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Scavenging dissolved oxygen via acoustic droplet vaporization

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ABSTRACT

Acoustic droplet vaporization (ADV) of perfluorocarbon emulsions has been explored for diagnostic and therapeutic applications. Previous studies have demonstrated that vaporization of a liquid droplet results in a gas microbubble with a diameter 5–6 times larger than the initial droplet diameter. The expansion factor can increase to a factor of 10 in gassy fluids as a result of air diffusing from the surrounding fluid into the microbubble. This study investigates the potential of this process to serve as an ultrasound-mediated gas scavenging technology. Perfluoropentane droplets diluted in phosphate-buffered saline (PBS) were insonified by a 2 MHz transducer at peak rarefactional pressures lower than and greater than the ADV pressure amplitude threshold in an *in vitro* flow phantom. The change in dissolved oxygen (DO) of the PBS before and after ADV was measured. A numerical model of gas scavenging, based on conservation of mass and equal partial pressures of gases at equilibrium, was developed. At insonation pressures exceeding the ADV threshold, the DO of air-saturated PBS decreased with increasing insonation pressures, dropping as low as 25% of air saturation within 20 s. The decrease in DO of the PBS during ADV was dependent on the volumetric size distribution of the droplets and the fraction of droplets transitioned during ultrasound exposure. Numerically predicted changes in DO from the model agreed with the experimentally measured DO, indicating that concentration gradients can explain this phenomenon. Using computationally modified droplet size distributions that would be suitable for *in vivo* applications, the DO of the PBS was found to decrease with increasing concentrations. This study demonstrates that ADV can significantly decrease the DO in an aqueous fluid, which may have direct therapeutic applications and should be considered for ADV-based diagnostic or therapeutic applications.

1. Introduction

The phenomenon of acoustic droplet vaporization (ADV), the acoustically mediated phase transition of liquid perfluorocarbon droplets into gas bubbles, is under investigation for several biomedical applications. Submicron-sized perfluorocarbon droplets extravasate from leaky tumor vessels, undergo ADV, and provide contrast on ultrasound images of cancerous tissue [1,2]. ADV-induced microbubbles generated from micron-sized droplets have also been investigated as point targets for phase aberration correction [3–5]. Further, contrast-enhanced photoacoustic images have been created using ADV to trigger the localized release of cardiogreen dye from a perfluoropentane (PFP) double emulsion [6].

ADV is also being explored for various direct and adjuvant therapeutic applications. Micron-sized perfluorocarbon microbubbles created via ADV have been shown to occlude capillary beds and arterioles, which can facilitate embolotherapy in cancer treatment [3,7,8]. Previous studies have also demonstrated ADV-mediated delivery of chemotherapeutic drugs such as paclitaxel [9], chlorambucil [10], and doxorubicin [11] loaded in perfluorocarbon droplets. Thermal ablation of cancerous lesions has been enhanced using perfluorocarbon droplets as cavitation nuclei during high intensity focused ultrasound (HIFU) exposure [12–14]. Further, ADV microbubbles can provide contrast-enhanced image guidance during treatment [12]. More recently, perfluorocarbon droplets have been shown to lower the acoustic power needed for HIFU-mediated lysis of blood clots relative to HIFU exposure without droplets [15]. Spatiotemporally-controlled ADV-mediated drug release from perfluorocarbon double emulsions has also been shown to regulate the mechanical properties of tissue-engineered scaffolds [16].

ADV results in a significant volumetric expansion of the perfluorocarbon. The ideal gas law was used to predict a volumetric

Abbreviations: ADV, acoustic droplet vaporization; PFP, perfluoropentane; DO, dissolved oxygen; PBS, phosphate buffered saline; EVA, ethyl vinyl acetate.

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expansion of a factor of 125 (i.e., a radial expansion of 5) [3], and the measured volumetric expansion of evaporating perfluoropentane was a factor of 151 (i.e., a radial expansion factor of 5.3) [3]. Kripfgans et al. [3] also computed a radial expansion factor of 5.9 which accounts for the diffusion of gases into and out of the microbubbles. Kang et al. [17] optically characterized the growth of the ADV-induced PFP microbubbles under various insonation parameters and measured the expansion factor due to ingassing to be between 2 and 3. Sheeran et al. [1] reported that the radial expansion factor of decafluorobutane droplets was 6 ± 1 in a degassed fluid (1.5 ppm of oxygen) and 10 ± 2 in an air-saturated fluid (6 ppm of oxygen). From these results, the amount of ingassing and thus the expansion factor appear to depend on the experimental conditions, potentially including the gas saturation of the fluid, formulation of the droplet, and concentration of the droplets. The diffusion of gases into perfluorocarbon-based ultrasound contrast agents has also been discussed in other studies [18–21]. Further, the high solubility of oxygen in perfluorochemicals [22,23] and the use of perfluorochemicals as blood substitute agents have been demonstrated in previous studies [24,25]. Johnson et al. [26] demonstrated that liquid PFP droplets alone can scavenge dissolved oxygen from the surrounding fluid. Culp et al. [27] subsequently demonstrated that a PFP emulsion can transport sufficient oxygen to ischemic tissue to decrease infarct volume in a rabbit stroke model.

Although these studies demonstrate that dissolved gases in a fluid can diffuse in and out of perfluorocarbon droplets, the change in dissolved gas in the surrounding fluid as a result of ADV has not been quantified. The current study reports on experimental measurements and numerical computations of the change in dissolved oxygen (DO) of a fluid containing PFP droplets before and after ultrasound exposure. PFP droplets diluted in saline were injected in a flow phantom containing inline dissolved oxygen (DO) sensors. The droplets were insonified using a single-element focused transducer at peak rarefactional pressures lower than and greater than the ADV acoustic pressure threshold amplitude. The effect of altering the size distribution and concentration of the droplets were investigated numerically. The phenomenon of ultrasound-mediated gas diffusion concomitant with the cavitation nucleation associated with ADV can have implications on current biomedical applications of ADV as well as serve as a potential strategy to scavenge gases *in situ*.

2. Materials and methods

2.1. Preparation of albumin-coated perfluoropentane (PFP) droplets

Albumin-coated PFP droplets were prepared based on a previously established protocol [3]. Briefly, 0.25 ml of dodecafluoropentane (Strem Chemicals, Newburyport, MA, USA) was added gravimetrically to 2 ml vials followed by the addition of 0.75 ml of 4 mg/ml bovine serum albumin (Sigma Aldrich, St. Louis, MO, USA) in phosphate buffered saline (PBS) (Sigma Aldrich). The vials were sealed with a rubber stopper, crimped, and placed on ice prior to amalgamation at 4800 rpm for 30 s in an amalgamator (WIG-L-BUG, Dentsply Rinn, Elgin, IL, USA) at 5 °C to obtain albumin-coated PFP droplets. The vials were refrigerated for at least 24 h before use. Vials were used within 2 days of being manufactured. The size distribution of the droplets was measured with a Coulter counter (Multisizer 4, Beckman Coulter Inc., Brea, CA, USA).

2.2. Experimental setup

The albumin-coated PFP emulsion was diluted in air-saturated PBS (1:30 v/v) and slowly drawn into a 60 ml syringe through an 18 G needle. Using a syringe pump, the droplets in PBS were pumped through an *in vitro* flow phantom (Fig. 1) at 5 ml/min. The flow phantom consisted of polyvinyl chloride tubing (McMaster-Carr, Aurora, OH, USA), in-line dissolved oxygen (DO) sensors (OXFTC, Pyroscience, Aachen, Germany) and ethyl vinyl alcohol (EVA) tubing (McMaster) immersed in a tank of degassed water maintained at 37 °C. ADV was induced by a single-element 2 MHz focused transducer (H106, Sonic concepts, Bothell, WA, USA) as the droplets flowed through the EVA tubing. Based on a previous study [28] the EVA tubing was used because it had an inner diameter of 1 mm which was comparable to the −6 dB elevational beamwidth of the 2 MHz focused transducer (1.1 mm), thin walls (0.38 mm), and high acoustic transmission coefficient (94%) to ensure uniform insonation of the droplets. The 2 MHz focused transducer had an aperture diameter of 6.3 cm and a focal distance of 6.4 cm.

B-mode images of the insonified droplets were acquired using an ultrasound research scanner (Vantage 256, Verasonics, Kirkland, WA, USA) equipped with a linear array transducer (L7-4, center frequency 5 MHz, Philips, Bothell, WA, USA), to monitor the formation of microbubbles [3]. DO in the fluid was measured over 120 s using in-line DO sensors located upstream and downstream of the insonation region. Based on a flow rate of 5 mL/min, fluid took approximately 20 s to travel from the ultrasound focus to the downstream DO sensor. The in-line DO sensors consisted of a luer lock flow-through cell and a fiber optic spot fiber (SPFIB-Bare, Pyro Science GmbH, Aachen, Germany) connected to an optical oxygen meter (FireStingO2, Pyro Science). The DO sensors were calibrated according to the manufacturer's instructions to measure 100% DO in air-saturated PBS at 37 °C. The effluent from the flow system was collected and diluted in PBS to obtain a final concentration of 1:8000 (v/v). The surviving droplets in the diluted effluent were measured in a Coulter counter (Multisizer 4, Beckman Coulter, Brea, CA, USA) equipped with a 30 μm aperture.

2.3. Ultrasound parameters

The acoustic output and the spatial beam profile of the 2 MHz transducer were calibrated up to 2 MPa peak rarefactional pressure using a 0.4 mm membrane hydrophone (Precision Acoustics, Dorchester, UK) mounted on a three-dimensional stepper-motor controlled system (Velmex NF90 Series, Velmex Inc., 291 Bloomfield, NY). A linear relationship between the voltage applied to the transducer and the peak rarefactional pressures below 2 MPa was obtained. A linear extrapolation of this relationship was used to estimate peak rarefactional pressures above 2 MPa [29,30]. The −3 dB focal volume of the 2 MHz transducer was 0.7 mm × 0.7 mm × 5.4 mm (azimuth × elevation × range), thus allowing a relatively uniform pressure field inside the EVA tubing (inner diameter 1 mm).

The acoustic droplet vaporization threshold was determined using established methods based on changes in echogenicity [3]. The peak rarefactional pressure of the 2 MHz transducer was ramped from 0 MPa to 12.2 MPa in steps of 0.6 MPa. The pulse repetition frequency was 100 Hz and the pulse duration was 5 μs. At each peak rarefactional pressure setting, a B-mode image of the droplets was acquired. The mean grayscale value was ascertained within a region of interest defined in the lumen downstream of the 2 MHz insonation location (Fig. 2a). A piece-wise linear fit of the mean grayscale value as a function of the peak rarefactional pressure was used to define the acoustic droplet vaporization threshold. The threshold was defined as the rarefactional pressure amplitude corresponding to the intersection between the first two lines of the piece-wise linear fit based on previous studies [3,31] (Fig. 2b). The threshold was determined for four vials of the PFP droplets (one measurement per vial) and the mean and standard