Detection of Stationary Microbubbles in Tissue Using Dual-Frequency Ultrasound

Benjamin R. Bollinger[1], Darin A. Knaus[3], Scott D. Phillips[3], Patrick J. Magari[3], Donna L. Alvarenga[2], and Jay C. Buckey[1,2]

[1]Thayer School of Engineering at Dartmouth College, [2]Dartmouth Medical School, and [3]Creare, Inc. (Hanover, NH 03755)

Overview

Many investigators have postulated that small bubbles (micronuclei) exist normally in tissue. The number and location of these micronuclei may be influenced by exercise and inactivity. They also may correlate with decompression sickness (DCS) risk. To date, these theories have not been verified since no techniques have existed to measure stationary microbubbles in tissue. Our recent results indicate that dual-frequency ultrasound can be used to detect stationary microbubbles in vivo. Dual-frequency ultrasound exploits the resonance properties of bubbles to detect and size them. To test the ability of dual frequency ultrasound to detect stationary bubbles we used ultrasonic contrast agent (USCA). Dual-frequency ultrasound was used to image USCA bubbles in-vitro (in a water tank) and in-vivo (after injection into muscle in an anesthetized swine). Small polymer microspheres were used as a control. The results showed that dual-frequency ultrasound could reliably detect bubbles both in-vitro and in-vivo. No signal was returned from the microspheres. We believe that this is the first demonstration of the ability to detect stationary microbubbles in tissue. The detection of stationary microbubbles during and following decompression could yield new insights on the progression and evolution of DCS, provide a metric for the effectiveness of DCS risk mitigation strategies, and provide real-time assessment of personal DCS risk. This could be important for future NASA operations since both ISS construction and lunar operations require extensive extravehicular activity (EVA). Improved bubble detection technologies could be used to evaluate and optimize pre-breathe strategies and monitor crewmembers in space.

Dual-frequency Ultrasound

The Creare Dual Frequency Instrument (CDFI) uses dual frequency ultrasound [1] to detect and size bubbles. The target area is driven with two frequencies, a lower "pump" frequency and a higher "image" frequency. Bubbles will resonate based on their size. If bubbles of the appropriate size are present, the pump frequency will cause them to resonate. These resonating bubbles will act as nonlinear mixers, and will emit the sum and difference of the two driving frequencies (Figure 1).

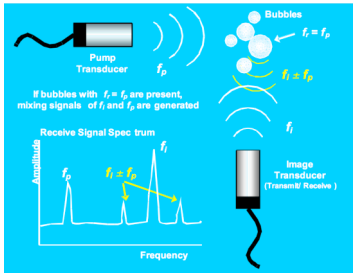

Figure 1: The dual-frequency ultrasound bubble detection method

Dual-frequency ultrasound has several advantages over other bubble detection methods. First, since it does not depend only on the linear reflection from individual bubbles, bubbles can be detected in areas with multiple other ultrasound reflectors. Second, the bubbles do not need to be in motion to be detected, as is the case for Doppler ultrasound. Finally, because the resonant frequency is size dependent, dual-frequency ultrasound can also provide size information.

Experimental Methods

Dual-frequency ultrasound bubble detection was implemented using a PC-based system. A National Instruments PXI system generated the signals and acquired the data under software control (LabView™) (Figure 2).

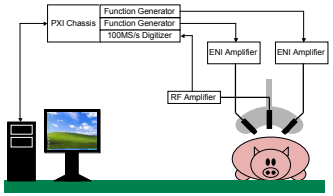

Figure 2: Schematic of the Creare Dual-frequency Instrument (CDFI), a dual-frequency ultrasound bubble detection device

An ultrasound contrast agent containing lipid-encapsulated bubbles (Definity®, Bristol-Meyers Squibb Medical Imaging) was used as a bubble standard. Solid polymer microspheres (polylactic acid particle standard, 2000 nm, Postnova Analytics) were used as a control. These microspheres are approximately the same size as the ultrasound contrast agent (USCA) bubbles and are strong ultrasound reflectors, but do not resonate. In-vitro experiments were performed by placing solutions of varying concentrations USCA or solid polymer microspheres (SPM) in a small rubber balloon and imaging the balloon in a water tank using dual-frequency ultrasound. In-vivo experiments were performed by injecting the same solutions into the thigh of an anesthetized swine. Multiple measurements were made using different dilutions of contrast agent and microspheres at various ultrasound powers.

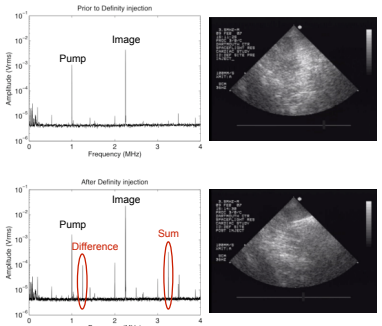

Figure 3: Measurements in the flank of an anesthetized swine before and after a 0.5cc injection of Definity® contrast agent microbubbles. On the left is the spectrum of the received signal using the CDFI. On the right are 2D (B-mode) images using a clinical ultrasound scanner (Sonos 1000)

Results

Initial experiments were run to validate the use of dual-frequency ultrasound for stationary bubble detection. In-vitro experiments showed that an fi = 5.0 MHz, fp = 2.25 MHz, and a receive transducer with a center frequency of 3.5 MHz, were ideal for detecting Definity. Figure 3 shows the results from a pilot experiment where 0.5 mL of undiluted Definity was injected in the thigh of an anesthetized swine and the site was imaged with the CDFI. No mixing signals were detected prior to injection, but strong mixing signals were detected afterward. The Definity, however, could not be distinguished from other sources of ultrasound reflection in tissue using clinical 2-D ultrasound. This demonstrated the ability of dual-frequency ultrasound to detect stationary bubbles in tissue.

Device Sensitivity

To characterize the concentration of microbubbles that can be detected in vivo we placed solutions of varying concentrations of USCA and SPM in small latex balloons in a water tank and imaged them using dual-frequency ultrasound. The same solutions were injected into the thigh of an anesthetized swine. In the balloons, a difference signal of 2.75 MHz was detected for USCA solutions of 10^9, 10^8, 10^7 particles/mL. No difference signal was detected for any of the other solutions. In tissue, no difference signal was detected at any site prior to injection. After injection, a difference signal was detected at the sites with 10^9, 10^8, and 5×10^7 particles/mL USCA. No difference signal was detected at the other sites. These results are summarized in figure 4.

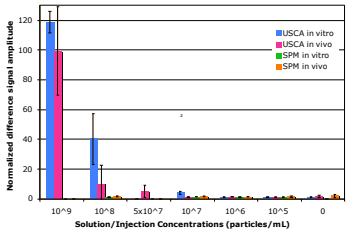

Figure 4: Difference signal obtained for different concentrations of ultrasound contrast agent (USCA) and solid polymer microspheres (SPM), both *in vitro* and *in vivo*. The error bars indicate +/- one standard deviation. The data for 10^8, and 5×10^7 particles/mL USCA in vivo exhibit large error bars because the received difference signal for these concentrations was transient, diminishing over the course of the measurements. We believe that this might be due to perfusion moving the USCA away from the measurement volume.

Transmit Pressure Measurements

To determine the minimum pressure required to obtain mixing signals from the different bubble concentrations, measurements were made using four different ultrasonic transmit pressures at each USCA and SPM concentration both in vitro and in vivo. The image and the pump transducers were driven to output 0.55, 1.12, 2.76, and 5.43 kPa RMS at their respective frequencies.

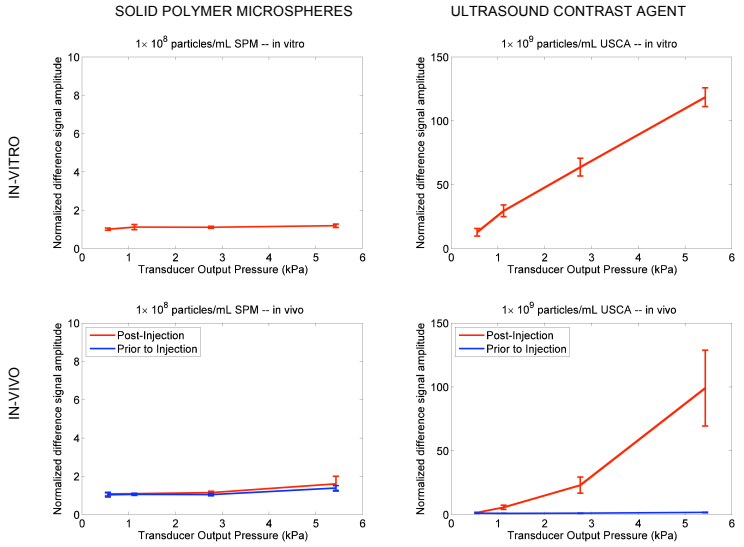

Figure 5: Dual-frequency ultrasound measurements *in vitro* and *in vivo* of stabilized bubble ultrasound contrast agent (USCA) and non-resonating solid polymer microspheres (SPM). The difference signal, normalized by the noise floor, is detected for USCA but not for SPM, and increases in amplitude with increasing transmit pressure.

Figure 5 shows the mixing signals received for each of the output pressures for the highest concentrations of USCA and SPM used, 10^9 and 10^8 particles/mL respectively. The received difference signal amplitude increases roughly linearly with increasing transmit pressure.

Discussion

This is the first demonstration that stationary microbubbles can be detected in tissue using dual-frequency ultrasound. Concentrations of USCA as low as 5×10^7 particles/mL were detected in tissue at peak powers lower than those typical of a clinical ultrasound scanner.

The CDFI bubble detection system is currently limited in sensitivity to detect lower concentrations of tissue bubbles by the noise floor of the system. Increasing the signal to noise should yield an increased ability to detect lower concentrations of tissue bubbles. This can be done by increasing the transmit pressures, which are currently limited by our amplifiers but are still less than typical clinical ultrasound.

Research is underway using the CDFI device to detect microbubbles that exist normally in tissue and decompression-induced bubble formation in tissue. Detecting native tissue bubbles is difficult since the concentration, locations and sizes of normally-occurring microbubbles in tissue is unknown. Theoretical models on DCS evolution that incorporate tissue microbubbles have used tissue bubble concentrations spanning 10 orders of magnitude [2]. Nevertheless this is the first demonstration that small stationary bubbles can be detected in tissue. This offers the possibility of research that could lead to a new understanding of the mechanics of bubble formation during activity and decompression.

Acknowledgements

This research was performed with the support of the NASA Space Biomedical Research Institute (NSBRI TD00402) and the Office of Naval Research (ONR N00014-02-1-0406).

References:
[1] V.L. Newhouse and P. M. Shankar. J. Acoust. Soc. Am. **75**, 1473-1477 (1984)
[2] M. A. Chappell and S. J. Payne. Phys. Med. Biol. **51,** 2321-2338 (2006)