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Efficient Generation of Cavitation Bubbles and Reactive Oxygen Species using Triggered HIFU Sequence for Sonodynamic Treatment

音響化学治療のための Triggered HIFU でのキャビテーション気泡と活性酸素生成の効率化

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1. Introduction

In sonodynamic treatment, first, sonosensitizer that produce reactive oxygen species (ROS) is injected into the body and reaches the target cancerous tissue. Next, cavitation bubbles are generated there, and finally, cancerous tissue is killed by generated ROS. It is considered that sonodynamic treatment may be able to improve treatment accuracy and increace treatable organs of HIFU treatment. In this study, Rose Bengal (RB) was employed as a sonosensitizer whose in vivo and in vitro bioeffects have been reported[1)]. Additionally RB not only promotes ROS generation chemically but also affects on cavitation behavior[1)].

For efficient sonodynamic treatment, we have been considering the exposure sequence to promote ROS generation efficiency. In our previous study[2)], it was demonstrated that triggered HIFU sequence, which consists of an extremely high intensity short pulse (triggered pulse) for cavitation cloud inecption[3)] immediately followed by a moderate intensity long burst (sustaining waves) for sustaining bubbles by volume oscillation, can generate ROS efficiently.

However, the behavior of cavitation bubbles that can generate ROS efficiently has not been much studied. In this study, to find out how triggered HIFU sequence effects on highly efficient ROS generation, the behavior of cavitation, generated in several concentrations of RB solution was observed by a high-speed camera. Moreover, the amount of generated ROS was also quantified.

2. Material and method

2.1 Experimental

As shown in **Figure 1**, an focused ultrasound transducer (Imasonic) and a sealed chamber were placed in a water tank filled with digassed water. The transducer had an outer diameter of 100 mm. At the center of the tranuducer, an ultrasonic probe was placed. The solution sealed in the chamber contained either 0, 1.0, or 10 mg/L of RB. It also contained 1 mol/L of potassium iodide (KI) for quantifying ROS. A high-speed camera captured cavitation bubbles from the side of the water tank at a PRF of 2.5 kps.

2.2 Exposed sequences

Three sequences, which had both triggered pulse and sustaining waves (TS sequence), only triggered pulse (T sequence), or only sustaining waves (S sequence) were employed.(**Figure 2**) The intensity and exposure duration of the triggered pulse were 20 kW/cm^2 and 100 µs, while those of the sustaining waves were 120 W/cm^2 and 50 ms, respectively. The utrasound frequency was 1 MHz. These sequences were continued for 5 minutes at a PRF of 10 Hz.

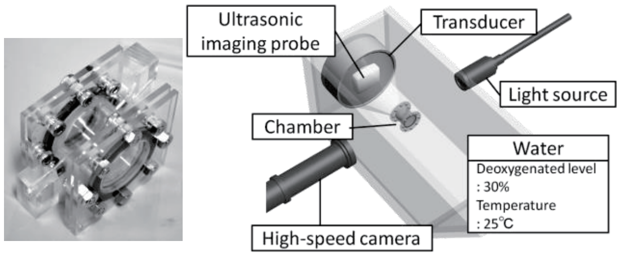

Fig. 1 Experimental devices and sealed chamber

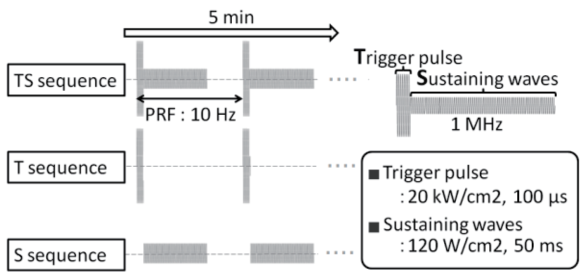

Fig. 2 Exposed sequences

2.3 ROS quantification

After HIFU exposure was finished, the amount of ROS was quantified. ROS oxidize an iodine ion and produce a triiodide ion that has a peak absorbance at 355 nm. Therefore, the amount of ROS can be quantified by comparing the absorbance at 355 nm before and after ultrasound exposure.

3. Result

Table I shows measured absorbance change at 355 nm. From this table, two things could be said.

First, in all of RB concentrations, TS sequence generated larger amount of ROS than T sequence while no ROS was generated by S sequence .

Second, positive RB concentration dependence was seen in both TS and T sequences.

Table I. Measured absorbance at 355 nm

	0 mg/L	1 mg/L	10 mg/L
TS	0.05	0.08	0.24
T	0.02	0.02	0.1
S	0	0	0

From high-speed camera pictures, mainly two features of cavitation behavior were observed.

First feature was that in TS sequence, cavitation cloud collapsed slower than in T sequence. It was suggested from **Figure 3**, high-speed camera pictures of cavitation cloud and 1 ms, 40 ms after cloud generation. The pictures at 1 ms and 40 ms were processed to binary images in order to quantitatively analyze bubbles easily. In collapse phase, in T sequence, cavitation cloud collapsed rapidly, and extremely small bubbles (suggested less than 10 μm) were remained, while in TS sequence, a greate number of bubbles still remained at 1 ms. Bubbles seemed to distribute like a stripe form.

Second one is that in TS sequence, the higher RB concentration became, the larger the number of sustained bubbles was. It was indicated by **Figure 4**. This graph was made by counting the number of bubbles from the binary pictures at 40 ms using ImageJ. The number of sustained bubbles in TS sequence has RB concentration dependence.

Besides, the amount of cavitation cloud did not seem to have RB concentration dependence.

4. Discussion

These two observed features of the bubble behavior may make the difference in ROS generation efficiency shown in Table I.

Firstly, one of the scenario to lead to the difference of ROS generation efficiency between TS and T sequence can be suggested as shown below.

A stripe form distribution which was seen in TS sequence after cloud collapse seemsed to agree well with negarive pressure distribution of sustaining waves around focal area of HIFU. At this time in the sequence, the large number of bubbles were oscillated violently. Furthermore, after collapsed, bubbles were still oscillated by sustaining waves. Thereby, ROS generation may be highly promoted in TS sequene.

Secondly, one of the reasons for the relationship between ROS generation efficiency and RB concentration may be explained by looking the number of sustained bubbles. It is considered that sustained bubbles are oscillated by sustaining waves and produse ROS. Therefore, the number of sustained bubbles may relate to the efficienfy of RB generation. Thus, increasing the number of sustained bubbles by adding RB cause higher efficiency of ROS generation.

However, in was also considered that RB promoted strongly through its chemical effect, because in T sequence, the amount of generated ROS had strong RB concentration dependence although the amount of cavitation cloud did not have it.

5. Conclusion

Sustaining waves in TS sequence made cloud collapse slower and oscillated sustained bubbles. RB had effect to increase the number of sustained bubbles. The two observed phenomena may be reasons for the promotion of ROS generation. However, it was also considered that chemical effect of RB also strongly affects on ROS generation.

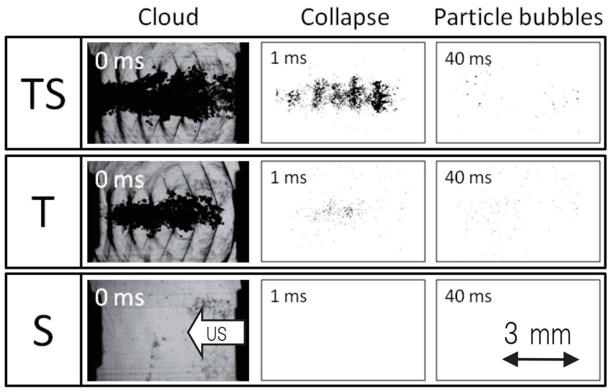

Fig. 3 High-speed camera pictures of cloud and detected bubbles pictures after cloud formation

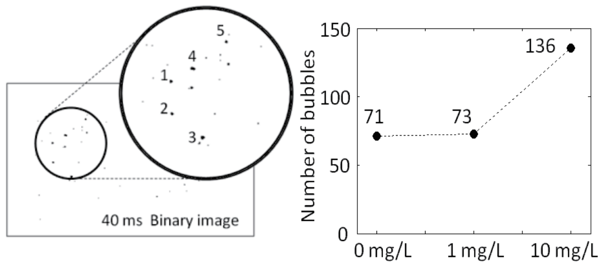

Fig. 4 An image of counting bubbles and counted the number of sustained bubbles at 40 ms (N=5, average)

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