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Viability validation of therapeutic cells according to surrounded amount of microbubbles and ultrasound exposure condition

表面に付着した微小気泡量と超音波照射条件に対する治療用細胞の生死検証

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1. Introduction

Recently, cellular immunotherapy has been recognized to be a new cancer therapy to reduce side effects as relapse and metastasis inhibitory effect, where the therapeutic cells are injected into the bloodstream. Since the dispersion of the cells in blood flow, there is a fundamental problem of the limitation of accumulation at the target area. To address this problem, a breakthrough idea has been proposed for *in vivo* delivery, which produces bubble-surrounded cells (BSCs) by attracting microbubbles to the surface of cells to reduce their density [1,2] and to be propelled using an acoustic radiation force. We confirmed that controllability is enhanced in BSCs compared with cells without bubbles [2,3]. Also, we confirmed that it is important to adopt the ultrasound exposure against conditions of flow velocity and concentration of the BSCs [4]. However, mechanical or biological damage to the cell, which were contained in a BSC, according to the conditions of ultrasound exposure has not been clarified. Therefore, in this study, we carried out the validation of cell viability versus various conditions of ultrasound exposure.

2. Methods

In this study, we have modified bubble liposomes (BLs) [5] with anti-CD8 antibody to covalently linked to the external surface of killer T-cells, which were derived from mouse. Fig.1 shows the microscopic images of BSCs using a confocal microscope, which were extracted from the suspension of BLs of 0.3 mg lipid/mL and the cells of 1.0 x 10^5/mL. There are two BSCs in the images, where the left one was obtained with optically filtered to only BLs (green), and the right was that to the cells (red) and superimposed onto the left. BLs are uniformly attached around the cell.

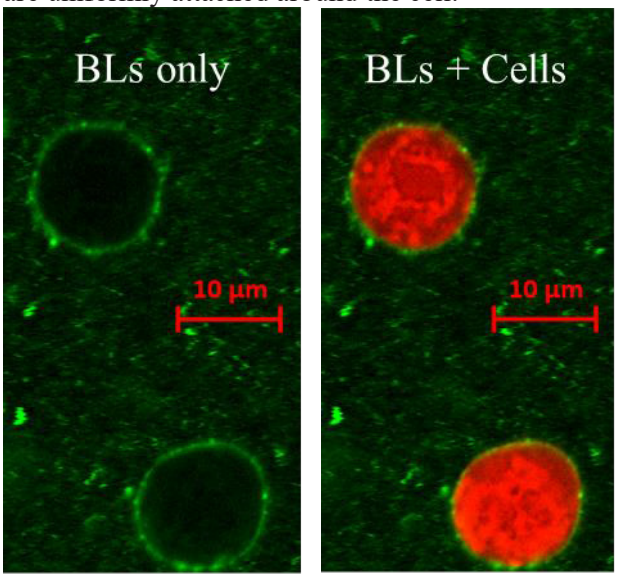

Fig.1 Confocal microscopic images of BSCs.

Fig.2 shows the experimental setup, where a suspension of BSCs was injected for 0.1 ml per well in a plate. An ultrasound transducer of 3 MHz was set at a distance l = 65 mm, which is corresponded to the focal distance, away from the center of the suspension. The condition of ultrasound exposure includes sound pressure, exposure time, and duty ratio of burst wave. Concentration of the cells was fixed to 1.0 x 10^5/mL. After the exposure, the cells were cultured in the well for 24 hours and then applied a colorimetric assay (Cell Counting Kit-8, 0.01 mL/well). After incubating in CO_2 for 4 hours at 37 ˚C, the absorbance in the well at 450 nm was measured. Finally, cell viability rate α was obtained using eq. (1).

$$\alpha = \frac{I_{Sample} - I_{Blank}}{I_{Control} - I_{Blank}} \times 100 \qquad (1)$$

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I_{Sample}: Absorbance of the suspension after ultrasound exposure
$I_{Control}$: Absorbance of the suspension without ultrasound exposure
I_{Blank}: Initial average absorbance without suspension

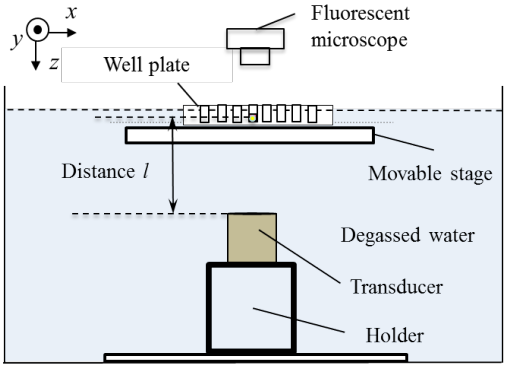

Fig.2 Experimental setup to expose ultrasound to suspension of BSCs in a well.

3. Results

Fig.3 shows the results of cell viability versus maximum sound pressure of ultrasound exposure with continuous wave (duty ratio of 100%), and exposure time of 60 s. Concentration of BLs was 0.3 mg lipid/mL, which was common on the comparison of the cells only. Cell viability was not affected by ultrasound exposure without BLs, whereas it was affected with BLs when the maximum sound pressure was 400 kPa-pp.

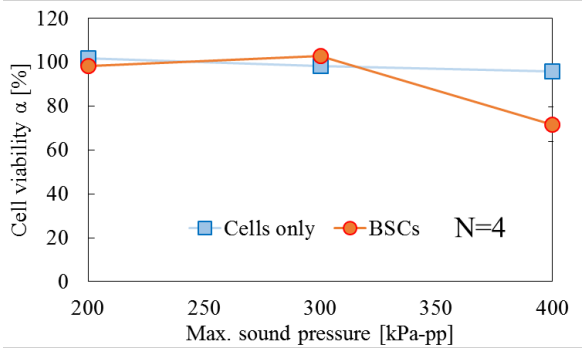

Fig.3 Cell viability versus maximum sound pressure with and without BLs.

Fig.4 shows the results of cell viability in BSCs versus net exposure time, which was calculated by multiplying total exposure time with duty ratio, for the comparison with burst waves. The maximum sound pressure of ultrasound was fixed with 400 kPa-pp. Concentration of BLs was 0.3 mg lipid/mL. With continuous wave, the cell viability decreased to 70% within the net exposure time of 20 s. In contrast, although there are much variations, a tendency that the cell viability decreased in proportion of net exposure time with burst wave, was confirmed.

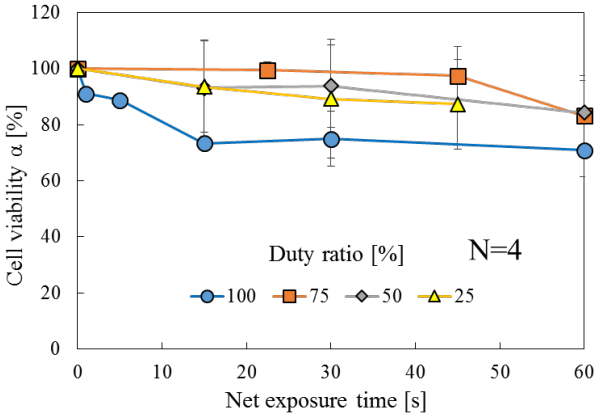

Fig.4 Cell viability versus net exposure time with continuous and burst wave.

Finally, cell viability with a parameter of BLs concentration is shown in Fig.5, where the maximum sound pressure of continuous wave was fixed with 400 kPa-pp. Cell viability clearly decreased in proportion to both of BLs concentration and exposure time. BLs concentration of 0.5 mg lipid/mL is more than the saturation of the adhesion on the cells [4]. From these results, surrounded amount of BLs assured to enhance the damage of the cells contained in BSCs upon ultrasonic exposure.

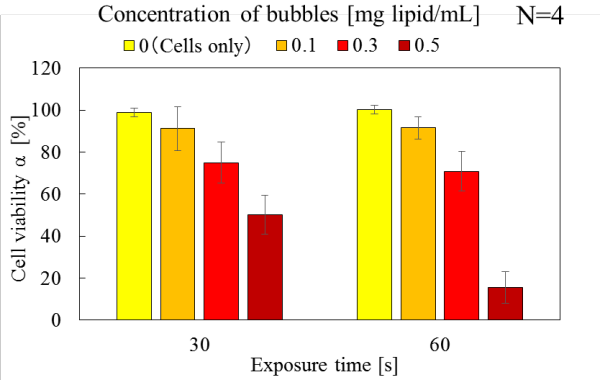

Fig.5 Cell viability versus exposure time of ultrasound and BLs concentration.

4. Conclusion

We have verified cell viability in BSCs with various conditions of ultrasonic exposure and BLs concentration. We have confirmed that the surrounded amount of BLs decreases the cell viability under continuous wave exposure rather than burst wave. In the next step, we are going to develop with a condition similar to *in vivo* environment.

References

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