Microfluidic production of micro- and nano- bubbles for medical applications.

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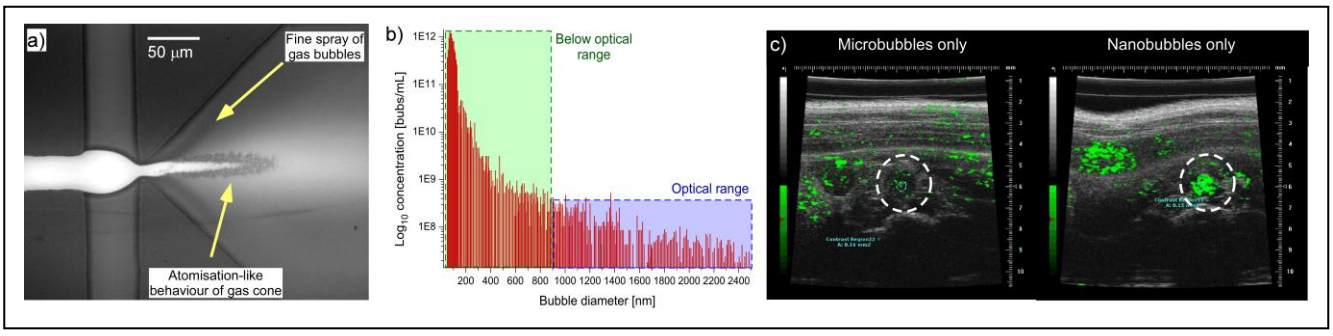
Lipid stabilized, micron sized gas-in-water emulsions have been utilised for many years as contrast agents for ultrasound (US). Due to their compressibility, sound waves are reflected and scattered from the gas / liquid interface, enhancing imaging. Recently, there has been interest in developing these microbubbles into a theranostic agent, one which can produce diagnostic information but also deliver a therapeutic payload to a diseased area such as a tumour.

Microfluidics has already been demonstrated for producing monodisperse microbubble populations by utilizing flow focusing geometries.[1,2] We have reported the use of a 3D expanding geometry for the preparation of high concentrations of therapeutic microbubbles using a new 'microspray' production regime**.** [3] Recently, the microspray production regime has been investigated as a potential method for producing nano-meter sized, 'ultra-fine' bubble populations.[4] The microspray regime produces bubbles in an atomization-like method generated by the high velocities inside the chip nozzle being subjected to a sudden and severe pressure drop as the outlet expands in the 3D planes (Figure 1a). As mentioned, this has successfully and robustly produced very high concentrations of microbubbles; however the histograms produced when counting optically indicated that a high number of bubbles were not being counted due to limitations in optical resolution. Alternative methods for sizing and counting detected large numbers of particles (10^{10}-10^{11} particles / mL) with diameters < 1µm (See figure 1b). In order to determine whether these particles were acoustically active (bubbles) or not (liposomes) the samples were investigated using a 15 MHz transducer and compared to commercially available contrast agents (Sonovue) and liposomes of a similar diameter. The sample showed large amounts of backscatter at higher frequencies compared to liposomes of a similar size, suggesting the presence of a gas core. Further *in vitro* investigations in mouse aorta compared using a 40 MHz transducer (figure 1c) showed excellent contrast intensity of the nanobubbles compared to the whole bubble sample, suggesting the presence of nanobubbles contributes significantly to the observed contrast intensity in contrast agents. In addition, these nanobubbles could improve image resolution and also have the potential to perfuse deeper into tissues for drug delivery purposes.



References

1. K. Hettiarachchi, E. Talu, M. L. Longo, P. A. Dayton and A. P. Lee, Lab on a Chip, **2007**, 7, 463-468.
2. E. Castro-Hernandez, W. van Hoeve, D. Lohse and J. M. Gordillo, Lab on a Chip, **2011**, 11, 2023-2029.
3. S. A. Peyman, R. H. Abou-Saleh, J. R. McLaughlan et al, Lab on a Chip, **2012**, 12, 4544-4552.
4. S. A. Peyman, J. R. McLaughlan, R. H. Abou-Saleh et al. Lab Chip, **2016**, 4, 679-687.