Ultrasound in Med. & Biol., Vol. 40, No. 6, pp. 1379–1384, 2014

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2013.11.025

● *Technical Note*

ON THE ACOUSTIC PROPERTIES OF VAPORIZED SUBMICRON PERFLUOROCARBON DROPLETS

Nikita Reznik,* Guillaume Lajoinie,† Oleksandr Shpak,† Erik C. Gelderblom,† Ross Williams,‡ Nico de Jong,§ Michel Versluis,† and Peter N. Burns*‡

*Department of Medical Biophysics, University of Toronto, Toronto, Ontario, Canada; †Physics of Fluids Group and MIRA Institute of Biomedical Technology and Technical Medicine, University of Twente, Enschede, The Netherlands; ‡Sunnybrook Research Institute, Toronto, Ontario, Canada; and §Biomedical Engineering Thoraxcenter, Erasmus Medical Center, Rotterdam, The Netherlands

(*Received* 23 *August* 2013; *revised* 19 *November* 2013; *in final form* 20 *November* 2013)

Abstract—The acoustic characteristics of microbubbles created from vaporized submicron perfluorocarbon droplets with fluorosurfactant coating are examined. Utilizing ultra-high-speed optical imaging, the acoustic response of individual microbubbles to low-intensity diagnostic ultrasound was observed on clinically relevant time scales of hundreds of milliseconds after vaporization. It was found that the vaporized droplets oscillate non-linearly and exhibit a resonant bubble size shift and increased damping relative to uncoated gas bubbles due to the presence of coating material. Unlike the commercially available lipid-coated ultrasound contrast agents, which may exhibit compression-only behavior, vaporized droplets may exhibit expansion-dominated oscillations. It was further observed that the non-linearity of the acoustic response of the bubbles was comparable to that of SonoVue microbubbles. These results suggest that vaporized submicron perfluorocarbon droplets possess the acoustic characteristics necessary for their potential use as ultrasound contrast agents in clinical practice. (E-mail: reznik@ohsu.edu) **© 2014 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Ultrasound contrast agents, Perfluorocarbon droplets, Droplet vaporization, High-speed imaging, Microbubble characterization.**

INTRODUCTION

Contrast-enhanced ultrasound is a well-established diagnostic imaging technique. Microbubble ultrasound contrast agents are small, encapsulated spheres of gas, typically 1 to 5 μm in diameter. Their size, similar to that of red blood cells, makes them a true blood pool agent, allowing for their use in a number of diagnostic applications, such as cancer detection (Burns and Wilson 2007), assessment of blood flow (Hudson et al. 2011) and blood pressure measurements (Tremblay-Darveau et al. 2014). However, their size is also a disadvantage for some applications, as microbubbles are confined to the blood vessels and cannot reach extravascular targets.

Submicron droplets of liquid perfluorocarbon (PFC) have been studied as a new generation of extravascular contrast agents for ultrasound. At a size of a few hundreds of nanometers in diameter, these droplets have the ability to extravasate selectively in regions of tumor growth and stay intravascular in healthy tissues, due to the enhanced permeability and retention effect (Maeda et al. 2000), effectively allowing for their passive targeting to tumors. The low-boiling-point droplets are acoustically inert, until exposed to a sufficiently high-intensity burst of ultrasound, at which point they vaporize to produce echogenic microbubbles (Reznik et al. 2011), stabilized by the coating material used to encapsulate their liquid droplet precursors for time scales of seconds to minutes (Reznik et al. 2012). Such extravascular contrast agents may be used for diagnostic ultrasound imaging to detect regions of tumor growth (Matsunaga et al. 2012; Williams et al. 2013) as well as for therapeutic applications, such as sensitizers for high-intensity focused ultrasound (HIFU) therapy (Phillips et al. 2013) and vehicles for drug delivery (Rapoport 2012).

For their successful application as ultrasound contrast agents (UCAs), upon vaporization, the droplets should produce echogenic bubbles that respond to ultrasound in a manner similar to the currently used microbubbles on

Address correspondence to: Nikita Reznik, Knight Cardiovascular Institute, Oregon Health & Science University, 3181 SW Sam Jackson Road, UHN62, Portland, OR 97239, USA. E-mail: reznik@ohsu.edu

clinically relevant time scales of hundreds of milliseconds. Although a number of studies have focused on the conditions necessary for droplet nucleation (Couture et al. 2012; Kripfgans et al. 2000; Reznik et al. 2011; Sheeran et al. 2011; Shpak et al. 2013a; Williams et al. 2013) and the vaporization process itself (Reznik et al. 2013; Sheeran et al. 2013; Shpak, et al. 2013b; Sheeran et al. 2014), only limited information is available regarding the acoustic properties of these newly created microbubbles.

In this report we conduct an initial study of the acoustic properties of individual vaporized PFC droplets. Using ultra-high-frame rate optical imaging, we examine the acoustic response of the fully vaporized microbubbles of different sizes to a 2.5-MHz ultrasound pulse of low intensity (mechanical index [MI] < 0.1) a few hundreds of milliseconds after the phase change. Specifically, we examine the change in bubble oscillation amplitude and non-linearity of oscillation as a function of bubble size. Furthermore, we study the influence of the presence of coating material on bubble oscillations and compare the non-linear acoustic response of these bubbles with that of commercially available SonoVue microbubbles, to assess whether the vaporized droplets possess adequate acoustic properties for their potential use as UCAs.

METHODS

Droplet preparation

Perfluorocarbon droplet emulsions were prepared by combining water, 5% v/v dodecafluoropentane (PFP, Fluoromed, Round Rock, TX, USA) and 0.8% v/v negatively charged fluorosurfactant Zonyl FSP (Sigma Aldrich, St. Louis, MO, USA) according to a previously established protocol (Reznik et al. 2013). The samples were coarse emulsified with a Vortex mixer (VM-300, Gemmy Industrial, Cherry Hill, NJ, USA) for 30 s. After the coarse emulsification, the solution was further emulsified with a tip sonicator (Sonifier 250, Branson, Branson, MO, USA) for 60 s at 80% duty cycle. Sizing of the droplets was performed with a Zetasizer nano-sizing system (Malvern, Worcestershire, UK). This method provided droplet samples that were polydispersed in size, ranging from 100 nm to 1 μm in diameter, with mean diameter of approximately 400 nm.

Droplet vaporization

A schematic of the experimental setup used for droplet vaporization and bubble characterization studies is provided in Figure 1. Both the high-intensity vaporization pulse and the low-intensity characterization pulses were sent from a 5-MHz-center-frequency Olympus transducer (A308S, Olympus, Quebec City, QC, Canada), driven by an arbitrary waveform generator (8026, Tabor, Tel Hanan, Israel) amplified by an E&I 350L (E&I, Rochester, NY, USA) power amplifier. The transducer output was calibrated with a 0.2-mm needle hydrophone probe (Precision Acoustics, Dorchester, UK). The transducer was focused on an Opticell containing the droplet sample. The droplet sample consisted of the originally prepared droplet solution, diluted 1:2000 in distilled water and degassed with a vacuum pump for at least 1 h. The Opticell was placed under a 60× water-immersion objective (NA = 1.00) of an Olympus BX-FM microscope, coupled to the Brandaris 128 ultra-high speed imaging system (Chin et al. 2003), providing a spatial resolution of 160 nm/pixel. The microscope objective was co-aligned with the transducer focus. The focal point was 50 μm above the wall of the Opticell, such that wall proximity would not have a significant effect on subsequent bubble oscillation (Garbin et al. 2007). The setup was placed in a tank filled with de-ionized water kept at a temperature of 37 ± 1°C. Droplet samples were vaporized with single ultrasound pulses, 10 cycles in length, and a peak negative pressure (PNP) of 3.5 MPa.

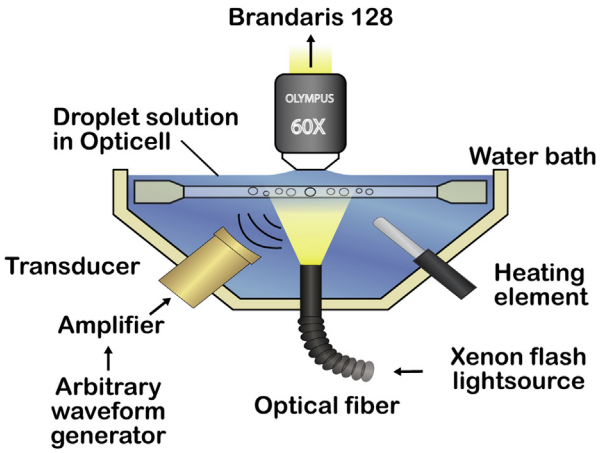

Fig. 1. Schematic diagram of the experimental setup. Acoustic high-intensity vaporization and low-intensity characterization pulses were sent from the single-element transducer. Detection was accomplished using a 60× objective of an Olympus microscope.

Vaporized droplet characterization experiment

After vaporization, a series of five low-intensity acoustic characterization pulses were sent. The pulses were 10 cycles in length, 2.5 MHz in center frequency and 116 kPa in PNP, corresponding to an MI of 0.07. The pulses were sent either 80 or 100 ms apart. As a result, the bubbles were observed at times ranging from 80 to 500 ms after vaporization, based on the pulse timing interval used for the specific trial. Bubble oscillations in response to the acoustic pulses were recorded optically in a series of movies of 128 frames each, recorded at 15 million frames per second (Mfps). For our analysis, we