MICROBUBBLE CONTRAST AGENTS FOR MEDICAL ULTRASOUND IMAGING: CURRENT ISSUES AND NEW DIRECTIONS

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Microbubbles have shown great promise for enhancing medical ultrasound imaging, with both potential and proven applications in Radiology and Cardiology and many other clinical areas. More recently the use of targeted agents for molecular imaging is being studied and the use of microbubble as mediators for the delivery of drugs and gene therapy is being investigated. In this presentation the basis for these different uses of microbubbles will be described and the challenges which are limiting the widespread acceptance and implementation of these techniques, will be outlined. These include issues relating to image artefacts, image quantitation and the complexity of interactions between microbubbles and both the ultrasound field and the human body.

1 INTRODUCTION

Microbubbles have, over recent years, been proven as effective contrast agents for diagnostic ultrasound imaging. After initial success as a means of enabling Doppler scans in patients who could not otherwise be imaged, these agents have found a broad range of uses in both radiology and cardiology. Many of the newer uses aim to provide the clinician with measurement of tissue perfusion and consequently the functional behaviour of tissues. As well as diagnostic uses, microbubble are also being investigated as therapeutic agents, in this case they are used as seeds for cavitation events and the resulting localised tissue damage can be used as an opportunity to delivery therapeutic drugs more efficiently to a localized region. They can also be used to encapsulate drugs or DNA.

In order to optimise both the diagnostic and the therapeutic applications of microbubbles a detailed understanding of their physical behaviour must be obtained. The interactions of microbubbles with sound, especially in a complex and dynamic *in-vivo* environment, is far from straightforward and the inherent non-linear properties of the microbubbles further complicates the situation.

Nevertheless, a number of researchers have developed models to describe microbubble dynamics and other researchers and commercial developers have used these models and additional experimental studies to devise sensitive bubble specific imaging modes. This paper describes experimental and clinical observations that we have used in our research to further illuminate the physical behaviour of microbubbles in an acoustic field.

2 EXPERIMENTAL METHODS AND OBSERVATIONS

2.1 *In-vitro* measurements from microbubble suspensions

In an ongoing series of experimental studies, we[1, 2] have investigated the acoustic behaviour of acoustic response suspension of microbubbles. Our results (Fig.1) have demonstrated that the attenuation of ultrasound due to the presence of microbubbles is highly pressure dependent and show how this attenuation peaks at a particular frequency (approximately 1.5 MHz for SonoVue® microbubbles). The pressure dependant component of the attenuation was shown to be much less at frequencies away from resonance. In a series of low pressure amplitude experiments (<100kPa peak negative pressure) no pressure dependence in the scattering component of the attenuation was detected. However, attenuation and scattering are not independent for a given bubble size and liquid.

Therefore, the scattering of microbubbles is almost certainly pressure-dependent to some extent but, within the sensitivity of our experiment and within the low pressure range used in this study, we were unable to measure this.

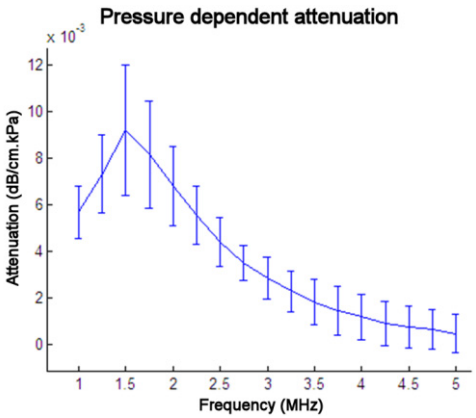

Figure 1: Pressure dependent attenuation coefficient for SonoVue® determined over a range of insonating frequencies. Note: the values shown correspond to a dilute suspension of 225µl/L. (adapted from[2]).

These observations have led other researchers[3, 4] to suggest a pressure threshold for the onset of microbubble vibration. This hypothesis has been demonstrated with high speed optical observations, and the mechanism is proposed as a means of enhancing the detection sensitivity of non-linear imaging modes using pulse amplitude modulation sequences. This is an example of how research into the basic physical interactions of sound and microbubbles can led not only to better understanding of the processes involved but also to ultimately provide a basis for improving the sensitivity of the imaging approaches that we use.

Our observations of the pressure dependence of microbubble attenuation have led us to propose a simple model for compensation of non-linear attenuation in microbubble ultrasound imaging, discussed in this issue[5] and[6].

2.2 High speed optical observations of single microbubbles in a sound field

The ability to visualize the physical behaviour of a single bubble in an acoustic field can provide essential information, which can contribute both to the understanding of the processes involved and to the development of new applications. Optical observations are particularly valuable in assessing the predictive value of physical models and can aid in the parameterisation of both new and existing

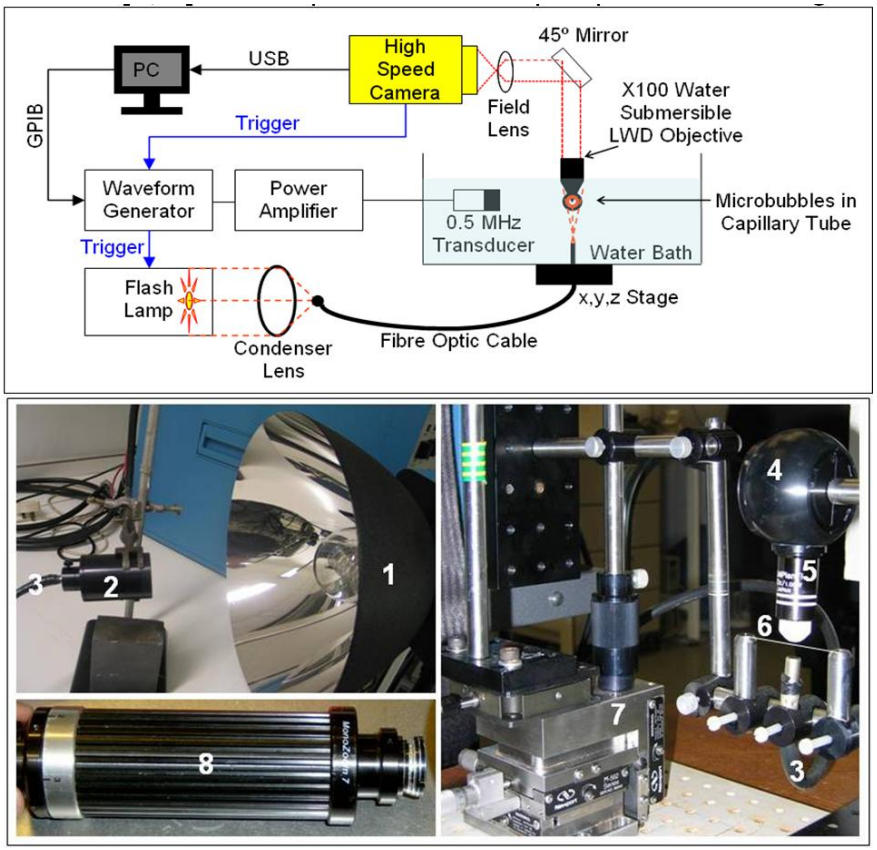

Figure 2: Experimental setup for high speed imaging of oscillating microbubbles. An ultrasound pulse produced by an arbitrary waveform generator is amplified to insonate a microbubble held in a cellulose capillary tube. The microbubble is imaged through a x100 long working distance objective. In the photographs: 1 = Flash lamp, 2 = Condenser lens, 3 = Fibre optic cable, 4 = 45° mirror, 5 = X100 microscope objective, 6 = Cellulose capillary tube held between 2 posts, 7 = x,y,z translation stage and 8 = Field lens. The transducer and water bath are not shown. Adapted from[13].

models developed. In a series of experiments we used the Cordin 550 high speed gas turbine camera to record bright field microscopy images of oscillating microbubbles. A broad range of experiments were performed to investigate the effects of pulse amplitude, length, encoding, and to investigate the effects of bubble-bubble interactions and bubble chemistry on the acoustic behaviour. Like those of other researchers our initial results with simple Gaussian modulated pulses demonstrated a number of inconsistencies between the observed and predicted behaviour of the bubbles[7, 8]. The experimental setup is presented in figure 2. Optical observations of a range of differently sized individual microbubbles were obtained at low insonating pressure amplitudes (<100 kPa). With the aim of parameterising the modified Rayleigh-Plesset model (as described by Hoff[9]), An example of the type of data obtained is presented in figure 3.

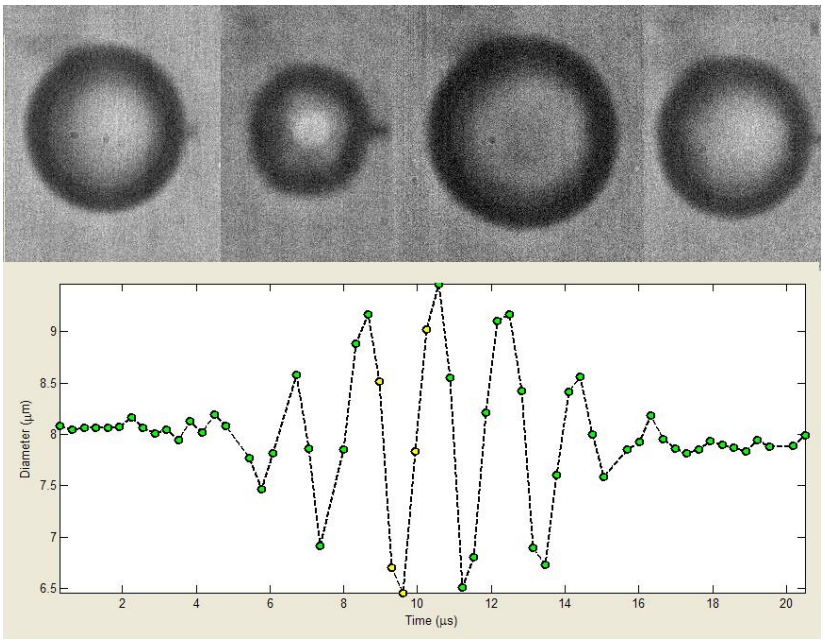

Figure 3: Example of the high speed optical obtained using the apparatus described in Fig. 2. The images show a SonoVue® bubble with initial diameter of 8µm, before, at min compression, at max expansion, and after insonation. The graph shows the radial trace from the 62 frames recorded by the camera.

It was found that even at the low pressures used, the measure data diverged from that predicted by the models. Many of the bubbles were seen to undergo some degree of shrinkage during insonation and, in addition, the modelling underestimated the microbubble oscillatory behaviour in the later stages of the insonation (see figure 4.). An analysis of the data from a range of bubble sizes showed that the shell parameters used in the model varied with the microbubble size, this is in agreement with the findings of other researchers [10, 11]. A more detailed analysis [12] suggests that the physical properties of the micro bubble (embodied by the shell parameters) are changing dynamically during the acoustic insonation. Figure 4 (right) shows a plot of the shell elasticity parameter for a single bubble obtained through fitting to only a localised region of the measured data. As the fitting window is moved along the measured data the shell parameter is seen to change. This suggests a physical change in the bubble shell, and could relate to the thresholding effect observed by [3]. By incorporating a variable shell parameter into the model in an *ad hoc* manner, the predictive value of the model was shown to improve [13]. Figure 5 shows two still frames from a sequence showing interaction between two microbubbles. Data such as these can be used investigate the effects of bubble concentration on their physical behaviour. In the following paper[14] we discuss further the challenges for modelling in the light of these experimental observations.

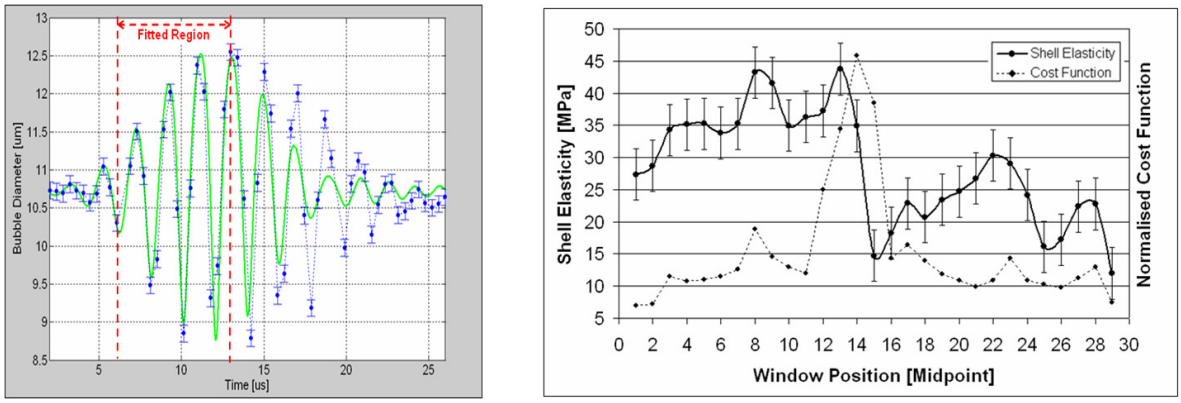

Figure 5: Left: A radial trace obtained from high speed microscopy, with the 'best fit' prediction from Hoff's modified Rayleigh-Plesset equation. Notice how the modeled response fits less well to the later part of the observed data. We hypothesize that this is due to physical changes in the bubble shell during insonation. Right: Results of fitting the model to small sub sections of the recorded for a single SonoVue® microbubble. The graph shows the elasticity value (left axis, solid line) and corresponding normalised cost function (right axis, dashed line) output from the optimisation for each position. The moving window encompassed 9 measured data points. The shell viscosity and shell thickness values were fixed at 1 Pa·s and 2.5 nm respectively. These results suggest that during the insonation there is a physical change in the shell properties. Adapted from[13].

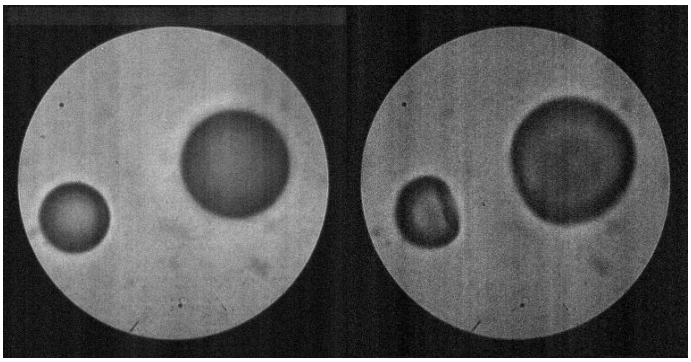

Figure 4: Two still frames from a high speed acquisition show the interaction of two bubbles close to one another. The bubbles have an 11 µm and a 7 µm initial diameter. The first frame is prior to insonation, while the second frame clearly shows the mutual effects of each bubble on the others' oscillation during insonation.

2.3 Clinical observations of artefacts

The non-linear pressure dependant attenuation of ultrasound by microbubbles is probably the key factor in limiting the acceptance of this approach for quantitative clinical research, such as monitoring patient's response to therapy or inter-patient comparisons. The basic work introduced in 2.1 above and described further in[5] led us to observe an interesting artefact in microbubble specific ultrasound images. The shadowing effect due to increased attenuation is a well known artefact with serious consequences for quantitation; however the opposite effect can also be seen in images where the ultrasound beam crosses regions of relatively high concentrations of microbubbles. Figure 6 shows an example in a patient, together with a recreation of the effect in a simple in vitro phantom. Our hypothesis is that the increased non-linear propagation due to the microbubbles in the path of the ultrasound leads to misclassification of echoes from linear scatterers at depth as non-linear. Through modelling we have confirmed that this is the case[15, 16] and in future work we hope to incorporate a compensation mechanism into the image formation process of the ultrasound imaging system.

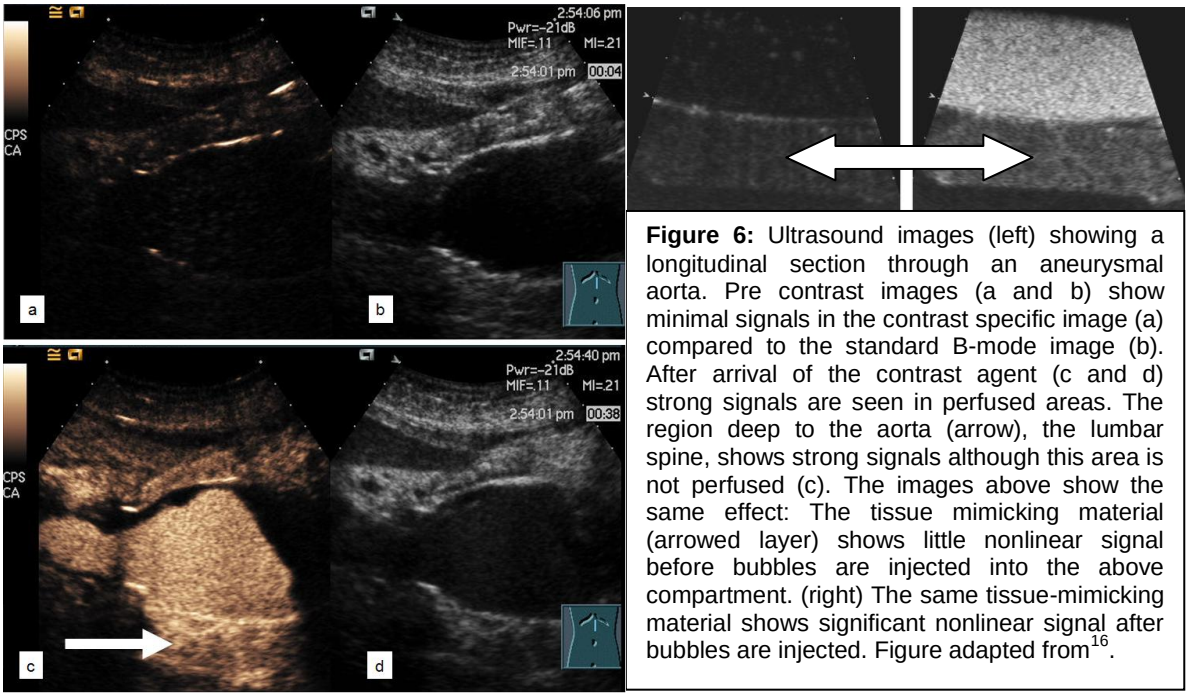

Figure 6: Ultrasound images (left) showing a longitudinal section through an aneurysmal aorta. Pre contrast images (a and b) show minimal signals in the contrast specific image (a) compared to the standard B-mode image (b). After arrival of the contrast agent (c and d) strong signals are seen in perfused areas. The region deep to the aorta (arrow), the lumbar spine, shows strong signals although this area is not perfused (c). The images above show the same effect: The tissue mimicking material (arrowed layer) shows little nonlinear signal before bubbles are injected into the above compartment. (right) The same tissue-mimicking material shows significant nonlinear signal after bubbles are injected. Figure adapted from[16].

3 CONCLUDING REMARKS

The success of microbubbles as ultrasound contrast agents lies in their highly non-linear response to ultrasound excitation. However, this non-linearity gives rise to complex behaviour. Individual bubbles, even those of the same size and coated with identical materials, can produce significantly different responses. The proximity of other bubbles can influence their oscillations and similarly nearby boundaries, such as the walls of blood vessels, also have an effect. The sound itself can be multiply scattered and the signals become more non-linear due to propagation through the bubble clouds.

In order to continue to devise and optimise new detection strategies[17, 18] for microbubble imaging an in-depth understanding of the acoustic response of microbubbles is required. Both bulk acoustic measurement and high speed optical observations have valuable roles to play in this process. The consequences for ultrasound imaging of the new phenomena observed through optical experiments such as, compression only oscillations, surface mode oscillations and the threshold effect mention above have not been investigated in detail. However, recent work by Sijl et al [19] has successfully combined optical and acoustical observation with this in mind.

Such an understanding is also crucial for the development of targeted microbubble imaging. Methods to distinguish the scattered signals due to attached bubbles from those freely circulating will benefit greatly from better understanding of the basic physical behaviour of microbubbles.

Experiments such as those described in this paper can also be used to investigate the effect of varying the chemical composition of the microbubble shell and have a role to play in microbubble design.

Finally, through detailed study of the physical interaction of sound with microbubbles a better understanding of the processes that lead to non-linear attenuation and propagation is becoming a reality. This will facilitate the use of microbubble ultrasound imaging as a quantitative clinical measurement tool, with obvious benefits for clinical research and ultimately patient care.

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