2Pa5-8

Study of culture conditions of vascular endothelial cells retained to vascular wall surface with microbubbles by acoustic radiation force

Shunya Watanabe[1†], Kota Konishi[1], Yoshiki Ito[1], Yoshitaka Miyamoto[2], Daiki Omata[3], Ryo Suzuki[3], Kohji Masuda[1]
([1] Graduate School of Bio-Applications and Systems Eng., Tokyo Univ. of Agriculture and Technology, [2] National Center for Child Health and Development, [3] Faculty of Pharma-Science, Teikyo Univ.)

1. Introduction

Recently, fabrication of artificial organs has attracted attention as an important technology for regenerative medicine. In some of those researches, 3D printing technology is applied to realize a spheroid and an organoid. To fabricate an artificial blood vessel including multiple bifurcations using vascular endothelial cells, construction of a tubular structure is difficult due to the requirement of multi-layered with the inside space. Therefore, although there are many attempts to fabricate artificial blood vessel with an arbitrary shape [1,2], they are still not for in vivo use in diameters smaller than 6 mm.

In our experiments on the control of cell dynamics by ultrasound using microbubbles, which we confirmed that the T-cells contained in BSCs (bubble-surrounded cells) [3,4] were retained by the acoustic radiation force on the vessel wall in flow under exposure of ultrasound [5]. Thus, we tried to use vascular endothelial cells with bubbles as an application of the retention of BSCs. In this paper, we report the our preliminary trial to culture the cells after the retention by acoustic radiation force, and to investigate the condition of acoustic field and surface on the retention for the proliferation of the cells.

2. Method

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 [5]. 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 [5]. 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. Thereafter, we prepared modified LBs by conjugating cyclic-RGD peptides [5], 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), a two-dimensional array transducer, a water tank, and the artificial blood vessel. The transducer, which has a central frequency of 3 MHz and 128 elements [5,6], was installed at the bottom of the water tank and targeted the observation area with a distance of l = 60 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 gel (Nitta Gelatin, Cellmatrix Type I-A) was coated with a thickness of 100 μm. In the following experiments, the concentrations of the cells and bubbles were fixed to be 1.0 x10^5/mL and 0.3 mg/mL in the suspension, respectively.

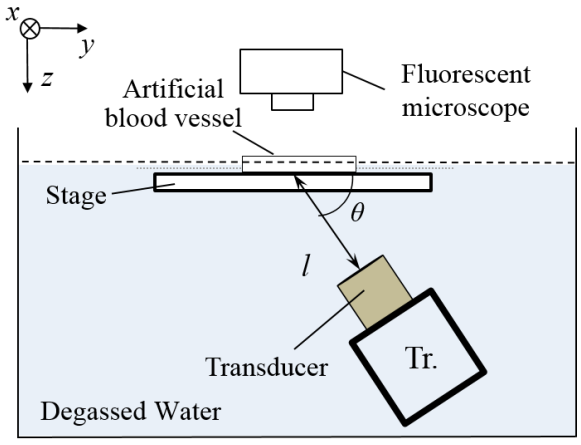

Fig.1 Schematic of the experimental setup.

Under the artificial flow of medium through the vessel, a 0.5-mL suspension of BSCs was

ultrason@cc.tuat.ac.jp

injected during ultrasound irradiation, where we applied the tempo-spatial division emission [5,6] with a duration of ultrasound emission of 60 s. The flow velocity was 10 m/s. Then the cloudy adhesion of BSCs on the upper wall of the path was observed in the middle of the observation area to evaluate the retention performance of BSCs. Figure 2 shows the positions of the focal points, which move repeatedly from f_1 to f_n while continuously switching the focal points every t_e seconds, which is a pseudo multifocal acoustic field.

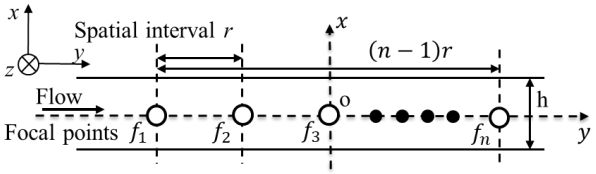

Fig.2 Schematic of a multifocal acoustic field.

Because the cells were fluorescently labeled using a cell tracker (Thermo Fisher SCIENTIFIC), the occupied area of retained cells can be calculated as S_{ret}. The vessel was placed for 1 h before filling with medium, and then cultured for 24 h in a CO_2 incubator to expect the cells to adhere on the collagen gel. To visually confirm the adhesion and viability of the cells, the cells were fluorescently stained with Calcein-AM solution (DOJINDO). Using the acquired images, the occupied area of the cultured cells was measured as S_{cul}.

3. Results

Figures 3 and 4 show the occupied area of the retained cells and the cultured cells, respectively, which include two types of acoustic fields with 1 and 2 focal points, where the spatial interval of the focal point of r = 2 mm with the maximum sound pressure in every focal point of 300 kPa-pp. In Fig. 3, it was confirmed that a wider area with the 2 focal points retained more cells compared to the 1 focal point. However, in Fig. 4, the occupied area of the cultured cells increased with 2 focal points compared with the retained situation, whereas it was decreased with 1 focal point. Figure 5 shows the comparison of the occupied area between the retained cells and the cultured cells. Using the multiple number of the focal points, the occupied area increased more than that with single focal points, which indicates the distribution of the acoustic intensity was dominant to retain more number of the cells as well as our preceding research [5]. Then the results newly noticed the possibility to discover appropriate conditions of multifocal acoustic fields for 3D fabrication of blood vessels.

4. Conclusion

In this research, we succeeded to culture the cells after the retention using acoustic radiation force on the bottom surface of the artificial blood vessel. The occupied area of the cultured cells varied according to the number of the focal points, where the condition of 2 focal points increased the cells.

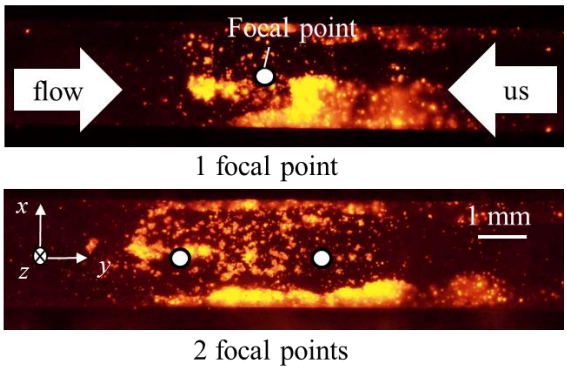

Fig.3 Occupied area of the retained cells through the artificial blood vessel.

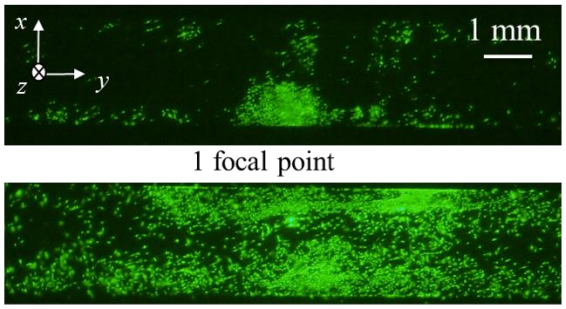

Fig.4 Occupied area of the cultured cells in 24 h after the retention.

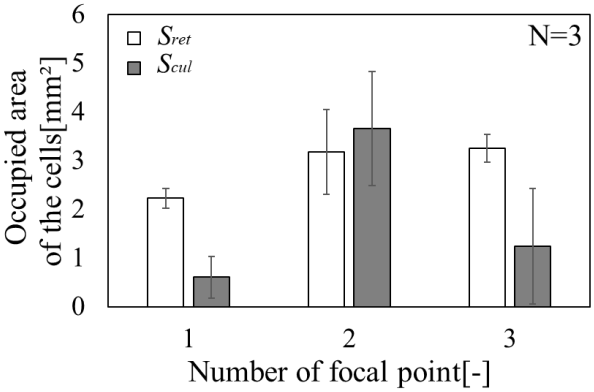

Fig.5 Comparison of the occupied area between the retained cells and the cultured cells.

References

[1] A. Hasan, et al : Biomed Microdevices. 2015
[2] T. Yue, etal : Micromachines. 2019
[3] R. Oitate, et al : Jpn. J. Appl. Phys. 2018
[4] M. Seki, et al : Jpn. J. Appl. Phys. 2019
[5] T. Chikaarashi, et al : Jpn. J. Appl. Phys. 2022
[6] H. Ushimizu, et al : Jpn. J. Appl. Phys. 2018