A Setup for the Assessment of the Effect of Tubular Confinement on the Acoustic Response of Microbubbles

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Abstract—**Ultrasound contrast agents are gas filled microbubbles which produced enhanced echoes in ultrasound imaging thus allowing the acquisition of detailed information on the path of blood. It is theoretically known that the size of a vessel affects the behavior of a microbubble, which could potentially be used to discriminate different sized vessels. This information would be useful in the monitoring of neovascularization in tumor growth or treatment. However, currently it is not possible to identify the vessel diameter by any means of signal processing of microbubble echoes. In order to assess microbubble behavior when confined in tubes we compared the acoustic backscatter from biSphere™ microbubbles both free in water and flowing in 200 μm diameter tubes that are similar in size to arterioles. Experimental systems that allow the interrogation of individual microbubbles were designed and modified to allow investigation of both free microbubbles and those in tubes. Unprocessed single microbubble RF data were collected, allowing the calculation of both the fundamental and second harmonic components of the backscattered signal. Microbubbles confined in tubes had lower amplitude response compared to unconfined microbubbles. On consecutive insonations of the same microbubble, free microbubbles produced echoes above noise more often than confined microbubbles. This setup may be used to investigate microbubble behavior in a range of smaller tubes with diameters similar to capillaries thus enabling signal processing design for vessel differentiation.**

I. Introduction

Contrast agents used for ultrasound imaging are microbubbles which strongly scatter the incident beam. They can be used to highlight areas of blood flow and perfusion [1] [2] or be molecularly targeted for site-specific imaging and therapy [3]. Perfusion imaging is now established in the clinic for liver pathology. However, robust tools for the quantitation of perfusion are not fully developed. This is despite the fact that current ultrasound imaging is capable of detecting single microbubbles very sensitively and current contrast imaging modes achieve high sensitivity at even very low acoustic pressures [4].

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Moreover, current imaging methods for ultrasound contrast agents do not distinguish between microbubbles in differently sized vessel environments. While the echoes from microbubbles from different vessel environment *in vivo* are available for processing, to our knowledge no systematic study of these echoes is available.

Capillaries and arterioles, that are less than several tens of micrometers are involved in diameter, are responsible for the exchange of substances between blood and tissue thus offering a window for assessing tissue function. For example, microvascular density is an established biomarker related to the progression of vascular related disease such as cardiovascular disease and cancer. In vitro, the amplitude of microbubble oscillation was reduced by more than 50% when the microbubble was positioned near a wall [5]. The effect of varying tube diameters on microbubble oscillations [6] has also been investigated. Expansion of lipid shelled microbubbles was less in narrower tubes compared to wider tubes. Due to damping of the movement of the microbubble shell, the threshold for fragmentation of lipid microbubbles is at higher acoustic pressures for microbubbles in narrower tubes [6, 7]. The investigation of the microbubble acoustics under different sized tube confinement is an obvious extension of these and as it will have immediate application to the signal processing. We have previously shown that studying the acoustics of single microbubbles offers useful data for the development of signal processing algorithms [8]. Single microbubble acoustics have, in addition, offered useful data that contribute to the understanding of microbubble properties [9-11]. To our knowledge there are no in vitro phantoms that successfully simulate in vivo vascular confinement [12] and here such a setup will be established. The aim of this communication is to compare the response of free, unconfined biSphere™ microbubbles to that of biSphere™ microbubbles flowing through a 200 μm diameter cellulose tube with the microbubbles in both situations being subjected to identical acoustic fields.

II. Materials and Methods

A. Ultrasound set up

Systems capable of studying individual confined and unconfined contrast agent microbubbles have been previously described [8,13]. Confinement was within a 200μm diameter cellulose tube. Both setups were used for collection of single microbubble data from biSphere™ microbubbles. BiSphere™ (Point Biomedical Corp, San

Carlos, CA, USA) is a rigid shelled microbubble with an albumin coating and contains nitrogen. The nature of the response of biSphere™ is that the rigid shell, on insonation, releases the gas through shell defects or by means of ultrasound induced cracking [14], resulting in the detection of a free gas microbubble in the vicinity of a rigid shell.

For all experiments described in this study a Philips Sonos 5500 (Philips Medical Systems, Andover, MA, USA) research machine was used with an S3 phased array transducer which allowed the capture of unprocessed RF data to allow detailed analysis of the backscattered signals. The scanner was driven at 1.6MHz in M-mode and acoustic data was collected at a distance of 7.5 ± 0.5 cm from the transducer, at which distance the transmitted and received acoustic fields were calibrated [13]. The region of interest was beyond the 6cm focal position and the beam width was 4.4mm. Peak negative acoustic pressures ranging from 300 – 1000 kPa were investigated. On placing water-filled cellulose tubes between the transducer and a 0.2 mm active element membrane hydrophone (Precision Acoustics, Dorset, UK) no difference was detected in the amplitude of the transmitted acoustic field. In addition, RF data of water-filled tubing was used to assess reflections due to the presence of the tube.

B. Data analysis and statistical comparison

The backscattered signal was stored and decomposed into fundamental and second harmonic components and the root mean square (RMS) backscattered pressure was calculated for each microbubble signal as previously described [11]. The rationale of using these two spectral components lies in the high sensitivity of detecting them in the current experimental setup. In addition, these components are used widely in the literature to describe the behavior of microbubbles. The duration (pulse length) of each echo was calculated and the lifetime of the microbubble was determined by measuring the duration of consecutive echoes from the same microbubble. Large numbers of RMS pressures were calculated and the statistical comparisons of data from microbubbles in the 200μm tube and unconfined microbubbles with no tube were made using a one dimensional Kolmogorov-Smirnov (K-S) test [15]. Both the fundamental and second harmonic components of the echoes in each experimental situation were compared. The K-S test assumes no prior knowledge of the nature of the distributions being compared.

III. RESULTS

A. Single microbubble backscatter

In total, echoes from over 550 individual microbubbles flowing though 200μm tubes were collected, for various acoustic pressures. Data for each acoustic pressure is shown in Fig. 1. Fig. 1 a-d show histograms of fundamental echo components and Fig 1 e-h are the second harmonic echo components, at different acoustic pressures. As the incident acoustic pressure was increased the fundamental and second harmonic response for all microbubbles increased. Both free and confined microbubbles followed a similar trend, however, the free microbubbles generally produced more echoes with a higher fundamental component at incident pressures of 300 and 550 kPa. As the incident acoustic pressure increases the distribution of echoes from free bubbles is wider than that of the confined microbubbles in both spectral components. The distributions of microbubble echoes from the two experiment situations were compared using the 1D K-S test. The fundamental response of the microbubbles in the 200 μm tube was significantly lower than that of the free microbubbles. The second harmonic response of free and confined microbubbles was significantly different for 550, 800 and 1000 kPa but not for 300kPa.

B. Lifetime of microbubble echo and duration of response

The percentage of microbubbles no longer present (no further echo above noise detected) after the 2nd insonating pulse are displayed in Fig. 2. For these calculations the full unprocessed RF echo comprising fundamental and harmonic components was used. As expected, for both confined and unconfined microbubbles, the number of microbubbles from which no echo was detected on the second insonation increased with acoustic pressure. However, a higher proportion of the microbubbles confined in the tube did not give consecutive echoes compared to the unconfined microbubbles.

We have assumed that when no echo above noise is detected that the microbubble is no longer responding to ultrasound. However, it maybe that the microbubble is not destroyed but is producing reduced amplitude oscillations below the noise level.

As the incident acoustic pressure increased there was an increase in the number of short duration echoes present on the first insonation. The short duration echo is due to cracking of the microbubble shell prior to gas release and these results confirm previous findings on the biSphere™ acoustic response [14]. The majority of echoes of short duration on the first insonation gave a full length echo (equal to the incident pulse length) on the second insonation. For confined microbubbles, of echoes at 550kPa which were less than 3.5μs in duration on the first insonation, 60% gave a full duration echo on their second insonation. For 800kPa 92% of short echoes on the first insonation were longer on the second insonation. For the free microbubbles these values were 94% and 90% respectively. As shown previously [14, 16], the short duration echo is associated with a free gas oscillation outside the microbubble shell following a crack that was induced by the pressure exerted by the gas trapped inside the bubble. Following this event the generated crack provides a permanent escape route for the gas and therefore the second echo displays mostly a full duration as above.

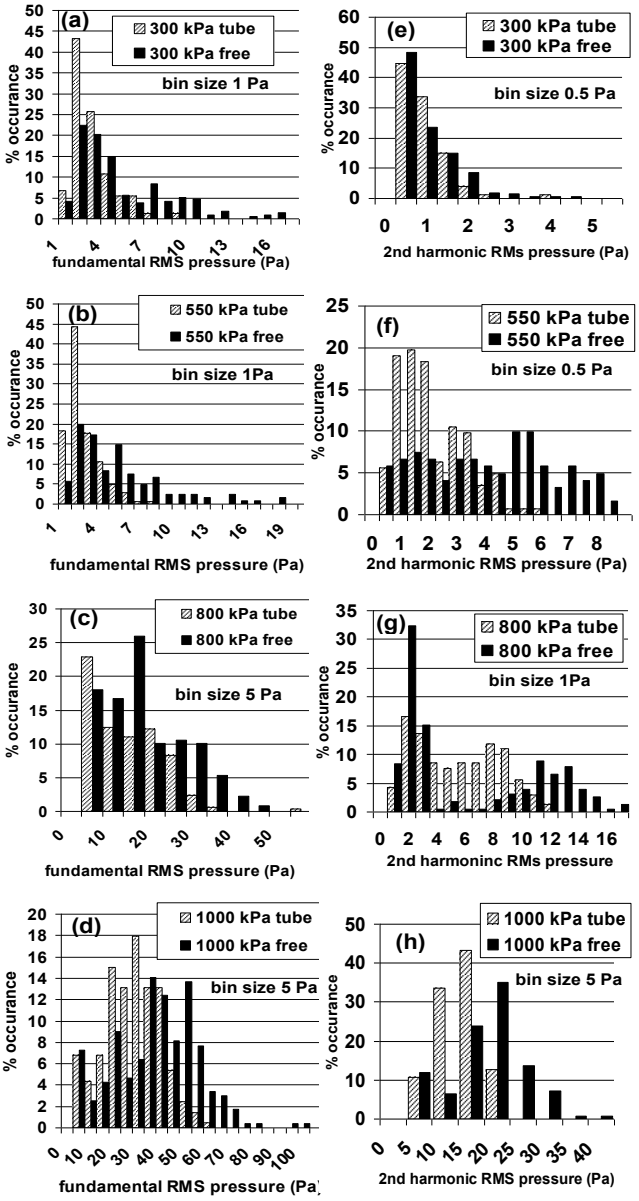

Figure 1. Histogram of distribution of number of (a-d) fundamental echoes and (e-h) 2nd harmonic echoes for first insonation.

IV. DISCUSSION

From the results presented here we have a comparison between the impact of two different environments on

microbubble behavior. Differences were observed in spectral signatures, echo duration and echo lifetime. Since the introduction of pulse sequences it is understood that all the above aspects of microbubble response may be important in the development of algorithms for selective microbubble detection. BiSphere™ microbubbles are not currently used clinically as their development was recently discontinued. This is partly due to their complex behavior in the presence of ultrasound and the difficulty in understanding how imaging applications can be developed in relation to it. In recent years it was shown that the shell of a subpopulation of biSphere microbubbles cracks in the presence of ultrasound,

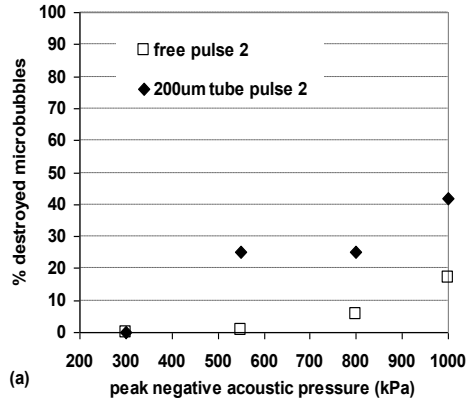

Figure 2. % of microbubbles destroyed on their second insonation for free and confined microbubbles

releasing gas and providing strong scatter signals of short duration [13,16]. The rest of the microbubble population provides scatter with duration similar to that of the transmitted ultrasound. This is due to their porous shells and release their gas without the requirement to create a shell crack [14]. There are also a number of other smaller sized biSphere™ microbubbles that do not respond to ultrasound [16]. The size distribution of these subpopulations is not fixed but is affected by ultrasound imaging parameters. In general the number of hard shelled microbubbles that scatter have been observed to increase with acoustic pressure [4]. It is further suggested here that the confinement provided by a 200μm tube surrounding the microbubble provides additional damping to the oscillation, thus reducing the amplitude of echoes. In addition to the confinement the different properties of biSphere™ microbubble subpopulations provides an intriguing potential for their use. For example from Fig. 1b and Fig. 1f it can be seen that for an incident field of peak negative pressure 550 kPa the fundamental echoes which differ most between confined and unconfined microbubbles are those between 1 Pa and 3 Pa as well as those over 7 Pa where no echoes from microbubbles flowing in the tube were detected. This demonstrates single microbubble acoustic measurements may enable the differentiation between microbubble subpopulations. Their scatter properties (including their progression) in response to a sequence of pulses may provide unique signatures that translate to elevated sensitivity in detecting them. Further work with more realistic confinement and/or selected microbubble dispersions (referring to size or other material property) may help identify signal processing strategies that are vessel size specific. More emphasis will be given in the future to extracting spectral or temporal signatures unique to microbubbles in small tubes in order to feedback on the design of signal processing algorithms that employ several pulses. It is known that in current signal processing methods tissue cancellation is more effective to perform compared to optimizing microbubble enhancement. This correlates well with the respective theoretical knowledge on scatter. Since a complete model of the behavior of microbubbles in an in vivo capillary is a challenge, the optimization of single microbubble signal processing in the same environment is a

recommended research avenue. The setup employed here can deliver such experimental data.

The differences noted in the proportion of short duration pulses between low and high peak negative acoustic pressures are attributed to the fact that each sample of biSphere™ contains both types of microbubbles: those that leak and those that crack. At lower acoustic pressures echoes are only detected from leaking microbubbles as the acoustic field is not enough to crack the shell [14]. The number of microbubbles that crack increases with increasing acoustic pressure. These microbubbles are thought to produce a shorter echo on first insonation as some of the energy from the incident 6 cycle pulse is used to crack the shell. In addition to the differences with acoustic pressure, the presence of shorter duration echoes is constant in the tube up to 1000 kPa while for free microbubbles, the proportion of shorter echoes increases between 550 and 800 kPa and then decreases for 1000 kPa. This difference in proportion of cracked microbubbles is suggested to be due to microbubbles insonated at 1000 kPa cracking very early, due to increased energy applied to the shell and hence the scattered echo on the first insonation has a longer duration pulse compared to a microbubble insonated at 550 kPa where the microbubble shell cracks later in the incident pulse resulting in a short duration echo [14].

In line with the above discussion, it is suggested that the presence of the tube dampens the effect of the incident pulse on the microbubble shell and thus, in the tube at 1000 kPa the time taken to crack the shell (and release free gas) is similar to 550 kPa. Finally, as shown above, the lifetime of the microbubbles in the tube is less than that of the free microbubbles, which may be associated with increased instability induced by the tube. Further to this, the majority of unconfined microbubbles that crack at 550 kPa provide full duration echoes in the second insonation. The presence of the tube reduces this proportion to 57%. It would be reasonable to hypothesize that once a crack has been created this facilitates a leaked gas oscillation on a second insonation. This does not agree with what has been observed in the tube environment and in addition at 1000 kPa in the unconfined microbubble environment.

The experimental procedure here provided a setting for the reproducible and reliable comparison between the two environments. Potential limitations in the comparison of the data provided by the two experimental setups include the differences between microbubble populations and between ultrasound beams in the different setups. The statistical analysis here enabled a comparison of microbubble signals without a prior assumption of their distribution and demonstrated that small but significant differences between the confined and unconfined microbubbles can be detected. Next steps in this research should investigate microbubble behavior in tubes closer to capillary diameters.

V. Conclusions

A system has been developed which allows the acoustic backscatter from a large number of single microbubbles in tubes to be assessed under a well characterized acoustic field. BiSphere™ microbubbles have generally lower amplitude scatter when confined in 200 μm cellulose tubes compared to unconfined ones. Persistence of the microbubble signal in consecutive pulses of ultrasound was higher in unconfined microbubbles. Further work will compare the response of different microbubble compositions confined in smaller diameter tubes. Signal processing development is facilitated when single microbubble echoes can be studied and the current setup offers such an opportunity.

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