What's in a name? Micro v. Nanobubbles in Ultrasound Imaging and Therapy

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Introduction

Microbubbles (1-10 μm in diameter) were initially developed as contrast agents for use in ultrasonic imaging due to their echogenicity and have subsequently been investigated in a range of therapeutic applications. Drawbacks of microbubbles include their relatively short circulation half-lives and inability to extravasate. Consequently, various sub-micrometre particles have been investigated in both diagnostic and therapeutic applications. Gas "nano" bubbles with diameters <1 μm have been widely explored and reported as being able to extravasate whilst remaining visible to conventional ultrasound imaging[1,2]. "Nano"bubbles have also been reported as effective agents for numerous therapeutic applications, e.g., for blood brain barrier permeabilisation, drug delivery and mechanical ablation. These results are surprising as theory suggests that sub-micrometre particles should not be able to exhibit comparable performance under the same ultrasound exposure conditions as microbubbles as either diagnostic or therapeutic agents. There is considerable variability, however, in the definition of "nano"bubbles in published studies and a wide range of techniques used to determine their size and concentration[3,4].

We investigated the following hypotheses to explain the observed acoustic responses from "nano"bubble suspensions: (i) the presence of pre-existing microbubbles as proposed by Myers et al. (2022)[3] (ii) coalescence resulting in the formation of microbubbles over time (iii) changes in the characteristic acoustic impedance of "nano"bubble suspensions at high bubble concentrations (iv) nonlinear propagation through high concentration bubble suspensions and/or (v) inconsistencies between expected and actual bubble size distributions and concentrations arising from challenges associated with measuring highly polydisperse bubble suspensions.

Methods

The acoustic responses of bubbles with diameters from 100nm to 10μm were modelled using a Rayleigh-Plesset type equation via both numerical and analytical solutions and these data were used to predict the attenuation and speed of sound in a bubble suspension[5]. Experimentally, several formulations of microbubbles (MB) and "nano"bubbles (NB) were fabricated[2,6]. These were then characterized with multiple methods: sub-micrometre bubble size was measured using Dynamic Light Scattering (DLS) on a ZetaSizer (Malvern, UK) and Transmission Electron Microscopy (TEM FEI Tecnai T12) using uranyl acetate staining. Micrometre and sub-micrometre bubble size and concentration were measured by Coulter Counter (Multisizer), light microscopy using a 40x objective (Leica, UK), or Nanoparticle Tracking Analysis using both Videodrop (Myriade, France) and Nanosight NT 300 (Malvern, UK) systems. Bubble suspensions were also imaged in both B-mode and contrast mode using a clinical ultrasound system with a 5-12MHz diagnostic ultrasound imaging probe (Philips L12-5A), and bubble oscillations were captured using high-speed imaging at 1 million frames per second (Shimadzu HPVx2) when driven at 0.5 MHz with peak negative pressures up to 1 MPa.

Results

Experimentally, there were considerable differences in the measured size distributions recorded from the different instruments. For NB, the modal diameter measured by the ZetaSizer was 200nm, by the Videodrop it was 235nm whilst the Nanosight reported 100nm. TEM indicated a range of sizes from 10s to 100s of nanometres, but it was not possible to obtain a sufficient number of images to generate a statistically meaningful size distribution. There was better agreement in the concentration measurements from the Videodrop and the Nanosight for the NB (1.94×10^{12} particles/ml and 8×10^{12} particles/ml respectively). None of these methods indicated the presence of any bubbles larger than 1 μm. Much better agreement was obtained for MB diameter measurements between the Multisizer and light microscopy than between the DLS, Nanosight and Videodrop, but neither is suitable for detecting bubbles smaller than ~500nm. These results are supportive of hypotheses (i) and (v) consistent with Myers et al (2022)[3].

Bubble stability was measured by recording the size distribution and concentration over time. The size and concentration of NBs were found to decrease by 18.5% and 19% respectively across 3 days when stored at 4ºC (Figure 1) but there was no evidence of rapid coalescence at either 20ºC or 37ºC when measurements were taken over 20 minutes in the Videodrop and NTA systems. Preliminary theoretical modelling of coalescence probability supported these findings, suggesting that hypothesis (ii) is not valid.

As expected, theoretical modelling predicted a substantial difference in scattering and attenuation coefficients between MB and NB for both linear and nonlinear propagation, with measurable scattering only being predicted at very high bubble concentrations for bubbles smaller than 400nm or frequencies above 15MHz. These results were in good agreement with the experimental ultrasound measurements. Negligible image contrast was observed under either B-mode or contrast imaging from NB suspensions, suggesting that neither hypothesis (iii) and (iv) was correct (Figure 2). Similarly, negligible bubble activity was observed under high-speed imaging from NB suspensions under exposure conditions associated with therapeutic applications and under which inertial cavitation was observed with MB.

Conclusions

The results of this study support the findings of Myers et al. that it is very difficult to accurately size a highly polydisperse bubble suspension and therefore difficult to produce bubble suspensions containing only bubbles of a particular size range without repeated filtration and/or centrifugation. There was no evidence to support the hypotheses of NB coaelscence or nonlinear effects at high NB concentrations being responsible for reported acoustic responses in either diagnostic or therapeutic applications. Whilst NB may offer advantages in terms of circulation time, extravasation and/or cellular fusion, higher pressure amplitudes and/or frequencies will be required to elicit an acoustic response and this may have important implications for bioeffects. The limitations of available bubble sizing methods need to be carefully considered in experimental design to avoid misinterpretation of results and to avoid errors in dose estimation. There is also a pressing need for a consensus on nomenclature for MB and NB as the latter currently encompass bubbles from 100-800nm, but this may correspond to a wide range of different bubble dynamics depending on the ultrasound exposure parameters.

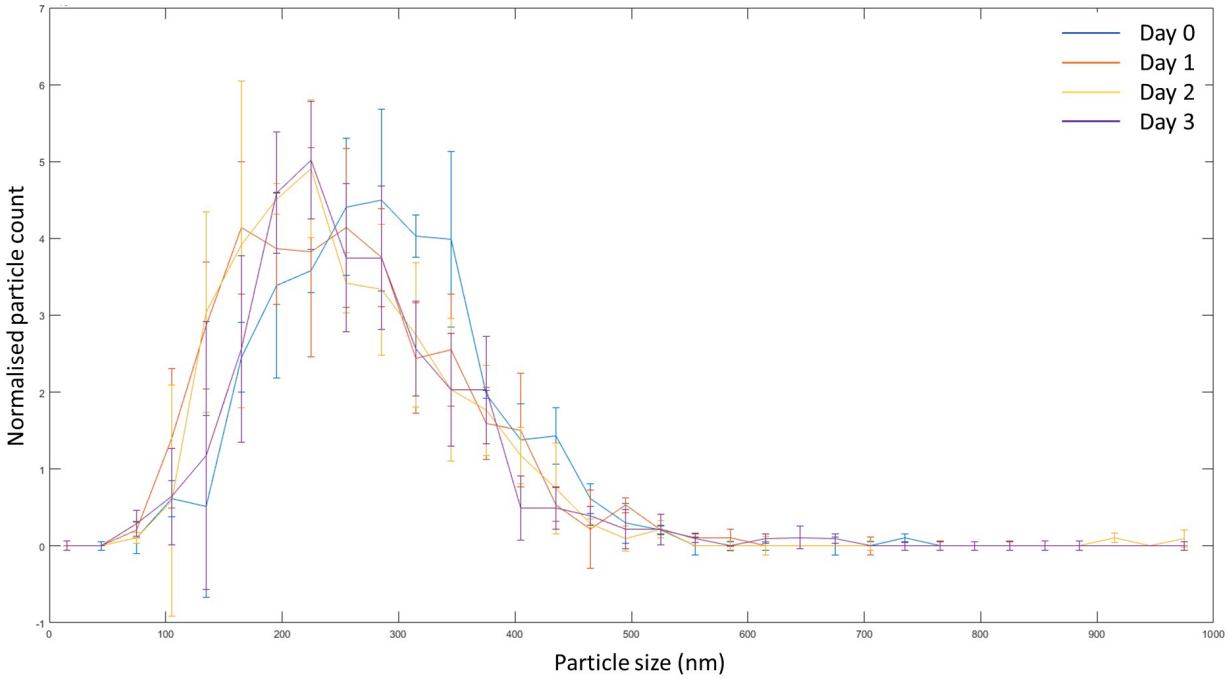

Figure 1. Size distribution of "nano"bubbles across multiple days (n=3, error bars represent standard deviation). There is a decrease in size and a more defined peak as the sample gets older but no evidence of substantial coalescence.

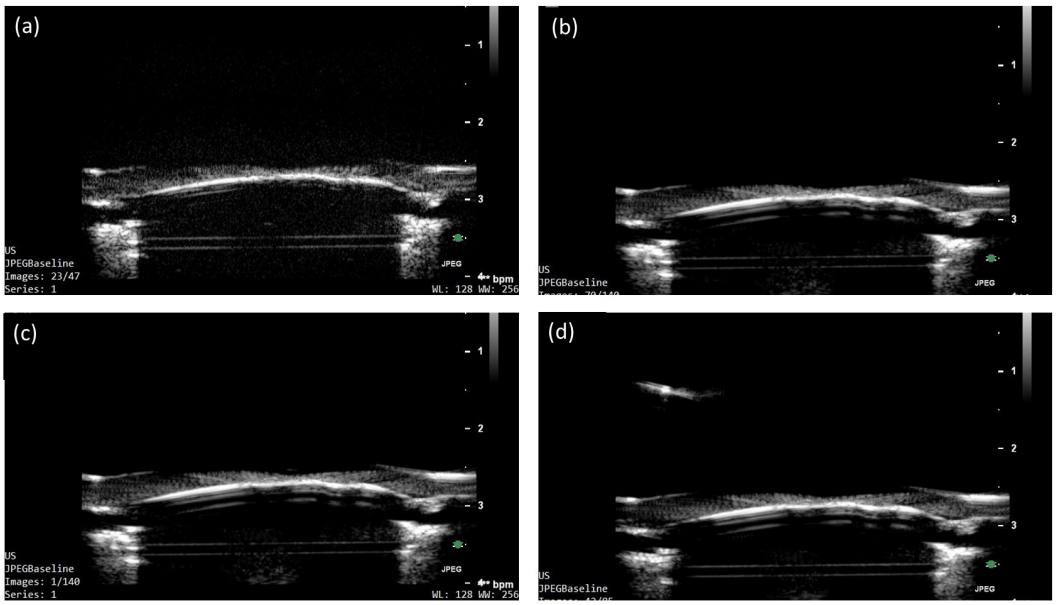

Figure 2. Images showing negligible detection of Contrast and B-Mode Ultrasound responses of water and "nano"bubbles in a tissue mimicking phantom. (a) and (b) show Contrast and B-Mode Ultrasound response of Milli-Q filtered water respectively. (c) and (d) shows Contrast and B-Mode Ultrasound response of NB suspensions respectively.

References

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