

## Effrct of the existence of red blood cell in trapping performance of microbubbles by acoustic radiation force

音響放射力によるマイクロバブルの捕捉性能における赤血球の影響

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### 1. Introduction

Making use of the phenomena that microbubble of  $\mu\text{m}$  order collapses after ultrasound emission near their resonant frequency, various types of therapeutic application have been proposed. By utilizing the effect of sonoporation, which allows uptake of large molecules into the cell, new methods for drug delivery or gene delivery are expected. The bubbles, which can contain the specified drug inside, have the possibility to correspond to various kinds of medications. However, because of the diffusion of bubbles after injection, it is difficult to concentrate the density of bubbles to enhance the uptake at the target area. If the density of bubbles inside human body can be controlled, the amount of drug would be minimum. Then we have noticed the acoustic radiation force [1], which is a physical phenomenon where an acoustic wave pushes an obstacle along its direction of propagation. Then though we have confirmed to be able to trap the bubbles against a water flow in the straight artificial blood vessel [2,3], the precise conditions for *in vivo* experiment were not investigated. In this paper, we introduce our attempt using the suspension of bubbles with actual red blood cells (RBCs) to investigate the behavior of bubbles in a closer condition to *in vivo* situation.

### 2. Principle

Assuming that the shape of microbubbles are spherical, the bubbles are exposed in spaciouly uniform ultrasonic wave, and the size of bubbles is much smaller than wavelength, an acoustic radiation force acts to the bubbles as per the following equation [3]:

$$F_{ac} = V\{D - (1 - \gamma)\}\nabla \langle K_E \rangle \quad (1)$$

where  $V$  is the volume of capsule,  $K_E$  is the mean density of kinetic energy,  $\gamma$  is the compression ratio

between the bubble and the medium, and  $D$  is the constant which depends on the density of the bubble and the medium around the bubble. Thus the direction of acoustic radiation force depends on the relation between  $D$  and  $\gamma$ .

When the bubbles are placed in a flow, a bubble should receive a flow resistance  $F_d$  according to the following equation:

$$F_d = 6\pi\mu u_r \quad (2)$$

where  $r$  is the radius of bubble,  $u_r$  is the flow velocity and  $\mu$  is viscosity coefficient of the medium. Thus, if the local acoustic radiation force to propel a bubble is greater than the flow resistance, the bubbles are pressed to the wall of blood vessel. Then the distribution along the wall is considered in either case of the following two types, which are shown in Fig.1 (a) and (b), respectively.

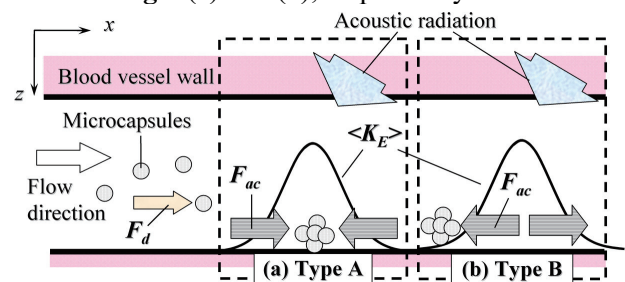


Fig.1 Two types of trapping microcapsules in flow by acoustic radiation force.

In the Fig.1 (a), which we defined as type A, the location of trapped bubbles is predicted in the middle of the energy distribution because the force works from lower to higher potential energy. In the Fig.1 (b), as type B, on the other hand, it is predicted away from the energy distribution.

### 3. Experiment

To observe the behavior of the microbubbles with the acoustic radiation force against flow, we have prepared an artificial blood vessel, which is made of polyethylene glycol (PEG), including a

straight path with the inner diameter of 2 [mm], as shown in **Fig.2**. To adjust the position and angle of ultrasound emission, 3-dimensional optical tracker system was introduced. We set the angle of the axis of the transducer as  $\theta = 60$  deg and focal point of ultrasound emission corresponds at the center of the observation area of an optical microscope. We prepared suspension of Sonazoid<sup>®</sup>, which has a phospholipid shell and perflubutane inside, and RBCs of pig. The density of the suspension was calculated by adjusting the volume of saline.

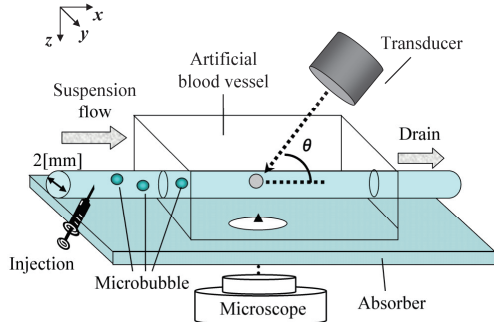


Fig.2 Schematic view of the experiment to trap microbubbles in flow.

#### 4. Results

We have examined by using three kinds of suspensions of microbubble only, RBCs only and composition of bubble and RBCs. **Fig.3** shows the microscope images after the suspension appeared in the observation area under ultrasound emission of central frequency of 3 [MHz] with flow velocity of 20 [mm/s]. Figs.3 (a), (b) and (c) show the transition when the suspension was the composition of bubble and RBCs. On the other hand, Fig.3 (d) shows when the suspension was bubbles only. The maximum sound pressure in the area was set to 300 [kPa]. The normalized distribution of sound pressure was calculated with Rayleigh equation and overlapped on Fig.3 (c) and (d).

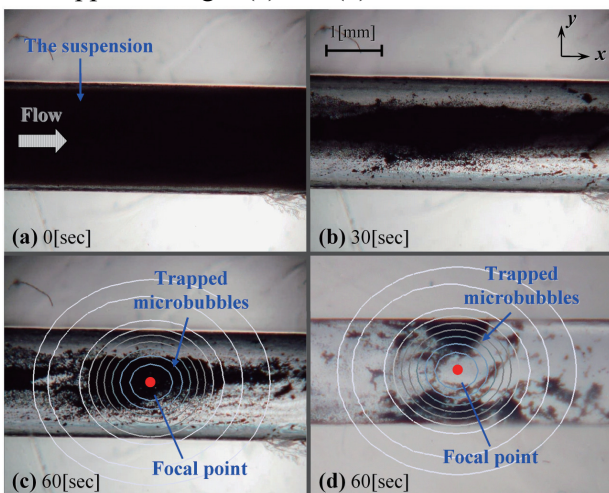


Fig.3 Transition of the observed area to confirm trapped microbubbles during ultrasound emission.

According to the distribution of the trapped bubbles, Figs.3 (c) and (d) are considered as type B and A in Fig.1, respectively. Also we compared the distribution of trapped bubbles among three kinds of suspensions. If the suspension was composed with only RBCs, significant trapped area was not confirmed. When RBCs were included with bubbles in the suspension, more trapped area of bubbles was confirmed than the suspension without RBCs. To evaluate quantitative amount of trapped bubbles, we defined trapped index by multiplying brightness difference and occupied area of bubbles. **Fig.4** shows the trapped index in 70 [sec] after the suspension appeared in the observation area during ultrasound emission. Here we prepared 10 times thicker suspension (RBCs 2) than the original density (RBCs 1) composed with bubbles. We confirmed that the trapped index increased by the existence of RBC and with proportion to density of RBCs.

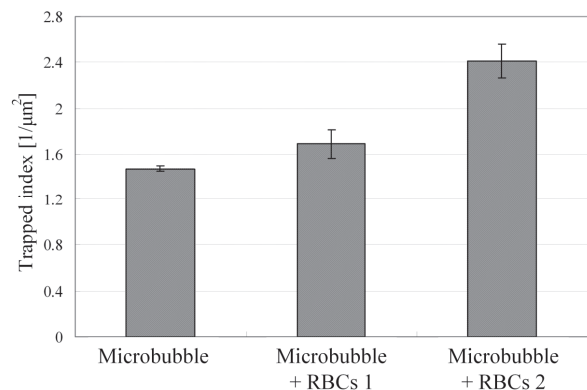


Fig.4 The trapped index of microbubbles versus types of suspension, where RBCs 2 indicates 10 times thicker than RBCs 1.

#### 5. Conclusions

In this study, we have experimented to trap microbubbles in flow with suspension of RBC in artificial blood vessel. We confirmed the bubbles are trapped to entire the vessel and classified two types of distribution. The amount of trapped the bubbles is confirmed to increase by the existence and density of RBC. We are going to apply the experiment by varying other parameters and *in vivo*.

#### References

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