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Dependence of Aggregate Formation of Microbubbles upon Ultrasound Condition and Exposure Time

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***Abstract*—We have previously reported our attempts to control microbubbles (microcapsules) behavior in flow by primary Bjerknes force to increase the local concentration of the bubbles at a diseased part. However, there was a limitation in efficiency to propel bubbles of μm-order size. Thus we consider that forming aggregates of bubbles is effective to be propelled before entering into an ultrasound field by making use of secondary Bjerknes force under continuous ultrasound exposure. In this study, we observed the phenomena of aggregates formation by confirming variation of diameter and density of aggregates under various conditions of ultrasound exposure. Then we elucidated frequency dependence of the size of aggregates of microbubbles.**

I. Introduction

MICROBUBBLES are known to form aggregates when they are put into an ultrasound field because secondary Bjerknes force, which acts attractive or repulsive between neighboring bubbles, is produced [1] by local condition of oscillation. Multiple bubbles form aggregate where they are oscillated in the same phase locally. The applications of this phenomenon are reported to sonoporation [2] and capillary embolization [3]. We have previously reported our attempt to propel microbubbles in flow [4,5] by a primary Bjerknes force [6,7], which is a physical phenomenon where an acoustic wave pushes an obstacle along its direction of propagation. However, because the primary Bjerknes force is proportional to square of the radius of a bubble, there was a limitation in efficiency to propel bubbles in blood flow when the size of a bubble is as small as red blood cell. Thus we consider that forming aggregates of bubbles is effective to be propelled before entering into an ultrasound field to receive more primary Bjerknes force.

For the case of micrometer-size microbubbles, upon ultrasound exposure, they are oscillated to produce Bjerknes force and to aggregate each other if the frequency of ultrasound is close to their resonance frequency. We already confirmed aggregate of bubbles in a straight flow when acoustic radiation force was produced in oncoming direction with MHz-order frequencies [8]. However, appropriate conditions of ultrasound had not been elucidated to form aggregates by secondary Bjerknes force. In this study, we have investigated the phenomenon of forming aggregates of bubbles and observed variation of diameter and density of aggregates under various conditions of ultrasound exposure. We used two kinds of microbubbles, which are F-04E (Matsumoto Oil, Co. Ltd) for mainly industrial use and Sonazoid® (GE Healthcare, Co. Ltd) for actual clinical use for comparison.

II. Principle

If two bubbles are located in an even ultrasound field and oscillated, secondary Bjerknes force was produced between the neighboring bubbles. Figure 1 shows transition to form aggregates of bubbles under ultrasound exposure.

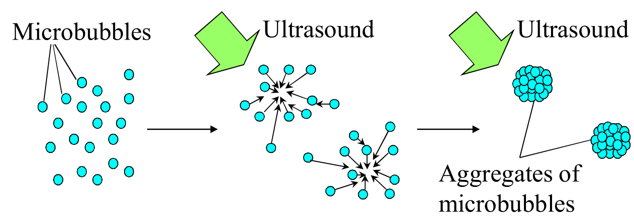

Fig.1. Transition to form aggregates of microbubbles under ultrasound exposure.

Assuming the shape of the aggregates is spherical, a primary Bjerknes force [9] acts to propel an aggregate in the direction of acoustic propagation as per the following equation,

$$F_r = \pi r^2 Y_p P \,, \qquad (1)$$

where P is the mean energy density of the incident wave, Y_p is a dimensionless factor called the radiation force function that depends on the scattering and absorption properties of the bubbles, and r is the equivalent radius of the aggregate of bubbles. Here if an aggregate can be regarded as a larger bubble, it would be easily propelled by less primary Bjerknes force. Thus it is very important to investigate behavior to form aggregates with various conditions of ultrasound.

III. Experiments

A. Observation of bubble behavior

Microbubbles named F-04E and Sonazoid are prepared.

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The microbubble F-04E has a shell made of poly(vinyl chloride) with a specific gravity of 0.0225 and an average diameter of 4 [µm]. It contains isobutene inside and is stable in room temperature. We selected only those microbubbles with a diameter less than 10 [µm] by using micro sieves. Figure 2 shows the microscopic image of F-04E with Sonazoid, which contains perfluorobutane inside with an average diameter of 2-3 [µm]. As we expected before [4], according to a mathematical simulation [10], the behavior of bubbles is affected by MHz-order frequencies. Also theoretical resonance frequency of F-04E is lower than that of Sonazoid because of the material of the shell and the size.

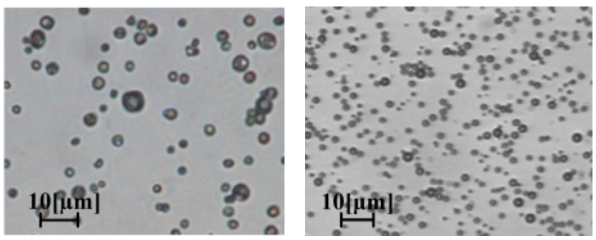

Fig.2. Microscopic image of F-04E (left) and Sonazoid (right)

We prepared a thin channel made of poly(ethylene glycol), which is placed in the bottom of a tank filled with water. Figure 3 shows the experimental setup. Suspension of bubbles is confined in the thin channel, size of which is approximately 4.0x50.0x0.3 [mm^3]. The channel was put in the bottom of a water tank, which was filled with degassed water. The physical axis of the transducer was set to go through the center in the *x-y* plane of the channel to locate focal point of ultrasound field in the observation area, size of which is approximately 1.5x1.5 [mm^2]. The behavior of bubbles was recorded optically using an inverted microscope (Leica, DMRIB) and a high-speed camera (Photron, 1024PCI). To synchronize the ultrasound exposure with optical observations, the trigger signal from the oscillator was connected to both of the transducer and the camera.

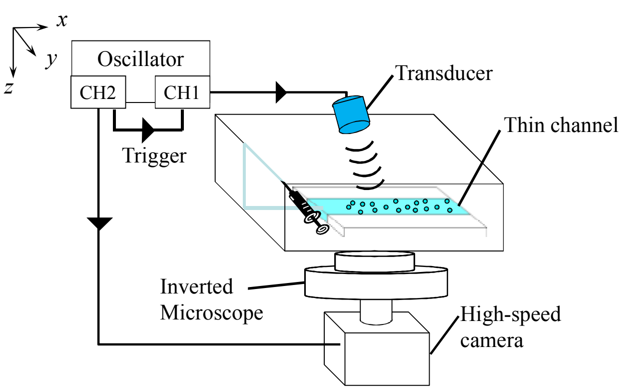

Fig.3. Experimental setup to observe aggregate formation of microbubbles.

Figure 4 shows the position configuration between the transducer and the thin channel. The angle of the axis of the transducer was set 70 [deg] to the *x-y* plane of the channel. We have prepared four kinds of transducers, which include a flat ceramic disc to emit plane wave of ultrasound with their center frequencies as 3, 5, 7 and 10 [MHz], respectively, to compare the effect between near and far from resonance frequencies. Measuring two-dimensional distribution of sound pressure in above four transducers, the half width of ultrasound beam was ranged between 4 and 5 [mm]. Therefore the observation area was adjusted to be included in the spot area of the ultrasound exposure.

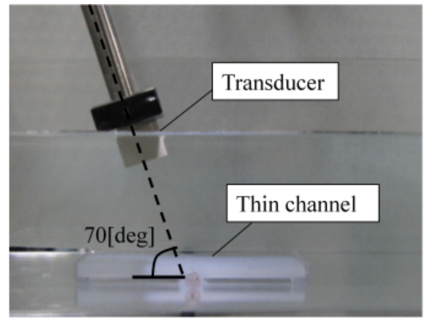

Fig.4. Position configuration between the transducer and the channel.

B. Evaluation of the size of the aggregates

When the exposure of continuous sine wave of ultrasound was started, formation of aggregates of bubbles was observed. Figure 5 shows the transition of the observation area when F-04E microbubbles were used with ultrasound conditions of central frequency of 3 [MHz] and maximum sound pressure of 100 [kPa]. Size variation of aggregate rapidly increased within 1.0 [sec] after the start of exposure.

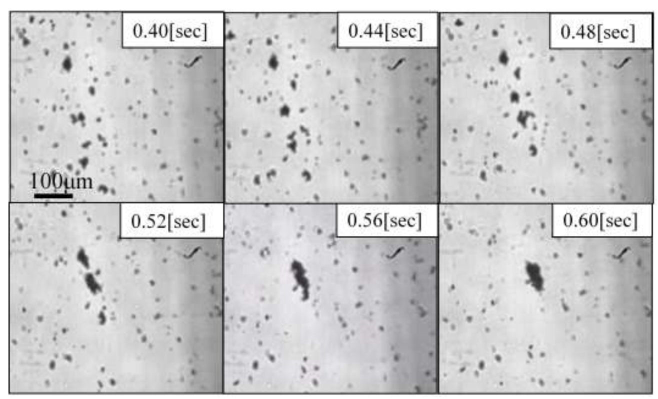

Fig.5.Transition of microscopic images of aggregate formation under ultrasound exposure(t=0 indicates the start of the exposure, central frequency of ultrasound: 3 [MHz] and maximum sound pressure: 100 [kPa]).

We have measured average diameters of the aggregates appeared in the frames by labeling method of image analysis. Figure 6 shows the method to measure the average diameter. To adopt various shape of a labeled area, at first the gravity point of the area was calculated. Then the ninety diameters in the area were calculated by fixing the gravity point and by changing the angle of 2 [deg] from the neighbor diameter. Finally the average diameter of the labeled area was obtained through multidirectional diameters to evaluate the size of the aggregate.

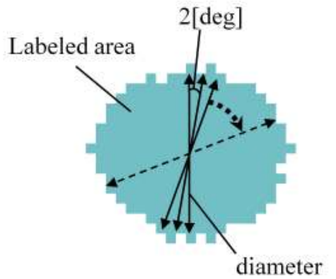

Fig.6. Calculation of the size of an aggregate by image analysis of a microscope image.

To evaluate the sizes of aggregates in a microscope image, the average diameters of all labeled area should be calculated. However, because unwanted shadows were included in images, which were produced by unevenness of wall in the thin channel or by angle of light source, they should be removed before measurement of the diameters of aggregates. Figure 7 shows the procedure to extract the bubbles, which contribute aggregate formation. Fig.7 (a) and (b) indicate the initial frame (initial status before ultrasound exposure) and an arbitrary chosen frame in the recorded file, respectively. In this experiment we recorded 3.0 [sec] of duration with 500 [fps] and among them 1500 [frames] were recorded.

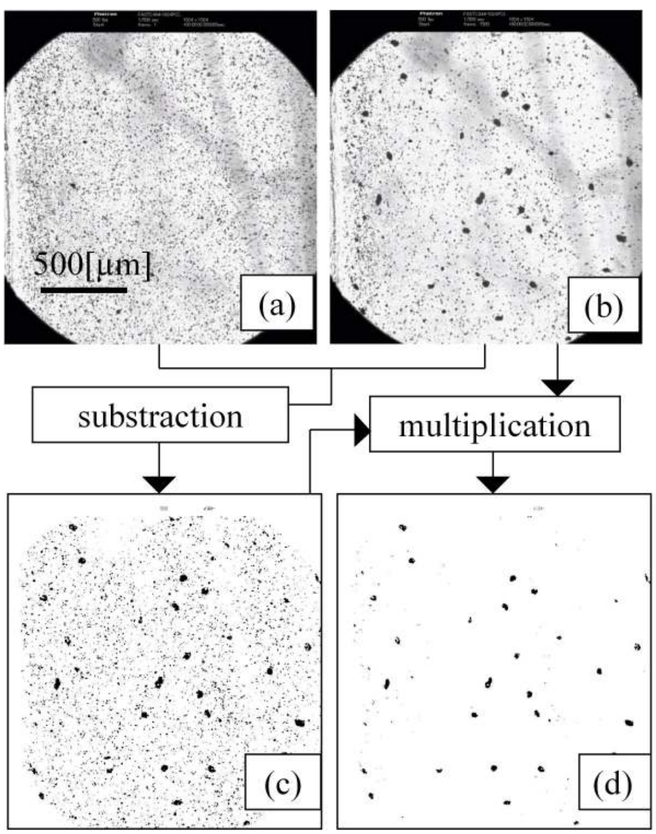

Fig.7. Extraction of microbubbles and their aggregates from microscope images, (a) initial frame, (b) an arbitrary chosen frame, (c) calculated mask image and (d) an example of obtained image.

Then image subtraction between Fig.7 (a) and (b) was calculated, where unwanted shadows indicate as the value of near zero, to create the binary mask image where background should be zero as Fig.7 (c). As mentioned in the chapter II, because the aggregates and bubbles are propelled by primary Bjerknes force, existence of bubbles was clearly distinguished from the background. Finally the mask image was multiplied to all of the frames to extract bubbles and their aggregates. Fig.7 (d) shows an example of obtained 1800 frames. Actually we applied noise reduction to the bubble-extracted images to erase small areas which average diameter is less than 10 [μm].

IV. Results

Figure 8 and 9 show bubble-extracted images by using microbubbles F-04E and Sonazoid, respectively, in 3 [sec] after starting ultrasound exposure with four kinds of central frequencies and maximum sound pressure of 100 [kPa]. From these images, greater aggregates were confirmed with lower frequencies in both types of microbubbles. Also comparing between F-04E and Sonazoid in the same frequencies, the size of aggregates of F-04E was almost two times larger than that of Sonazoid.

We investigated the time variation of the average diameter of aggregates under each condition. Figure 10 shows the average diameter of aggregates of F-04E versus time after starting ultrasound exposure. The each line indicates the average of 3 trials. Here the sizes of aggregates were confirmed to increase according to the exposure time of ultrasound. Also the size of aggregates became greater with lower frequencies but reached to their saturation quicker with higher frequencies.

Figure 11 shows the average diameter of aggregates of Sonazoid versus time after starting ultrasound exposure. The same tendency was obtained as Fig.10, except the aggregates with the central frequency of 5 [MHz] were greater than that with 3 [MHz]. Regarding the central frequency, more bubbles were formed aggregates when it was close to the resonance frequency, which is supposed to be around 5[MHz]. We have considered that the result reflects the effect of the resonance frequency of bubbles. When ultrasound with the central frequency of 3 [MHz] was emitted, more isolated bubbles were observed.

From those results, when the central frequency is closer to their resonance frequency, the bubbles would form larger aggregate and to receive more primary Bjerknes force. In the case of F-04E aggregates after ultrasound exposure of 3[MHz], the size became about 6 times larger than that of single bubbles. Then 36 times greater with additional primary Bjerknes force will be promising because it is proportional to square of the radius of bubbles. We are going to investigate behavior of aggregates of bubbles more precisely under various conditions of ultrasound to realize aggregate formation in a fast velocity of blood flow. Meanwhile we are going to develop a method to identify the precise location of the focus of ultrasound *in vivo* by detecting and constructing three-dimensional shape of blood vessel.

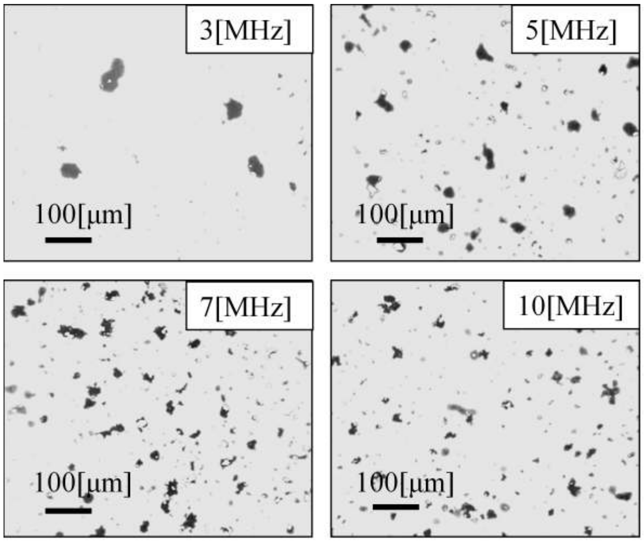

Fig.8. The bubble-extracted images using microbubbles F-04E in 3 [sec] after exposure of continuous ultrasound of 100 [kPa].

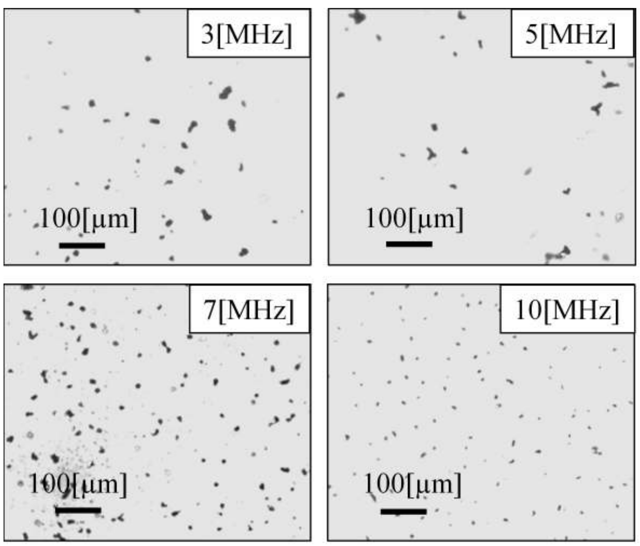

Fig.9. The bubble-extracted images using microbubbles Sonazoid in 3 [sec] after exposure of continuous ultrasound of 100 [kPa].

V. Conclusions

In this study, we observed aggregate formation of microbubbles using continuous wave with central frequencies between 3 and 10 [MHz]. We confirmed that the size and the saturation time of the aggregates depend on the central frequency, which is dominant to the resonance frequency of bubbles. For further analysis, appropriate conditions to realize active control of the microbubbles *in vivo* should be elucidated, which indicates combination of both conditions to form aggregates of bubbles and to propel the aggregates.

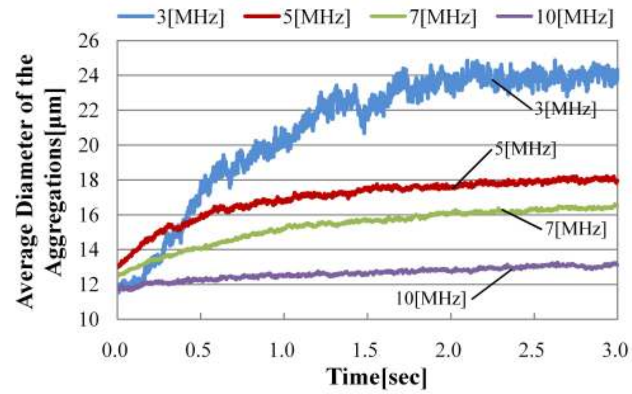

Fig.10. Time variation of the average diameter of aggregates of microbubble F-04E with maximum sound pressure of 100 [kPa].

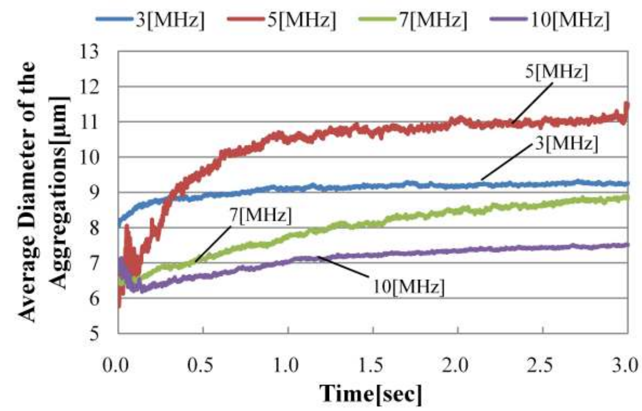

Fig.11. Time variation of the average diameter of aggregates of microbubble Sonazoid with maximum sound pressure of 100 [kPa].

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