1Pa5-5

Development of efficient method of generating reactive oxygen species by expanding cavitation region using ultrasound focus scanning

Shotaro Miyake[1†], Shin-ichiro Umemura[2,3], and Shin Yoshizawa[1,2,3] ([1]Grad. School Eng., Tohoku Univ.; [2]Grad. School Biomed. Eng., Tohoku Univ.; [3]Sonire Therapeutics)

1. Introduction

HIFU is a minimally invasive treatment method in which ultrasound is irradiated from outside the body and focused on a small region inside the body without incisions. In the focal region, cavitation bubbles can be generated. Cavitation is a phenomenon in which bubbles are generated, oscillate, and may violently collapse. The oscillation and collapse of cavitation bubbles can produce thermal, mechanical, and chemical effects on the surrounding tissue. The chemical effect, focused on in this study, is the effect caused by the high temperature and pressure inside the bubble due to its volumetric contraction, which functions as a reaction field where reactive oxygen species (ROS) can be generated by decomposing water molecules. ROS have a cell-killing effect, which can be utilized in a treatment method called sonodynamic therapy[1].

In this study, we experimentally investigated method to efficiently generate ROS. By improving the efficiency of ROS production, it is expected to increase the treatment throughput while reducing the thermal effects on normal tissues.

2. Materials and Methods

Fig. 1 shows the experimental setup. A 128-channel array transducer (Japan probe) with a diameter of 147.8 mm and a focal length of 120 mm was used for HIFU irradiation. The transducer was driven by a staircase-wave driving system (Asahi TU-TX02) at a frequency of 1 MHz. A polyacrylamide gel phantom was placed in a water tank and used as the target for irradiation. Photographs were taken in a dark room to prevent the influence of external light. The exposure time of the DSLR camera was set to 30 s.

The HIFU focal points and exposure sequence are shown in **Fig. 2**. The irradiation time for the first focal point A was 90 μs to increase the probability of cavitation bubble cloud generation, and the irradiation time for the second and subsequent irradiations was 10 μs. The sequence shown in **Fig. 2** was used as 1 cycle, and 90 cycles were irradiated for 300 ms per cycle. Acoustic intensity in all irradiations was constant at 96 kW/cm^2. The ultrasound sequence with scanning the focus toward the transducer was created to utilize the mechanism of 'shock scattering'[2][3], in which a cavitation bubble cloud is formed by reflected waves from individual bubbles.

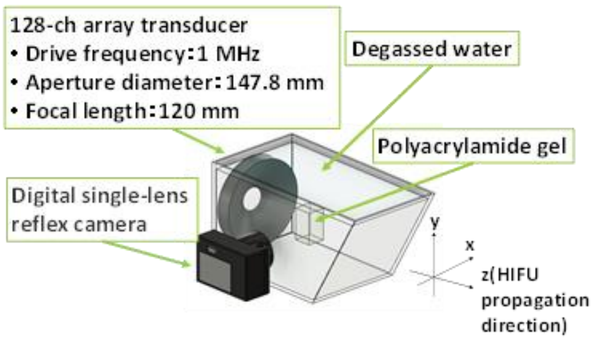

Fig. 1 Experiment setup

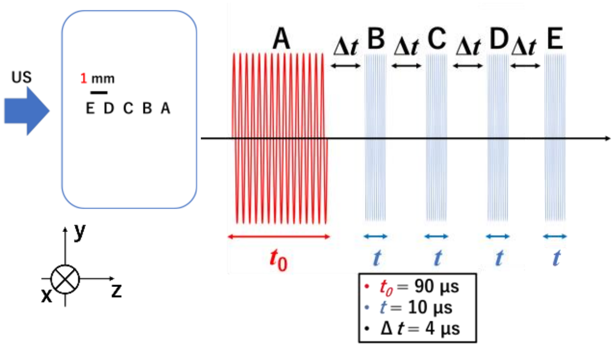

Fig. 2 HIFU focuses and exposure sequence

'Shock scattering' is based on the idea that a single bubble generated by the negative pressure of ultrasound plays the role of free-end reflection, and that the nonlinearly distorted incident wave with high positive pressure is reflected at the free end, generating a strong negative pressure, which in turn generates additional bubbles to form a cloud. By setting the focal points as described above, it is thought that the cloud generated immediately before can be used as a reflector to generate further clouds and ROS more efficiently.

ROS generation was evaluated using luminol chemiluminescence. Reactive oxygen species produced by ultrasound irradiation have a strong oxidative effect. Since luminol emits blue light when oxidized, the reaction between luminol and reactive oxygen species was photographed in a dark room, and the blue component was extracted from the images. Their values were used as brightness values to evaluate the amount of ROS production[4].

3. Results and Discussion

Experimental results are shown for the cases with one focal point and five focal points. Fig. **3** shows the brightness and **Fig. 4** shows the amount and efficiency of ROS production. The efficiency was obtained from the brightness values accumulated within the ROI in the image and divided by the input energy. The brightness and efficiency were normalized by those of one focus. The efficiency of the ROS production increased by a factor of about 1.3 in the case with scanned five focal spots, compared to the case with one focal spot.

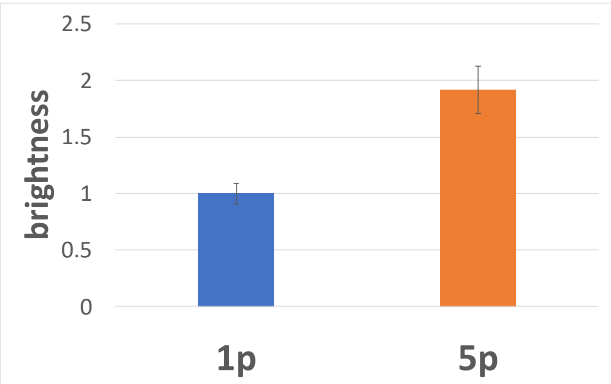

Fig. 3 ROS generation and brightness

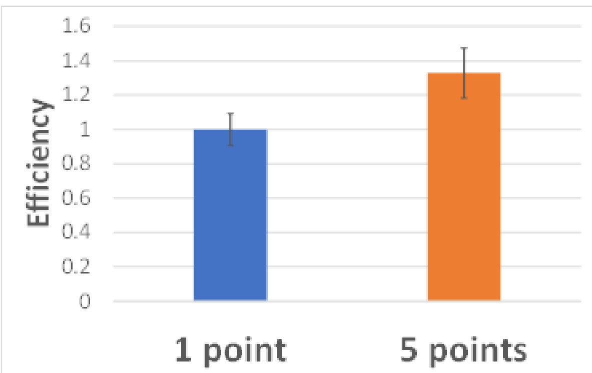

Fig. 4 ROS generation and efficiency

The images after extracting the blue component from the captured images, shown in **Fig. 5**, demonstrates that the cloud grew from the first cloud to the left side of the image (transducer side) when scanning the focus for five points. ROS emission from the second and later focal points was relatively uniform; in both cases, seen is a dark-colored area, corresponding to the left edge of the cloud at the first focal point. Although the cause has not been clarified at this time, it is thought to be due to the long irradiation time at the first focal point, and it is possible that reactive oxygen species can be generated more uniformly by setting appropriate parameters of ultrasound duration.

Further studies are needed to determine the possibility of expanding the cloud area and generating reactive oxygen species more efficiently by further increasing the number of focal points and optimizing the sequence.

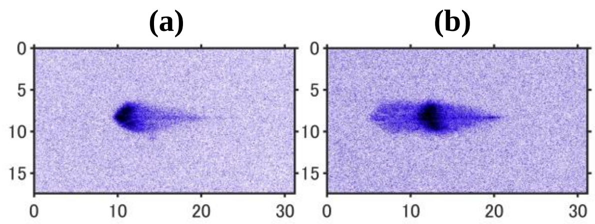

Fig. 5 Image after extracting blue component.
(a) After irradiation of only 1 focal spot
(b) After irradiation of 5 focal spots
(Unit : mm)

4. Conclusion

In this study, we experimentally investigated a method to efficiently generate ROS by setting multiple focal points in the direction of ultrasound propagation and expanding the region of cavitation cloud generation. The five-point irradiation was more efficient than the single-point irradiation in generating ROS. However, there are still some points to be improved, such as the uniformity in brightness within the region, which possibly will be improved by setting more appropriate irradiation parameters.

References

1. Guo-Yun Wan *et al.*: Cancer Biol. Med., 326, (2016)
2. A. D. Maxwell *et al.*: J Acoust. Soc. Am., 130, (2011).
3. Yige Li *et al.*: IEEE, VOL. 66, NO. 7, 1185, (2019)
4. D. Mashiko *et al.*: Jpn. J. Appl. Phys. 57 07LF13, (2018)