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Mechanical bioeffects of acoustic droplet vaporization in vessel-mimicking phantoms

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ABSTRACT

This study investigated the mechanical bioeffects exerted by acoustic droplet vaporization (ADV) under different experimental conditions using vessel phantoms with a 200-μm inner diameter but different stiffness for imitating the microvasculature in various tumors. High-speed microscopy, passive cavitation detection, and ultrasound attenuation measurement were conducted to determine the morphological characteristics of vascular damage and clarify the mechanisms by which the damage was initiated and developed. The results show that phantom erosion was initiated under successive ultrasound exposure (2 MHz, 3 cycles) at above 8-MPa peak negative pressures (PNPs) when ADV occurred with inertial cavitation (IC), producing lesions whose morphological characteristics were dependent on the amount of vaporized droplets. Slight injury occurred at droplet concentrations below $(2.6 \pm 0.2) \times 10^6$ droplets/mL, forming shallow and rugged surfaces on both sides of the vessel walls. Increasing the droplet concentration to up to $(2.6 \pm 0.2) \times 10^7$ droplets/mL gradually suppressed the damage on the distal wall, and turned the rugged surface on the proximal wall into tunnels rapidly elongating in the direction opposite to ultrasound propagation. Increasing the PNP did not increase the maximum tunnel depth after the ADV efficiency reached a plateau (about 71.6 ± 2.7% at 10 MPa). Increasing the pulse duration effectively increased the maximum tunnel depth to more than 10 times the diameter of the vessel even though there was no marked enhancement in IC dose. It can be inferred that substantial bubble generation in single ADV events may simultaneously distort the acoustic pressure distribution. The backward ultrasound reinforcement and forward ultrasound shielding relative to the direction of wave propagation augment the propensity of backward erosion. The results of the present work provide information that is valuable for the prevention or utilization of ADV-mediated mechanical bioeffects in clinical applications.

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1. Introduction

Low-boiling perfluorocarbons (PFCs), such as perfluorobutane (PFB) and perfluoropentane (PFP), can exist in a superheated state without spontaneous vaporization after being emulsified into droplets of micron to submicron sizes with surfactants such as albumin, phospholipids, or polymer [1–3]. Exposure to ultrasound pulses with peak negative pressures (PNPs) above particular levels can trigger the nucleation and growth process of gas pockets inside these droplets, after which the droplets rapidly become gaseous bubbles that are 3.5–5.5 times larger in diameter than their initial sizes [4–6]. The ultrasound-triggered droplet-to-bubble transition, known as acoustic droplet vaporization (ADV), has presented both diagnostic and therapeutic usefulness in several studies thanks to the ability to produce substantial bubbles from low droplets doses. Bubbles generated by ADV can produce local ultrasound contrast enhancement as point sources for the correction of transcranial ultrasound phase aberrations [7,8]. In therapeutic applications, they can cause tumor starvation as gas emboli by reducing the blood supply to tumors, or to increase the efficiency of ultrasound ablation by increasing the amount of cavitation nuclei in the ultrasound-irradiated region [9–11]. The capabilities to conjugate with targeting ligands and encapsulate chemotherapeutic agents have rendered superheated droplets a robust theranostic system for ultrasound-controlled drug delivery [12–17]. Since liquid-based agents have longer lifespan due to the high resistance to external stress, they are promising alternatives to conventional bubble-based agents in therapeutic applications such as BBB disruption, targeted chemotherapy, and ultrasound enhanced ablation [18,19].

The key mechanism by which low-boiling PFCs can be stabilized in a superheated state as spherical droplets is the exertion of

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Laplace pressure, which is a function of surface tension and droplet size [20]. Smaller droplets have much higher ADV thresholds and produce smaller bubbles than larger ones [6,21]. For micron PFP droplets, the ADV thresholds may fall within 4–9 MPa and decrease with increasing acoustic frequency and pulse duration [1,21,22]. Once ADV is initiated by internal gas nucleation, a droplet can rapidly grow in volume with a wall velocity of tens to hundreds of meters per second [4,5,23,24]. This process may involve the similar phenomena that have been reported for bubble-based agents to cause mechanical bioeffects such as pore formation, endothelial cell damage, hemolysis, and capillary rupture [25], but may pose greater risk to tissue damage under much more intense ultrasound exposure conditions. The indication of inertial cavitation (IC), i.e., the emission of wideband backscattered echoes, can be observed at certain pressure levels above its ADV threshold, indicating the occurrence of violent inertial collapse of gas vapor [21]. The ADV of droplets attaching to single cells has been shown to cause mechanical stretching to tear off and/or permeabilize the cell membranes [16]. The volume expansion can also burst the walls of capillaries with diameters less than tens of micrometers *in vivo* [26]. The presence of droplets can significantly lower the minimum PNP required to perform histotripsy for effective tissue erosion [27]. The possibility to cause and/or augment mechanical bioeffects raises the safety issues about the use of ADV in clinical applications; however, so far only a few studies are available, and none of them focuses on vascular bioeffects. From the perspectives of diagnosis and therapeutics, imaging should be done with reduced bioeffects, and treatment efficacy should be exerted in limited regions with minimized side effects in collateral tissues. Understanding the characteristics and control of the mechanical bioeffects is of great importance for the correct use of ADV in different applications.

The cavitation of bubbles can deform a vessel structure by varying the lumen volume, and exert strong mechanical stresses on the vessel wall by generating shock waves and liquid jets [28,29]. *Ex vivo* and *in vivo* studies have shown these effects to injure vascular endothelial cells or completely rupture vessel walls [25,30]. When the PNP exceeds tens of megapascals, bubble clouds may be generated even without the presence of preexisting bubbles, simultaneously exhibiting IC strong enough to cause tissue disintegration [31]. The morphology and dimensions of produced lesions have been studied using tissue-mimicking phantoms and found to be similar to those produced in kidney tissues [32]. The microscopic observation have shown that the development and progression of lesions is the consequence of tunneling by single bubbles [33], which may be affected by several phenomena: (i) primary acoustic radiation forces pushing bubbles in the direction of ultrasound wave propagation and secondary acoustic radiation forces attracting bubbles to adjacent bubbles or boundaries [34], (ii) the absorption and partial reflection of ultrasound energy shifting the geometric focus to the prefocal region and shielding the postfocal region from ultrasound exposure [35], (iii) the transient reinforcement of PNP due to the constructive interference between backscattered shocks and incident waves [31]. Tissue geometries may also play an important role since they may also contribute to the partial reflection of ultrasound energy as well as the enhanced retention of bubble nuclei near tissue interfaces [36]. These phenomena complicate the damaging effects of cavitating bubbles.

To improve the understanding of mechanical bioeffects of ADV, particularly in the early stage where damage forms at vascular levels, we conducted high-speed photography to monitor the microscopic integrity of vessel phantoms during ADV. The morphological characteristics and progression of phantom damage were recorded optically after the onset of ADV. The dependencies of damage severity on droplet concentration, phantom stiffness, and acoustic parameters such as PNP, pulse duration, and pulse repetition frequency (PRF) were investigated. Passive cavitation detection and ultrasound attenuation measurement were conducted to clarify the mechanisms by which the damage was initiated and developed. The results of the present work provide information that is valuable for assessing and controlling the biosafety of ADV in clinical applications.

2. Methods

2.1. Droplet preparation

Droplets were composed of PFP (bulk boiling point of 29 °C) coated with phospholipids and cholesterol. These materials were purchased from ABCR (Karlsruhe, Germany), Avanti Polar Lipids (AL, USA), and Sigma–Aldrich (MO, USA). First, a lipid–cholesterol mixture containing 47.5 mol% cholesterol, 47.8 mol% distearoylphosphatidylcholine, and 4.7 mol% distearoyl-phosphatidylethanolamine-poly(ethylene glycol) 2000 was prepared in a 2 mL vial. One milliliter of phosphate buffered saline (PBS) was added to the vial to dissolve the thin film in a sonication bath at 60 °C. After the vial had cooled to 4 °C, 100 μL of PFP was infused into the vial with a 100 μL gastight syringe (HA-81000, Hamilton Co., Tokyo, Japan). Note that this process must be performed in an ice bath to minimize the spontaneous evaporation of PFP out of the vial. The vial was then sealed and sonicated at 20 °C for 5 min to produce a droplet emulsion. To remove nonencapsulated materials, the emulsion was processed with four 30-s washing/centrifugation cycles at 2000*g* with clean PBS. The final emulsion was stored at 4 °C overnight to allow the clearance of unstable droplets that could undergo spontaneous vaporization. The droplet yield was measured with a Coulter counter (model Multisizer 3, Beckman Coulter Inc., CA, USA) within the size range of 0.7–18.0 μm.

2.2. Vessel phantom preparation

Phantoms were composed of 0.75, 1, and 2 g of agarose powder (UltraPureTM Agarose, Invitrogen Corp., Carlsbad, CA, USA) dissolved in 100 mL of hot water. A cellulose tube with an outer diameter of 200 μm (Spectrum Labs, CA, USA) was placed in the agar solution, and was then removed after the solidification of agar to form a vessel phantom. The agar composition was varied to produce phantoms with various stiffness for imitating different biological tissues. The stiffness was measured by Young's modulus with a material testing system (LPX, Lloyd Instrument, England). During the measurements, a flat metal disk was driven by a linear motor to compress a phantom specimen on a flat plate. The applied stresses deformed the phantom structure at a strain rate of 0.02 s^{-1}. The Young' modulus of the specimen was computed from the slope of a stress–strain curve recorded in the system. For phantoms with agar percentages of 0.75%, 1%, and 2%, the Young's moduli were measured to be 25.5 ± 2.41, 55.4 ± 2.97, 240.1 ± 37.4 kPa ($N = 3$), respectively, corresponding to the stiffness of different tumor tissues in breast, liver, prostate, and uterus [37]. The measured Young's moduli matched well with those reported in the literature [33,38].

2.3. High-speed photography of lesion formation

High-speed photography was performed in an integrated acousto-optical system, as illustrated in Fig. 1(a). A 2-MHz high-intensity focused ultrasound (HIFU) transducer (model SU-101, Sonic Concepts Inc., WA, USA) was fixed in a custom-designed water tank attached to an inverted microscope (model IX71, Olympus Corp., Tokyo, Japan). The transducer was confocally positioned with a waterproof objective (model Achroplan 40X, Carl Zeiss Ltd., Tokyo, Japan). An arbitrary waveform generator (model AWG 2005,

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