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● *Original Contribution*

QUANTIFYING ACTIVATION OF PERFLUOROCARBON-BASED PHASE-CHANGE CONTRAST AGENTS USING SIMULTANEOUS ACOUSTIC AND OPTICAL OBSERVATION

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Abstract—Phase-change contrast agents in the form of nanoscale droplets can be activated into microbubbles by ultrasound, extending the contrast beyond the vasculature. This article describes simultaneous optical and acoustical measurements for quantifying the ultrasound activation of phase-change contrast agents over a range of concentrations. In experiments, decafluorobutane-based nanodroplets of different dilutions were sonicated with a high-pressure activation pulse and two low-pressure interrogation pulses immediately before and after the activation pulse. The differences between the pre- and post-interrogation signals were calculated to quantify the acoustic power scattered by the microbubbles activated over a range of droplet concentrations. Optical observation occurred simultaneously with the acoustic measurement, and the pre- and post-microscopy images were processed to generate an independent quantitative indicator of the activated microbubble concentration. Both optical and acoustic measurements revealed linear relationships to the droplet concentration at a low concentration range $<10^8$/mL when measured at body temperature. Further increases in droplet concentration resulted in saturation of the acoustic interrogation signal. Compared with body temperature, room temperature was found to produce much fewer and larger bubbles after ultrasound droplet activation. (E-mail: Mengxing.tang@imperial.ac.uk) © **2015 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Perfluorocarbon droplet, Acoustic droplet vaporization, Quantification, Phase change, Microbubble, Contrast agent, Temperature, Concentration.**

INTRODUCTION

The use of microbubbles as a contrast agent for medical ultrasound has enabled a range of applications in medicine (Cosgrove 2006). The routinely adopted diagnostic applications include using microbubbles as a blood pool marker for endocardial border delineation (Elhendy et al. 2004; Kaufmann et al. 2007) and liver vasculature imaging (Cosgrove 2007; Oldenburg et al. 2005; Vilana et al. 2006). Many other diagnostic applications also look promising, such as contrast-enhanced ultrasound imaging of the spleen (Harvey et al. 2005) and kidney (Cosgrove and Chan 2008; Quaia et al. 2003), as well as detection of neovascularization and atherosclerotic plaques (Coli et al. 2008; Feinstein 2006) in the coronary and carotid arteries. Recent studies also promoted the use of microbubbles for quantitative (Sboros and Tang 2010; Tang et al. 2011; Wei et al. 1998), targeted and molecular imaging (Klibanov 2007). In addition to the aforementioned diagnostic applications, microbubbles are also considered for use as gene and drug delivery vehicles (Lentacker et al. 2006; Unger et al. 1998) and thermal ablation enhancers (Coussios et al. 2007; Stride and Coussios 2010) for ultrasound therapy.

Limitations of microbubble-mediated ultrasound techniques include the rapid dispersion and clearance of microbubbles *in vivo* and the incapability of interrogating or delivering drugs within the interstitial space of solid tumors because of the enhanced permeability and retention effect (Hobbs et al. 1998). To extend their use in the extravascular space, there have been studies on phase-change contrast agents (PCCAs) since 1995 (Albrecht et al. 1996; Forsberg et al. 1995). The concept underlying PCCAs is

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the vaporization of nanoscale droplets into microbubbles by ultrasound (*termed acoustic droplet vaporization*) after their permeation through blood vessels, for example, into the interstitial space of tumors. During the past two decades, there have been many studies on the application of nanodroplets in vascular imaging (Correas et al. 2001; Kasprzak and Ten Cate 1998), molecular recognition for cancer detection (Lin and Pitt 2013; Sheeran et al. 2013b), drug delivery (Rapoport 2012; Rapoport et al. 2011) and enhanced tumor ablation (Zhang and Porter 2010; Zhang et al. 2011). In addition, droplets have been found to have unique applications, which microbubbles may not, in ultrasound aberration correction (Haworth et al. 2008), vascular occlusion (Samuel et al. 2012; Zhang et al. 2010) and contrast-enhanced photo-acoustic imaging (Strohm et al. 2012; Wilson et al. 2012).

In 2011, a new decafluorobutane-based PCCA was developed that has been found to be not only more uniform and smaller (peak size: 200–300 nm), but also stable. It was also found to be sufficiently labile to vaporization by a clinical ultrasound pulse at body temperature (Sheeran et al. 2011). High-speed microscopic images illustrated that once the droplets were vaporized, the particle usually expanded approximately five times in diameter, with the exception of some large outliers that resulted from bubble fusion, a secondary effect of the ultrasound vaporization pulse, and/or a secondary effect of the pressurization procedure in droplet preparation (Lin and Pitt 2013; Sheeran et al. 2011, 2013a). The same studies also indicated that, with a stronger ultrasound activation pulse, the microbubbles produced tended to shift to a smaller-sized population, and although the ultrasound pressure needed for droplet activation increased with ultrasound frequency, the mechanical index, which is more relevant to clinical implementation, decreased with ultrasound frequency (Sheeran et al. 2011, 2013a).

Some fundamental research on acoustic characterization of the ultrasound activation of droplets has been reported. For example, the degree of inertial cavitation during acoustic droplet vaporization was previously studied (Fabiilli et al. 2009; Giesecke and Hynynen 2003). Evidence from cavitation detection suggested that the phase transition usually occurred before the existence of inertial cavitation. The acoustic signature of the acoustic droplet vaporization was reported in Sheeran et al. (2014). The acoustic signal produced by single-droplet vaporization was found to be distinct from the typical microbubble and tissue scattered echo signal. Results also indicated that monitoring growth of the newly generated microbubbles may allow differentiation of converted droplets from the surrounding stable microbubbles by tracing the change in scattered sound power at fundamental and harmonic frequencies (Reznik et al. 2011). Furthermore, uniform activation of nanodroplets was achieved *in vivo*, and a 16- to 20-dB increase in contrast was characterized by comparing the linear intensity of two ultrasound images pre- and post-droplet activation in a rat kidney (Puett et al. 2014). Although the relationship between microbubble concentration and scattered acoustic power was described previously (Lampaskis and Averkiou 2010), the relationship between droplet concentration and bubble concentration after vaporization has not been studied previously and needs to be investigated because of the additional complexity and uncertainty of the droplet–bubble conversion. In this study, we establish the relationships between droplet concentration and simultaneous optical and acoustic measurements acquired pre- and post-droplet activation on laboratory phantoms.

METHODS

Droplet preparation

Phase-change contrast agents were produced using the "microbubble condensation" method described by Sheeran et al. (2011). Briefly, lipid-coated, decafluorobutane-filled microbubbles were first produced in 2-mL sealed vials according to the formulation and procedure described (Sheeran et al. 2011). Microbubbles (gaseous state) were condensed to nanodroplets (liquid core) by gently swirling the vials in a -7°C bath while pressurizing 40 mL room air into vials through a syringe connected to a 25G needle. In Figure 1 are microscopic images of the microbubble emulsion before and after condensation at the top and bottom planes of the hemocytometer. Both samples were diluted to 1:20 and allowed to stand for 5 min before the images were acquired to allow for stratification, if it did occur. Figure 1(a, c) illustrates that before condensation, microbubbles were observed only at the top plane because of their buoyancy. Immediately after condensation, a majority of the microbubbles disappeared from the top plane (Fig. 1b). The remaining large microbubbles in Figure 1b were most likely a result of a small number of large outlier droplets that were relatively easily vaporized even without an ultrasound activation pulse. This could be due to the smaller Laplace pressures on the larger droplets. Droplets, being comprised of dense liquid decafluorobutane (1.517 g/mL), settled to the bottom of the hemocytometer (indicated by the *arrows* in Fig. 1d). By subtracting the microbubble concentration measured after condensation ($\sim 5 \times 10^7$ bubbles/mL) from that measured before condensation ($\sim 6 \times 10^9$ bubbles/mL) using the protocol described in Sennoga et al. (2010), the concentration of the droplet emulsion was estimated to be on the order of $\sim 5.5 \times 10^9$ droplets/mL. The microbubbles that remained in the droplet emulsion were not separated out in