Ultrasound in Med. & Biol., Vol. ■, No. ■, pp. 1–10, 2016

0301-5629/$ - see front matter

http://dx.doi.org/10.1016/j.ultrasmedbio.2016.09.003

● *Technical Note*

MORE THAN BUBBLES: CREATING PHASE-SHIFT DROPLETS FROM COMMERCIALLY AVAILABLE ULTRASOUND CONTRAST AGENTS

Paul S. Sheeran,*† Kimoon Yoo,* Ross Williams,* Melissa Yin,* F. Stuart Foster,*† and Peter N. Burns*†

*Physical Sciences Department, Sunnybrook Research Institute, Toronto, ON, Canada; and †Department of Medical Biophysics, University of Toronto, Toronto, Canada

(*Received* 5 *March* 2016; *revised* 30 *August* 2016; *in final form* 6 *September* 2016)

Abstract—Phase-shift perfluorocarbon droplets have been investigated for over 20 years as pre-clinical ultrasound contrast agents with distinctive advantages in imaging and therapy. A number of formulation strategies exist, each with inherent advantages and limitations. In this note, we demonstrate a unique opportunity: that phase-shift droplets can be generated directly from commercially available microbubbles. This may facilitate pre-clinical and translational development by reducing the in-house synthesis expertise and resources required to generate high concentration droplet emulsions. Proof-of-principle *in vitro* and *in vivo* is given using droplets created from Definity and MicroMarker. The results demonstrate the role of perfluorocarbon choice in the trade-off between thermal stability and vaporization threshold, and suggest that commercial microbubbles with decafluorobutane cores may be ideal for this approach. (E-mail: pssheeran@gmail.com) © **2016 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Perfluorocarbon, Ultrasound contrast agents, Phase-shift droplets, Acoustic droplet vaporization, Definity, MicroMarker.**

INTRODUCTION

Clinical ultrasound contrast agents take the form of gas-filled microspheres, commonly referred to as microbubbles. In the past two decades, investigations into alternative contrast agents have increased substantially in order to address the fundamental limitations of microbubbles for specific diagnostic and therapeutic applications. Phase-shift perfluorocarbon droplets are perhaps the most extensively researched alternative and are designed to exist metastably in the liquid state under a desired degree of superheat until additional energy in the form of ultrasound or heat nucleates the core and the particles vaporize to the gas state, expanding volumetrically. This approach provides the benefits of increased circulation lifetime, relative ease of generating stable particles at the nanoscale, the ability to generate microbubbles "on-site" through externally applied ultrasound and the possibility of accessing the extravascular space of cancerous tissues (Kripfgans et al. 2000; Rapoport 2012; Sheeran and Dayton 2012a).

Ultrasonically activated phase-shift droplets have made slow progress into the clinic. Many fundamental mechanisms of droplet vaporization and expansion have only recently been revealed (Doinikov et al. 2014; Shpak et al. 2014), and much work remains in characterizing bioeffects, pharmacokinetics and dosing before use in humans can be considered. A variety of formulation strategies exist, including amalgamation/homogenization/mechanical agitation (Kawabata et al. 2005; Kripfgans et al. 2000), sonication (Fabiilli et al. 2010; Matsuura et al. 2009; Rapoport et al. 2009), microfluidics (Bardin et al. 2011; Seo et al. 2010) and extrusion past porous membranes (Giesecke and Hynynen 2003; Sheeran et al. 2011b). More recently, Sheeran et al. (2011a, 2012b) developed a "microbubble condensation" technique to create phase-shift droplets in which volatile compounds are first encapsulated as microbubbles and are then condensed to the liquid state by decreasing the ambient temperature and/or increasing the ambient pressure. Researchers have demonstrated that microbubble condensation can be coupled with other particle generation techniques such

Address correspondence to: Paul S. Sheeran, 2075 Bayview Ave, Room S640, Toronto, ON M4N 3M5, Canada. E-mail: pssheeran@gmail.com

as microfluidic generation of microbubbles in order to refine droplet size distributions (Seo and Matsuura 2012; Seo et al. 2015).

Each of these formulation strategies has advantages and disadvantages with regard to clinical applicability, resource requirements, and the necessary level of expertise in interfacial chemistry/colloid science (Sheeran et al. 2016b). In this note, we demonstrate that generating phase-shift droplets by microbubble condensation may provide a unique advantage in that it allows for the creation of droplets directly from existing commercial clinical and pre-clinical contrast agents, providing high yield emulsions with minimal resource requirements and without need for in-house bubble synthesis. Here, droplets are created directly from the clinical contrast agent Definity (Lantheus Medical Imaging, Billerica, MA, USA) after formation of bubbles by mechanical agitation, and the pre-clinical contrast agent MicroMarker (Bracco, Geneva, Switzerland and VisualSonics, Toronto, Canada), formed following reconstitution of a lyophilized emulsion. Proof-of-principle is given *in vitro* and *in vivo* on clinical and pre-clinical ultrasound machines.

MATERIALS AND METHODS

Droplet emulsion preparation

Phase-shift droplets were generated from two commercially available phospholipid-encapsulated microbubble contrast agents. Definity, the most commonly used clinical ultrasound contrast agent in North America, has a gaseous octafluoropropane core (boiling point −36.7°C), while MicroMarker, a pre-clinical contrast agent designed for small-animal imaging, has a core composed of nitrogen and decafluorobutane (boiling point −2°C). Both agents have a distribution peak diameter below 1 μm and a volume-weighted peak diameter below 5 μm (Helfield et al. 2012; Raymond et al. 2014).

Definity microbubbles were formed by activating vials (stored at 4°C) by mechanical agitation according to standard protocols. Octafluoropropane droplets were generated from this suspension with a similar approach to that described in Sheeran et al. (2012b). First, the vial was cooled to −10°C in an isopropanol bath maintained at temperature with dry ice, followed by pressurization of the headspace with room air to approximately 170 kPa above atmospheric pressure over the course of 2 min. MicroMarker microbubbles were prepared by resuspending the lyophilized agent with 0.5 mL of decafluorobutane gas-equilibrated saline. Decafluorobutane droplets were generated by cooling the resulting suspension to −8°C in the isopropanol bath and pressurizing the headspace with room air to approximately 100 kPa above atmospheric pressure over the course of 2 min in order to condense the decafluorobutane component of the core and expel the nitrogen component (condensation of a pure decafluorobutane core can be accomplished by cooling alone). The droplet suspensions were then centrifuged at 1000 rpm for 2 min at 4°C to separate droplets from microbubbles remaining in the solution, and were held in a centrifuge tube on ice for up to 1 h before testing.

Particle sizing

Example distributions of droplets generated by condensing Definity and MicroMarker microbubbles were measured using a Nanosight LM10 (Malvern Instruments, Inc., Malvern, UK). The droplet suspensions were diluted 1/200 and 1/100 in room-temperature saline for octafluoropropane droplets (generated from Definity) and decafluorobutane droplets (generated from MicroMarker), respectively. Five 30-s videos of non-overlapping volumes were collected for each dilution, analyzed and averaged to generate representative distributions. This process was repeated three times by preparing new dilutions from the same vial, and the results averaged for a final representation of the vial size distribution and particle concentration.

In vitro *experimental design*

An *in vitro* vessel flow phantom was constructed by connecting a 6.4-mm diameter (0.24 mm wall thickness) latex tube (Penrose Drain; Medline Industries, Inc., Mundelein, IL, USA) to polyvinyl chloride tubing secured by a custom acrylic holder that was submerged in a large heated water bath. The flow circuit consisted of a 1.5 L sample container held 30–40 cm above the large heated water bath. Temperatures between 25°C and 42°C were maintained in the large water bath by an immersion circulator/heater, and in the upper sample container using a small fish tank heater with an adjustable voltage regulator.

Droplet dilutions were imaged and vaporized in the vessel phantom by fixing an ultrasound imaging probe to a mechanical arm and submerging the probe to image the latex tube lengthwise in a set position. Droplets created from the clinical microbubble Definity were tested using a Philips iU22 clinical scanner with an L9-3 linear array probe (Philips Ultrasound, Bothell, WA, USA). Standard B-mode imaging settings were used to capture videos of the samples (mechanical index [MI] = 0.01, 68 fps). The imaging focus was placed in the center of the tube at 2.2 cm depth. Sample flow in the circuit was stopped and droplets were vaporized by manually triggering a single flash frame—conventionally used to destroy contrast agents for the purposes of volume-flow and molecular imaging (Hudson et al. 2015; Wilson and Burns 2010)—over a range of preset nominal mechanical indices between MI = 0.05 and MI = 0.97 (the highest output