1P5-22

Proceedings of Symposium on Ultrasonic Electronics, Vol. 35 (2014) pp. 167-168
3-5 December, 2014

Quantitative Assessment of Reactive Oxygen Sonochemically Generated by Cavitation Bubbles

キャビテーション気泡の音響化学反応により発生した活性酸素量の定量的評価

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1. Introduction

In high-intensity focused ultrasound (HIFU) treatment method, ultrasound is generated outside the body and focused at the malignant tissue and induces irreversible changes to the tissue. The thermal bioeffect of ultrasound has been considered to be the primary mechanism of such changes.

Cavitation bubbles are microbubbles generated by highly negative pressure, and they are known to enhance the HIFU treatment using the thermal bioeffect of ultrasound.

Cavitation bubbles also work as chemical reactor which can induce chemical bioeffects. The treatment method using such chemical bioeffect has been sugggested and called sonodynamic treatment.

A chemical, that can be ultrasonically activated to induce the sonochemical bioeffect is called a sonosensitizer. Typically, it is activated through the collapse of acoustic cavitation and, when activated, it generates radicals such as singlet oxygen, OH radical, and so on, which can induce irreversible changes to the tissue. By using sonochemical and thermal bioeffect together, cancerous tissue can be treated nearly comletely, and it might reduce the possibility of recurrence of cancer.

Rose Bengal (RB) is such a sonosensitizer in the xanthene dye family, whose in vivo and in vitro bioeffects have been reported. It has also been reported that RB can reduce the cavitation threshold.[1-3)]

In this study, cavitation bubbles were generated in varies concentrations of RB, and the relation between the behavior of cavitation bubbles and the amount of radicals was investigated by observing cavitation bubbles using a high-speed camera and quantifying the generated radicals using KI dosimetry.

2. Material and Method

2.1 Experimental setup

An experimental set up of this study is shown in **Fig.1**. An focused ultrasound array transducer (Imasonic) and a sealed chamber were placed in a PMMA water tank. The water tank was filled with degassed water, whose DO level and temperature were approximately 30% and 25°C, respectively. The transducer had outer and inner diameters of 100 and 36 mm.

The sealed chamber was made of a PMMA pipe, and a poly-ethylene film was stretched at each end of the pipe to let ultrasound propagate inside the chamber. A volume of the chamber was approximately 4 ml. The solution sealed in the chamber contained either 0, 1.0, 2.5, 5, or 10 mg /L of RB and of potassium iodide (KI). Their DO level was around 30 %.

A high-speed camera (Shimazu) was set to observe the behavior of the cavitation bubbles. Its frame rate and exposure time was 500 kfps and 250 ns, respectively.

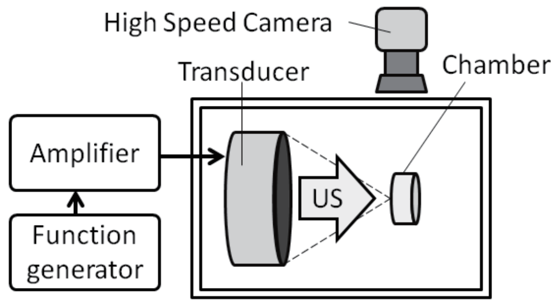

Fig.1 Experimental setup

2.2 Experiment

Ultrasound was irradiated by the transducer and cavitation bubbles were generated in the chamber. The ultrasonic exposure sequence consists of an extremely high intensity short pulse (trigger pulse) immediately followed by a moderate intensity long burst (sustaining burst). The intensity and exposure duration of the trigger pulse were 40 kW/cm^2 and 100 µs, while those of the sustaining burst were 500 W/cm^2 and 100 ms, respectively. The ultrasound frequency was 1.2 MHz.

In this study, the amount of OH radicals were estimated. Although singlet oxygen radicals are known to be generated, to detect them is difficult because their lifetime is extremely short.

In order to assess the amount of OH radicals, the KI dosimetry[4] was employed. In this dosimetry, OH radicals attack KI liberating iodine. Iodine also reacts with some amount of remaining KI to form I^{3-}.The extent of iodine liberation during the reaction was estimated using a UV/VIS spectrophotometer to measure the absorbance at 355 nm wavelength. The amount of the iodine liberation depends on the supply of OH radicals, which should depend on the efficiency of the sonochemical reaction.

The amount of OH radicals estimated by this method was compared with high-speed camera pictures at each concentration of RB.

3. Results and Discussion

High-speed camera pictures are shown in **Fig.2**. Bubble clouds were observed at 0, 1.0, and 2.5 mg/L of RB. However, at 5 and 10 mg/L, a large cloud was not formed although scattered cavitation bubbles were observed.

The differences of absorbance at 355 nm wavelength before and after ultrasonic irradiation at each RB concentration are shown in **Fig.3**. In all cases, the absorbance at 355 nm wavelength increased by ultrasonic exposure, At RB concentrations up to 2.5 mg/L, the difference between before and after ultrasonic exposure increased at 355 nm wavelength as the RB concentration increased. In contrast, at 5 and 10 mg/L, the difference was even smaller than 0 mg/L.

The observed RB concentration dependence of active oxygen generation is quite consistent with that of microbubble cloud generation. The reason for the observed dependence is considered as follows, assuming that a large cloud of cavitation microbubbles is essential for efficient sonochemical generation of active oxygens. Above a certain RB concentration, microbubbles are generated in a widely scattered form due to the low cavitation threshold reduced by RB. These microbubbles scatter ultrasound energy and inhibited the formation of a large microbubble cloud. The tested range of RB concentration is known to be not too high to chemically inhibit the reaction itself.

4. Conclusion

In this study, the behavior of cavitation microbubbles and the amount of generated OH radical in five concentrations of RB were compared. The result the amount of cavitation bubbles and concentration of RB are factors that determines the amount of generated active oxygen. Moreover, the results also suggest that in order to generate active oxygens at a high efficiency, the effect of RB on cavitation cloud formation can be even more important than its effect as a chemical sensitizer.

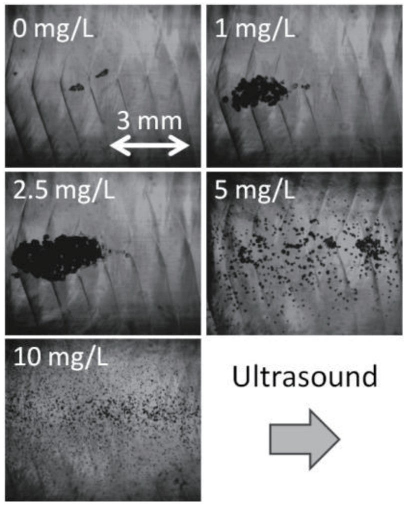

Fig.2 High-speed camera pictures at each concentration of RB

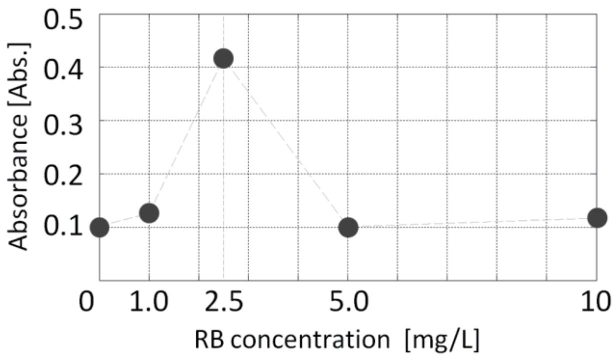

Fig.3 The differences of absorbance at 355 nm wavelength before and after ultrasonic irradiation at each concentration of RB

Acknowledgement

The authors would like to thank Mr. Kawabata of Central Research Laboratory, Hitachi, Ltd. Special thanks also go to Prof. Matsuura, and T Associate Prof. Katagiri, Tohoku Univ. This research was supported by a Grant-in-Aid for Scientific Research from the Japan Society for the Promotion of Science (JSPS).

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