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Single microbubble acoustics in small tubes

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ABSTRACT

Ultrasound microbubble contrast agents can be used to image blood flow in vessels. Small blood vessels form around arterial plaques and in tumours, by angiogenesis, and microbubbles can be imaged in these vessels to aid diagnosis. Currently no specific ultrasound imaging techniques exist which can distinguish between microbubbles in large or small vessels, however *in vitro* it has been shown that microbubbles in small tubes have a different acoustic response to those in larger tubes. Imaging techniques optimised for large or small vessels, slow flowing or attached microbubbles would help microbubbles be used to their full potential. A system for the investigation of single microbubbles was modified to include tubes. Definity and biSphere microbubbles were studied in 200μm cellulose and 50μm acrylic tubes. Data for free bubbles subject to the same acoustic field was also available for comparison. For all microbubbles a 6-cycle pulse of transmit frequency 1.6MHz was used with peak negative acoustic pressures of 550 kPa. The fundamental and harmonic backscattered pressures were calculated. For rigid shelled biSphere in the 200μm tube, the mean fundamental RMS pressure was 2.1 $\pm$ 1.3Pa compared to 4.7 $\pm$ 3.7Pa for free microbubbles. For softer shelled Definity the mean harmonic RMS pressure for free microbubbles was 3.2$\pm$ 1.2Pa and 7.54 $\pm$ 3.2Pa in the 50μm tube. The results demonstrate that the origin of strong harmonic signatures from microbubbles in vivo is partly due to the presence of vessel walls, and that it is possible to detect small changes in microbubble behaviour. This provides valuable information on the acoustic response of microbubbles in tubes that can be used to develop better signal processing algorithms.

BACKGROUND

Contrast agents for medical ultrasound imaging are in the form of micron sized bubbles which enhance the scattering of the ultrasound beam. They can be used as blood pool agents, tracing the path of the blood through the circulatory system. In some situations the microbubbles will be in narrow vessels. It has been demonstrated that when a microbubble is near a boundary, such as a vessel wall, its response to ultrasound changes (Garbin et al. 2007). An imaging method capable of detecting whether scattered ultrasound is from a microbubble in a large blood vessel or a narrow one may have diagnostic advantages. Monitoring changes in the microvasculature can help with diagnosis of disease, and optimising the imaging of microbubbles will aid this diagnosis. In addition, microbubbles targeted to specific markers in vivo may allow molecular imaging or therapeutic treatment with ultrasound (Unger et al. 2004). Imaging techniques to distinguish between a microbubble bound to a vessel wall compared to a microbubble flowing slowly through the vessel is necessary to use this application to its full potential. A method has been developed which allows investigation of the acoustic reponse of single attached microbubbles (Butler et al. 2007). Comparison of this with microbubbles in narrow tubes will help in distinguishing behaviour specific to the microbubble location.

The aim of this work was to develop a system that could be used to assess the acoustic response of microbubbles in different sized tubes. Any behaviour noted in microbubbles in narrow tubes which is not present in wide tubes, or in the absence of tubes, may be utilised to develop signal processing techniques for location specific imaging.

MATERIALS AND METHODS

A system for the investigation of single microbubbles was already in place (Sboros et al. 2003) and further modified to include tubes. Cellulose tubes, taken from a dialysis filter (Gambro, Cambridge, UK) of 200 μm diameter and 50μm diameter acrylic tubes (Paradigm Optics, Vancouver, WA, USA) were used. A diagram of the system is shown in Fig 1. Flow through the tubes was achieved by a gravity feed. In instances when microbubble flow was reduced by using a 50μm tube a syringe driver was used to provide flow.

All microbubbles were insonated with 6-cycle pulses of transmit frequency 1.6 MHz and peak negative acoustic pressure 550 kPa. Unprocessed backscatter data was obtained from a Philips Sonos 5500 research ultrasound machine with

S3 phased array transducer. The fundamental and harmonic components of backscattered pressure were calculated.

A high concentration solution of microbubbles was used to align the transducer over the tube. This flow was imaged and the region with maximum backscatter taken to be the centre of the flow. An image of a high concentration of microbubbles is shown in Fig 2.

To ensure backscattered signals were received from individual microbubbles a very dilute solution of contrast agent was used. In addition, to ensure that the first time the microbubble was insonated it was in the region of interest, the time between consecutive imaging frames was 2 seconds, leaving time for any microbubbles beyond the region of interest to flow through and for fresh microbubbles to reach the region of interest. Microbubble concentration was deemed appropriate when microbubble signals were detected in less than 1 in 3 frames.

Data was collected from rigid shelled BiSphere microbubbles and softer lipid shelled Definity microbubbles in both 200 μm and 50 μm tubes. In addition, data from microbubbles free in solution, i.e. untubed microbubbles, was also collected.

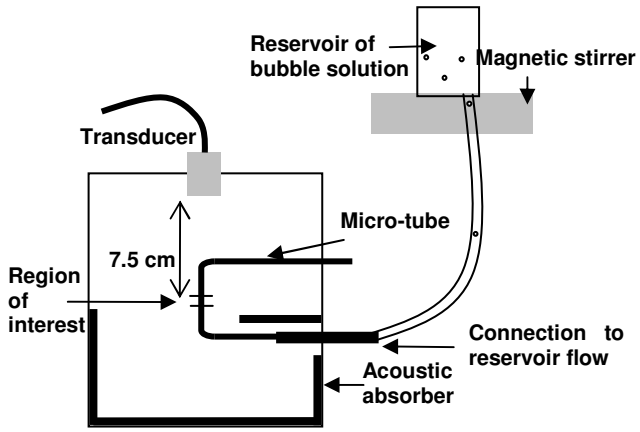

Figure 1 Schematic diagram of the experimental set up of tube mounted in a Perspex tank.

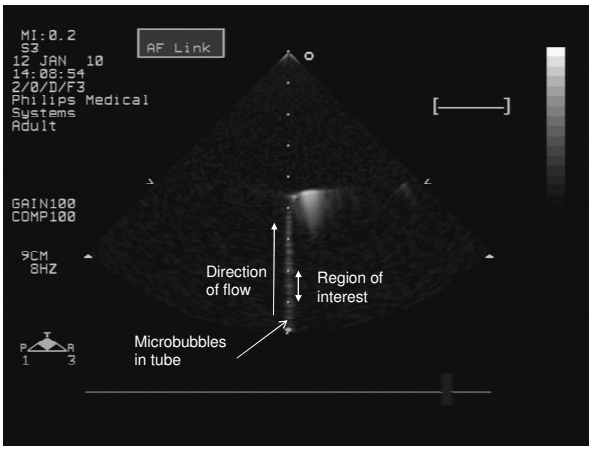

Figure 2 High concentration of Definity microbubbles flowing through a tube of 50 μm diameter. This was used for visualising the location of the tube during alignment.

RESULTS

Single microbubble signals were detected for biSphere and Definity insonated at 550 kPa peak negative pressure. A total of over 900 bubble signals were detected. The fundamental and harmonic components were extracted from each backscatter signal. A typical backscattered signal is shown in Fig. 3a with the fundamental and harmonic components in Fig 3b and 3c.

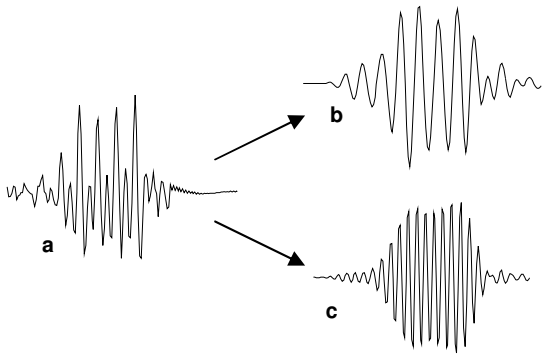

Figure 3 a) Backscattered signal from single biSphere microbubble in a 200μm diameter tube: b) is the fundamental and c) is the harmonic component of the backscattered signal.

The mean backscattered pressures from the fundamental and harmonic components are given in Table 1. For biSphere the mean RMS fundamental pressure for the 200 μm tube is less than that of the free bubbles. For Definity there is a smaller difference in the mean values for free bubbles and those in 200 μm tubes. For both Definity and biSphere in the 50 μm tubes, the RMS harmonic backscatter is increased compared to microbubbles in 200 μm tubes.

There were no significant differences seen in the size distributions of the free microbubbles and microbubbles flow throught the 200 μm tube.

DISCUSSION

The differences in mean backscattered pressures from Definity and BiSphere are due to the nature of the microbubbles. Definity comprises fluorocarbon gas surrounded by a soft lipid shell that will oscillate when subject to an acoustic field. BiSphere microbubbles are nitrogen filled and rigid shelled with a soft albumin coating. BiSphere microbubbles scatter ultrasound when the nitrogen within is released through shell deformities or on shell cracking, hence the backscattered signal is from gas released from the shell. Therefore two different microbubble behaviours in small tubes were investigated.

The mean bubble diameter of Definity is 1-3 μm and the mean diameter of biSphere is approximately 3μm. For microbubbles in the centre of a 200 μm tube, it is thought that since the size of the tube is much larger than the microbubbles, there should be minimal difference in the response of a microbubble in the 200 μm tube compared to free (untubed) agent. This was not

Table 1 Values for harmonic and fundamental components of RMS backscatter pressure from single BiSphere and Definity microbubbles in 200 µm and 50 µm tubes. The bracketed values are one standard deviation

	Fundamental component of backscatter			**Harmonic component of backscatter**		
	Free	**200µm tube**	**50 µm tube**	**Free**	**200µm tube**	**50 µm tube**
	n= 121	n= 142	n= 56	n= 121	n= 142	n= 56
BiSphere Mean RMS pressure (standard deviation) Pa	4.7 (3.7)	2.1 (1.3)	3.7 (2.7)	4.0 (2.3)	1.9 (1.2)	6.4 (4.4)
	n=94	n=292	n=270	n=94	n=292	n=270
Definity Mean RMS pressure (standard deviation) Pa	3.7 (3.7)	3.0 (2.6)	2.9 (1.6)	3.2 (1.2)	3.2 (1.1)	7.5 (3.2)

observed: there was a difference in the mean values of the backscattered pressure from 200 µm tubed and untubed bubbles, particularly for biSphere. At the smaller tube size of 50 µm, the harmonic content of the backscattered signal was larger than for the 200 µm tubed and untubed bubbles.

Suggested reasons for the difference are the presence of the tube. For the 200 µm tube, microbubbles flowing through a tube were visualised on a microscope and the majority of the microbubbles were flowing through the centre of the tube. The presence of the tube is thought to reduce the amplitude of the microbubble oscillations (Caskey et al. 2005; Sassaroli and Hynynen 2006). This could be the reason for the reduced mean backscatter pressure in the 200µm tube, however for the 50µm tube the mean backscatter pressure increases. This may be in part due to the filtering of the size of microbubbles flowing through the tube in addition to the narrow tube.

The 200 µm tube was a soft, cellulose tube while the 50 µm tube was a rigid acrylic tube. The different materials may have affected the acoustic field inside the tubes.

CONCLUSION

A system has been developed that allows investigation of the acoustic response of single microbubbles in narrow tubes. Two different types of microbubble have been investigated and differences have been noted in the response of microbubbles in 200 µm and 50 µm tubes in addition to differences in the response of untubed microbubbles and microbubbles in 200 µm tubes. These differences may be used to develop signal processing techniques to identify microbubbles in vessels of difference sizes.

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