Proceedings of Symposium on Ultrasonic Electronics, Vol. 36 (2015)
5-7 November, 2015

Effect on red blood cell membrane induced by pulsed ultrasound

パルス超音波が赤血球膜に及ぼす作用

Masato Imura[1†], Kentaro Tani[1], Daisuke Koyama[1] and Yoshiaki Watanabe[1] ([1]Doshisha Univ.)
居村　真人[1†]，谷　健太朗[1]，小山　大介[1]，渡辺　好章[1] ([1]同志社大学)

1. Introduction

With recent development of ultrasonic diagnosis and treatment techniques, safety on a living body and the effect by high-intensity ultrasound should be considered. Effect on blood by high-intensity ultrasound can be evaluated by measuring the amount of protein flowing out from the inside of red blood cells [1]. Our group has reported the quantitative evaluation of the effects on blood by a standing-wave ultrasound field in the frequency range from tens kHz to 1 MHz by free hemoglobin (FHb) concentration [2]. Pulsed ultrasound is used in ultrasonic diagnosis techniques. In this report, we evaluated the effect on blood by pulsed ultrasound, through the observation of the shape of red blood cells.

2. Hemolysis

Hemolysis is the phenomenon in which the interior of the hemoglobin flows out to the red blood cells outside by the damage of the red blood cell membrane. Hemolysis can be evaluated by the total amount of FHb, and higher FHb concentration means higher degree of hemolysis. The main causes of hemolysis are physical impacts such as pressure and mechanical stress, and chemical elements, and the hemolysis has a risk to cause complications such as renal failure. Plasma component in blood is colorless and transparent and changes to red by the FHb when hemolysis occurred. It is possible to measure the amount of FHb through the absorbance of the plasma component by using a spectrophotometer (540 nm).

3. Experimental methods

Figure 1 shows the experimental system. An ultrasonic cell consists of an aluminum cylinder (length: 80 mm; inner diameter: 50 mm; outer diameter: 60 mm) and a circular ultrasound PZT transducer (thickness: 2 mm; thickness resonant frequency: 1 MHz). In order to prevent the effect by heat of the excited transducer during ultrasonic exposure, 135 mL of degassed water was poured into the ultrasonic cell. An aluminum cylinder (inner diameter: 10 mm; outer diameter: 12 mm) was used as a container of blood, and a thin parafilm was attached at the bottom of the cylinder. The container of blood was immersed in water on the central axis of the ultrasonic cell. 1.0 mL of bovine blood (Nippon Bio-test Laboratories ins.) was used for evaluation and adjusted by Alsever's solution for preservation solution (the mass ratio of 1:1). By applying the continuous sine-wave signal, an ultrasonic standing-wave field is generated in the blood sample. The blood samples were exposed to ultrasound with the maximum sound pressure amplitude of 24.9 kPa for 1 minute. The transducer was excited with 50- and 100-cycle burst-drives with several duty ratios ranged from 20 to 80% by changing the PRT in each condition. For comparison, the continuous drive was also performed. All the experiments was conducted with the blood temperature at approximately 25℃. After ultrasound exposure, the blood sample were centrifuged for 5 minutes with 2000 G by a centrifuge separator (MILLPORE, CHIBITAN-R) to separate the supernatant that consists mainly of plasma and precipitates component including

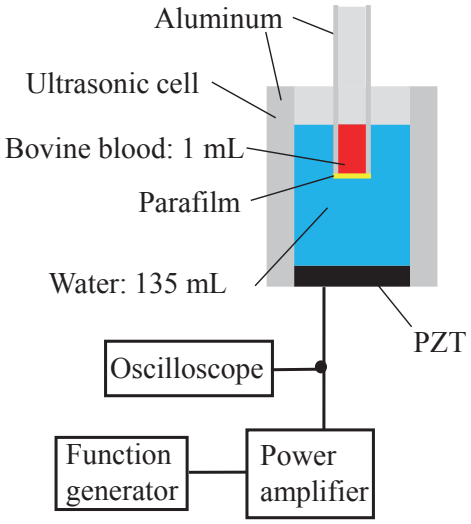

Fig. 1 Experimental system.

dkoyama@mail.doshisha.ac.jp

normal red blood cells. 10 μL of the supernatant was sampled and sandwiched between a slide glass and a cover glass, and observed by an optical microscope. This experimental procedure from the sample preparation to the optical observation was repeated four times in each condition.

4. Results

The temperature rises of the blood sample in each condition were within 5 ℃; the blood temperature did not rise up to 40℃ at which the protein will be denatured. Therefore, in these experiments, the temperature rise had little influence on red blood cells. **Figure 2** shows photographs of supernatants after ultrasound exposure in each condition. Comparing with the supernatant before ultrasound exposure (control), the color of the supernatant with 50 cycle and duty ratio of 80% turned to red obviously, and this implies that the blood sample is hemolyzed.

Figure 3 shows the representative microscopic images of red blood cells in (a) the blood before ultrasound exposure and (b) the supernatant after ultrasound exposure. Since the red blood cells are hardly present in the supernatant after centrifugation, the red blood cells in Fig. 3(b) are referred to "ghost red blood cells"[3], which are empty cell membranes without contents of hemoglobin. In the microscopic observation, the transmitted light intensity through the ghost red blood cell is larger than that through the normal red blood cells because the light is not refracted at the cell surface. **Figure 4** shows the changes in the number of ghost red blood cell per 1 μL in each condition. The number of ghost red blood cells increased with the duty ratio of the ultrasonic irradiation and had the maximum value with the 50 cycle and duty ratio of 80%. Although the tendency that the hemolysis increases with the duty ratio can be seen, further study is required since the dispersions are quite large.

5. Conclusion

In this report, we examined the red blood cell damaged by ultrasound exposure using bovine blood. As a result of microscopic observation of the supernatant after the ultrasound exposure, ghost red blood cells were observed. The number of ghost red blood cells increased with the duty ratio of the ultrasonic irradiation.

6. Acknowledgement

This work was partially supported by a research grant from Bilateral Joint Research Project by JSPS.

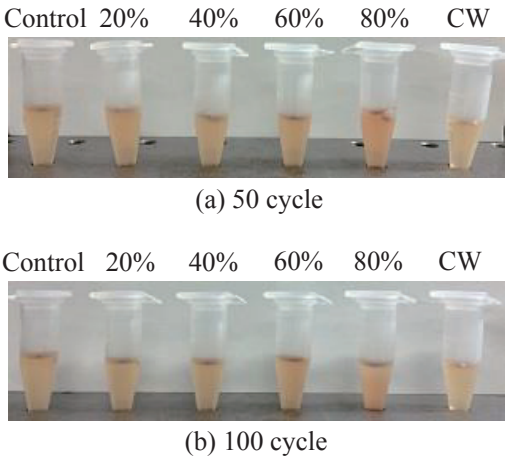

Fig. 2 Photographs of the supernatant at (a) 50 and (b) 100 cycles.

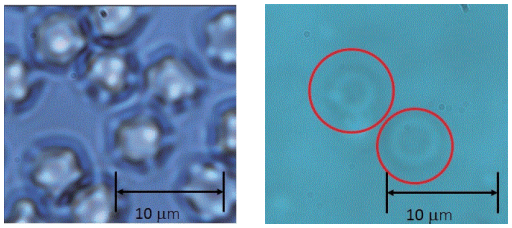

Fig. 3 Microscopic images of (a) red blood cells and (b) ghost red blood cells.

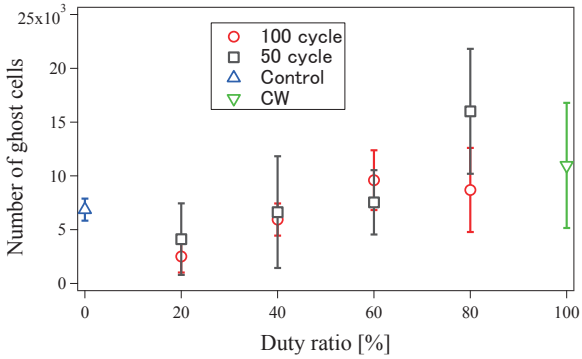

Fig. 4 Relationship between the duty ratio and the number of ghost red blood cells.

References

1. S. Kawan, M. Kobayashi and M. Okujima, Technical report of IECE. US 97(333), 37-43, 1997.
2. K. Mino, M. Imura, D. Koyama, M. Omori, S. Kawarabata, M. Sato and Y. Watanabe, Ultrasound Med. Biol., 41(2), 465-471, 2015.
3. M. Nose, S. Amagaya and Y. Ogihara, Chemical & pharmaceutical bulletin. 37(12), 3306-3310, 1989.