

# Dissolved Oxygen Scavenging by Acoustic Droplet Vaporization using Intravascular Ultrasound

Kevin J. Haworth  
Department of Internal Medicine  
University of Cincinnati  
Cincinnati, USA  
kevin.haworth@uc.edu

Bryan H. Goldstein  
Division of Cardiology  
Cincinnati Children's Hospital  
Medical Center  
Cincinnati, USA

Karla P. Mercado-Shekhar  
Department of Internal Medicine  
University of Cincinnati  
Cincinnati, USA

Rohan Srivastava  
Medical Sciences Program  
University of Cincinnati  
Cincinnati, USA

P. Arunkumar  
Department of Internal Medicine  
University of Cincinnati  
Cincinnati, USA

Haili Su  
Department of Internal Medicine  
University of Cincinnati  
Cincinnati, USA

Ellena M. Privitera  
Medical Sciences Program  
University of Cincinnati  
Cincinnati, USA

Christy K. Holland  
Department of Internal Medicine  
University of Cincinnati  
Cincinnati, USA

Andrew N. Redington  
Division of Cardiology  
Cincinnati Children's Hospital Medical Center  
Cincinnati, USA

**Abstract**—Modification of dissolved gas content by acoustic droplet vaporization (ADV) has been proposed for several therapeutic applications. Reducing dissolved oxygen (DO) during reperfusion of ischemic tissue during coronary interventions could inhibit reactive oxygen species production and rescue myocardium. The objective of this study was to determine whether intravascular ultrasound (IVUS) can trigger ADV and reduce DO. Perfluoropentane emulsions were created using high-speed shaking and microfluidic manufacturing. High-speed shaking resulted in a polydisperse droplet distribution ranging from less than 1 micron to greater than 16 microns in diameter. Microfluidic manufacturing produced a narrower size range of droplets with diameters between 8.0 microns and 9.6 microns. The DO content of the fluids was measured before and after ADV triggered by IVUS exposure. Duplex B-mode and passive cavitation imaging was performed to assess nucleation of ADV. An increase in echogenicity indicative of ADV was observed after exposure with a clinical IVUS system. In a flow phantom, a 20% decrease in DO was measured distal to the IVUS transducer when droplets, formed via high-speed shaking, were infused. In a static fluid system, the DO content was reduced by 11% when droplets manufactured with a microfluidic chip were exposed to IVUS. These results demonstrate that a reduction of DO by ADV is feasible using a clinical IVUS system. Future studies will assess the potential therapeutic efficacy of IVUS-nucleated ADV and methods to increase the magnitude of DO scavenging.

**Keywords**—acoustic droplet vaporization, intravascular ultrasound, oxygen, dissolved gas, phase-shift emulsion, microfluidics

## I. INTRODUCTION

Acoustic droplet vaporization (ADV), the ultrasound-mediated phase-transition of perfluorocarbon droplets into gas microbubbles, has been studied for nearly two decades [1]. Several groups have noted that following the phase-transition of perfluorocarbons from a liquid to gas, other dissolved gases

(e.g., nitrogen and oxygen) diffuse into the microbubbles [1]-[4]. The concomitant reduction of dissolved oxygen (DO) in the surrounding fluid is due to a difference in the partial pressures in the perfluorocarbon microbubble and the surrounding fluid post ADV.

Radhakrishnan et al. [5] demonstrated that a reduction in the partial pressure of oxygen in a fluid from 100% to 25% (relative to air saturation) can be achieved with ADV. More gas is scavenged as the number of droplets undergoing ADV increases. Reducing the available dissolved gas content within the blood may be advantageous for numerous applications such as decompression sickness (nitrogen), acidosis (carbon dioxide), carbon monoxide poisoning, or acute oxidative stress (reoxygenation/reperfusion injury). Acute oxidative stress is known to contribute to the reperfusion injury that follows treatment for an ischemic event such as myocardial infarction [6] and stroke. Percutaneous transluminal coronary angioplasty and stent angioplasty is the standard of care for acute myocardial infarction, with intravascular ultrasound (IVUS) commonly used to assess the coronary artery before and after intervention. Furthermore, transcatheter therapy for embolic stroke is being used increasingly. Consequently, the objective of this study was to determine if ADV could be nucleated by a clinical IVUS system and induce a reduction in the partial pressure of oxygen in a fluid. This objective was tested using both a static system and a flow phantom.

## II. MATERIALS AND METHODS

### A. Droplet Manufacturing

#### 1) High-speed Shaking

Droplets were manufactured using high speed shaking as previously described [1], [7]. Briefly, 0.25 mL (0.425 g) of perfluoropentane (FluoroMed, Round Rock, USA) was added gravimetrically to a 2 mL serum vial. Subsequently, 0.75 mL

Research was supported by award K25HL133452 from the National Institutes of Health, award 16SDG27250231 from the American Heart Association, and a University of Cincinnati Interdisciplinary Award.

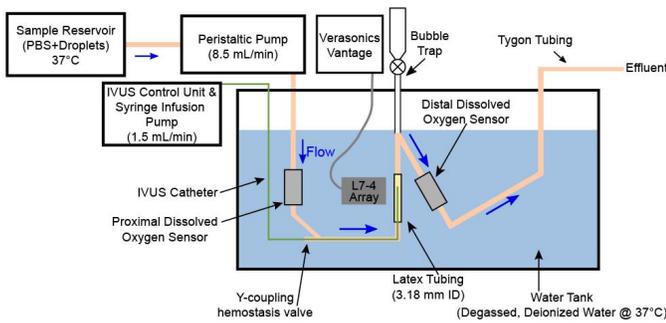


Fig. 1. Schematic of the flow phantom used to assess the ability of an intravascular ultrasound device operating at 40 MHz to nucleate acoustic droplet vaporization and dissolved gas scavenging.

of 4 mg/mL bovine serum albumin (Sigma-Aldrich, St. Louis, USA) in 0.01 M phosphate-buffered saline (PBS, Sigma-Aldrich) was added and the vial was capped, crimped, and shaken at 4800 RPM for 30 s (Wig-L-Bug, Dentsply Rinn, York, USA). Droplets were stored at 5°C between 12 hours and 7 days. Prior to use, large droplets were removed using centrifugation [7]. Initially, 1 mL of the droplet emulsion was added to 3.8 mL of PBS in a 15 mL conical centrifuge tube and centrifuged for 1 min at 70 $\times$ g. Of the supernatant, 3.456 mL was reserved and centrifuged for 5 min at 5000 $\times$ g. 1.310 mL of the pellet was reserved and resuspended in PBS. The droplet concentration and size distribution were measured using a Multisizer 4 Coulter counter (Beckman Coulter Inc., Brea, USA).

## 2) Microfluidic Manufacturing

Droplets were extruded using a microfluidic chip (Dolomite, Royston, United Kingdom) with 14  $\mu\text{m} \times 17 \mu\text{m}$  channels similar to the methods described by Moncion et al. [8]. The chip has three inlet ports. The center inlet port had perfluoropentane pumped through it at a flow rate of 1  $\mu\text{L}/\text{min}$ . The two outer ports were infused with a 62.5 mg/mL solution of Pluronic F68 (CAS#9003-11-6, Sigma-Aldrich) in PBS at a flow rate of 5.5  $\mu\text{L}/\text{min}$ . Droplets were collected from the outlet and sized with a Multisizer 4 Coulter counter.

## B. Ultrasound Exposure Systems

Droplets were exposed to ultrasound using a clinical coronary IVUS system (iLab, Boston Scientific, Marlborough, USA) in either a sample holder with static fluid or a flow phantom. DO was measured before and after ultrasound exposure using DO sensors (Pyroscience GmbH, Aachen, Germany).

### 1) Flow Phantom

A schematic of the flow phantom is shown in Fig. 1. PBS at 37°C was pumped through the flow phantom at 8.5 mL/min using a peristaltic pump. High-speed shaken and centrifuged droplets were injected into the system using a syringe pump at 1.5 mL/min via the flush port of the intravascular (IVUS) catheter (Opticross™, 3.0 Fr, 40 MHz coronary imaging catheter, Boston Scientific). The total volumetric flow rate exiting the system was 10 mL/min. The 40.3 MHz IVUS transducer was driven using an iLab system and had a manufacturer-reported derated peak negative pressure of 1.37 MPa at a distance of 0.75 mm from the transducer

(location of maximum intensity). Flow-through DO sensors (Pyroscience GmbH) were placed proximal and distal to the active element of the IVUS transducer. A bubble trap was placed proximal to the second (distal) DO sensor as large numbers of microbubbles were observed to interfere with the optics of the DO sensor. ADV was monitored using both B-mode imaging and passive cavitation imaging with an L7-4 linear array (Philips, Bothell, USA) connected to a Vantage 256 ultrasound research scanner (Verasonics, Kirkland, USA). Passive cavitation images were formed using a delay, sum, and integrate algorithm [9]. Summation was performed over the frequency band of 2–6 MHz to identify the location of IVUS emissions. An increase in echogenicity was used to confirm ADV [1].

### 2) Static Exposure System.

Droplets manufactured with microfluidics were phase-transitioned using the same IVUS catheter. Droplets were diluted in static PBS to a volumetric concentration of  $6.0 \times 10^8 \mu\text{m}^3/\text{mL}$  (0.06% v/v). One milliliter of the diluted droplet solution was reserved in a 15 mL centrifuge tube as a non-IVUS exposed control. The other 1 mL was slowly drawn into a 6.0 Fr guide catheter. The IVUS catheter (3.0 Fr) was inserted into the guide catheter, the acoustic output was turned on and was passed through the guide catheter 6 times. The fluid in the guide catheter was slowly withdrawn into a 1 mL syringe, minimizing exposure to the air. The DO of the IVUS exposed sample and non-IVUS exposed control were measured for approximately 30 s using a needle DO sensor (OXR430-UHS, Pyroscience GmbH).

## III. RESULTS

### A. Droplet Size Distributions

The normalized number-weighted and volume-weighted size distributions of the droplets are shown in Fig. 2. The magnitude of DO scavenging is dependent on the total volume of perfluoropentane phase-transitioned [5]. For reference, the size distribution of droplets manufactured using high-speed shaking but before centrifugation (black) are shown in addition to the size-isolated droplets (blue) and the droplets manufactured with a microfluidic chip (green). High-speed shaking produced droplets ranging from less than 1  $\mu\text{m}$  in diameter to greater than 16  $\mu\text{m}$  in diameter. A centrifugation protocol removed droplets greater than approximately 6  $\mu\text{m}$  in diameter. The volume fraction of perfluoropentane droplets in PBS before centrifugation was  $3.9 \times 10^{10} \mu\text{m}^3/\text{mL}$  and  $7.1 \times 10^9 \mu\text{m}^3/\text{mL}$  after centrifugation. Centrifugation results in 82% of the perfluoropentane volume lost.

In contrast, microfluidic droplet manufacturing resulted in a narrower droplet size distribution between approximately 8.0  $\mu\text{m}$  and 9.6  $\mu\text{m}$  in diameter. Thus, subsequent size-isolation was not needed and the corresponding loss did not occur. The number and volume fractions of perfluoropentane droplets in PBS collected from the effluent of the microfluidic chip was  $1.53 \times 10^8$  droplets/mL and  $6.42 \times 10^{10} \mu\text{m}^3/\text{mL}$ , respectively. Sufficient quantities of droplets could be produced for all the static experiments in under 2 h of manufacturing time.

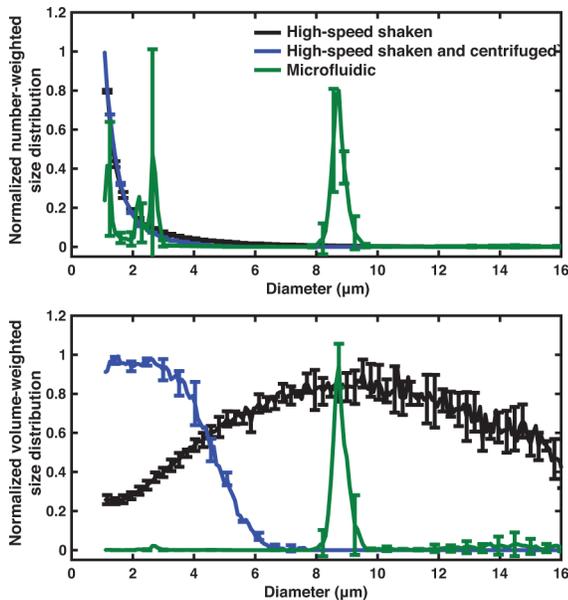


Fig. 2 Normalized (A) number-weighted and (B) volume-weighted size distribution of the droplets manufactured via high speed shaking (black), high-speed shaking with size-isolation via centrifugation (blue), and microfluidic manufacturing (green).

### B. IVUS-nucleated Acoustic Droplet Vaporization

Transverse B-mode images of the flow phantom and IVUS catheter with droplets infused are shown in Fig. 3A and 3B with and without IVUS acoustic output, respectively. Overlaid passive cavitation images show the beamformed acoustic emissions using a color map. Hyperechoic microbubbles are visible distal to the IVUS transducer only when exposed to ultrasound, indicating that ADV was nucleated.

### C. Dissolved Oxygen Scavenging

#### 1) Flow Phantom

The dissolved oxygen content of the fluid was measured in

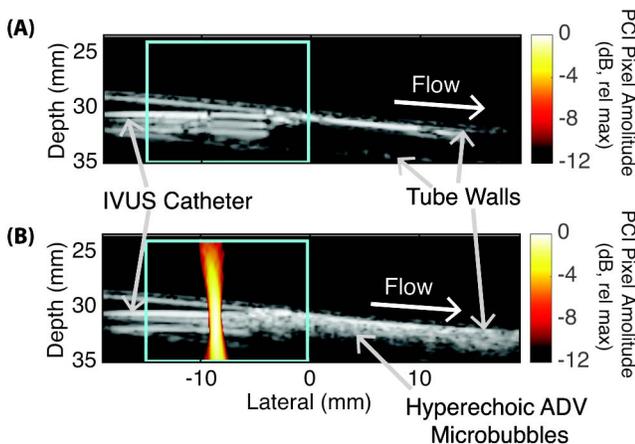


Fig. 3 Duplex B-mode and passive cavitation image of the flow phantom tube with the IVUS catheter inserted. (A) Distal to the IVUS catheter is relatively hypoechoic without IVUS exposure. (B) With IVUS exposure, the lateral location of the element can be identified from the passive cavitation image. Hyperechoic ADV microbubbles are observed distally.

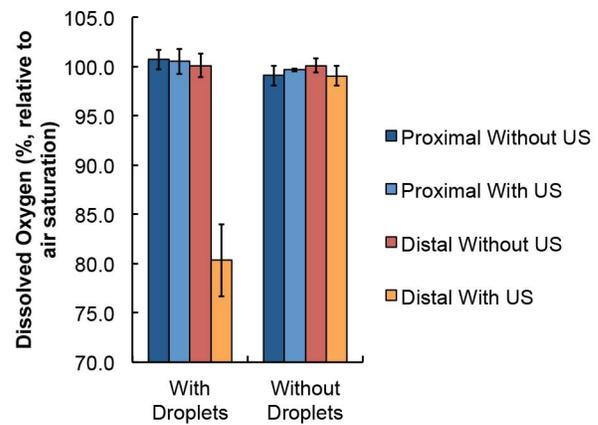


Fig. 4 The average DO content measured by the proximal (light blue and dark blue) DO sensor and the distal (red and orange) DO sensor with and without IVUS exposure. A decrease in DO is measured only distal to the IVUS transducer when droplets are present. Error bars represent the standard deviation of the measurements.

both the proximal and distal sensors for three trials with droplets and two trials without droplets. The average DO content measured by each sensor for each experimental condition is shown in Fig. 4. For all trials with droplets, a decrease in DO was measured by the distal sensor approximately 10 s after the IVUS was initiated, which was the time needed for the converted droplets to flow from the IVUS transducer to the DO sensor. When droplets were not present in the fluid, the DO did not change significantly when the IVUS acoustic output was turned on. For all trials, the proximal DO sensor did not detect a substantial change in DO for the duration of the experiments.

#### 2) Static Exposure System

The DO content of the fluid not exposed to IVUS was  $91.6\% \pm 0.2\%$  (relative to air saturation). The DO content of the fluid exposed to IVUS was  $80\% \pm 4\%$ . The reduction in DO was significantly different based on a Student's t-test ( $p = 0.02$ ).

## IV. DISCUSSION

It has been shown that an off-the-shelf clinical coronary IVUS system can nucleate ADV to induce oxygen scavenging. The magnitude of scavenging is less than reported elsewhere [5], indicating that modification may be needed to increase the fraction of perfluoropentane droplets undergoing ADV, or to increase the concentration of perfluoropentane droplets in the fluid. The former may be achievable by modifying the ultrasound pressure amplitude or frequency.

Oxygen scavenging was demonstrated with droplets formed via high-speed shaking followed by centrifugation and via a microfluidic chip. The droplets formed using microfluidics have a narrower size distribution. The vaporization threshold and vaporization efficiency are a function of droplet diameter [5], [7], [10], [11], and narrower size distributions may provide more control over droplet vaporization.

Pulmonary capillary diameters range from approximately 2 to 14  $\mu\text{m}$  in diameter [12]. Thus, microfluidic droplets with a mean diameter of 8.9  $\mu\text{m}$  may get caught in capillary networks [5]. *In vivo* studies will need to be performed to understand

whether larger droplets are beneficial (by increasing oxygen scavenging) or adverse (by causing vascular embolization). There is a potential to optimize the droplet size. Both Martz et al. [11] and Fabiilli et al. [13] have demonstrated that a higher flow rate through a microfluidic chip results in smaller droplet diameters. Exploration of alternate flow parameters in the microfluidic chip, or modification of the channel sizes in the chips, may enable the production of smaller droplets that would not embolize the capillaries.

There are several limitations to our study. First, the static fluid was not maintained at 37 °C for the duration of the study. The droplet vaporization threshold increases with decreasing temperature [14]. Therefore the magnitude of dissolved gas scavenging in the static fluid studies may be lower than would be expected at 37 °C. Spontaneous vaporization was observed in the control sample, which may explain why the measured DO was 91.6% rather than 100%. Finally, we used saline to perform these studies; further experiments with blood will need to be performed to reveal the dynamics of oxygen scavenging in more complex fluids.

In summary, our proof-of-principle experiments confirm the feasibility of using a clinical IVUS system to scavenge oxygen with ADV. This technology could be used to avoid reperfusion injury at the time of transcatheter treatment of ischemia-reperfusion syndromes.

#### ACKNOWLEDGMENT

The authors gratefully acknowledge Mario Fabiilli, PhD at the University of Michigan for conversations regarding microfluidic droplet manufacturing.

#### REFERENCES

- [1] O. D. Kripfgans, J. B. Fowlkes, D. L. Miller, O. P. Eldevik, and P. L. Carson, "Acoustic droplet vaporization for therapeutic and diagnostic applications," *Ultrasound Med Biol*, vol. 26, no. 7, pp. 1177–1189, 2000.
- [2] P. S. Sheeran, V. P. Wong, S. Luo, R. J. McFarland, W. D. Ross, S. Feingold, T. O. Matsunaga, and P. A. Dayton, "Decafluorobutane as a Phase-Change Contrast Agent for Low-Energy Extravascular Ultrasonic Imaging," *Ultrasound Med Biol*, vol. 37, no. 9, pp. 1518–1530, 2011.
- [3] N. Reznik, R. Williams, and P. N. Burns, "Investigation of vaporized submicron perfluorocarbon droplets as an ultrasound contrast agent," *Ultrasound Med Biol*, vol. 37, no. 8, pp. 1271–1279, 2011.
- [4] S.-T. Kang, Y.-L. Huang, and C.-K. Yeh, "Characterization of acoustic droplet vaporization for control of bubble generation under flow conditions.," *Ultrasound Med Biol*, vol. 40, no. 3, pp. 551–561, 2014.
- [5] K. Radhakrishnan, C. K. Holland, and K. J. Haworth, "Scavenging dissolved oxygen via acoustic droplet vaporization," *Ultrason Sonochem*, vol. 31, pp. 394–403, 2016.
- [6] J. L. Zweier, "Measurement of superoxide-derived free radicals in the reperfused heart. Evidence for a free radical mechanism of reperfusion injury.," *J. Biol. Chem.*, vol. 263, no. 3, pp. 1353–1357, 1988.
- [7] K. P. Mercado, K. Radhakrishnan, K. Stewart, L. Snider, D. Ryan, and K. J. Haworth, "Size-isolation of ultrasound-mediated phase change perfluorocarbon droplets using differential centrifugation," *J Acoust Soc Am*, vol. 139, no. 5, pp. EL142–EL148, 2016.
- [8] A. Moncion, M. Lin, E. G. O'Neill, R. T. Franceschi, O. D. Kripfgans, A. J. Putnam, and M. L. Fabiilli, "Controlled release of basic fibroblast growth factor for angiogenesis using acoustically-responsive scaffolds.," *Biomaterials*, vol. 140, pp. 26–36, 2017.
- [9] K. J. Haworth, K. B. Bader, K. T. Rich, C. K. Holland, and T. D. Mast, "Quantitative Frequency-Domain Passive Cavitation Imaging.," *IEEE Trans Ultrason Ferroelectr Freq Control*, vol. 64, no. 1, pp. 177–191, 2017.
- [10] M. L. Fabiilli, K. J. Haworth, I. E. Sebastian, O. D. Kripfgans, P. L. Carson, and J. B. Fowlkes, "Delivery of Chlorambucil Using an Acoustically-Triggered Perfluoropentane Emulsion," *Ultrasound Med Biol*, vol. 36, no. 8, pp. 1364–1375, 2010.
- [11] T. D. Martz, P. S. Sheeran, D. Bardin, A. P. Lee, and P. A. Dayton, "Precision Manufacture of Phase-Change Perfluorocarbon Droplets Using Microfluidics," *Ultrasound Med Biol*, vol. 37, no. 11, pp. 1952–1957, 2011.
- [12] J. C. Hogg, "Neutrophil kinetics and lung injury.," *Physiological Reviews*, vol. 67, no. 4, pp. 1249–1295, 1987.
- [13] M. L. Fabiilli, J. Silpe, C. Rush, D. Lemmerhirt, E. Tang, G. Vasey, and O. D. Kripfgans, "High throughput production of uniformly-sized fluorocarbon emulsions for ultrasonic therapy using a silicon-based microfluidic system," *Ultrasonics Symposium (IUS), 2014 IEEE International*, pp. 1770–1773, 2014.
- [14] M. L. Fabiilli, K. J. Haworth, N. H. Fakhri, O. D. Kripfgans, P. L. Carson, and J. B. Fowlkes, "The role of inertial cavitation in acoustic droplet vaporization.," *IEEE Trans Ultrason Ferroelectr Freq Control*, vol. 56, no. 5, pp. 1006–1017, 2009.