Observations of Insonified Contrast Agents *In-Vitro* and *In-Vivo*

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**Abstract:** Although ultrasound contrast agents play an increasingly important role in medical imaging, their exact behavior during insonation is not well known. With the use of optical microscopy, we observe the change in radius of microbubbles during insonation, as well as the effects of ultrasonic radiation force. During insonation, the radius of the bubble can fluctuate up to 15% with the compressional and rarefactive pressure cycles of the ultrasonic pressure wave. This change in radius is directly related to the bubble's compressibility, which determines the echogenic properties of the bubble, as well as the magnitude of the radiation force experienced by the bubble in an ultrasound field. We also demonstrate that primary acoustic radiation force has a significant effect on microbubbles. The radiation force generated by a 5 MHz transducer driven at a pulse repetition frequency of 10 kHz can deflect contrast agents to the wall of an 80-micron vessel *in-vivo*, with an acoustic pressure of 800 kPa. Such manipulation of the contrast agents may be desired in drug delivery applications, as it provides a method of concentrating bubbles in a specific area.

**INTRODUCTION**

It has already been shown that microbubble contrast agents have great potential in clinical ultrasound. However, current imaging techniques are far from utilizing the full potential of these agents. In order to design an imaging system which can separate echo signals from contrast agents and surrounding tissue, we must have a better understanding of the behavior of contrast agents when they are insonified. In this paper, the observation of microbubbles fluctuating in radius during insonation is documented. The change in radius of a microbubble during the compressional and rarefactive cycles of the ultrasonic pressure wave is related to the compressibility of the microbubble. Compressibility determines the magnitude of radiation force experienced by the microbubbles. Acoustic radiation force has previously been shown to displace compressible microbubbles *in-vitro*, and may have potential applications in manipulating microbubbles such as assisting targeted agents (1). The displacement of contrast agents to the side of a vessel wall is shown *in-vivo*.

**EXPERIMENTAL SYSTEMS**

In order to study the fluctuations in radius of contrast agent microspheres during insonation, we have coupled a video microscopy system and an ultrasound system. The microbubbles are attached to a polystyrene plate, which is placed under the microscope. A water bath serves as a coupling medium between the bubbles and an ultrasonic transducer. A Panametrics V391 500 kHz transducer with a 2 inch spherical focus was used to insonify the microbubbles. In order to magnify the bubbles enough for video analysis, the microscope was equipped with a 100x water immersion objective. An arbitrary waveform generator (Tektronics AWG2021) was used in conjunction with an RF power amplifier (ENI 325LA) to energize the transducer. The video system consisted of a high speed flash strobe (EG&G 2611) which was optically coupled to the microscope with a fiber optic cable and condenser. The strobe was triggered from a high speed camera (Kodak Motioncorder Analyzer). The combination of the strobe and camera allowed capture of 600 frames per second with 1 microsecond temporal resolution.

Radiation force *in-vivo* was observed with the use of intra-vital microscopy. For these studies, surgical preparation allowed microscopy of the vasculature of the cremaster muscle of a living mouse. A special water bath system allowed an ultrasonic transducer (Panametrics V309) to be coupled to the animal. Approximately 300 µL of a FITC-labeled, perfluoropropane filled, albumin-shelled contrast agent was injected into the animal through the jugular vein. These bubbles could be observed flowing through the microvasculature using epifluorescence. Transducers were energized by a Ritec SP-801A square wave pulser.
OBSERVATIONS AND DISCUSSION

During insonation with a 500 kHz sinewave at approximately 400 kPa, we observe that MP1950 (phospholipid shell, perfluorobutane core) undergoes a change in radius during the compressional and rarefractional cycles of the ultrasound pressure wave. Figure 1 shows three images of an MP1950 bubble during insonation. In 1a, the bubble is being compressed by the pressure wave, in 1b, the bubble is at rest, and in 1c, the bubble is expanding in the pressure wave rarefraction. Analysis using NIH image indicated that the bubble shrinks by approximately 9%, and expands by approximately 6%. MP1950 bubbles will continue to oscillate in this manner unless there is a significant increase in the acoustic pressure. An OptisonTM (albumin shell, perfluoropropane core) bubble subjected to the same conditions behaves quite differently. During a compressional cycle, an Optison bubble will wrinkle, and then return to its original shape at rest. Initially, the Optison bubble will not expand. Eventually (above a minimum acoustic pressure), the albumin shell will break down and will become more flexible. After this occurs, the albumin-shelled bubble may exhibit expansion as well as compression.

Intra-vital microscopy of vasculature in the mouse cremaster muscle shows that without ultrasound, contrast agents flow in the circulatory system in a similar manner to blood cells. We have shown that with the use of acoustic radiation force, a portion of the contrast agents in the flow stream are displaced to the vessel wall. Figures 2b and 2c show blood flow in an 80-micron diameter vessel approximately two minutes after a solution of FITC-labeled bubbles was injected into the animal. The fluorescently-labeled contrast agents appear as white dots in the flow stream. Figure 2b shows the vessel before insonation, and figure 2c shows the vessel during insonation. A 5 MHz transducer was used to insonify the tissue at approximately 800 kPa and 10 kHz pulse repetition frequency (PRF). The combination of high PRF and medium acoustic power caused the highly compressible microbubbles to be displaced to the vessel wall away from the transducer by radiation force.

CONCLUSIONS

By observing the behavior of microbubbles during insonation, we can determine properties such as compressibility and evaluate the influence of the shell. Such an evaluation is useful, since compressibility determines the magnitude of radiation force experienced by the microbubbles. Our experiments indicate that radiation force can displace contrast agents to the wall of an 80-micron vessel with a moderate acoustic pressure and high PRF. Observation of radiation force in-vivo indicates that there is potential for using radiation force as a bubble manipulation technique in-vivo.

REFERENCES