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Experimental Investigation of Effect of Ultrasonic Duty Cycle on Generation of Reactive Oxygen Species for Highly Efficient Sonodynamic Treatment

高効率な音響力学治療にむけた超音波照射時間率が活性酸素種生成に与える影響の検討

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1. Introduction

HIFU (high-intensity focused ultrasound) is one of the non-invasive modalities for cancer treatments. In this treatment, ultrasound generated outside a body is focused on a target tissue in the body. The intense ultrasonic energy in the focal region makes it possible to coagulate cancer tissues.

Sonodynamic treatment (SDT) is also a non-invasive treatment using ultrasound, where cancer is treated by reactive oxygen species (ROS) generated by the oscillation and collapse of cavitation bubbles, which are induced by ultrasound at a sufficiently high intensity. When cavitation bubbles collapse, the temperature and pressure inside the bubble become extremely high[1)] due to adiabatic compression. This provides a reaction field for the generation of ROS (e.g. hydroxyl radical, hydrogen peroxide, and singlet oxygen) from water molecules. Sonosensitizers such as certain porphyrins and anthracyclines can enhance the ROS generation when exposed to ultrasound. Cancer cells can be treated by not only heat induced by HIFU but also cytotoxicity of such ROS.

The size of the region treated by SDT in an HIFU exposure is small, typically in the order of a mm. This provides a good spatial selectivity of the treatment, but at the same time results in a long treatment time when the tissue to be treated is much larger than it. Therefore it is important to improve the efficiency of the ROS generation to reduce the treatment time.

In this study, the effect of ultrasonic sequences on the generation of ROS was investigated using sonochemiluminescence.

2. Material and Method

2.1 Trigger HIFU sequence

In this study, 'Trigger HIFU sequence[2)]' was used to generate and oscillate cavitation bubbles efficiently. The sequence consists of "trigger pulse" and "sustaining burst". Firstly the trigger pulse at an extremely high intensity is irradiated for the generation of bubble clouds through the shock scattering mechanism[3)]. Immediately after that, the sustaining burst at a moderate intensity follows for the continuous oscillation of bubbles.

2.2 Sonochemiluminescence

To visualize the region of the chemical reaction induced by ultrasound, sonochemiluminescence has been used in many previous studies[4,5)]. In this study, luminol chemiluminescence was used to analyze the region and amount of ROS generation. Luminol is excited by hydroxyl radicals, and it emits blue light at 425 nm[4)] when it returns to the ground state. The sonochemiluminescent reaction was analyzed in this study based on the hypothesis that the intensity of emitted light is linearly proportional to the amount of ROS[5)] though the exact process of the luminol sonochemiluminescence has not been clarified.

2.3 Experimental setup

The experimental setup is shown in **Fig. 1**. An acrylic tank with a 128-channel 2D-array transducer was filled with degassed water. The transducer with a diameter of 120 mm and a focal length of 147.8 mm was driven at 1 MHz. The dissolved oxygen saturation of water at 25°C was 18-22%. HIFU was irradiated to a polyacrylamide gel which had been soaked in luminol solution (6 mM Na_2CO_3 and 0.7 mM luminol) for 24 hours being shaded and cooled at about 5°C. A single lens reflex camera (Nikon D500) was used to shoot the light emitted from the gel at a shutter speed of 30 s and an ISO sensitivity of 5000.

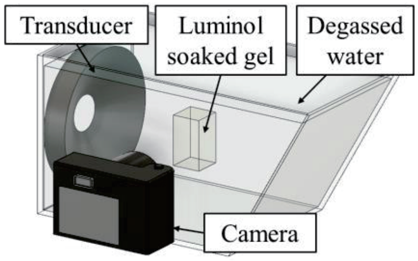

Fig. 1 Experimental setup

2.4 HIFU exposure sequence

The exposure sequence of HIFU is shown in **Fig. 2**. The number beside the waveform corresponds to the focal point presented in **Fig. 3**. In this study, HIFU was focused on two separate positions with the same distance from the geometric focus in the vertical direction. Firstly a trigger pulse was irradiated to focus ① for 0.1 ms. As soon as the trigger pulse finished, a sustaining burst was irradiated to focus ② for 10 ms. After a certain length of interval time t_1, another trigger pulse and a sustaining burst were sequentially transmitted to focus ③ and ④, respectively, followed by the interval time t_2 completing a cycle of the sequence. t_2 was equal to t_2 in this study. The repetition frequency ranged from 3.1 to 49.5 Hz and the cycle was repeated 90 times. The intensity of the trigger pulse and sustaining burst was 50 kW/cm^2 and 0.25 kW/cm^2, respectively.

The brightness of the emitted light in the images was integrated and compared for the intervals (t_1 and t_2) of 0, 5, 30, and 150 ms and the distance between two focal point (d_f) of 2.5 mm and 3.0 mm.

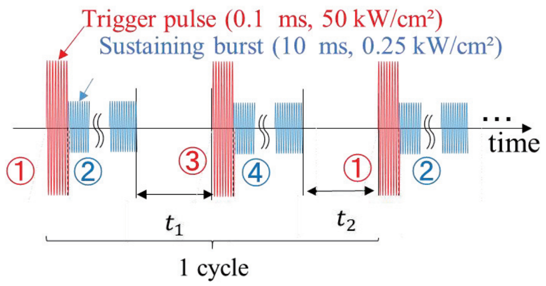

Fig. 2 HIFU exposure sequence

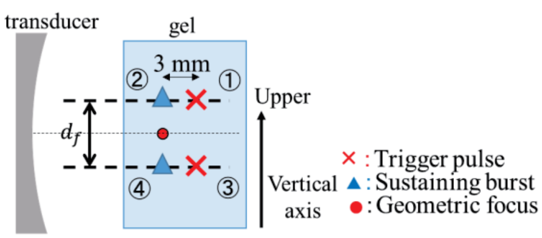

Fig. 3 Focal point in gel

3. Results and Discussion

Fig. 4 shows the integrated brightness of the light emitted from the luminol-soaked gel. At the interfocal distance of 3 mm, as the interval increased, the integrated brightness increased. At the interfocal distance of 2.5 mm, the brightness at intervals of 30 and 150 ms were comparable. It is considered that during a long interval, small cavitation bubbles which scattered the incoming trigger pulse were dissolved more, resulting in the better energy efficiency of ROS generation. However, a longer interval slows the rate of ROS generation. With no intervals, the integrated brightness was approximately 70% of that with an interval of 30 ms. This means that the ROS generation rate without intervals was 3-4 times higher than that with an interval of 30 ms.

In the previous study[5)], the integrated brightness was compared by changing the interval t_1 while the repetition frequency was kept constant at 3 kHz, i. e. $t_1 + t_2$ was kept constant. The results of this study enabled to discuss the effect of the intervals on the ROS generation efficiencies regarding energy and time more clearly.

When the intervals was increased from 30 to 150 ms, the integrated brightness was further increased at an interfocal distance of 3.0 mm but not at 2.5 mm. This was probably because the side lobes of HIFU, focused at the neighboring focus, may have elongated the life of HIFU-scattering small cavitation bubbles when the interfocal distance was 2.5 mm

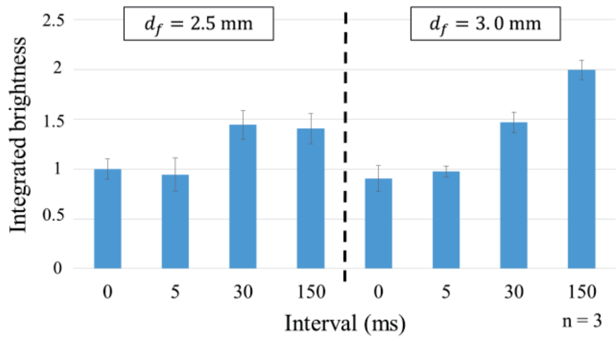

Fig. 4 Comparison of integrated brightness with several intervals

4. Conclusion

By changing the interval times in the HIFU sequence, the efficiency of ROS generation was compared. The energy efficiency was improved as interval gets longer, while highest ROS generation rate was obtained with no intervals in terms of time efficiency.

References

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