3P5-10

Comparison of damage in vascular endothelial cells on basement membrane according to surrounding microbubbles and irradiation direction of ultrasound

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1. Introduction

In recent years, cell immunotherapy, which use the patient's own immune cells, has been attracting attention as a new cancer treatment. However, there is a problem that immune cells injected into blood vessels are dispersed in the bloodstream and resulting low accumulation of immune cells at the target site. As a solution, our laboratory proposes an *in vivo* delivery system that using microbubbles and acoustic radiation force. Our group had previously reported a technique of active cell control based on the formation of bubble-surrounded cells (BSCs) in which bubbles are attached to the cells via appropriate ligands [1,2]. In this process, there is a concern about cell damage caused by cavitation of bubbles surrounding cells. To examine the effects of damage on cells, our previous study [3] compared the cell viability using not only attached bubbles but also floating bubbles, where the results indicated that attached bubbles to cells might have a protective effect to the cells. However, the presence condition of the cells in the previous experiments was floating in medium, which was different from a situation of *in vivo* blood vessel. Therefore, we investigated the effect of the cells adhered on wall according to ultrasound irradiation directions, which are the direction toward to the wall, and the direction from behind the wall, with various presence situations of bubbles.

2. Methods

In this experiment, we used bovine carotid artery vascular endothelial cells (HH cells) and lipid bubbles (LBs), where we call the modified bubbles with cRGD peptides that bind specifically to the cells as LBs (+). In contrast, the bubbles without modification are called as LBs (–). In the bottom of artificial blood vessel made of PDMS, which has a rectangular cross-section of width 2.0 mm and height 1.0 mm, collagen film was coated 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 on vessel wall. Then, the vessel was filled with LBs suspension to be set on the stage of the experimental setup shown in **Fig. 1**. In the bottom of water tank, which was filled with degassed water that was kept at 37 ˚C, an ultrasound transducer to emit plane wave with a center frequency of 3 MHz was installed. The distance between the vessel 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, and the maximum irradiation time is 60 s. After ultrasound exposure, the vessel was moved to a CO_2 incubator to culture the cells for 1 hour. The difference of the situations of ultrasound irradiation direction is shown in **Fig. 2**.

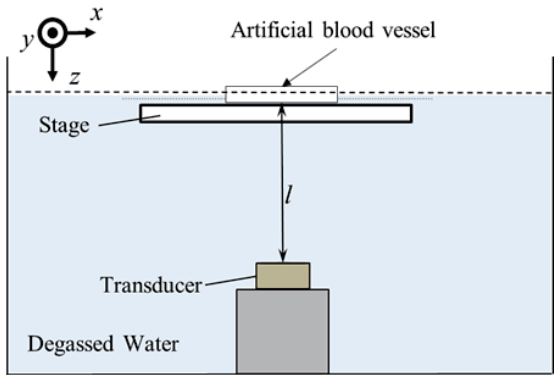

Fig.1 Experimental setup to expose ultrasound to cells in an adherent condition in an artificial blood vessel.

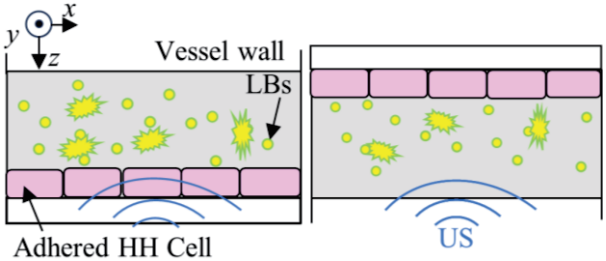

Fig.2 Situations of ultrasound irradiation direction to adherent cells in the vessel (Left: the direction from behind the wall, Right: the direction toward the wall).

After the incubation, living cells were stained with Calcein-AM and dead cells were stained with propidium iodide (PI). After removing a solution, fluorescent images were acquired using a

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fluorescence microscope (Olympus, BXFM) and a digital camera (Olympus, DP74). The number of cells were measured using analysis software (NIPPON ROPER, Image pro plus) to derive cell viability [3].

3. Results

Fig. 3 shows the fluorescent images including living and dead cells with the ultrasound irradiation direction from behind the wall, where other conditions are the combinations between maximum sound pressure of 200 or 400 kPa-pp and LBs (–) concentrations of 0.3 or 0.5 mg/mL. Although the number of living cells (green) occupied the entire image, the lower the maximum sound pressure, the greater the number of dead cells (red) near the center of the images (focal point).

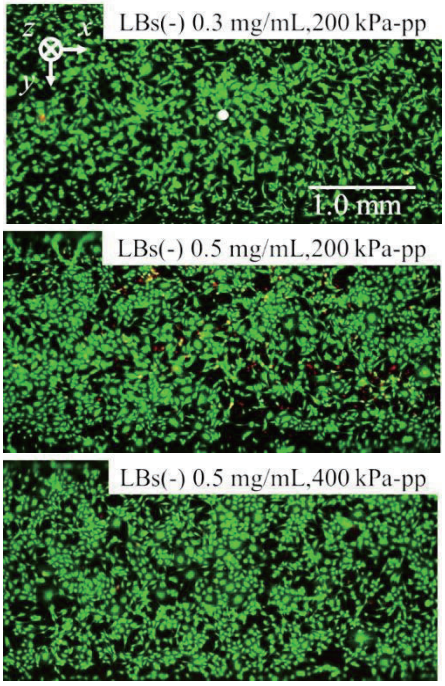

Fig.3 Fluorescent images of adherent cells after ultrasound exposure (living cells: green, dead cells: red)

Fig. 4 shows the cell viability with respect to the sound pressure at LBs concentration of 0.5 mg/mL with the ultrasound irradiation direction from behind the wall. In LBs (–), cell viability increased in proportion to maximum sound pressure, whereas there was no variation in LBs (+). **Fig. 5** shows the comparison of cell viability between two ultrasound irradiation directions with the presence of LBs (–) at the maximum sound pressure of 300 kPa-pp**.** The results showed that cell viability decreased in proportion to the concentration of LBs (–), which suggests a similar tendency with the results using floating cells [3]. However, comparing with ultrasound irradiation directions, higher cell viability was confirmed with the direction from behind the wall, which suggests that there was less damage on the cells since the LBs were propelled away from the wall.

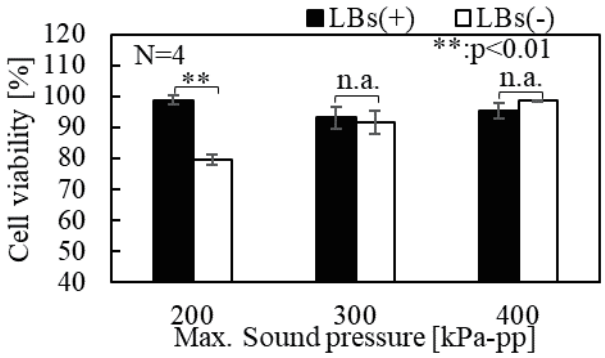

Fig. 4 Comparison of cell viability between LBs (+) and LBs (–) at 0.5 mg/mL according to sound pressure.

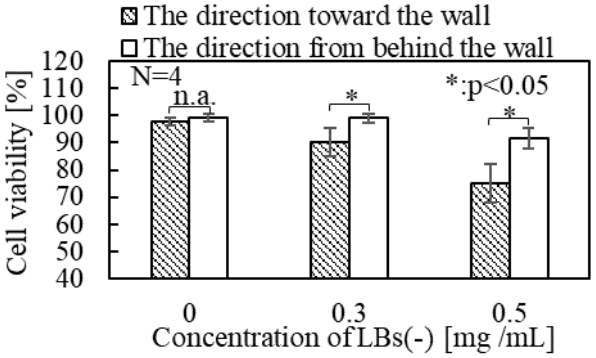

Fig. 5 Comparison of cell viability between the ultrasound irradiation directions with LBs (–).

4. Conclusions

We have verified the effects on cells adhered on a wall with various experimental conditions; ultrasound irradiation directions, types of LBs, and their concentrations. The results suggested the confirmation that LBs (+) have a potential to protect the cells as well as our preceding results with floating cells. Also, higher cell viability was confirmed with the direction from behind the wall than the direction toward the wall. Therefore, to minimize cell damage on inner surface of blood vessel, those results will be reflected to ultrasound irradiation strategy in a future *in vivo drug* delivery.

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References

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