3P5-7

Experimental study of damage on vascular endothelial cells according to microbubble concentration and ultrasound exposure

微小気泡濃度と超音波照射に対する血管内皮細胞の損傷に関する実験的検討

Tatsuya Saito[1†], Masakazu Seki[1], Takuya Otsuka[1], Naoya Kajita[1], Yoshitaka Miyamoto[2], Johan Unga[3], Kazuo Maruyama[3], Ryo Suzuki[3], Kohji Masuda[1] ([1]Graduate School of BASE, Tokyo Univ. of Agriculture and Technology; [2]National Center for Child Health and Development ; [3]Faculty of Pharma-Sciences, Teikyo Univ.)

齊藤 達也[1†] 関 政和[1] 大塚 拓也[1] 梶田 直哉[1] 宮本 義孝[2] Unga Johan[3] 丸山 一雄[3] 鈴木 亮[3] 桝田 晃司[1] ([1]東京農工大学大学院生物システム応用科学府 [2]国立研究開発法人国立成育医療研究センター研究所 [3]帝京大学薬学部)

1. Introduction

In recent years, 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. There is a fundamental problems of the limitation of accumulation at the target area to disperse the cells in blood flow. To address this problem, we are researching on ultrasonic therapy using bubble-surrounded cells (BSCs) [2,3] containing therapeutic cells and thin catheters [4]. However, upon controlling BSCs and a catheter as shown in Fig.1, the effect of ultrasound exposure on the vessel wall has not been clarified.

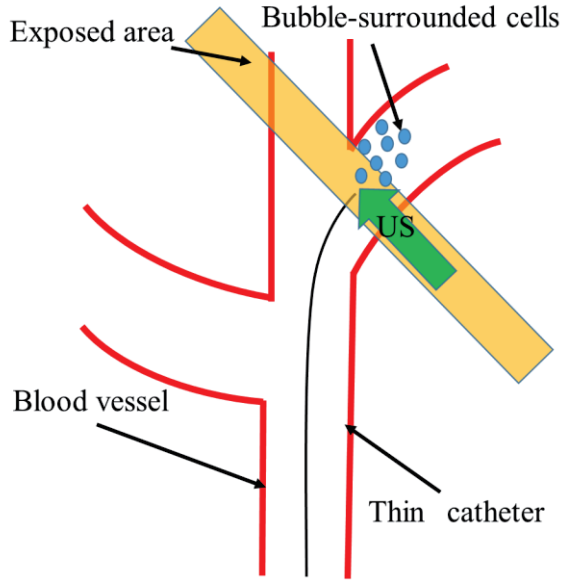


Fig.1 The concept of treatment with acoustic radiation force

ultrason@cc.tuat.ac.jp

Therefore, in this study, we carried out the validation of the vascular endothelial cells viability versus presence of microbubbles and various conditions of ultrasound exposure.

2. Methods

In this study, we have used lipid bubbles in recent years (LBs) [5] and normal vascular endothelial cells extracted from bovine carotid artery. The following experiment was conducted for the two cases using the cells with and without LBs floating around the cells. The LBs concentration was 0.3 mg/mL at which the amount of LBs was saturated when BSCs were prepared by stirring the cells and LBs with the ligand in the previous study.

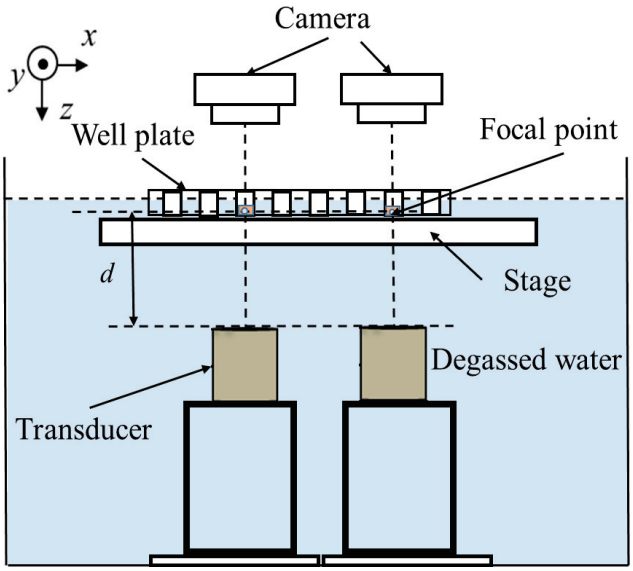


Fig.2 Experimental setup to cell viability.

Fig.2 shows the experimental setup. We filled the tank with degassed water and the

temperature was kept constant at 37 ˚C in a thermostatic bath. A suspension which had concentration of 1.0×10^5/mL cells were injected for 0.1 mL per well in a plate. An ultrasound transducer was set at a distance d = 65 mm, which was corresponded to the focal distance, away from the center of the suspension under ultrasound exposure with frequency of 1 and 3 MHz. Maximum exposure time was 60s and maximum sound pressure was 400kPa-pp. After the exposure, the cells were cultured in CO_2 incubator for 24 hours at 37 ˚C and then applied a colorimetric assay (Cell Counting Kit-8, 0.01 mL/well). After incubating for 4 hours, the absorbance in the well at 450 nm was measured. Finally, cell viability rate α was obtained using eq. (1).

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

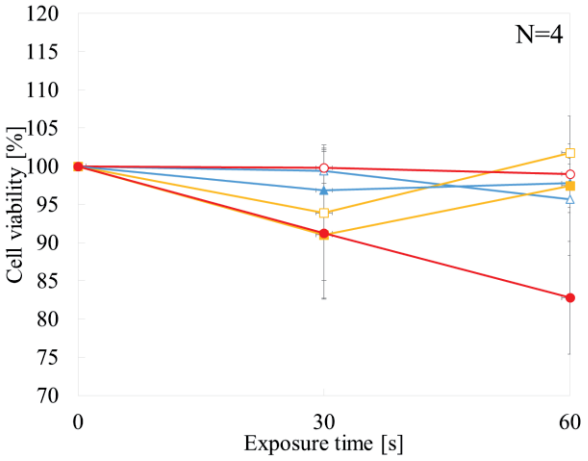
In eq. (1) $I_{Contlrol}$ is absorbance of the suspension without ultrasound exposure, I_{Blank} is initial average absorbance without suspension, I_{Sample} is absorbance of the suspension after ultrasound exposure.

3. Results

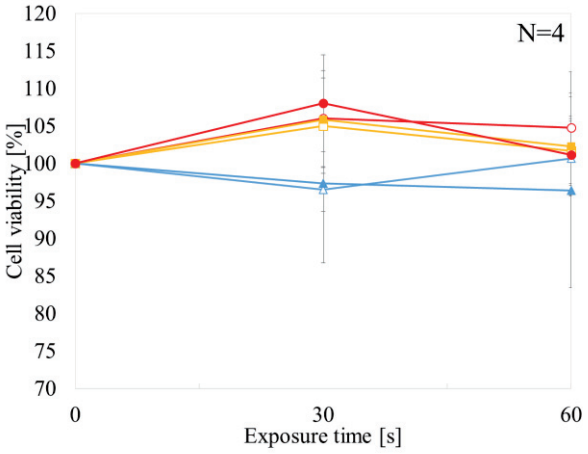
Fig.3 (a) shows the results of cell viability versus exposure time when we irradiated 1MHz ultrasound using experimental setup as Fig.2. In the case of cells only, the survival rate was almost 100% regardless of the exposure time, and no damage due to ultrasound exposure was observed. But when LBs were present, the cell viability tended to decrease in proportion to the exposure time, especially at a sound pressure of 400 kPa-pp.

Next, Fig.3 (b) shows the results of cell viability when we irradiated 3 MHz ultrasound. In the case of 3 MHz, the cell viability rate decreased to a maximum of 4% under the conditions with a LBs concentration of 0.3 mg/mL, a sound pressure of 200 kPa-pp, and an exposure time of 60 s, and there was no significant change in the survival rate. From these results, it was found that the damage to cells was larger when 1 MHz ultrasonic waves were exposure in the presence of LBs compared to 3 MHz.

The difference in the cell viability between two frequencies was caused by sound pressure distribution. The 1 MHz sound pressure distribution was wider than the 3 MHz sound pressure distribution, so it is considered to have a large effect on cells and LBs. From the results of a similar research using fibroblasts and osteoblasts, the cell viability was higher with 3 MHz [6], and it can be said that the same tendency was observed.



(a)



(b)

Max Sound Pressure [kPa-pp]	Concentration [mg/mL]	
	0	0.3
200	△	▲
300	□	■
400	○	●

Fig.3 Viability variation of the cells under ultrasound exposure with (a) 1 MHz and (b) 3 MHz.

4. Conclusion

We have verified cell viability of vascular endothelial cells in response to ultrasound exposure. When 1 MHz ultrasound was irradiated in the presence of LBs, the cell viability decreased to 82% under conditions of 400 kPa-pp and exposure time 60s. In the next step, we are going to work on conditions for extending exposure time and cell viability in BSCs with LBs attached to cells.

References

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