Comparison of damage in vascular endothelial cells surrounded by microbubbles under ultrasound irradiation according to presence condition of the cells

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1. Introduction

In recent years, cell immunotherapy, in which therapeutic cells are injected into the bloodstream, has been attracting attention as a new cancer treatment method in order to reduce side effects such as recurrence and suppression of metastasis. However, there is a problem that the injected cells are dispersed in the bloodstream. As a solution, our laboratory proposes an *in vivo* delivery system that induces and retains therapeutic cells to an arbitrary position in blood vessel network. Thus, we have been conducting research for ultrasound therapy using bubble-surrounded cells (BSCs) [1,2] or a thin catheter [3,4]. Considering those conditions, because we have ever examined damage of vascular endothelial cells in a floating condition [5] not in an adherent condition, there was a limitation to assume that of the blood vessels. Thus, we investigated the cell viability under similar condition of ultrasound exposure in an adherent condition.

2. Methods

In this experiment, bovine carotid artery vascular endothelial cells (HH cells) were used as the target cells, and lipid bubble liposomes (LBs) were used, an artificial blood vessel which has a square cross-section of width 2.0 mm and height 2.0 mm and made of PDMS was used. In the bottom of the vessel, collagen film was formed as a basement membrane. Then the cells were seeded with a cell concentration of 300 cells/mL and cultured in a CO_2 incubator at 37 °C for 24 h to achieve a conditions of cell adhesion. Then, the vessel was filled with LBs concentration to be set on the stage of the experimental setup shown in **Fig. 1**. The water tank was filled with degassed water, and an ultrasound transducer with a center frequency of 3 MHz was set at the bottom of the water tank. The distance between the path and the transducer was l = 65 mm, which corresponds to the near-field limit of the transducer. The maximum sound pressure was limited to 400 kPa-pp. After ultrasound exposure, the cells in the artificial blood vessel were incubated in a CO_2 incubator for 1 hour. The situation of the cells in the vessel was shown in **Fig. 2**, where the cells were propelled to the vessel wall by acoustic radiation force, and meanwhile, exposed the destruction of LBs in other surface.

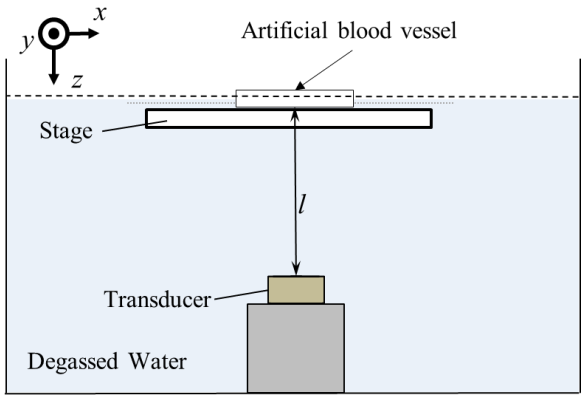

Fig.1 Experimental setup to expose ultrasound to cells in an adherent condition in an artificial blood vessel.

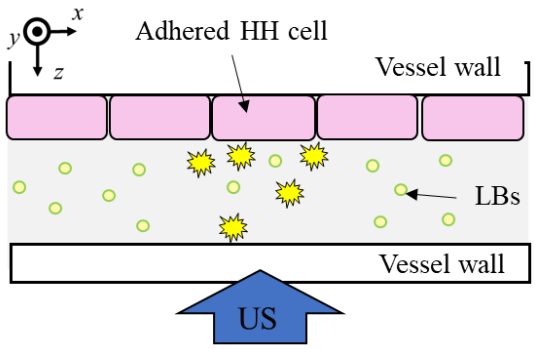

Fig.2 Situation of the adherent cells in LBs suspension in the vessel.

After the incubation, the vessel wes once washed by PBS to inject a solution of Calcein-AM and propidium iodide (PI) solved in PBS before the incubation for 20 min. After removing a solution, fluorescent images were acquired using a fluorescence microscope (Olympus, BXFM) and a digital camera (Olympus, DP74). The number of

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cells were measured using analysis software (NIPPON ROPER, Image pro plus) to derive cell viability.

3. Results

Fig. 3 shows the fluorescent images with the different conditions of ultrasound exposure, which are the maximum sound pressure of 200, 400 and 400 kPa-pp at LBs concentrations of 0.3, 0.3 and 0.5 mg/mL, respectively, with a continuous wave and a fixed exposure time of 60 s. Most cells were living (green), where the beam width of the ultrasound exposure was 2 mm. The number of dead cells (red) were seen with higher sound pressure.

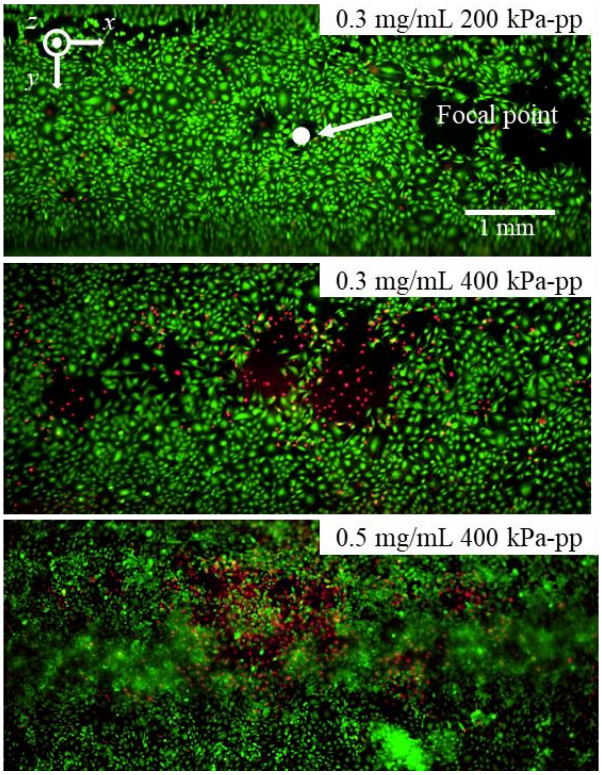

Fig.3 Fluorescent images of adherent cells after ultrasound exposure (living cells: green, dead cells: red)

Fig. 4 shows the cell viability with respect to the sound pressure according to the parameter of the LBs concentration. Cell viability decreased in proportion to the sound pressure and the LBs concentration. Also, there was no significant difference in cell viability between the sound pressures of 300 and 400 kPa-pp, which might be a immunity to sound intensity. **Fig. 5** shows the comparison of the cell viability between adhering and floating, as the condition of the presence of cells, at the LBs concentration of 0.3 mg/mL, where the latter results were derived from [5]. Higher cell viability can be expected with the adhering situation than the floating situation**.**

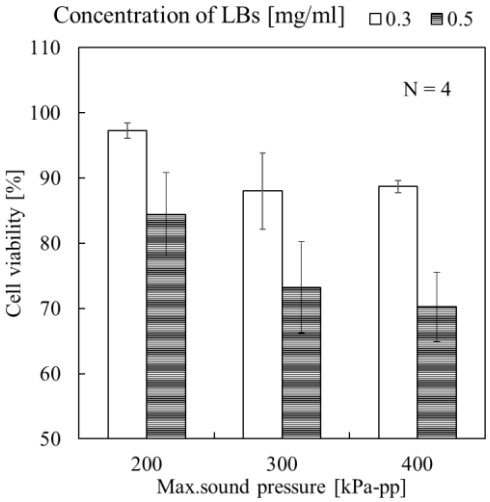

Fig.4 Comparison of cell viability with adhering cells according to sound pressure.

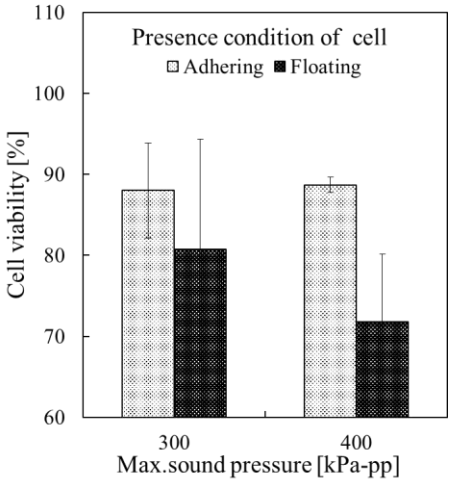

Fig.5 Comparison of cell viability between the adhering and the floating conditions of the cells.

4. Conclusion

We have verified the effects on cells under ultrasound exposure with different presence conditions of the cells. Although we confirmed the decrease of the cell viability according to the sound pressure and LBs concentration as well as our preceding research, less damage to the cells in adhering condition was significantly found. We are going to verify the cytoprotective effect of LBs adhering to the cells [5] in the adhesion condition of the cells.

References

1. M. Seki, et al, JJAP, 58, 2019.
2. T. Chikaarashi, et al, JJAP, 61, 2022
3. H.Ushimizu, et al, JJAP, 57, 2018.
4. J. Takano, et al, JJAP, 59, 2020.
5. Y. Ito, et al, JJAP, 61, 2022.