

Cavitation Induction with Phase Change Nano Droplet 相変化ナノ液滴を用いるキャビテーション生成

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1. Introduction

In ultrasound echography, microbubbles are the most commonly used contrast agent. They show high echogenicity and characteristic non-linear acoustic responses, enabling their acoustic echo signals to be distinguishable from other signals by using appropriate ultrasound exposure sequences and filters.

Recently, it has been revealed that microbubbles have possibilities to be used as 'sensitizers' for HIFU therapy because they have been found to enhance temperature rise induced by tissue absorption of ultrasound energies. [1-3].

However, a serious problem in utilizing microbubbles is that their sizes (several microns in diameter) are too big to leak into tissues from blood vessels when administered intravenously.

To solve the problem, we are developing a nano-sized ultrasound contrast agent which turns into microbubbles upon the exposure of ultrasound pulses and refers it as phase change nano droplet (PCND).

We aim to administer PCND and change their phase to gas and produce microbubble only at the target inside body. Then we could visualize the target if the droplets are accumulated, and we could then further expose therapeutic ultrasound such as HIFU for site-specific treatment. Because it is an 'on-demand' generation of microbubbles and not requires the 'incubation time', almost all bubbles are usable for therapy

In our previous work, we investigated the effect of PCND on accelerating temperature rise induced by ultrasound exposure *in vitro* and *in vivo*. It was found that PCND is effective for the HIFU sensitizer with 2-MHz ultrasound exposure.

In this study, we performed preliminary experiments on the possibility of utilizing low frequency ultrasound (around 1 MHz) with PCND, aiming to apply PCND as a sensitizer not only for HIFU but also for cavitation therapy of cancer.

2. Materials and Methods

PCND preparation

The preparation procedure of PCND was described elsewhere [4]. Briefly, DPPC liposome was prepared and the liposome was further emulsified at

high pressure (20 MPa) in the presence of perfluorocarbon liquids. The size distribution of PCND was measured with a LB-550 (Horiba, Ltd., Kyoto, Japan) dynamic light-scattering size analyzer. The mean diameter of the PCND was about 0.2 μm . Gel phantom was prepared basically the same procedure as previously reported. In preparation, 5-% albumin solution is included in the gel as the indicator of thermal coagulation.

Experimental setup for ultrasound exposure

In this study, both *in vitro* and *in vivo* experiments were carried out with the same setup as previously reported[5]. Focused ultrasound transducer (2.2 MHz with a diameter of 35 mm or 1.1 MHz with a diameter of 48 mm) were submerged in water tank filled with degassed water kept at 37 °C. Specimen (polyacrylamide gel containing the above mentioned nano droplet or mice injected nano droplet from a tail vein) was placed at the focus of the transducer. Ultrasound was exposed for 20 s. *In vivo* experiments were carried out with anesthetized CDF1 mice bearing Colon 26 tumor tissues implanted subcutaneously.

3. Results and Discussion

Figures 1 and 2 show optical change in gel phantom with PCND exposed to 2.2 and 1.1 MHz ultrasound, respectively. The gels are basically transparent and contain 5% albumin solution which produces white opaque regions on thermal coagulation. In preliminary experiments using thermocouples, it was confirmed that albumin in this gel coagulates at 55 °C. In each figure, white regions can be observed at 5 seconds and the white region increased as exposure time proceeded. When 2.2 MHz ultrasound was used (Fig. 1), prolonging the exposure time affects much in brightness increase but not drastically on the size of the white (coagulated) region. On the contrary, when 1.1 MHz ultrasound was used (Fig. 2), the size of the white region increased as the exposure time exceeded. In the white regions at 5 seconds, bubbles are likely to present because 'dancing' of white sub-regions were observed.

It was found that ultrasonically induced temperature rise can be enhanced with not only

with 2-MHz ultrasound but also 1.1-MHz ultrasound. Though, the way how enhancement is different.

Such difference can be interpreted by assuming that the numbers of microbubbles are almost constant when 2.2 MHz ultrasound is used and increases as exposure time prolongs when 1.1-MHz ultrasound is used. With such an assumption, process in Figs. 1 and 2 can be interpreted that dominant factor for the temperature elevation in Fig. 2 is the thermal diffusion and that in Fig. 3 is the number of microbubbles.

To further investigate if such assumption is valid and cavitation is involved in increasing the number of bubbles, acoustic emission signals from gel phantoms were measured and the frequency spectra were obtained. When 2.2-MHz ultrasound was used, the presence of PCND did not significantly affect the spectra components. On the contrary, when 1.1 MHz ultrasound was used, the presence of PCND resulted in drastic increase in fractional harmonic components. Not only half and its higher order harmonics, which typically shown when cavitation phenomena are induced, but other fractional harmonics are observed.

Above results suggest that when PCND are used in combination with 1.1-MHz ultrasound, cavitation is induced while not significantly induced when 2.2-MHz ultrasound is used. The result is consistent with results in Figs. 1 and 2.

It is well known that when cavitation is induced, mechanical and chemical bioeffects are also induced as well as thermal ones. The spectrum suggests that the cavitation is violently induced thus mechanical or chemical bioeffects are expected. This result indicates that when PCND is used in combination with low frequency ultrasound, multiple therapy mechanisms work for destroying target tissues. Therapy system with multiple mechanisms will contribute to widen the applicable targets because such system will less affected its therapeutic effects on the vascularity of target tissues.

The result that PCND can make a 'hole' in tumor tissue is promising that PCND will show very strong anti-tumor effects but at the same time suggesting a potential problem of it that the tissue interfaces influence the therapeutic effects of PCND combined ultrasound therapy because the hole was firstly made at the surface of the tissue not at the focus inside tissue.

Suppressing the influence of tissue interfaces would be a very crucial factor for the developing of PCND-aided low frequency ultrasound therapy.

Localizing the effects of PCND-aided ultrasonic bioeffects only at the target would be possible by utilizing focused ultrasound.

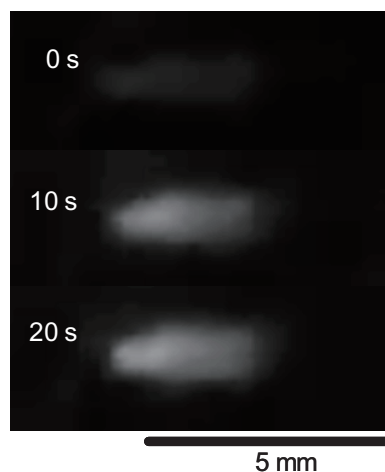


Fig. 1 Optical change in gel phantom on ultrasound exposure (Freq.: 2.2 MHz, Intensity: 1.0 kW/cm² PCND conc. : 4 mg/ml)

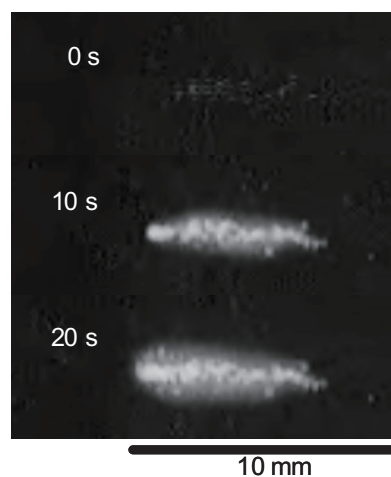


Fig. 2 Optical change in gel phantom on ultrasound exposure (Freq.: 1.1 MHz, Intensity: 0.6 kW/cm² PCND conc. : 4 mg/ml)

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