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Effect of Number Concentration of Contrast Agent Microbubbles in a Microchamber on Shell Disruption

マイクロチャンバーにおける造影マイクロバブル群の数濃度がシェル崩壊に及ぼす影響

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1. Introduction

Ultrasound-assisted drug delivery system (DDS) using microbubbles as drug carriers is a non-invasive treatment that reduces adverse side effects of drugs and is independent of the tumor shape. In this system, carrier particles (encapsulated microbubbles) loaded with drugs are injected into the blood vessel, and drug release is triggered by the mechanical stimulus of ultrasound irradiation at the lesion site.

In order to effectively disrupt microbubbles, the sound frequency needs to correspond to the resonance frequency of the microbubbles[1)]. However, the resonance characteristics of the microbubble clusters depend on the number concentration[2)]. Yoshida et al. calculated the sound attenuation of a suspension of Sonazoid® microbubbles and revealed that the attenuation of the population increases as the number concentration increases[3)]. However, the disruption property of Sonazoid® has not been addressed.

In order to evaluate the effectiveness of disruption of Sonazoid®, we observed ultrasonic disruption of Sonazoid® microbubbles insonified by high intensity focused ultrasound (HIFU) in a microchamber, and measured the number of disrupted microbubbles. The experiment was performed by varying the number concentration of Sonazoid® suspension and the sonication frequency.

2. Materials and Methods

2.1 Microbubble preparation

A suspension of microbubbles (Sonazoid®, Daiichi Sankyo) was synthesized by mechanical agitation and diluted by deionized water. Various concentration of the suspension was made by controlling the volume ratio of water and the microbubble suspension. After preparation, the suspension was injected into a slide-type microchamber (SD100, Nexcelom bioscience) to distribute the microbubbles in a two-dimensional space. As shown in **Fig. 1**, the chamber slide consists of two plastic plates laminated together. A microchamber of 0.1 mm in height has inlet/outlet ports at both ends for injecting the sample.

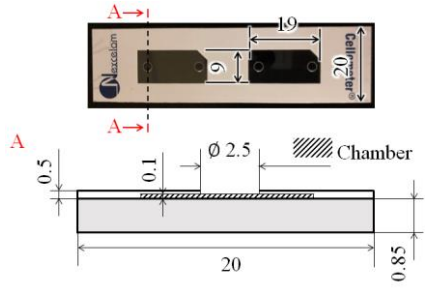

Fig. 1　Schematic of the slide-type microchamber.

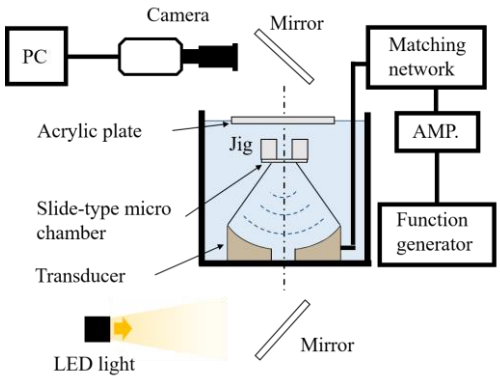

Fig. 2　Experimental setup.

2.2 Experimental setup

As shown in **Fig. 2**, the HIFU transducer (H-102, Sonic Concepts) was submerged in the water tank, and a slide-type microchamber was placed on the top of the coupling cone. In addition, a jig was placed on the chamber slide to fix its position during sonication. Furthermore, a transparent acrylic plate was placed above the chamber slide to flatten the water surface. A 7 cycles-sinusoidal pulse at 1 MHz or 3 MHz was generated by a signal generator (WF1973, NF) at 5 V peak to peak voltage, amplified 20 times by a power amplifier (HSA4101, NF), and was input to the HIFU transducer. A backlight optical image of the sample was obtained before and after sonication by the microscopy system.

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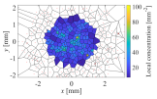

Fig. 3 A typical Voronoi diagram calculated from a microscopic image experimentally obtained after sonication (Fig. 4(c)).

2.3 Image processing

Image analysis was performed by utilizing an image processing software (ImageJ). The recorded image was converted into a binary image, and positions of gas bubbles were detected. As shown in **Fig. 3,** Voronoi cells which divide the whole image into several small parts were generated with respect to the bubble positions (red points). The local concentration of the disrupted microbubbles was defined as the inverse of the area of the Voronoi cell. In the present study, we only focused on the bubble cluster inside the irradiation area.

3. Results and Discussion

When microbubbles are disrupted by sonication, the encapsulated gas leaks out and increases its volume gradually due to mass diffusion, as seen in **Fig. 4**. We defined a disruption concentration as the average local concentration of the Voronoi cell in the sonication area. **Fig. 5** shows the result of the disruption concentration of Sonazoid® microbubble suspension as a function of the nuclei concentration (before sonication). For 1 MHz sonication, the disruption concentration is proportional to the concentration and higher than that by 3 MHz ultrasound. On the other hand, for sonication at 3 MHz, disruption only occurred in a particular concentration.

The dominant reason for the high disruption concentration at 1 MHz may be the existence of transient cavitation during sonication, mainly generated in the lower frequency. In 3 MHz sonication, the microbubbles oscillate as stable cavitation and disrupt only at the on-resonance condition. It concludes that effective microbubble shell disruption can be achieved at low-frequency ultrasound irradiation and with high nuclei concentration of Sonazoid® suspension.

4. Conclusion

In order to effectively disrupt Sonazoid®, a sonication experiment was performed utilizing HIFU while varying the concentration of suspension and frequency of ultrasound. In this experiment, a high concentration of Sonazoid® suspension sonicated by low-frequency ultrasound enhances the disruption of microbubbles effectively.

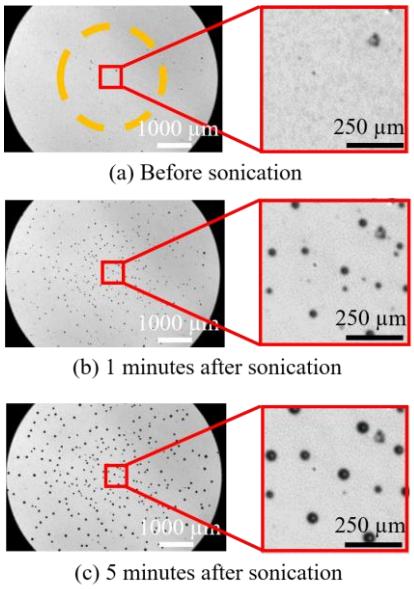

Fig. 4 Representative images of Sonazoid® microbubbles (a) before sonication, (b) 1 minute after sonication, and (c) 5 minutes after sonication. The sound frequency is 1MHz. The yellow dashed line indicates the sonication area.

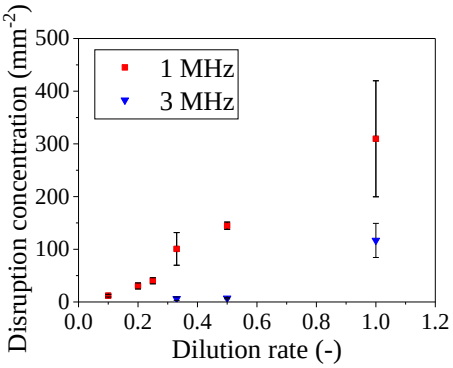

Fig. 5 Number concentration of the disrupted micro-bubbles. The horizontal axis was normalized by the concentration of undiluted suspension.

Acknowledgment

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References

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