ULTRASONIC DISRUPTION OF COMPOSITE LIPID-COATED MICROBUBBLES

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Introduction

There is considerable interest in the ability of ultrasound (US) to disrupt various biocompatible materials, with an eye to localized drug delivery applications. Although thermal mechanisms have shown the most promise [1], it remains important to characterize the direct effects of ultrasonic pressure waves on various potential carriers, both for the fundamental physical insights that may be obtained and to increase the arsenal of medical tools available to treat disease. Carriers that are most responsive to ultrasonic pressure waves inevitably include (either deliberately or inadvertently[ref lipos]) trapped gas, as the acoustic impedance mismatch between aqueous material and gas is large. These carriers may be used simultaneously for contrast enhancement, a further potential advantage.

We have studied the response of composite lipid-coated microbubbles to 1.1 MHz ultrasound, by synchronizing a microscope/CCD camera with the electronics used to generate single (~5 cycle) ultrasound pulses. The microbubbles are formed by using a laboratory probe sonicator, positioned at the interface between a suspension of liposomes and a gaseous headspace containing perfluorobutane (PFB). PFB is commonly used in preparing such ultrasound-responsive carriers, owing to its very low solubility in water, which stabilizes the bubbles well even in the absence of a complete surface coating. We found that the response of these carriers depends on the specific phospholipids used in their coat, a result that is somewhat surprising in light of the temperatures reached on adiabatic compression.

Experimental

Lipids were obtained from Avanti Polar Lipids (Alabaster, AL) and PFB from Synquest (Alachua, FL.) Coated microbubbles in phosphate buffered saline were formed [2] with composite lipid shells containing 90 mol% phospholipids and 10 mol% polyethylene glycol (PEG) lipids. (PEG-lipids are generally included to improve biocompatibility and circulatory retention [1], but also may improve ultrasound responsivity [3]. Bubbles were diluted into PBS and imaged at the top surface of a sealed plastic cuvette, immersed in a water tank, using a 20X long-working distance objective (Mitutoyo) and a CCD camera (DFK 31BU03, The Imaging Source, Charlotte, NC), synchronized to take an image after every second US pulse. 1.1 MHz US pulses (3 μs FWHM, 400 kPa peak pressure) were applied using a focused transducer (H-101, Sonic Concepts, Bothell, WA). We estimate the amplitude at the samples to be ~280 kPa, owing to reflections.)

Results and Discussion

Bubbles larger than about 10 microns in diameter showed no apparent response to the US pulses. Smaller bubbles showed various responses that appeared to depend primarily on the choice of the coating phospholipid; typical size traces are shown in Fig. 1.

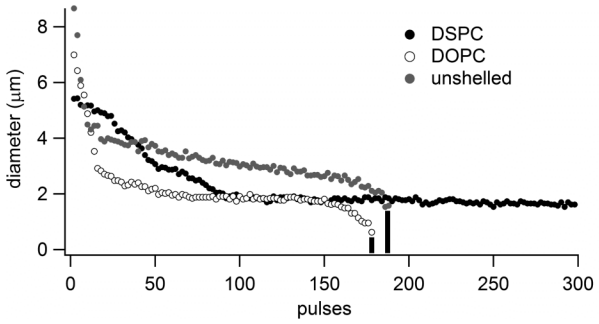

Fig. 1. Diameter of composite lipid-coated microbubbles after exposure to ultrasound pulses.

Disteroyl phosphatidylcholine (DSPC) is a lipid with two saturated chains that forms a solid phase (in liposomes) below 55°C, and on Langmuir monolayers as well [4]. Bubbles coated with this lipid, in addition to the 10 mol% PEG-lipid, shrank dramatically on exposure to US, but quickly reached a stable diameter of ~ 2 μm and did not change further. Bub-

bles coated with dioleoyl phosphatidylcholine (DOPC) also showed rapid shrinkage to a (quasi-) stable size, but almost always (20/23) vanished in a dramatic dissolution or disruption event after > 50 pulses. Uncoated PFB bubbles showed an initial rapid shrinkage, followed by a phase of slower shrinkage, ending in complete dissolution.

The fact that all these bubbles shrink in ultrasound may seem in contradiction to "rectified diffusion" models, in which bubbles grow owing to the easier diffusion of dissolved gas *to* the bubble when it is large and at low pressure (low chemical potential), compared with the diffusion of bubble gas from the bubble when it is small and at high pressure (high chemical potential). However, the PFB gas was not equilibrated with the PBS solutions, and the bubble shrinkage likely reflects the dissolution of some of the gas into the buffer.

Large bubbles (> 10 μm) generally did not respond to the ultrasound pulses, regardless of coat. The resonant frequencies for bubbles larger than 10 μm (and smaller than 2 μm) are far below (above) the 1.1 MHz driving frequency, which may play a role in their stability. Nonetheless, the dramatic differences in the terminal behavior of bubbles with different coats warrants further investigation.

To this end, we have modeled the bubble behavior, using a modified Herring equation [5]. (This deviates from Rayleigh-Plesset in the incorporation of radiative damping that may be important when wall velocities approach sound speed.) The results are shown in Fig. 2, for a 3 μm radius bubble. The top panel shows the driving pressure used in the model, compared with experimental driving pressure measured with a hydrophone. As is commonly observed, bubbles are compressed to very small radii and very high temperatures; this was found regardless of the surface pressure/tension used in the model. The internal temperature of the bubble was found to be far in excess of any relevant lipid melting transition. When a variable surface pressure was included (to model the variation of surface tension with lipid coat density), the peak surface pressure was several hundred mN/m, far in excess of the collapse pressure of any lipid monolayer.

Conclusion

The modeling studies, coupled with the distinctly different experimental results for DSPC vs DOPC/uncoated bubbles, strongly suggest that lipids are always shed, *at least transiently*, from the surface of these bubbles. It would follow that bubble stability is then related to the ability of lipid fragments to rapidly re-coat the expanding bubble. Why a saturated lipid can do this (apparently) more effectively than an unsaturated one is unclear, but may be related to formation of different micellar intermediates.

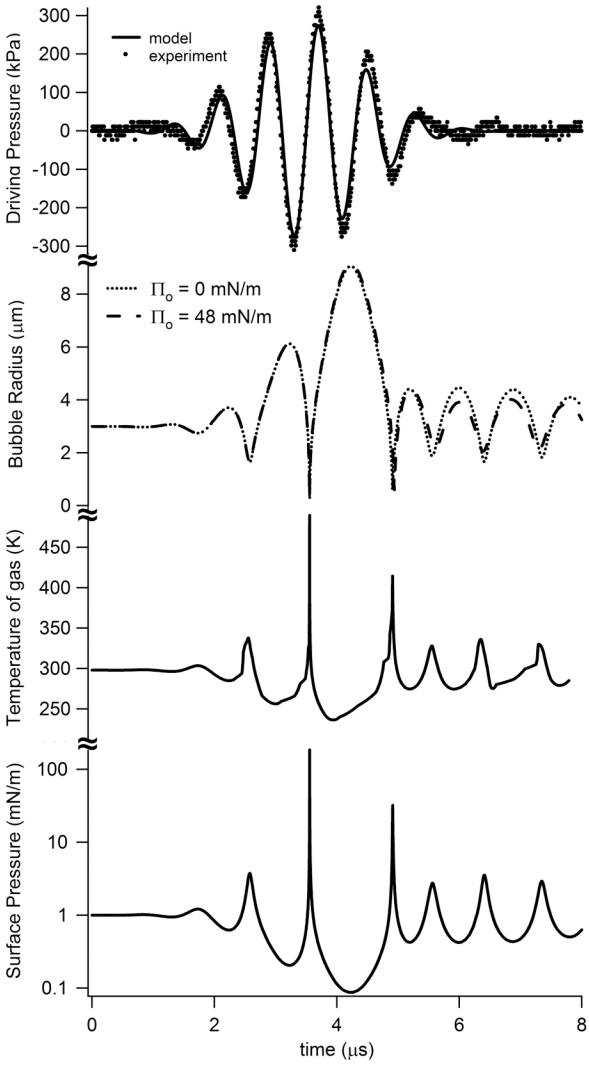

Fig. 2. Numerical solution of the Herring equation.

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