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Effect of microbubble vibration on HeLa cells under ultrasound irradiation

超音波照射下におけるマイクロバブルの振動が HeLa 細胞に与える影響

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1. Introduction

To reduce adverse side effects in medication, the temporal and spatial control of drug in live body is required. In ultrasound drug delivery system, microbubbles decorated with surfactants can be adsorbed to only the target tissues and deliver the medicine through blood flow[1]. The bubbles can achieve the local drug release with the collapse of bubbles under sonication. When the microbubbles adhere to target cells, the microjet or shock wave generated by the collapse of bubbles enable the perforation through the cell membrane (sonoporation)[2], resulting in effective drug administration.

To establish the safety criteria and realize the accurate medication, the effect of microbubble vibration and collapse on cells should be evaluated quantitatively. In this report, the effect of bubble vibration and collapse on adhesive cells were investigated by in vitro ultrasound experiments and the rate of dead cells were evaluated.

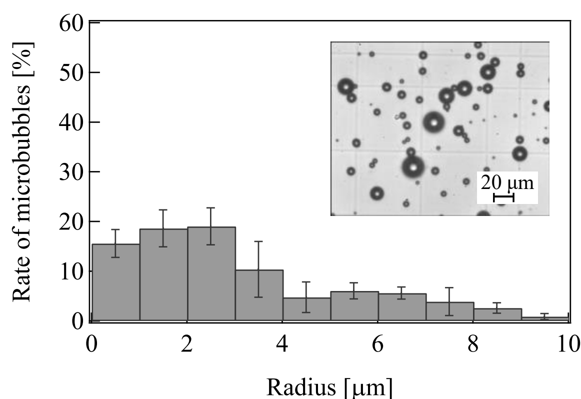
2. Methods

2.1 Fabrication of microbubbles

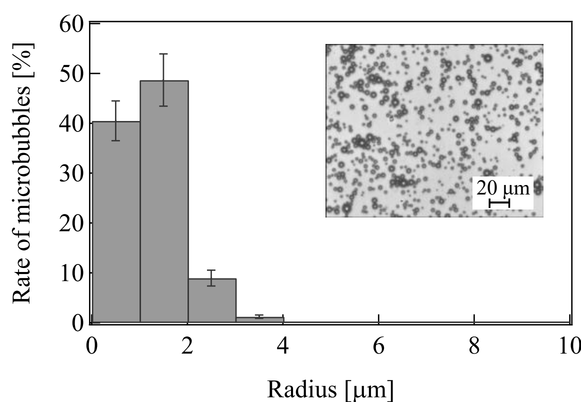
Phospholipid solution composed of L-α-phosphatidylcholine-distearoy1 (DSPC, 15 mg), 1, 2-distearoyl-sn-glycero-3-phosphoethanolamine (DSPE, 10 mg), PEG-monostearate 40.E.O. (10 mg), and phosphate buffered saline (PBS, 10 mL) was prepared. A syringe was filled with 1 mL of the solution, and another syringe was filled with 2 mL of fluorocarbon (C_4F_8 of 8% and N_2 of 92%). By connecting the two syringes and stirring 30 times by hands, microbubble solution was fabricated.

2.2 Size distribution of microbubbles

The size distribution of microbubble can be controlled by the stirring conditions and the buoyancy of microbubbles. Two bubble samples with different size distribution were prepared. The solution with larger bubbles was fabricated through the difference of buoyancy (sample 1). The solution with smaller bubbles was fabricated by stirring the bubble solution by an ultrasonic homogenizer for 1 minute (sample 2). **Fig. 1** shows the size distributions of bubbles in two samples. The error bars express the standard deviation for three times.



(a) Sample 1



(b) Sample 2

Fig. 1 Size distributions of DSPC microbubbles.

2.3 Experiment for sonication

Fig. 2 shows the experimental setup for ultrasound irradiation. A water tank was filled with degassed water, and a PZT ultrasound plane

transducer (diameter: 32 mm and resonance frequency: 1.0 MHz) was employed. HeLa cells purchased from the Institute of Physical and Chemical Research, Japan, were used as adhesive cells. The HeLa cells were cultured on a slide glass coated with collagen in an incubator for 48 hours. The slide glass was installed at the glass bottom of a culture dish, and 5 μL of propidium iodide (PI) solution and 3 mL of culture medium were added to the dish to measure the viability of the cells under fluorescent observation. 50 μL of the microbubble solutions were added to investigate the effects of microbubbles, and the dish was installed at the water surface above the ultrasound transducer. Pulsed sinusoidal signal with 100 cycles at 1 MHz was input to the transducer, and the maximum negative sound pressure at the bottom of the dish was controlled to be 1.0 MPa.

3. Results and discussion

Fig. 3 shows the rates of dead cells in cases without (control) and with microbubbles (sample 1 and 2 in Figs. 1(a) and (b), respectively). The error bars express the standard deviation for 4 trials. Comparing with the control, the rates of dead cells increased significantly in the cases with the microbubbles. These results imply that the bubble vibration and collapse under ultrasound irradiation induced the shock wave and microjet and affected the cell viability. It is well known that the vibrational amplitude of bubbles is maximized under the resonance condition, and the resonance radius of an air bubble at 1.0 MHz is approximately 2.4 μm [3]. Therefore, the microbubbles in sample 2 with the average radius of 1.3 μm gave higher rate of the dead cells compared with that with sample 1. It should be noted that the error bar in the results with sample 2 was increased dramatically as shown in Fig. 3. Miller *et al*. reported that the damage of cells could not confirmed under sonication when the distance between the cells and microbubbles was more than 1 mm [4]. The distance between the cells and bubbles is one of the important factors and should be taken into account to evaluate the effects on cells precisely.

4. Conclusion

In this report, the effects of microbubble vibration on HeLa cells under ultrasound irradiation was evaluated under fluorescent observation. It was found that the microbubbles with the resonance size induced the shock wave and the microjet and enhanced the rate of dead HeLa cells.

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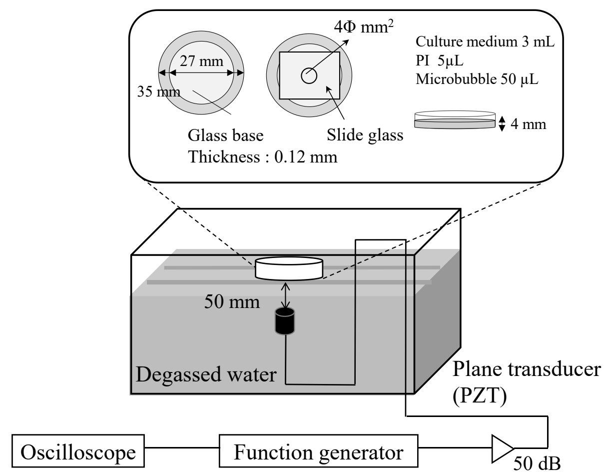

Fig. 2 Experimental setup.

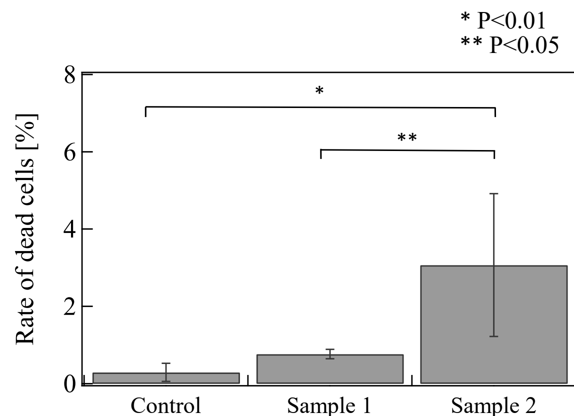

Fig. 3 Rates of the dead cells without (control) and with microbubbles (sample 1 and sample 2) at 1.0 MHz and 1.0 MPa.

Acknowledgment
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References

[1] Alexander L. KIibanov,"Targeted delivery of gas-filled microspheres, contrast agents for ultrasound imaging", Advanced Drug Delivery Reviews 37 pp. 139-157, 1999
[2] Nobuki Kudo, Katsuyuki Yamamoto, "Sonoporation with Microbubbles Exposed to Pulsed Ultrasound", Seitaiikougaku 43 (2) :231-237, 2005 (in Japanease)
[3] Hiraku Tabata, Shu Suematsu, et al.…"Acoustic destruction of microcapsules and release of internal substance" IEICE Technical Report US2012-74（2012－11）
[4] Miller DL, Quddus J: "Sonoporation of monolayer cells by diagnostic ultrasound activation of contrast agent gas bodies." Ultrasound Med Biol.26 (4) 661-667, 2000.