Quantitative analysis of bubble-surrounded cells retained on vessel wall by acoustic radiation force

音響放射力により壁面捕捉された細胞-微小気泡凝集体の定量解析

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1. Introduction

Recently, cellular immunotherapy [1] 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. Because of 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 lipid bubble [2] as microbubbles to the surface of cells to reduce their density and to be propelled using an acoustic radiation force. We confirmed that BSCs was retained by a single focal point acoustic field under flow condition [3,4]. In addition, we have confirmed that emission of a multi-focal sound field using tempo-spatial division increases the retained area. In order to realize the cell delivery system, it was necessary to study the shape of the sound field with higher efficiency for retention under different flow velocity and sound pressure conditions. In our preceding attempt, we evaluated the retained area of BSCs by thresholding brightness in microscopic images. However, the evaluation using retained area of BSCs was quantitatively insufficient because the spread of retained areas depends on the conditions of acoustic field. Therefore, the method of evaluation has to be improved to measure the number of retained cells using brightness analysis of microscopic images. In this study, we calibrated the correlation between the number of the cells and brightness before the experiments using various multi-focal acoustic fields with various flow velocities.

2. Methods

In this experimental, we used CD8 positive T lymphocytes (T-cells) [2], whish have the size ranged around 10-2 μm and were dyed with tetramethyl rhodamine. We also prepared anti-CD8 antibody-modified bubbles liposome, where the antibody was covalently linked on the surface [2] to form BSCs. First, to elucidate the correlation between number of T-cells and brightness on an microscopic image derived from fluorescence intensity, a small polycarbonate channel with a thickness of 1.0 mm covered by a PDMS sheet was prepared. Then, the relationship between injected concentration of BSCs and the brightness was measured using a fluorescence microscope (Olympus BXFM) and a digital camera (Olympus DP74).

To evaluate quantitative amount of the retained BSCs, the experimental setup shows in **Fig.1** as well as our preceding research [3]. An artificial blood vessel, which has a square cross-section of width 1.0 mm and thickness 1.0 mm and, made of polyvinyl alcohol, was placed at the water surface. A 2-dimensional array transducer, which has a central frequency of 3MHz and 128 elements, was installed in a water tank targeting at a distance of l = 60 mm and an elevation angle of θ = 60 deg.

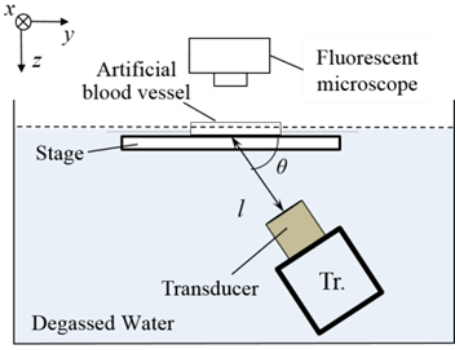

Fig.1 Experimental setup to expose ultrasound to suspension of BSCs in an artificial blood vessel

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The duration of ultrasound emission was 30 s after the injection of BSCs suspension of 0.5 mL through the artificial blood vessel. The retained amount of BCSs was obtained by converting from the brightness of acquired images using image analysis software (NIPPON ROPER, Image pro plus).

3. Results

The correlation between number of T-cells N_k and brightness I_k on microscopic images is shown in **Fig. 2**. When N_k was less than 800 /ml, I_k was increased in proportion to N_k, whereas I_k saturated when N_k was more than 800 /ml. From this result, we executed the following experiment concerning the maximum concentration of T-cells.

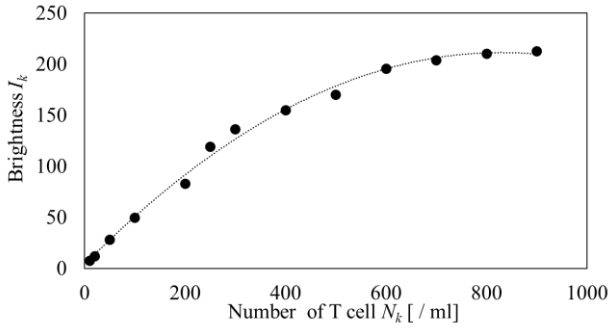

Fig.2 Correlation between number of T-cells and brightness

Fig.3 shows the microscopic images, which retained BSCs with maximum sound pressure 400 kPa-pp. BSCs were clearly retained flow velocities of 5 and 10 mm/s, whereas much lower brightness was confirmed with 30 mm/s.

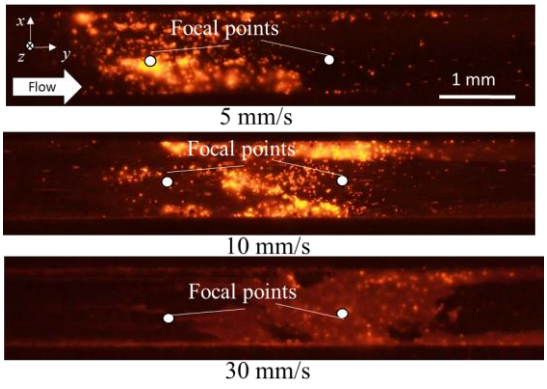

Fig.3 Microscopic images to visualize retained BSCs after 30 s of emission

Fig.4 shows the retained number of T-cells contained in BSCs, which was calculated from the brightness on microscopic images, versus number of focal points with flow velocity of 5,10,20 and 30 mm/s. In lower flow velocity, the number of the retained cells were increased, whereas there is an optimum focal points with higher flow velocity.

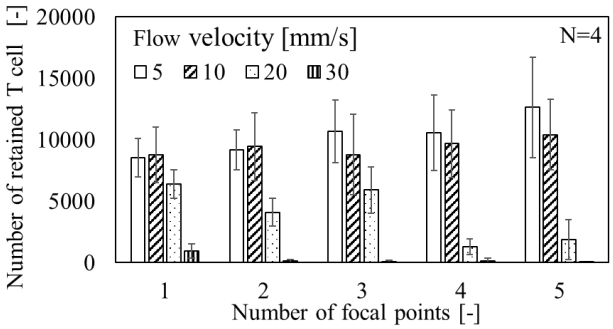

Fig.4 Number of retained T-cells contained in BSCs versus number of focal points

Fig.5 shows the average number of retained T-cells divided by emission area of ultrasound, which was defined by the shape of acoustic field base on beam widths and the number of focal points. From these results, there is a possibility of effective retention for ultrasound emission condition to adopt to a flow condition.

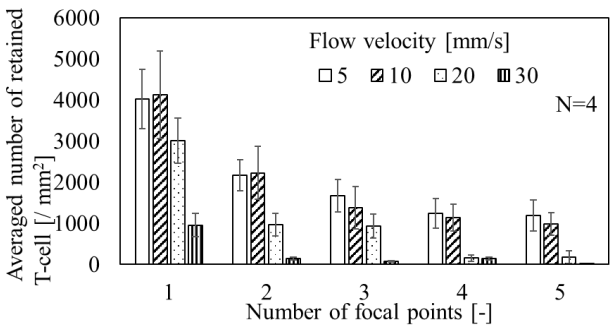

Fig.5 Averaged number of retained T-cells in BSCs versus number of focal points

4. Conclusion

We confirmed the correlation between number of T-cells and brightness. Next, We have verified number of retained T-cells in BSCs with various conditions of flow velocity and focal points. It was confirmed that the variation of number of retained T-cells according to ultrasound condition and flow velocity to apply to further experimental design.

References

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