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Validation of damage on vascular endothelial cells under ultrasound exposure according to adhered density of bubbles

血管内皮細胞に付着した微小気泡の密度に対する超音波照射下での細胞損傷の検証

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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]. However, despite there are various interaction conditions between vascular endothelial cells and bubbles, e.g. bubbles are floating near the cell, or adhering on the cells, validation of cell damage under ultrasound exposure was not sufficient. Therefore, we investigated the cell viability versus various conditions of bubbles adhesion with ultrasound exposure.

2. Methods

In this study, bovine carotid artery vascular endothelial cells (HH cells) were used as the target cells, and lipid bubble liposomes (bubbles) were used. On the surface of the bubbles a cRGD peptide ligand can be modified to specifically adhere to the cells. Here we define four situations between the cells and the bubbles as shown in **Fig. 1**, where "No bubble" indicates the cells only. Other situations are following; "with surrounding bubbles (SB)" as the bubbles are floating without adhesion on the cells, "with adhered bubbles (AB)" as the bubbles contact on the cells without floating, and "with surrounding and adhered Bubble (SAB)" as the bubbles are both adhered and floating. In the situation of SB, bubbles without a ligand modification was used. The method of BSCs production and validation of damage on the cells were executed as well as our preceding researches, which includes ultrasound exposure method and the viability using CCK-8 [2].

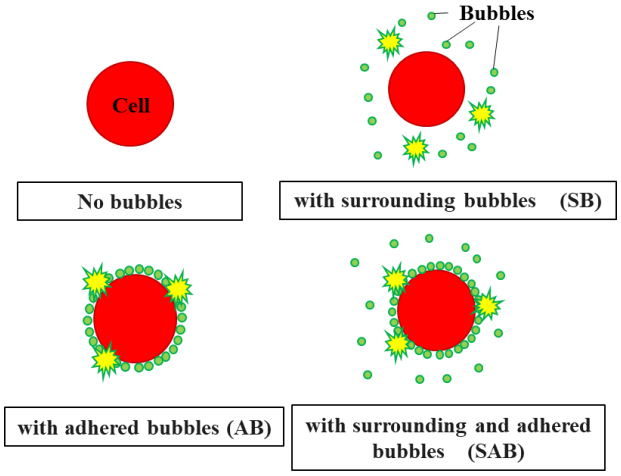

Fig.1 Four situations between the cells and the bubbles.

The waveform of the exposed ultrasound was a burst wave with a maximum sound pressure of 400 kPa-pp and a duty ration of 60%. The concentration of bubbles was established as 0.1 or 0.3 mg/mL, where the concentration of the cells was fixed to 1.0×10^5 cells/mL.

Here we define the distance between floating bubbles from the lipid concentration as shown in **Fig. 2**, as the cell concentration of X [/mL], one side length of the cube α [μm] as $\alpha = \sqrt[3]{1/X}\times10^3$, and the radius of the cell β [μm]. Also, the distance between the neighboring cells is considered to be

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equal to α because of the size of the cubes. Then, considering the number of bubbles in a cube, and assuming the number concentration of bubbles is Y [/mL], the number of bubbles in a cube centering a cell is $N_b = Y / X$. Based on those assumption, the distance between the floating bubbles γ [μm] is given by the following equation.

$$\gamma = \sqrt[3]{(\alpha^3 - 4\pi\beta^3/3)/N_b} \quad (1)$$

Using γ, which is the distance between the cubes centered on the bubble, the distance between a cell and a bubble should be $\gamma/2$.

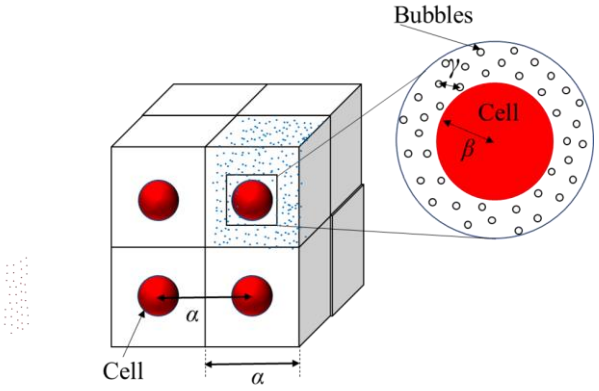
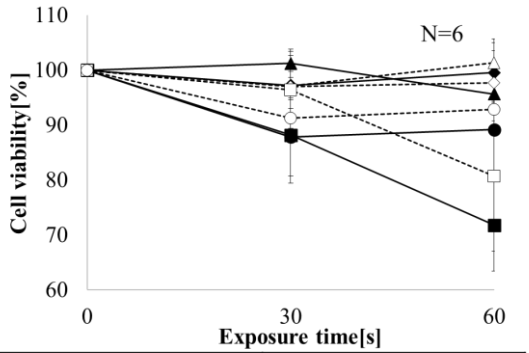


Fig.2 Schematic of distance between bubbles

3. Results

Fig. 3 shows the experimental results of cell viability with respect to the irradiation time, where a continuous wave with a sound pressure of 400 kPa-pp at a frequency of 3 MHz was applied. Comparing four situations, the most severe damage was caused to the cells with SB situation, where the distance between bubbles was γ = 2.24 μm. Meanwhile, comparing AB with SB and SAB situations, the adhered bubbles were considered to protect from the damage caused by floating bubbles.



	Bubbles adhesion to cells			
Max sound pressure [kPa-pp]	NB	AB	SB	SAB
300	◇	△	□	○
400	◆	▲	■	●

Fig.3 Comparison of cell viability in four situations between the cells and the bubbles.

Fig. 4 shows the cell viability with respect to the irradiation time with AB situation according to the parameter of bubble concentration. Because there was no significant difference between two concentrations, the adhered bubbles would not affect to cell viability.

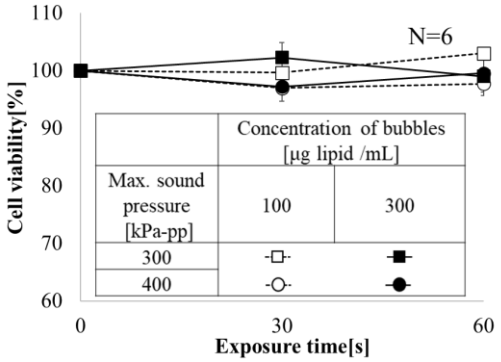


Fig.4 Comparison of cell viability with AB situation according to bubble concentration.

Fig. 5 shows the cell viability with respect to the distance between floating bubbles of SAB and AB , where a continuous wave with a sound pressure of 400 kPa-pp at a frequency of 3 MHz was applied for 60 sec. Cell viability decreased with increasing inter-bubble distance, but SAB was up to 27% more protective of cells against SA.

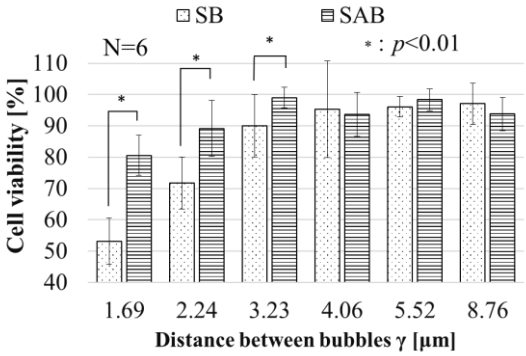


Fig.5 Comparison of cell viability with SAB and AB situation according to distance between floating bubbles

4. Conclusions

We verified the viability of vascular endothelial cells considering four situations between the cells and the bubbles. The floating bubbles cause more damage on the cells rather than the adhered bubbles. We are going to apply this results for future experimental design.

References

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