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Study to form microbubbles aggregations as carrier for cell therapy and its fluorescence imaging evaluation

細胞治療のためのキャリアとしての微小気泡凝集体の形成法と蛍光観察評価

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1. Introduction

Recently, a cellular immune therapy is operated by injecting specially activated therapeutic cells into bloodstream, which is expected as a new treatment method for cancer. However, because the therapeutic cells spread with blood flow, there are some concerns, which are side effect to normal area and limitation of accumulation to the target area. Therefore, reliability of the cellular therapy will be enhanced if a control method of therapeutic cells in blood flow would be developed. Meanwhile, we have previously reported our attempts for active control of microbubbles in artificial blood vessels, which represents active induction through paths[1,2], and production of aggregations[3] of microbubbles by making use of Bjerknes forces. Then we newly propose a method to form microbubble aggregation including therapeutic cells as a carrier for the cellular therapy, as shown in Fig.1. Microbubble aggregations are produced around a therapeutic cell by secondary Bjerknes force before injecton in bloodstream. Then the aggregations are propelled to be induced to the target area in blood by primary Bjerknes force, where ultrasound transducers are located on body surface. Here we consider that the aggregations including therapeutic cells are propelled and moved by acoustic forces, whereas individual cells are not moved because of their density. In this paper, we report our experiment to form microbubbles aggregations including lymphocytes as example of therapeutic cells.

2. Method

We have extracted lymphocytes, which diameter is about 10-15 μm, from blood of rat to dye with CellTracker orange CMRA for monitoring the location of lymphocytes. Also we prepared suspension in normal saline with lymphocytes and Sonazoid microbubbles, where their concentrations are 0.84×10^5/ml and 1.6 μl/ml, respectively.

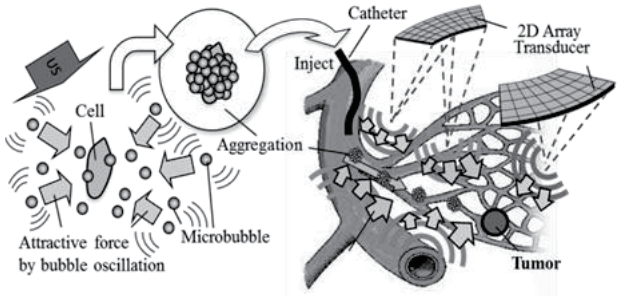

Fig.1 Goal of the research.

Fig.2 shows the experimental setup to form microbubbles aggregation with lymphocytes. We have produced a plate, which has a hemispheric well with diameter of 7 mm and depth of 3 mm, using poly(ethylene glycol) monomethacrylate (PEGMA). Placing the bottom of the plate soaked in water, a concave ultrasound transducer, which frequency range can be varied between 3 and 7 MHz, was set in the bottom of the water tank. The distance between the surface of the transducer and the center of the well was set to be 60 mm, where the well is included in the focal area in the above frequencies.

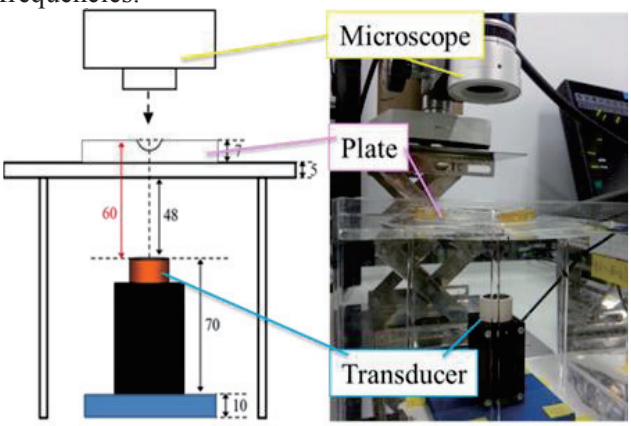

Fig.2 Experimental setup.

A microscope was set to observe formation of aggregations in the well from the top of the plate under ultrasound exposure. After ultrasound

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emission, the suspension was sampled to confirm aggregations including lymphocytes using a fluorescent microscope.

3. Results

We have put suspension of 100 µl into the well to expose continuous wave of ultrasound for 30 s with maximum sound pressure of 100 kPa-pp. Fig.3 shows the microscopic images of a sample of microbubbles aggregation. Though an aggregation was confirmed in Fig.3(i) in visible light, existence of lymphocytes was not confirmed. In Fig.3(ii), a lymphocyte was visualized under excitation light with wavelength of 548 nm, where fluorescence emits reflection light with wavelength of 576 nm. A lymphocyte was confirmed to be included in a microbubbles aggregation.

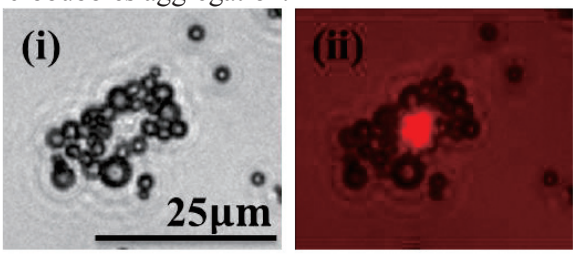

Fig.3 Detection of a lymphocyte included in microbubbles aggregation using fluorescence excitation.

Fig.4 shows the distribution of number of aggregations versus the mean diameter of aggregation, where totally 450 aggregations were examined from 5 samples in suspension. We have confirmed the same tendency to our previous report [4], which showed that the size of aggregation decreased in proportion to the central frequency. Though many aggregations less than 10 µm were produced with 7 MHz, lymphocytes were hardly detected. We found lymphocytes in aggregations, which size is more than 20 µm with frequency of 3 and 5 MHz. However, collapsed or expanded microbubbles were also found with lower frequencies.

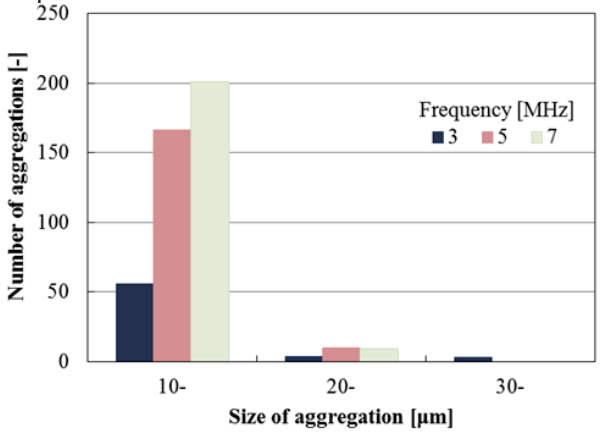

Fig.4 Distribution of number of aggregations according to their size.

Fig.5 shows the cell content ratio in aggregations versus the central frequency. With frequency of 7 MHz, cell content ratio was 0.95 %, which indicates the size of aggregations were insufficient to include lymphocytes. On the other hand, with frequency of 3 MHz, though number of aggregation is 4 times less than with frequency of 7 MHz, cell content ratio in aggregation was 7.9 %. From those results, selection of central frequency is important to form microbubble aggregations including lymphocytes.

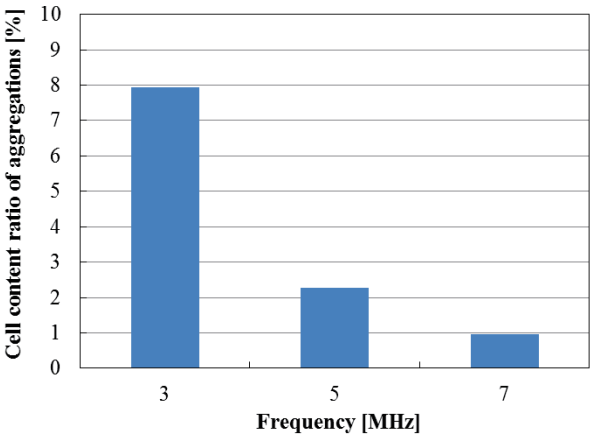

Fig.5 Cell content ratio in aggregations vs central frequency of ultrasound.

4. Conclusions

In this study, we have preliminary investigated a method to form microbubble aggregations including cells under ultrasound exposure for the future cellular therapy. We confirmed lymphocytes were included in Sonazoid microbubble aggregations using fluorescence observation. The number and size of aggregations showed dependency on the central frequency, where the cell content ratio was inverse proportion to number of aggregations. We are going to investigate further quantiative experiment using various microbubbles and therapeutic cells.

Acknowledgment

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References

1. Masuda K, et al.: Jpn. J. Appl. Phys., Vol.50, 2011, 07HF11
2. Shigehara N, et al.: Jpn. J. Appl. Phys., Vol.52, 2013, 07HF15
3. Demachi F, et al.: Seitai-ikougaku, Vol.51, No.6, 2013, 374-383 [in Japanese]
4. Koda R, et al.: Proc. of IEEE Engineering in Medicine and Biology Society, 2011, 5589-5592