3P5-5

Viability variation of T-cells under ultrasound exposure according to adhesion condition with microbubbles

微小気泡との接着状況に対する超音波照射下の T 細胞の生存率の変化

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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. To disperse 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]. So far, mechanical or biological damage to the cell, which were contained in a BSC, according to the conditions of ultrasound exposure, has been studied [5]. However, when frequency is changed and when bubbles are near a cell and not on a cell, damage to the cell has not been clarified. Therefore, in this study, we carried out the validation of cell viability versus various conditions of ultrasound exposure and adhesion condition with microbubbles.

2. Methods

In this study, we used lipid bubble (LBs) [6] and killer T-cells, which were derived from mouse. The suspension was made by stirring LBs of 0.3 mg lipid/mL and the cells of 1.0 x 10^5/mL. Fig.1 shows two images, where the left one shows bubbles are near a cell, and the right shows bubbles are on a cell [5]. In the following experiment, when cells are only or bubbles are near a cell, we experimented.

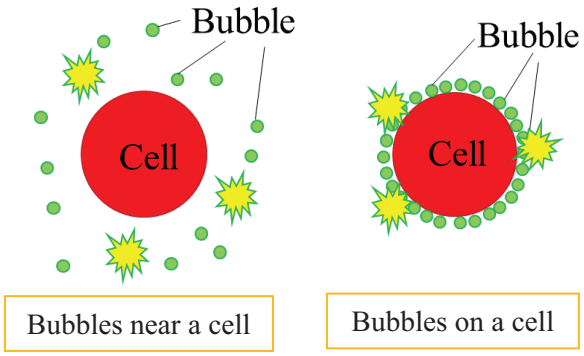

Fig.1 The relation between a cell and bubbles

A suspension of cells and bubbles was injected for 0.1 ml per well in a plate. An ultrasound transducer of 1 or 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 water temperature of the thermostatic bath filled with deaerated water was set to 37 ° C. The condition of ultrasound exposure included sound pressure, exposure time. Concentration of the cells was fixed to 1.0 x 10^5/mL. Fig.2 shows a well of microscopic images before and after ultrasound exposure. 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).

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

I_{Sample}: Absorbance of the suspension after ultrasound exposure

$I_{Control}$: Absorbance of the suspension without ultrasound exposure

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I_{Blank}: Initial average absorbance without suspension

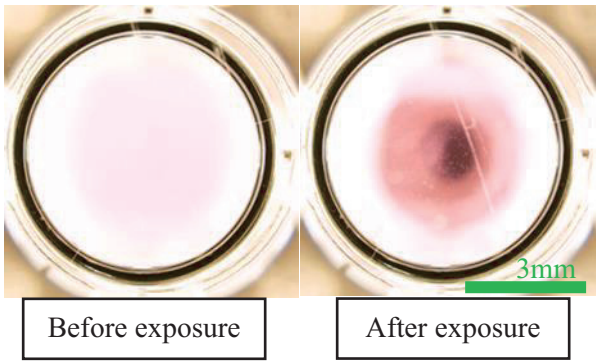

Fig.2 A well before and after ultrasound exposure

3. Results

Fig.3 shows the results of cell viability of 1 MHz versus ultrasound exposure time with continuous wave. Concentration of LBs was 0.3 mg lipid/mL, which was common on the comparison of the cells only. The legend on the Fig.3 shows each concentration of bubbles and the maximum sound pressure. Cell viability was affected by maximum sound pressure and concentration of bubbles. The lowest cell viability was 66.7% when the LBs concentration was 0.3 mg/mL and the maximum sound pressure was 400 kPa-pp.

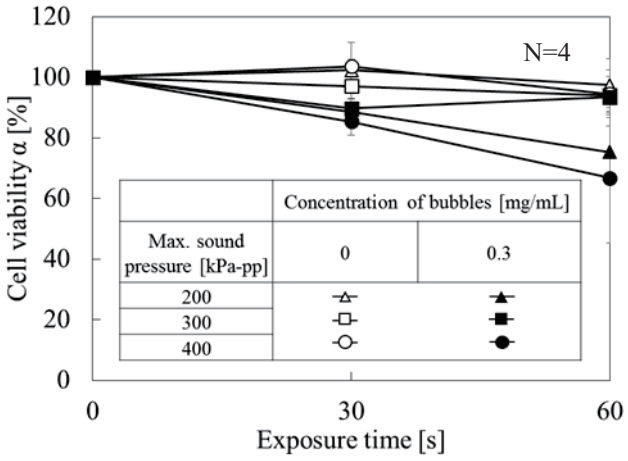

Fig.3 Cell viability at 1 MHz versus exposure time with and without LBs

Fig.4 shows the results of cell viability of 1 MHz versus ultrasound exposure time with continuous wave. The legend on the Fig.4 is the same as Fig.3. Cell viability was affected by maximum sound pressure and concentration of bubbles. The lowest cell viability was 66.7% when the LBs concentration was 0.3 mg/mL and the maximum sound pressure was 400 kPa-pp.

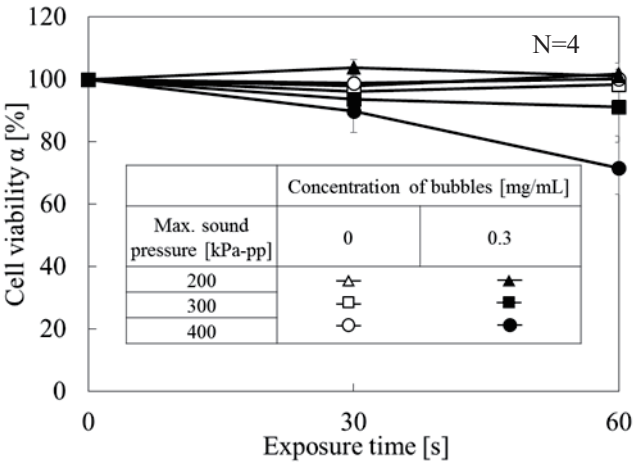

Fig.4 Cell viability at 3 MHz versus exposure time with and without LBs

In addition, Fig.5 shows the difference of cell viability according to adhesion condition with microbubbles. The exposure conditions were the maximum sound pressure of 400 kPa-pp, the frequency of 3 MHz, the exposure time of 30 s, and the microbubble concentration of 0.3 mg/mL. There was a tendency that the cell viability in case of presence of bubbles near a cell was higher than that on a cell [5].

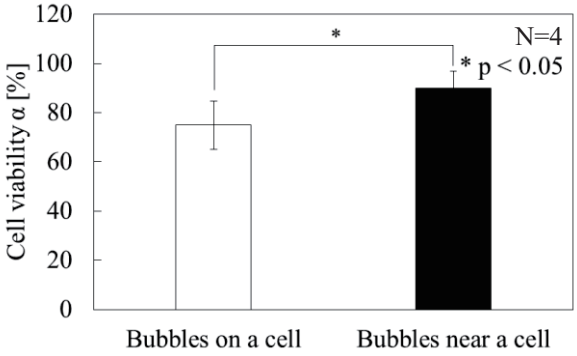

Fig.5 Cell viability at 3 MHz according to adhesion condition with microbubbles

4. Conclusion

We have verified cell viability with various conditions of ultrasound exposure and LBs concentration. We have confirmed that cell viability tended to decrease with increasing exposure time, maximum sound pressure, and LBs concentration at both 1 and 3 MHz frequencies.

References

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