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The lifetime evaluation of vapourised phase-change nano-droplets

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ABSTRACT

Phase-change nano-droplets (PCNDs) are sub-micron particles that are coated with phospholipid and contain liquid-state perfluorocarbons such as perfluoropentane (boiling point = 29 °C) and perfluorohexane (boiling point = 57 °C), which can vapourise upon application of ultrasound. The bubbles generated by such reactions can serve as ultrasound contrast agents or HIFU sensitisers. However, the lifetime of bubbles generated from PCNDs on μs-order is not well known. Knowledge of the condition of PCND-derived bubbles on μs-order is essential for producing bubbles customised for specific purposes. In this study, we use an optical measurement system to measure the vapourisation and stability of the bubbles (bubble-lifetime) as well as the stability-controlling method of the nucleated bubbles on μs-order while changing the internal composition of PCNDs and the ambient temperature. PCND-derived bubbles remain in a bubble state when the boiling point of the internal composition is lower than the ambient temperature, but lose their optical contrast after approximately 10 μs by re-condensation or dissolution when the boiling point of the internal composition is higher than the ambient temperature. We reveal that the superheating condition significantly affects the fate of vapourised PCNDs and that the bubble-lifetime can be controlled by changing both the ambient temperature conditions and the internal composition of PCNDs.

1. Introduction

Medical applications of ultrasound started with imaging, and the technology is still commonly used in clinical settings. Recently, high-intensity focused ultrasound (HIFU) [1,2], which has therapeutic applications has been attracting considerable attention because it can be conducted minimally invasively or non-invasively.

However, problems exist for medical applications of ultrasound. For example, detection of tumours is an extremely common problem in diagnostic ultrasound [3]; in conventional ultrasonic images, it is difficult to identify boundaries between normal and diseased tissues as there may be only a low contrast between them. HIFU treatment also has the following drawbacks. First, ultrasound is scattered and absorbed by fat, skin and other tissues while propagating to a target region, which leads to energy deficiency when treating the region. Second, to gain a high-intensity acoustic profile at the focus, unintended sites such as skin might get burned because an approaching path overlaps at the skin in each sonication. Therefore, HIFU is pulsed to avoid near-field heating and cooling time is set between subsequent exposures of HIFU sequences [4]; this cooling time makes the treatment time significantly longer.

Microbubbles have the potential to solve these issues. The response of microbubbles to ultrasound is non-linear and that of tissues to ultrasound is almost linear; thus, microbubbles can be differentiated from the surrounding tissues, indicating their potential to be used as an ultrasound contrast agent [5]. In addition to imaging purposes, microbubbles can act as a HIFU sensitiser. The HIFU thermal coagulation effect can be accelerated by the presence of microbubbles [6–9]. Several studies on phantom [10,11], *ex vivo* [12] and *in vivo* [13,14] experiments have reported that there is good correlation among the generation of acoustic cavitation, the temperature-rise ratio and the peak temperature. These results indicate that the presence of bubbles can improve the treatment efficiency and reduce the treatment time under HIFU.

However, applications of microbubbles are limited because of their size and fragility to ultrasound. They cannot permeate

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through gap junctions in the endothelial cells making up the vessel wall of cancerous tissue sites, which restricts their use only to intravasculature. Intending to reach the extravascular tissue sites, Kawabata et al. [15] proposed that nano-sized particles be used for permeation and changed in size once they reach the extravascular targeted tissue sites. Phase-change nano-droplets (PCNDs), also known as acoustic nano-droplets, are sub-micron-sized particles, small enough to permeate through the vascular endothelial cell gap junctions of cancerous tissue sites. PCNDs are coated with phospholipid or encapsulated with protein or polymer and contain liquid-state perfluorocarbons (PFCs) inside. Commonly used PCNDs include perfluoropentane (PFP, boiling point = 29 °C) and perfluorohexane (PFH, boiling point = 57 °C), and that Kawabata et al. [15] showed that the boiling point can be modulated by mixing of these specific PFCs. The major difference between these three types of PCNDs is their boiling point. Application of ultrasound to a PCND can vapourise the PFC inside a PCND and transform it into a microbubbble. PCNDs are actively being researched as extravascular ultrasound contrast agents [16–19], HIFU sensitisers [20–22] and extravascular drug carriers [16,23,24], and vapourisation for methods such as sonoporation [25,26] or the blood–brain opening [27] are also being investigated.

While various studies have shown the potential for medical applications of PCNDs, the underlying mechanism of the droplet vapourisation process and the stability of the generated bubbles are not well known. Resent research has advanced the knowledge of the vapourisation mechanism. Shpak et al. [28] showed a superheated droplet vapourised by the focusing of a nonlinear wave within the droplet. They have also shown detailed droplet vapourisation dynamics in Ref. [29]. Reznik et al. [30] discussed the paths that newly generated bubbles might take, and Sheeran et al. [31] discussed several of the bubble phenomena that occur within an ultrasound pulse. After exposure to vapourisation ultrasound pulses, the behaviour of the vapourised PCND can be assumed to fall into one of two groups; the short or the long-lifetime group. Each group can be sub-classified into two cases: the recondensation and dissolution cases for the short-lifetime group, and the remaining and coalescence cases for the long-lifetime group. Optical studies, especially those using high-speed imaging, are being actively conducted to study the vapourisation processes and properties of the generated bubbles. However, μs-order optical observation of the vapourisation process has rarely been conducted, and most previous studies have focused on the resulting bubbles (time scale = ms to minute order). Several studies [29,32,33] have taken μs or ns-order measurements of larger droplets (μm-size droplets), which are thought to experience many of the same phenomena as nm-size droplets. There might be differences in size dynamics due to the effect of Laplace pressure, heat transfer and rectified heat transfer [29]. Thus, the stability of bubbles generated from PCNDs at μs-order is not well known. Besides the formation of stable bubbles, as indicated in a previous vapourisation acoustic measurement study [34], there is a significant possibility that the bubbles re-condense into liquid droplets on μs order. Knowledge of the conditions of PCND-derived bubbles on μs order is essential for producing bubbles for specific purposes using PCNDs. Several authors [30,31] have conducted high-speed optical imaging on vapourisation of submicron-sized droplets. To the authors' knowledge, Reznik et al. [30] were the first authors that succeeded in μs-order optical observation of the vapourisation processes of PFP PCNDs (ranging from 100 nm to 1 μm in diameter with a mean diameter of approximately 400 nm) at an ambient temperature condition of 37 °C. They imaged the initial vapourisation process within 10 μs at 10 million frames per second (Mfps), and reported that there were two states after the formation of the bubble; one that remained as a stable bubble and one that disappeared within approximately 10 μs. They focused on observation of individual and identical bubbles. Moreover, they used only one type of PCND. It is important to conduct research on the vapourisation behaviour as an entity and for different types of PCNDs.

Moreover, there is a significant demand for the stability-control of PCND-derived bubbles. For application as an ultrasound contrast agent or for sonoporation, the recondensation case is most desirable because vapourisation is thought to be repeated and reiterated injections of PCNDs can be avoided. In contrast, the long-lifetime group also can be used as ultrasound contrast agent with using conventional ultrasound-imaging techniques such as the pulse-inversion method. For application as a HIFU sensitiser, the remaining and coalescence cases are desirable because one wants to maintain the HIFU-sensitising effect for a long period of time during HIFU-ablation therapy. We assumed that the boiling point of the internal composition (PFC) of the PCND would be correlated with the stability of the PCND-derived bubble; the PCND-derived bubble is in a stable state when the boiling point is lower than the ambient temperature, whereas it is in a meta-stable state when the boiling point is higher than the ambient temperature.

In this study, we used an optical measurement system to measure the vapourisation and the stability (bubble-lifetime) and to investigate the stability-controlling method of nucleated bubbles at microsecond order while changing the internal composition of the PCNDs and the ambient temperature.

2. Materials and methods

2.1. Experimental set-up

The experimental set-up consisting of the optical system, the ultrasound system, and the target gel phantom with PCNDs was developed as illustrated in Fig. 1(A) to observe the vapourisation of PCNDs and measure the bubble-lifetime.

2.2. PCNDs and gel phantoms

Three different types of PCNDs, whose internal compositions were PFP, PFH and a mixture of the two (PFP:PFH = 1:1), were provided by Central Research Laboratory Hitachi (Tokyo, JAPAN). The method of preparation of PCNDs can be found in Ref. [15,35]. A laser-diffraction particle-size analyser (Beckman-Coulter LS13320) was used to measure the particle-size distribution of the PCND suspensions. The mean diameter of each type of PCND was 380 ± 70, 370 ± 70 and 290 ± 130 nm, respectively. The droplet concentrations were estimated using the total volume of PFC used for PCNDs and the size distribution of each droplet [30]. As a result, PFP, PFH and PFH–PFP mixture droplet concentrations were 4×10^{14}, 4×10^{14}, and 7×10^{14} droplets/ml, respectively. The main known difference between the three types of PCNDs is the boiling points of their internal compositions; the boiling point of PFP is 29 °C, that of PFH is 57 °C, and that of the mixture is 40 °C (calculated from Raoult's law and Clausius–Clapeyron Equation). The three different types of PCNDs were embedded into the polyacrylamide gel separately to simulate PCND accumulation in a tumour. PCNDs are expected to flow inside blood vessels or accumulate at the extravascular tumour tissue sites [19,36] by the enhanced permeability and retention effect [37]. We intended to simulate those accumulated at tumour tissues by incorporating PCNDs inside the polyacrylamide gel, as did Kawabata et al. [34].

The method for preparation of the polyacrylamide gel solution can be found in the literature [38,39]. PCNDs were embedded just before the fixation by ammonium peroxodisulfate solution. The final relative concentration of PCNDs to gel solution was set at 0.2% (v/v). Two types of gel solution (with and without PCNDs) were prepared. First, the gel solution without PCNDs was poured