ON-CHIP PRODUCTION OF NANOMETER SIZED 'ULTRA FINE' BUBBLE POPULATIONS

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ABSTRACT

Microbubble (MB) contrast agents have been used for many years as image enhancers for medical Ultrasound (US). Ultra-Fine bubble (UFB) populations of bubbles <1 μm in diameter are a relatively new technology that has found use as highly effective 'eco' cleaning agents. High-resolution US imaging is another potentially exciting area for UFB. This paper reports the on-chip production of UFB populations with a diameter of ~ 500 – 700 nm at a concentration of 10^{10} bub / mL. These UFB showed US scattering at higher frequency fields and enhanced contrast when imaging in *in vivo* mouse models.

KEYWORDS: Ultra-Fine bubbles, Ultra-Sound, Contrast agents

INTRODUCTION

Lipid stabilized, micron sized gas-in-water emulsions have been utilised for many years as contrast agents for US. When subjected to an US wave, the bubbles oscillate due to the compressibility of their core and shell. Oscillations cause large amounts of US scatter and therefore enhance an US image. The resonant frequency of a bubble depends on many physical parameters, including the bubble size. Generally, the smaller the bubble: the higher the resonant frequency. A relatively new area of bubble technology is the use of UFB as eco-cleaners in flotation technology in mining and microfabrication. However, another new and potentially exciting use of UFB is in high-resolution US imaging, due to their small size their resonant frequency is much higher than that of MB which better suits the high-frequency transducers used in pre-clinical and clinical US. Microfluidics has already been demonstrated for producing monodisperse MB populations by utilizing flow focusing geometries.[1,2] Production regimes of this type offer control over bubble size, but is limited to 1-2 μm by the width of the nozzle and production of low concentrations of bubbles (<10^7 bub / mL). Last year, we reported the use of a 3D expanding geometry for the preparation of high concentrations of MB contrast agents (10^9 – 10^{10} bub / mL) using a new 'microspray' production regime.[3,4]

Recently, the microspray production regime has been investigated as a potential method for producing UFB populations. We present size and concentration data of bubble populations, their response to high-frequency US fields and preliminary *in vivo* US imaging in mouse models. To our knowledge this is **the first microfluidic example of its kind.**

EXPERIMENTAL

MBs were prepared from a mixture of lipids DPPC and DSPE-PEG2000. All lipids were purchased from Avanti Polar Lipids (Alabaster, Al, USA) and all other reagents from Sigma-Aldrich, UK unless stated otherwise. The protocols for lipid preparation and MB formation were followed according to previously published methodologies.[3] The microchips were fabricated by Epigem Ltd (Redcar, UK) in polycarbonate and SU-8. The depth of the chips was 25 μm, with an additional layer of 25 μm in the outlet for 3D expansion (50 μm in total). Acoustic backscatter from the bubble populations was measured using a focused 15 MHz single element US transducer (V313, Olympus KeyMed Ltd, UK). The US transducer was connected to a pulser/receiver (5072PR, Olympus KeyMed Ltd, UK), which generated a broadband US pulse. Upon reception these signals were 1 MHz high pass filtered and amplified by a 20 dB pre-amplifier. 500 pulses were recorded at a pulse repetition frequency (PRF) of 0.5 kHz for each bubble population and were processed using Matlab (MathWorks Inc, USA). The received signal spectra were averaged in the frequency domain to reduce the variance of the experimental results due to external

noise sources. For *in vivo* imaging, 4x CD1 nude male mice were imaged with the VisualSonics Vevo770 High Frequency Ultrasound (HFUS) and anaesthetised with isofluorane in medical air. The aorta of the mouse was identified and arterial flow confirmed with Pulsed Waved (PW) Doppler imaging. A 50 µL bolus of ~1x10^8 bubbles were administered via tail vein catheter at a rate of 0.6 mL / min. Imaging in Contrast B-mode was analysed with VisualSonics software to determine the contrast intensity over time.

RESULTS AND DISCUSSION

Figure 1a shows the microscopy image of microspray production inside the chip. Lipid solutions focused the gas through a ~10 µm nozzle at high flow velocities (~1.5 m / s) and high pressures (15 psi) and then subjected to a downstream 3D expansion in the outlet.

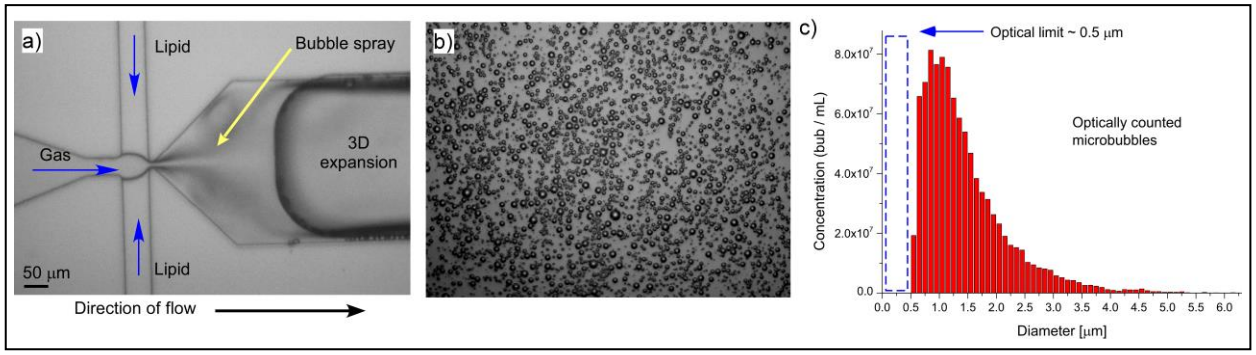

Figure 1: a) Microspray production regime showing 3D expansion in outlet. b) Typical optical image of MBs produced by regime. c) Size histogram of MBs showing the size cut-off in optical counting.

This pressure drop causes the atomization-like behavior of the gas cone, causing a spray of bubbles to break off in the outlet. Figure 1b shows an optical image of MBs produced by the microspray regime and figure 1c was the resultant size histogram from the sample. The histogram shows the high concentration of MBs in the size range ~1 to 6 µm, however the histogram shape appears cut off on the left hand side, which would correspond to optical resolution cut-off. This suggested that there was a population of bubbles less than 1 µm in size that were too small to be resolved and counted optically.

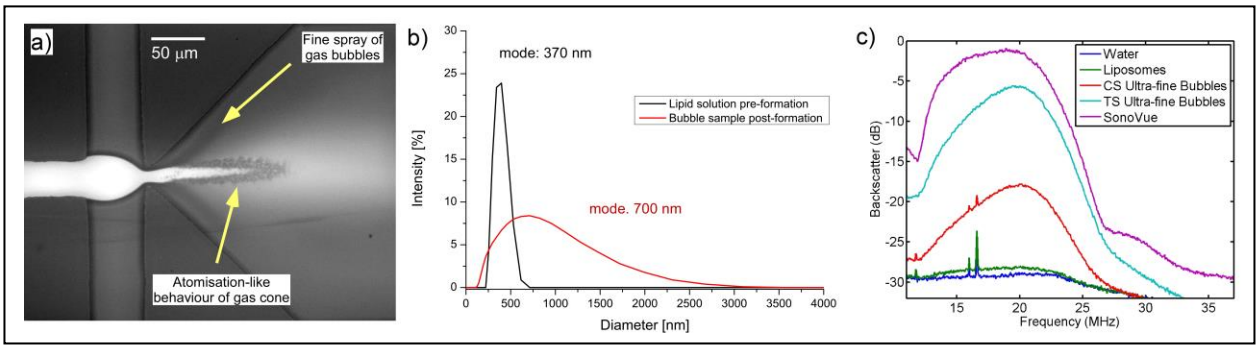

Figure 2: a) Atomisation-like behavior of the gas cone during microspray production. b) Sizing data for subnatant of separated bubble samples. c) US backscatter from samples using a 15 MHz transducer.

Figure 2a shows an image of the gas cone at 90x magnification, MBs were seen breaking off towards the tip of the cone however toward the neck of the nozzle a very fine spray of bubbles was also observed. Samples of bubbles from the chip were subjected to two size separation techniques, one relying on the natural buoyancy of the bubbles in which larger bubbles rise more rapidly than smaller bubbles (TS-separated) and the second being by gentle centrifugation (CS-separated). Larger MBs were removed from

the top of both samples and the subnatant containing the UFB retained. Figure 2b shows the size of the UFBs when measured on a DLS with a mode size of approximately 700 nm. The lipid solutions used in production will also contain vesicles in the order of nm that have not incorporated into bubble shells. The US scatter from the samples was investigated and compared to commercial MB samples (SonoVue) and a sample of vesicles in order to distinguish the UFBs from the vesicles in the samples. Figure 2c shows the US backscatter from the four samples using a 15 MHz transducer. Firstly, both UFB samples showed significantly higher backscatter compared to the vesicle control, indicating the particles sized in the samples are mostly UFBs. Secondly, the MB sample showed a broad backscatter signal over a range of frequencies (~15 – 25 MHz) however, the UFB samples show a more confined backscatter at higher frequencies (~20 – 25 MHz). This shift in the peak suggests the presence of high numbers of UFBs in the sample giving rise to the backscatter signal at higher frequencies, which is in-line with theory.

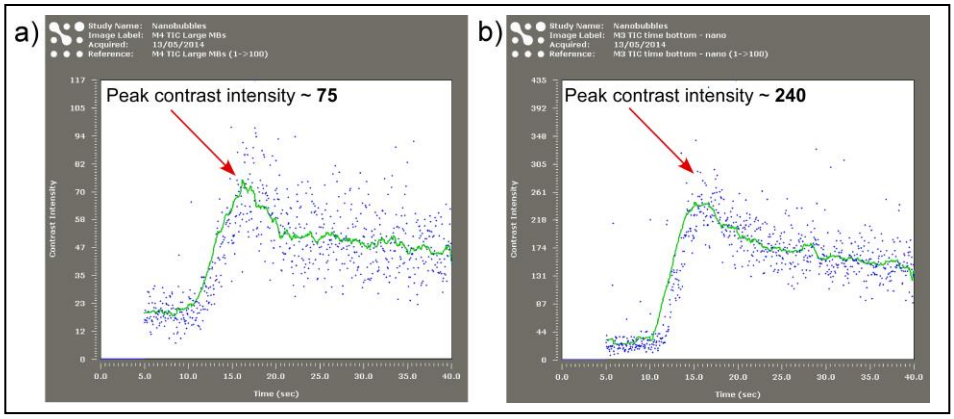

Figure 2:a) In vivo wash in curve for MBs only showing peak contrast intensity of ~75. b) Wash in curve for UFB subnatant showing peak contrast intensity of ~240.

The potential use of UFBs as high-resolution contrast agents for US was also investigated *in vivo*. Figure 3 shows the wash in curves for bubble samples in mice aorta. Figure 3a shows the signal received from MBs only (bubbles removed from top of separation vial) and 3b shows the signal received from the UFBs in the subnatant. The UFBs show significantly higher contrast (~ 240) than the microbubbles alone (~ 75), indicating that the overall contrast observed from the contrast agent at pre-clinical US frequencies has a huge contribution from the presence of UFBs.

CONCLUSION

UFBs have been produced for the first time on-chip using a microspray flow regime in a 3D expanding chip. These UFBs have shown to have a higher resonant frequency than MBs and show excellent *in vivo* US signal. UFBs show great potential as contrast agents for higher resolution US imaging.

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