow microspheres with controlled fragility for medical use. U.S. Patent 6 776 761 B2. Aug.. 2004
- [42]. Sboros V. Response of contrast agents to ultrasound. *Adv. Drug Deliv. Rev.* 2008; 60(10):1117–1136. [PubMed: 18486270]
- [43]. Yuen HK, Princen J, Illingworth J, Kittler J. Comparative study of Hough transform methods for circle finding. *Image Vis. Comput.* 1990; 8(1):71–77.
- [44]. Gentle, JE. *Statistics and Computing.* Springer; New York, NY: 1998. Numerical linear algebra for applications in statistics.

- [45]. DiPietroPaolo D, Miller H-P, Nolte G, Ern S. Noise reduction in magnetocardiography by singular value decomposition and independent component analysis. *Med. Biol. Eng. Comput.* 2006; 44(6):489–499. [PubMed: 16937200]
- [46]. VanBaren P, Ebbini ES. Multipoint temperature control during hyperthermia treatments: Theory and simulation. *IEEE Trans. Biomed. Eng.* 1995; 42(8):818–827. [PubMed: 7642196]
- [47]. Böhmer M, Chlon C, Raju B, Chin C, Shevchenko T, Klibanov A. Focused ultrasound and microbubbles for enhanced extravasation. *J. Control. Release.* 2010; 148(1):18–24. [PubMed: 20600402]
- [48]. Leighton, TG. *The Acoustic Bubble*. Academic; New York, NY: 1994.
- [49]. Allen JS, Kruse DE, Ferrara KW. Shell waves and acoustic scattering from ultrasound contrast agents. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control.* 2001; 48(2):409–418. [PubMed: 11370354]
- [50]. Church CC. The effects of an elastic solid surface layer on the radial pulsations of gas bubbles. *J. Acoust. Soc. Am.* 1995; 97(3):1510–1521.
- [51]. Ammi A, Cleveland R, Mamou J, Wang G, Bridal S, O'Brien W. Ultrasonic contrast agent shell rupture detected by inertial cavitation and rebound signals. *IEEE Trans. Ultrason. Ferroelectr. Freq. Control.* 2006; 53(1):126–136. [PubMed: 16471439]

Fig. 1.

(a) Schematic of the experimental setup for the overpressure study. Ultrasound contrast agents were subjected to overpressure in a glycerol-filled, cylindrical test chamber while being imaged with a video microscope. (b) A sample image and (c)–(e) illustration of the steps involved in the image-processing algorithm.

Fig. 2.

Schematic of the subharmonic-study experimental setup. A dilute solution of ultrasound contrast agents (UCAs) was injected into a test tank via a 200- μm needle. The focus of a 20-MHz transducer was aligned 2 mm downstream. Individual UCAs were subjected to 20-MHz tonebursts and backscatter signals were recorded.

Fig. 3.

Histograms (12 bins) of ultrasound contrast agent (UCA) (a) diameter and (b) rupture pressure. Each UCA population consisted of at least 300 UCAs. All five Philips UCA populations exhibited a Gaussian size distribution centered around the nominal mean diameter of 2 μm ; the Point UCA population exhibited a polydisperse size distribution that was centered around its nominal mean diameter of 3.4 μm . Rupture pressure histograms indicated that different UCA populations exhibited different rupture thresholds. The Point UCAs had the lowest threshold for rupture; the Philips UCAs with a shell-thickness-to-radius ratio (STRR) of 100 nm/ μm had the highest rupture threshold. Only 50% of these UCAs ruptured when subjected to up to 330 kPa of overpressure. UCAs that did not rupture at overpressures below 330 kPa are categorized as NR.

Fig. 4.

Mean ultrasound contrast agent (UCA)-rupture pressure with respect to shell-thickness-to-radius ratio (STRR). The box plot was obtained by performing one-way analysis of variance (ANOVA). The mean rupture pressure for each UCA population was statistically different from mean rupture pressures of other UCAs (indicated by *). The rupture pressure was linearly related to STRR. 📷

Fig. 5. Representative waveforms of the backscattered ultrasound in response to (a) 10-cycle and (b) 20-cycle excitations. The envelope of the signals highlights the subharmonic component. (c) and (d) Corresponding power spectra. Substantial subharmonic activity was detected in many of the backscatter events.

Fig. 6.

(a)–(e) Singular-value decomposition (SVD)-derived subharmonic score (SHS) for each ultrasound contrast agent (UCA) population for (dashed line) 10-cycle excitation and (solid line) 20-cycle excitation. Each data point represents a mean of SHS values obtained from six 500-UCA data sets. The error bars indicate the standard deviation. For all UCAs, the 20-cycle excitation produced greater values of SHS than the 10-cycle excitation. The Point UCAs (a) produced the lowest SHS, indicating relatively weak and infrequent subharmonic events. The Point SHS indicated that peak subharmonic activity occurred at approximately 0.8 MPa for both pulse durations. All four Philips UCAs exhibited significantly higher values of SHS than the Point UCA. In the pressure range that was investigated, none of the Phillips UCAs indicated a peak of SHS. The subharmonic onset was estimated from the pressure required to reach an SHS of 10 times the initial value by fitting the curves to a power law and are indicated by the vertical black lines.

Fig. 7.

Diameter of the ultrasound-induced destruction zone of the polymer-shelled ultrasound contrast agents (UCAs) with respect to incident acoustic pressure. (Data for the figure courtesy of Böhmer *et al.* [47].) The damage area increased with increase in incident pressure, but decreased with an increase in shell-thickness-to-radius ratio (STRR).

Fig. 8.

Theoretically predicted scattering cross-section of the ultrasound contrast agent (UCA) populations for two cases: (a) nominal shell thickness inferred using shell-thickness-to-radius ratio (STRR) and the measured mean UCA size, i.e., an intact, homogenous UCA; and (b) a shell of effective bending thickness estimated by fitting the overpressure-rupture data to the Ru model (3), i.e., an inhomogeneous UCA with shell imperfections. The UCA parameters employed for these calculations are presented in Table I. The resonance frequencies of the Philips UCAs obtained for the homogenous shell are up to 4 times greater than the excitation frequency of 20 MHz. When estimated using the inhomogeneous shell, the resonance frequencies shift close to 20 MHz. The resonance frequency of the homogenous Point UCAs was estimated to be 24 MHz, which indicated that the Point UCA might require a lower acoustic pressure to induce nonlinear oscillations.

TABLE I

Effective Bending Thickness Obtained by Fitting Ultrasound Contrast Agent (UCA) Rupture Data to the Ru Model (3).

UCA type	Nominal STRR (nm/ μm)	Mean radius (μm)	Shell thickness (nm)	Effective bending thickness (nm)
Point	7.5	1.9	14.5	9.4
Philips	30	1.1	31.6	4.8
Philips	40	1.0	40.2	4.7
Philips	65	1.3	82.2	6.4
Philips	100	1.2	121.5	6.6

In addition to the nominal values of STRR and the measured mean radius for each UCA population, the following values of the shell parameters were employed in (3): $E = 1.35$ GPa, $G = E/(2(1 + \nu))$, $G_t = 0.4G$, $\mu = 0.4$, and $\nu = 0.4$.

STRR = shell-thickness-to-radius ratio.