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Experimental conditions for efficient retention of vascular endothelial cells on channel wall using microbubbles and acoustic interference

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1. Introduction

In recent years, the fabrication of artificial organs has shown promise as an important technology for regenerative medicine, where not only 3D printing technology but also formation of two-layered microtubes [1)] were utilized to fabricate an artificial blood vessel using vascular cells. However, a limitation of those method is its ability to construct characteristics of blood vessels. To solve this issue, we formed bubble-surrounded cells (BSCs) to use microbubbles to attach on the surface of the cells with ultrasound exposure to control cell dynamics in flow channel [2)]. In our previous trials, the shape of the retained cells depended on the sound pressure distribution, which localized and showed ring shape centering the focal points. Therefore, we formed an interferential acoustic field using multiple transducers to disperse the distribution of retained cells on the inner wall of flow channel.

2. Methods

In this research, we employed bovine-derived carotid epithelial HH cells (cells, hereinafter) obtained from the Japan Cell Research Bank. They were cultured at 37 °C and a CO_2 concentration of 5 %, using Eagle's minimal essential medium with 10% fetal calf serum [2)]. When the culture reached confluence, it exhibited a typical cobblestone structure. Additionally, we used lipid bubbles (LBs), containing perfluoropropane (PFP, C_3F_8) gas and composed of DSPC and DSPE-PEG [2)]. They were dissolved in mixed organic solvents (each containing 4 mL of chloroform) and then 4 mL phosphate buffered saline (PBS) was added into the lipid solution and sonicated before the removal of the organic solvent via evaporation. The obtained LBs had an average diameter of 100 nm and were encapsulated with the phosphate buffer solution in a liposome. Then, we prepared modified LBs by conjugating cyclic-RGD peptides [3)], which covalently adhere to vascular endothelial cells via DSPE-PEG on the LB surfaces.

Fig. 1 shows the experimental setup to observe the behavior of the BSCs in flow under ultrasound exposure including a fluorescence microscope (Olympus, BXFM with DP74), ultrasound transducers, a water tank, and the artificial blood vessel. A pair of identical ultrasound transducers (central frequency of 3 MHz) to emit plane wave [4)] was installed at the bottom of the water tank and targeted the observation area with a distance of d = 65 mm. The elevation angle θ was set to 60°, such that the irradiation area of the acoustic field was included in the observation area. The artificial blood vessel, which was made of PDMS and has a rectangular cross section with a width of 2.0 mm and a height of 1.0 mm, was placed at the water surface. In the bottom of the vessel, collagen film (Nitta Gelatin, Cellmatrix Type I-C) was coated. In a prepared suspension of 0.5 mL, the concentrations of the cells and LBs were fixed to be 1.0 x10^5/mL and 0.3 mg/mL, respectively.

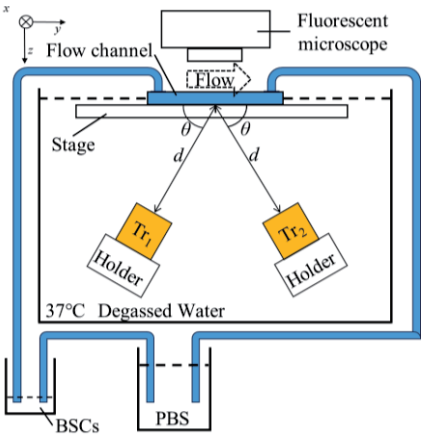

Fig. 1 Experimental setup with two transducers.

When the two transducers were driven together, a standing wave was produced in the thin channel. Here, the transducers Tr_1 and Tr_2 emit sinusoidal waves of ultrasounds f_1 and f_2, respectively, which travel in opposite directions to form a periodical variation in y-direction. The distribution of the acoustic field can be calculated as the summation of f_1 and f_2:

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$$f_1 = A_1 \sin[\omega t - kx], \quad (1)$$
$$f_2 = A_2 \sin[\omega t + kx], \quad (2)$$
$$f = f_1 + f_2 = 2A_1 \sin \omega t \cos kx + (A_2 - A_1) \sin[\omega t + kx], \quad (3)$$

where A_1 and A_2 ($A_1 < A_2$) are the amplitudes 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. Therefore, obtained acoustic field consists of the combination between the standing wave and the travelling wave.

The distribution of acoustic intensity on the channel varies according to the magnitude of sound pressure. To evaluate retained cells quantitatively, we defined and calculated the applied acoustic intensity of the interference acoustic field, where the distribution was divided into small grid with a width w [2]. Also, defining mean sound pressure as P_{ij} [Pa-pp] in a small area, the applied acoustic power E can be calculated by

$$E = \sum_i \sum_j w^2 \frac{1}{2\pi} \int_0^{2\pi} \frac{\left(\frac{P_{ij}}{2} \sin\theta\right)^2}{Z} d\theta$$

$$= \frac{w^2}{8nZ} \sum_i \sum_j P_{ij}^2, \quad (4)$$

where Z is an acoustic impedance of the medium. To normalize acoustic power by the applied area, as well as SATA (Spatial average temporal average) intensity, we defined the applied acoustic intensity as $I_{SATA} = E/S$ to compare between various acoustic fields, where S indicates the area of the acoustic field more than -20 dB of the maximum sound pressure. In the following calculation, $w = 0.5$ mm was used.

3. Results

Table 1 shows the conditions of three acoustic fields we prepared. There was no travelling wave in the condition D_2, whereas the standing wave and travelling wave were combined in the conditions of D_1 and D_3. **Fig. 2** shows the outlooks of the retained BSCs in 60 s after starting ultrasound exposure. The I_{SATA} of these acoustic fields were almost similar. Comparing the distributions of the cells, interference fringes were clearly seen in all conditions. However, occupied area of retained cells was superior in the condition D_1, where the sound pressure produced by Tr_1 was located upstream of the observation area.

Table 1 Conditions of three acoustic fields

Conditions	$2A_1$ [kPa-pp]	$2A_2$ [kPa-pp]	I_{SATA} [mW/cm^2]
D_1	250	100	299
D_2	200	200	301
D_3	100	250	299

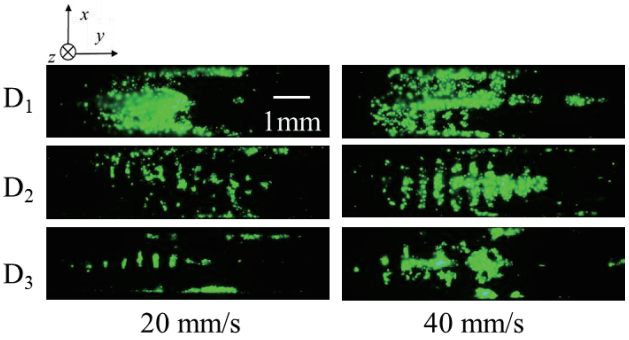

Fig.2 Outlook of retained BSCs after 60 s of exposure of interference acoustic field.

Fig. 3 shows the temporal variation of the occupied area the retained cells in three acoustic fields with two flow velocities of 20 and 40 mm/s. These results show that the retained cells increased and saturated within 60 s. However, the occupied area decreased in higher flow velocity of 40 mm/s, which indicates the adhered cells were detached due to flow resistance. Comparing three acoustic fields, the condition D_1 showed more retained area than other two conditions. These results suggest that there is a possibility of appropriate conditions of acoustic field for effective retention according to flow velocity and concentration of the cells.

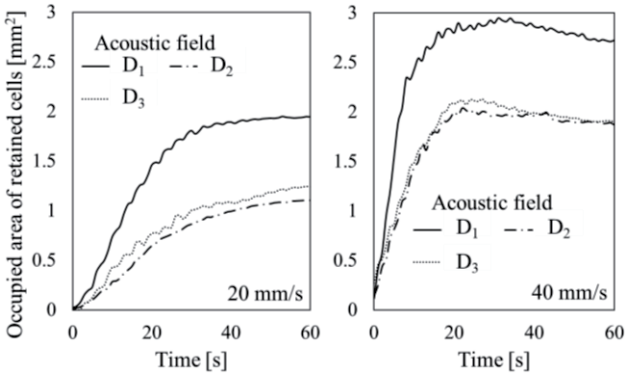

Fig.3 Time variation of occupied area of retained cells.

4. Conclusion

In this research, we succeeded to retain the cells using interference acoustic field produced by multiple sound sources. The retained area varied by acoustic conditions in the same acoustic intensity. We are going investigate appropriate condition for an effective retention of the cells.

References

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