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Study of acoustic field sweeping for active induction of bubble-surrounded T-cells

T 細胞を包含した微小気泡凝集体の誘導制御のための音場形成および走査条件の検討

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1. Introduction

Recently, cellular immunotherapy [1,2] has been recognized as a new cancer therapy, where the therapeutic cells are injected into the bloodstream. However, because of the dispersion through the blood stream, the number of the cells reached to the target area is quite lower than the injected amount. Therefore, *in vivo* cell delivery system, which is realized by attracting nanobubbles on the surface of cells to reduce their density, is discussed using acoustic radiation force for dynamic control of cells [3]. Controlling of therapeutic cells using ultrasound as our preceding researches with bubbles will become a key technique for an effective therapy of the cells. We have ever produced aggregations of bubble liposomes (BLs) including cells [4] and verified the adhesion of BLs on cells according to ultrasound exposure. Also, we researched reactions and persistence of the aggregations, according to the acoustic field sweeping, especially standing wave in still water [5]. However, behavior of the aggregations in flow under ultrasound exposure was not confirmed. In this study, we introduce our attempt to actively induce aggregations using a bifurcation in a thin channel by sweeping a standing wave field.

2. Experimental methods

We have newly introduced CD-8 positive T lymphocytes (T-cells) for future therapeutic applications and dyed them with tetramethyl rhodamine to distinguish in the fluorescence observation. Bubble-surrounded T-cells (BSCs) were produced with the bubble liposome (BLs) by our conventional method [4,5]. Fig.1 shows the experimental setup to observe the behavior of the BSCs under ultrasound exposure, which consists of an industrial microscope (BXFM, Olympus). A thin channel with a bifurcation, which was made of poly(vinyl alcohol) (PVA), was placed on the stage for position adjustment. In the bottom of the water tank, which was filled with degassed water, two identical ultrasound transducers (central frequency of 5 MHz) to emit plane wave were set with elevation angles of θ = 30 deg. Distances between the channel and the transducer are $d_1 = d_2 = 65$ mm. Maximum sound pressure was established to 100 or 200 kPa-pp, where the half width of the sound pressure distribution was 5 mm. When the two transducers were driven together, a standing wave was produced to form interference fringes in the channel. The position relationship between the bifurcation and ultrasound is shown in Fig.2, where the width of the paths are $w_1 = 1.4$ mm and $w_3 = 1.0$ mm, respectively. The axes of the transducer make the intersection at the central axis of the upper path with the distance of $w_2 = 2.5$ mm from the bifurcation point.

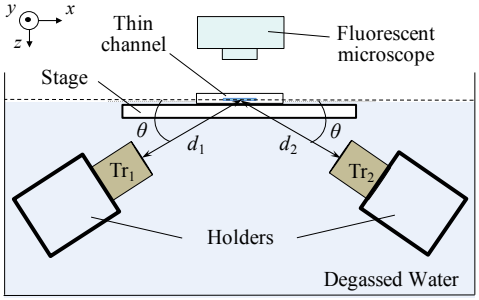

Fig. 1 Experimental setup with two transducers.

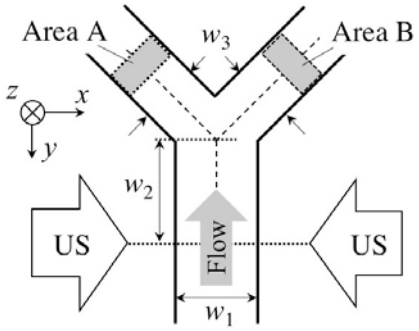

Fig. 2 Schematic of the bifurcation, the evaluation areas and ultrasound emission.

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The transducers Tr_1 and Tr_2 emit sinusoidal waves of ultrasounds f_1 and f_2, respectively, to form a standing wave as the following equations:

$$f_1 = A \sin[\omega t\text{-}kx + \delta(t)], \quad (1)$$

$$f_2 = A \sin[\omega t + kx\text{-}\delta(t)], \quad (2)$$

where A is the amplitude of continuous ultrasound, ω is the angular frequency, k is the wave number of $k = 2\pi\cos\theta/\lambda$, and λ is the wavelength. Also, $\delta(t)$ indicates a phase shift. When $\delta(t)$ was established to simply increase, the nodes in standing wave move in the x-direction with the node velocity, which can be calculated by k and the slope of $\delta(t)$ [5].

To evaluate the induction rate, we adopted our conventional method [6] by comparing the brightness difference between the areas A and B, which were established in the lower paths after the bifurcation point.

3. Results

The suspension of BSCs was produced from $1.0\text{x}10^5$ /ml of T-cells with 0.3 mg lipid/ml of bubbles. Flow velocity through the channel was fixed at 30 mm/s. Figure 3 shows the microscopic image when the suspension of BSCs were injected under exposure of continuous ultrasound with maximum sound pressure of 200 kPa-pp for each transducer with a node velocity of 5 mm/s in x-direction. It was confirmed that the BSCs were clearly induced to the path of Area B by the brightness variation compared with Area A.

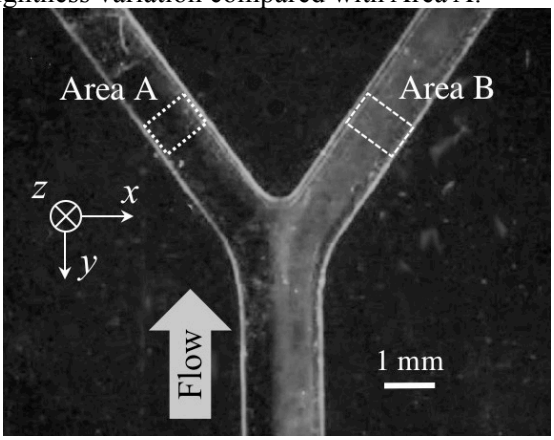

Fig.3 Microscopic image when BSCs were induced to the path of Area B.

We have verified the same procedure with various node velocities of 1, 5, and 10 mm/s. Fig.4 shows the induction rate versus the node velocity with comparison between BSCs and the cells without bubbles. It is obvious that BSCs are superior in the controllability to the cells without bubbles in every node velocity. Regarding BSCs, despite it was confirmed that the induction rate increased in proportion to the maximum sound pressure, there seems to be an optimum node velocity between 1 and 10 mm/s, which might be derived from not only flow velocity but also the magnitude and distribution of sound pressure.

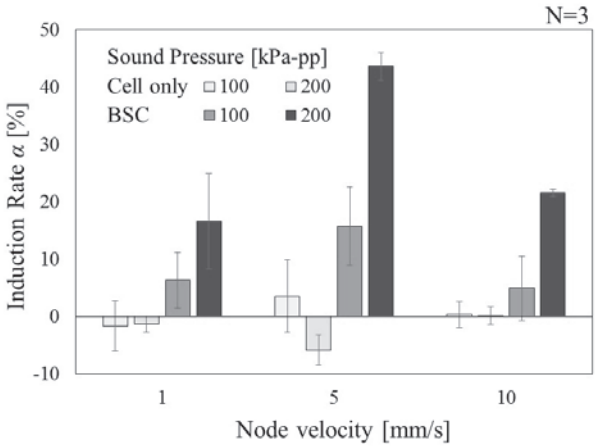

Fig.4 Induction rate versus the node velocity of standing wave according to the maximum sound pressure and existence of bubbles.

4. Conclusions

We realized that BSCs can be inducted to the desired path in a bifurcation by sweeping the nodes in standing wave field. The advantage of BSCs were confirmed for active induction in flow. Also, we found that the induction performance of the BSCs was affected by the multiple conditions including node velocity and sound pressure of the standing wave field. We are going to continue towards future *in vivo* applications.

Acknowledgment

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