

## Validation of tracking performance of cell-microbubble aggregations versus variation of acoustic field

超音波音場分布の変化に対する細胞－微小気泡凝集体の追従性能の検証

Riki Oitate<sup>1†</sup>, Akie Shimomura<sup>1</sup>, Hikaru Wada<sup>1</sup>, Takashi Mochizuki<sup>1</sup>, Kohji Masuda<sup>1</sup>, Yusuke Oda<sup>2</sup>, Ryo Suzuki<sup>2</sup>, and Kazuo Maruyama<sup>2</sup> (<sup>1</sup>Graduation School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, Japan, <sup>2</sup> Faculty of Pharma-Sciences, Teikyo University, Japan)

追立理喜<sup>1†</sup>, 下村明栄<sup>1</sup>, 和田洗<sup>1</sup>, 望月剛<sup>1</sup>, 榊田晃司<sup>1</sup>, 小田雄介<sup>2</sup>, 鈴木亮<sup>2</sup>, 丸山一雄<sup>2</sup> (<sup>1</sup> 東京農工大学大学院生物システム応用科学府, <sup>2</sup> 帝京大学薬学部)

### 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 microbubbles 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 microbubbles will become a key technique for an effective therapy of the cells. We have ever produced aggregations of bubble liposomes (BLs) including cells and verified the adhesion of BLs on cells according to ultrasound exposure [4]. Also, we confirmed the initial motion velocity of aggregations was in proportion to the applied acoustic energy [5]. However, reactions of the aggregations according to the exposed frequency of ultrasound exposure were not experimented. In this study, we observed the behavior of the aggregations using various frequencies of ultrasound standing wave.

### 2. Experimental methods

We have produced BLs-surrounded cells (BSCs), which are defined as the aggregations of BLs including Colon-26 cells with the same method of the preceding study [4]. The BLs and cells were independently dyed with DiO and tetramethyl rhodamine, respectively, to distinguish each other in the fluorescence observation. Fig.1 shows the experimental setup to observe the behavior of the BSCs under ultrasound exposure, which consists of an industrial microscope (BXFM, Olympus), fluorescence mirror, and mercury lamp. A thin channel, which is produced using poly(ethylene glycol) monomethacrylate (PEGMA), and has a

width of 2 mm and a thickness of 200  $\mu\text{m}$ , is put on the stage below the objective lens. In the bottom of the water tank, which is filled with degassed water, two identical ultrasound transducers were set with elevation angles of  $\theta = 30$  deg. Distances between thin channel and the transducer are  $d_1 = d_2 = 65$  mm. Also we prepared three pairs of identical transducers with their central frequencies of 3, 5, and 7 MHz.

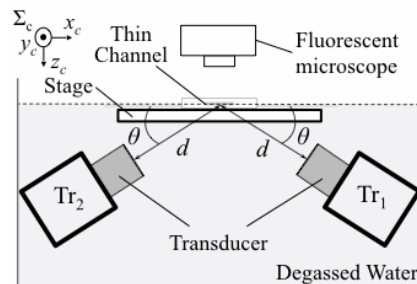


Fig.1 Experimental setup to observe behavior of the BSCs using two transducers and fluorescent microscope.

We have produced the BSCs suspension using ultrasound exposure with a sound pressure of 100 kPa-pp, an exposure time of 30 s, a BLs concentration of 0.33 mg lipid/mL, and the cell concentration of  $0.77 \times 10^5/\text{mL}$  in phosphate-buffered saline [4]. For the reference, when ultrasound emission started using two transducers, where the transducers emitted the sound pressure more than 100 kPa-pp in phase, the BSCs gradually formed multiple lines with the interval corresponded to the distance of half wavelength in  $y$ -direction, which indicates the interference fringes of the produced standing wave. In the following experiments, we have fixed the maximum sound pressure of 150 kPa-pp in each transducer to minimize the destruction of BLs, where the two transducers have the identical frequency properties.

The BSCs suspension was injected to fill with the thin channel in Fig.1. As the initial step, standing wave was produced to make the interference fringes, which determine the initial position of BSCs, where the ultrasound exposure time was less than 20 s. In the next, after confirming the BSCs were stood in the

lines, the acoustic field was periodically varied to move the line of BSCs, where the phases in  $Tr_1$  and  $Tr_2$  were varied in conjunction with each other. In the following experiment, one cycle of the variation was set as 4 s, where the phase in  $Tr_1$  increased for 2 s and decreased for 2 s, whereas the phase in  $Tr_2$  decreased for 2 s and increased for 2 s. Accordingly, the observation area was recorded by the microscope to analyze the behavior of BSCs.

### 3. Results

When the variation of acoustic field was started, the BSCs in the multiple lines were individually moved along the  $x$ -direction in the observation area. Fig.2 shows the averaged distance of BSCs from their initial positions versus the time of ultrasound exposure of the above periodic acoustic field. Fig.2 (a), (b), and (c) correspond to the central frequency of the transducers of 3, 5, and 7 MHz, respectively. In the all frequencies, the motion of BSCs tended to follow the variation of the movement of the acoustic field. We found a clear following motion of BSCs in 7 MHz, whereas less motions were confirmed in 3 and 5 MHz. To verify the behavior of BSCs, we also experimented the same procedure with only the cells in the suspension without BLs. The motions of BSCs were obviously increased by attaching BLs on the cells. Here it should be mentioned that the exposure time to determine the initial position of the cells was at most 60 s due to the low response of the cells without BLs compared with that of BSCs.

Fig.3 shows the total displacement of averaged distance of BSCs for three cycles (12 s). As the result, motion performance of BSCs with 7 MHz were three times higher than that with the other frequencies, where the cells without BLs were hardly moved by the frequency of 7 MHz.

### 4. Conclusions

In this study, we experimented a following performance of BSCs according to the periodic variation of acoustic field. We realized a distinct effect of controllability of BSCs with the frequency of 7 MHz of ultrasound exposure. We are going to verify with more conditions of acoustic field.

### Acknowledgement

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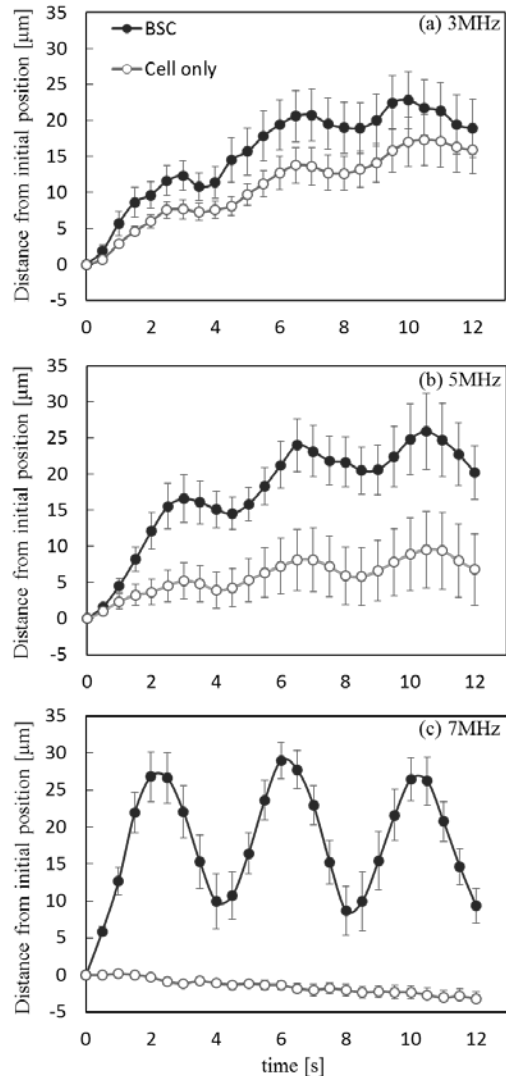


Fig.2 Time variations of distances from the initial position of BSCs according to the variation of the ultrasound standing wave.

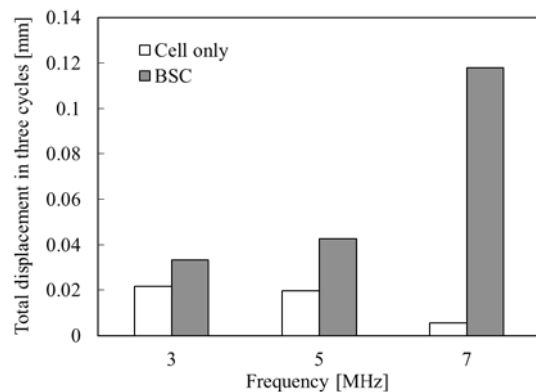


Fig.3 Total displacement of BCS during three cycles (12 s) of variation of ultrasound standing wave.