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● *Technical Note*

"COMPRESSION-ONLY" BEHAVIOR OF PHOSPHOLIPID-COATED CONTRAST BUBBLES

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Abstract—Ultrasound contrast agents oscillate approximately linearly up to a certain pressure range where nonlinearity sets in. Nonlinear microbubble oscillations are exploited in ultrasound pulse-echo imaging as this improves the contrast-to-tissue ratio. Here we report the observation of a highly nonlinear response of phospholipid-coated contrast agents at pressures as low as 50 kPa, termed "compression-only" behavior, where the microbubbles only compress, yet hardly expand. Time-resolved bubble dynamics recorded through ultra high-speed imaging revealed that nearly 40% of the coated bubbles show "compression-only" behavior. (E-mail: n.dejong@erasmusmc.nl) © 2007 World Federation for Ultrasound in Medicine & Biology.

INTRODUCTION

Ultrasound contrast agents (UCAs) are small encapsulated microbubbles (mean diameter ~3 μm) that are used to enhance the diagnostic quality of medical ultrasound images. Current imaging modalities for contrast agents, such as power modulation (Eckersley et al. 2005) and pulse inversion imaging (Burns et al. 2000), are based on nonlinear bubble responses. The nonlinear bubble response is used to discriminate between contrast agent and tissue. For small acoustic pressures, up to 20 kPa, the bubble response is predominantly linear (Hoff 2000). At elevated pressures, nonlinear responses start to occur; the compression phase differs from the expansion phase. At acoustic pressures above ~100 kPa, the nonlinearity increases, accompanied by changes in the bubble shape and dimension, resulting in bubble destruction in the extreme case.

The backscattered signal from a nonlinearly oscillating bubble contains not only the fundamental (transmitted) frequency, but also harmonic frequencies, at twice the fundamental frequency (second harmonic), or even higher multiples of the insonation frequency (third, fourth, etc.). This nonlinear scattering effect is not displayed by tissue and it, therefore, offers a possibility to separate the response of the bubble from that of surrounding tissue. Currently, mainly the second harmonic scattering of the bubbles is adopted in commercial diagnostic ultrasound machines. There are two main reasons for this. First, the second harmonic scattering from the bubbles is the most intense compared with higher harmonic scattering. Second, the frequency bandwidth of current imaging array transducers is, in general, limited to 70% to 80% of the center frequency and, therefore, does not allow operation of higher harmonic imaging (e.g., at the third harmonic) without a substantial sacrifice of resolution and sensitivity. These factors have rendered the imaging of such higher harmonic frequencies alone almost inconceivable. Using higher harmonics, a new imaging technique termed "superharmonic imaging" has been proposed by Bouakaz et al. (2002), where selective imaging of higher harmonics showed improved image quality and contrast detection. Superharmonic imaging showed as much as 50 dB in contrast-to-tissue ratio.

The ultrasound imaging techniques proposed to date exploit nonlinear oscillations of contrast agent microbubbles as they promote the contrast-to-tissue ratio. Nonlinearity, however, sets in at relatively high acoustic pressures. Here, we report the observation of a highly nonlinear response of phospholipid-coated contrast agents at moderate acoustic pressure levels (as low as 50 kPa), termed "compression-only" behavior, where the microbubbles only compress, yet hardly expand.

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METHODS

SonoVue (Bracco Research SA, Geneva, Switzerland) or BR-14 contrast bubbles were prepared in the vial. Both types of bubbles present a phospholipid coating; SonoVue contains SF_6 gas, whereas BR-14 contains perfluorocarbon gas, which is even less soluble in water. The contrast bubbles were fed through a capillary fiber inside a small water-filled container (Fig. 1). An Olympus microscope with a 60× high-resolution water-immersed objective (NA = 0.9) and a 2× magnifier produced an image of the contrast bubbles. The image was then relayed to the high-speed framing camera Brandaris-128 (Chin et al. 2003). A focused broadband single-element transducer was mounted at 75 mm from the capillary. A Tektronix AWG 520 arbitrary waveform generator provided an ultrasound burst that was amplified by an ENI A-500 amplifier. The bubble response was investigated with an ultrasound burst of eight cycles at a frequency of 1, 1.8 or 4 MHz and pressures of 50, 100 or 200 kPa. The Brandaris camera was operated at a framing rate of 15 million frames per second, thereby resolving the insonified microbubble dynamics.

A diameter-time curve is measured for each bubble using image analysis software developed in-house (van der Meer et al. 2006). The boundary detection algorithm for the diameter determination is based on a minimum-cost analysis (MCA) using the gray-scale slope along the radial direction as the cost function. The diameter of the bubble is defined as the diameter of a circle with an area equal to the area enclosed by the detected boundary. The diameter is then plotted against time to produce a diameter–time curve. From the diameter–time curve, the relative expansion E and the relative compression C of the bubble was deduced. E is defined as the absolute expansion (maximum diameter minus initial diameter) divided by the initial diameter. C is defined as the absolute compression (initial diameter minus minimum diameter) divided by the initial diameter.

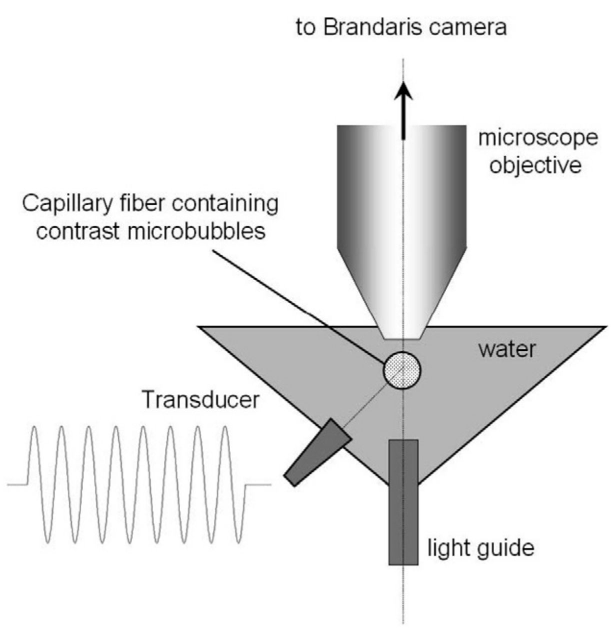

Fig. 1. Schematic view of the experimental set-up.

RESULTS

A total of 101 observations of oscillating bubbles was recorded at an acoustic pressure covering the range of linear behavior (50 kPa), moderately nonlinear behavior (100 kPa) and, for 200 kPa, the start of bubble destruction. Figure 2 shows a typical example of a regular bubble (top panel) and a bubble showing compression-only behavior (bottom panel) insonified at a frequency of 1.8 MHz. The regular bubble has an initial diameter of 3.5 μm and shows a relative expansion amplitude E, which is equal to the relative compression amplitude C. The fast Fourier transform (FFT) of the diameter–time curve shows a dominant peak at the insonifying frequency, whereas higher harmonics are below the noise level. In the bottom panel of Fig. 2, the diameter–time curve is displayed for a 2.0-μm diameter bubble showing compression-only behavior. While the bubble is subjected to a symmetric sinusoidal ultrasound wave, it only compresses and hardly expands beyond its initial diameter. Furthermore, the shape of the diameter-time curve in the compression phase of the ultrasound shows sharp edges, very different from the regular bubble response, which is shown in the upper part of the figure. Accordingly, the FFT of the diameter–time curves shows high second and even third harmonics, which are only 10 dB below the fundamental component.

From the relative expansion and compression of the bubble, E and C, respectively, the ratio E/C was calculated. Figure 3 shows the scatterplot for E/C as a function of the diameter for the three acoustic pressures (50, 100 and 200 kPa) and for the three frequencies (1, 1.8 and 4 MHz). This results in nine subplots containing the 101 observed bubbles. The value of E/C for bubbles vibrating symmetrically will be close to unity. For bubbles displaying “compression-only” behavior, the value of E/C will be close to zero. In the scatterplot, the data points with an E/C value <0.5 are labeled “compression-only” bubbles and are indicated with filled circles. The open circles are used for bubbles with an E/C value >0.5. Although the dataset is sparse for some cases, *e.g.*, only two bubbles in Fig. 3d, it can be concluded that “compression-only” behavior can be observed for pressures as low as 50 kPa. Furthermore, all bubbles in our dataset with a diameter <4 μm show “compression-only” be-

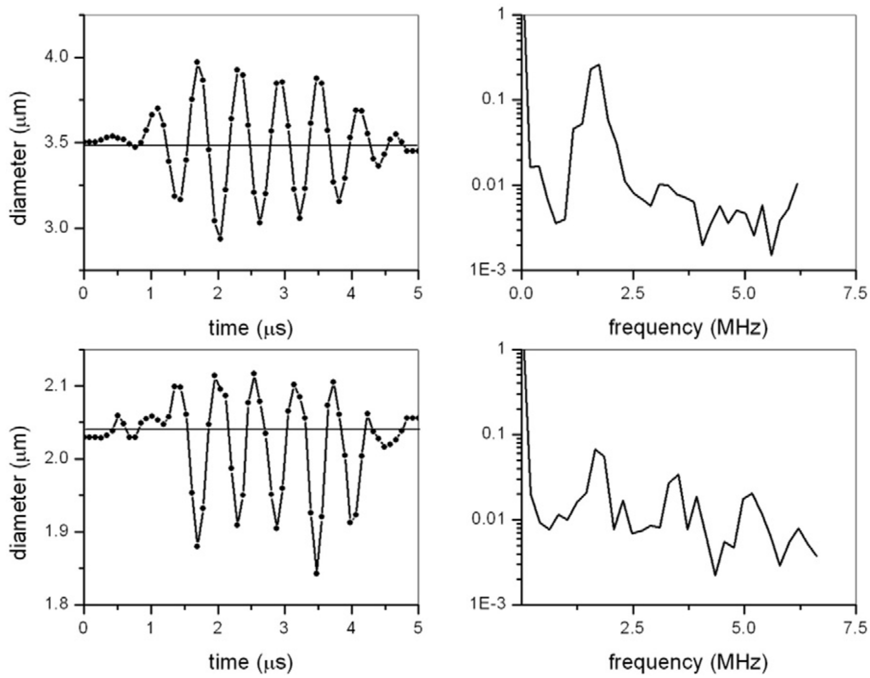

Fig. 2. Top: regular oscillation behavior of a coated microbubble of 3.5 μm in diameter. The bubble was insonified with a gated six-cycle sine-wave burst at 1.8-MHz center frequency at a pressure of 100 kPa. Left panel: the diameter–time curve produced from optical high-speed imaging data. Right panel: the FFT of the diameter-time curve. Bottom: compression-only behavior of a coated microbubble of 2.0 μm. The bubble was insonified with a gated six-cycle sine-wave burst at 1.8-MHz center frequency at a pressure of 100 kPa. Left panel: diameter–time curve produced from optical high-speed imaging data. Right panel: the FFT of the diameter–time curve.

havior at 1-MHz driving frequency. Of the total set of 101 bubbles, ~40% showed compression-only behavior.

We also include a numerical simulation of *E/C* as a function of the bubble diameter. The bubbles were subjected to the same burst as in the experiment with the same frequencies and acoustic pressures. *E* and *C* were determined from the synthetic diameter–time curves, which were calculated from the elastic shell model of de Jong et al. (1992). The shell parameters for this model were taken from the available experimental data on bulk SonoVue as presented by Gorce et al. (2000), assuming a surface tension σ of 0.07 N/m, a shell stiffness of S_p = 1.1 N/m and a shell friction of $S_f = 0.27 \times 10^{-6}$ kg/s. By comparing the experimental data with the simulations, it follows that "compression-only" behavior must be explained by a more sophisticated shell model, possibly including shell buckling and rupture (Marmottant et al. 2005).

DISCUSSION AND CONCLUSION

The "compression-only" behavior of phospholipid-coated bubbles was discovered in the course of an optical ultra high-speed contrast imaging study on individual SonoVue and BR-14 bubbles and was analyzed retrospectively. Acoustically, such a bubble behavior will prove difficult to detect. First, acoustic studies on the behavior of single microbubbles are difficult to perform. Moreover, because the resolution of ultrasound is much lower than the bubble diameter, multiple bubbles will be sampled simultaneously, and acoustically it will be very difficult to discriminate the compression-only behavior from a nonlinear vibration. From the optical analysis, it was found that 40% of the bubbles showed compression-only behavior. It is to be expected that samples with a much higher fraction of "compression-only" bubbles can be achieved, if well-defined preparation conditions are met. For example, it is speculated that a small shrinkage of the bubble after preparation will induce "compression-only" behavior. One of the possible explanations for the bubble's behavior is the buckling of the phospholipid shell (Marmottant et al. 2005). Shell buckling will prevail if gas diffuses from the core into the liquid, thereby suppressing bubble expansion in the rarefaction phase. Therefore, in addition to the parameters controlled in this study, *i.e.*, frequency and acoustic pressure, the time elapsed after preparation of the agent, which was typically between a few minutes or as long as one day (in a clinical setting, the contrast agent should be administered within 6 h after preparation), may contribute significantly to an enhanced "compression-only" effect.

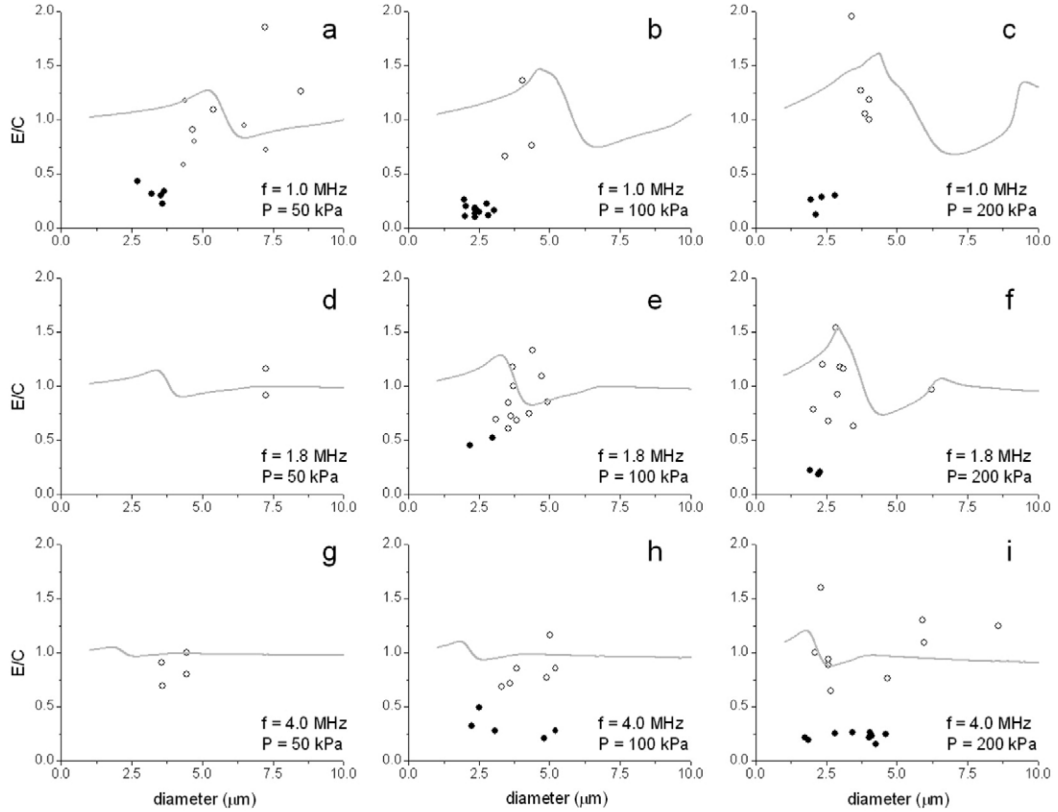

Fig. 3. Expansion over compression, termed *E/C*, as a function of the bubble diameter for three frequencies (1, 1.8 and 4 MHz) and at three acoustic pressures (50, 100 or 200 kPa). Filled circles: bubbles showing compression-only behavior with *E/C* <0.5. Open circles: bubbles with *E/C* >0.5. Solid line: numerical calculation of *E/C* as a function of bubble diameter. The simulation was performed using the elastic shell model by de Jong et al. (1992). The shell parameters were derived from bulk SonoVue bubbles as characterized by Gorce et al. (2000).

The "compression-only" behavior is highly nonlinear and has been observed at acoustic pressures as low as 50 kPa. Current methods for contrast imaging are all based on the nonlinear behavior of the bubbles as it results in the optimal contrast-to-tissue ratio. Future research should reveal the increase in sensitivity using coated bubbles displaying the "compression-only" behavior and detection methods such as pulse inversion (Burns et al. 2000) and radial modulation imaging (Bouakaz et al. 2006).

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