34th Annual International Conference of the IEEE EMBS
San Diego, California USA, 28 August - 1 September, 2012

Observation of flow variation in capillaries of artificial blood vessel by producing microbubble aggregations

Kohji Masuda, *Member, IEEE,* Nobuhiko Shigehara, Ren Koda, Nobuyuki Watarai, Seiichi Ikeda, Fumihito Arai, *Member, IEEE*, Yoshitaka Miyamoto, and Toshio Chiba

***Abstract*—Microbubbles form their aggregations between the neighboring microbubbles by the effect of secondary Bjerknes force under ultrasound exposure. However, because of the difficulty to reproduce a capillary-mimicking artificial blood vessel, the behavior of aggregations in a capillary has not been predicted. Thus we prepared artificial blood vessels including a capillary model, which was made of poly(vinyl alcohol) (PVA) by grayscale lithography method, with minimum diameter of the path of 0.5 mm. By using this model we investigated the possibility of artificial embolization, where the microbubble aggregations might block entire vessels not to penetrate flow in downstream. Confirming that the sizes of flown aggregation were greater than the section area of the minimum path in the capillary model, we investigated the probability of path block in it. As the results we confirmed the probability increased in proportion to sound pressure and inversely to flow velocity. We are going to investigate with more kinds of parameters to enhance the possibility of artificial embolization.**

I. Introduction

It is known that microbubbles form their aggregations by secondary Bjerknes force, which acts as attractive or repulsive force between the neighboring microbubbles, under ultrasound exposure. By making use of microbubble aggregations, the effects of ultrasound therapy would be expected by accelerating the temperature increase in thermal therapy [1,2] and inducing sonoporation [3] to allow the uptake of larger molecules into cells in physical drug delivery [4,5]. Though the mechanism to form aggregation is very complex, because of the connection among various parameters, various researches related to microbubble aggregations were experimentally and theoretically investigated [6-11]. However, because of the difficulty to produce a capillary-mimicking artificial blood vessel, the behavior of aggregations in a capillary, e.g., probability to obstruct in bloodstream, shape destruction of the aggregation, etc, has not been predicted. If there is a possibility that the aggregations might block entire vessels, it can be applied as a novel therapeutic method of an artificial embolization near the target area of a tumor. Otherwise it might be also possible to control microbubble aggregations under the condition that the embolism won't be produced.

*Reserach supported by the Japan Society for the Promotion of Science (JSPS) through the Funding Program for Next Generation World-Leading Researchers (NEXT Program).

K.Masuda, N.Shigehara, R.Koda and N.Watarai are with Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan (e-mail: ultrason@cc.tuat.ac.jp).

S.Ikeda and F.Arai are with Graduate School of Mechanical Engineering, Nagoya University, Nagoya, Aichi 464-8601, Japan.

Y.Miyamoto and T.Chiba are with the National Center for Child Health and Development, Setagaya, Tokyo 157-8535, Japan.

We have ever reported our attempts to control microbubbles using the primary and secondary Bjerknes force to elucidate the conditions in sound pressure, central frequency of ultrasound, and flow velocity for active path selection in water flow [12,13] and trapping in blood flow [14] of microbubbles using artificial blood vessels with a simple shape. In those experiment we have experimentally found that there was an upper limitation of the size of microbubbles aggregations, which were propelled to the wall by primary Bjerknes force, and finally they flaked off the wall and flied to downstream. Thus we have considered that it is important to confirm the behavior of the flown aggregations if there is a capillary in the lower course, before application to living organs. From this point of view, we designed and fabricated a capillary-mimicking artificial blood vessel to observe the behavior of microbubbles aggregations with the primary and secondary Bjerknes force. In this paper, we describe the experiment to investigate the influence of the flown aggregations in an artificial capillary and discuss the possibility for an artificial embolization.

II. Experimental Methods

A. Experimental setup

We used the microbubble Sonazoid®, which has a phospholipid shell and perfluorobutane inside with an average diameter in the range from 2 to 3 μm. The suspension of microbubbles was prepared just before the experiment at a concentration of 0.3 μL/kg, which was diluted 50 times from the original preparation of Sonazoid, because the diffusion in the human body was considered. To observe the behavior of the microbubbles, we have prepared transparent artificial blood vessels, which are explained in the next section. Figure 1 shows the experimental setup. It was fixedly floated from the bottom of a water tank, which is filled with water, to prevent multiple reflections of ultrasound between the artificial blood vessel and the bottom of the tank.

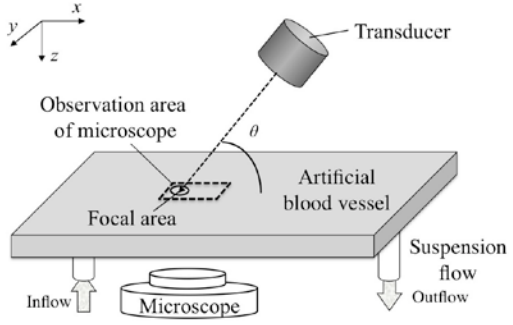

Figure 1. Schematic of the experiment with the artificial blood vessel, the microscope and the ultrasound transducer.

We used an optical microscope (Omron KH-7700) to observe the interested area in the blood vessel. The source of light is located above the water tank. The two-dimensional position of the blood vessel can be adjusted by an *x*-*y* stage. The position and the angle of the ultrasound transducer can be adjusted in three-dimension [14] by using an optical tracker system. We set the focal area of sound field to be included in the observation area, where the angle of the axis of the transducer was set at $\theta = 60^{\circ}$ to avoid physical interference of both the source of light and the edge of the tank. We prepared an ultrasound transducer including a concave ceramic disc to emit focused ultrasound, whose central frequency was 3 MHz, because we have already confirmed that aggregations of microbubbles can be produced and trapped in the middle of a straight path by using the same transducer [14].

B. Artificial blood vessels

We have prepared the following two types of artificial blood vessels, which were the straight path model and the capillary model. The straight path model was made of poly(ethylene glycol) (PEG), which sound velocity (1540-1560 m/s) and density (1.27 g/mL) are similar to that of water. The external size was 85 x 55 x 10 mm^3 and the inner diameter of straight paths was 1 mm. This model is used for calculation of the size of microbubble aggregation under various conditions of ultrasound emission.

On the other hand, the capillary model was made of the mixture material of wax and poly(vinyl alcohol) (PVA) because of the fabrication method of grayscale lithography [15], which is a novel method to be able to fabricate the material, whose sound velocity (1520-1550 m/s) and density (1.2 g/mL) are also similar to that of water, with a high spatial resolution of 100 µm. We have designed the capillary model as shown in Fig.2. The external size was 180 x 70 x 8 mm^3. The inflow path of 2 mm was repeatedly divided into two lower courses to constitute the artificial capillary until the middle of the model, where the minimum diameter was 0.50 mm. After the middle of the model they converged toward the outflow of the model in the opposite way from the inflow to the middle. In this model the diameters of the paths in the same *x*-coordinate are uniform and any *y*-directional cross sections of all the paths are circle. The diameters and the section areas corresponding from A to E in Fig.2 are shown in Table 1 to guarantee a constant flow velocity in any part of the model. The configuration between the transducer and the artificial blood vessel is shown in Fig. 3.

C. Calculation of the size of flown aggregation

When the suspension of microbubbles was injected into the artificial blood vessel under continuous ultrasound emission, we have ever confirmed that cloudy aggregations of microbubbles were observed and varied according to conditions of the ultrasound, whereas no significant aggregations were observed in the case without ultrasound [14]. Considering the angle of the axis of the transducer q, the distribution of sound pressure in the observation area was calculated using the Rayleigh equation to adjust the focal area on the microscope image. The size of an aggregation, which was considered to be sticking to the vessel wall, increases according to the time of ultrasound emission until it would flake off the wall and fly to downstream.

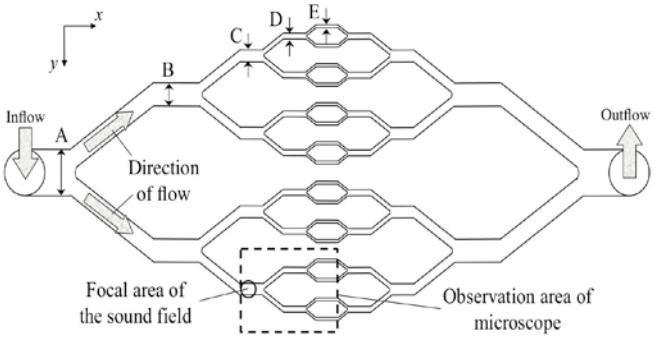

Figure 2. Design of the artificial blood vessel of the capillary model.

TABLE I. THE DIAMETERS AND THE SECTION AREAS OF THE CAPILLARY MODEL, WHICH CORRESPONDS FROM A TO E IN FIG.2.

	A	B	C	D	E
Diameter [mm]	2.00	1.41	1.00	0.71	0.50
Section area [mm^2]	3.14	1.56	0.785	0.395	0.196

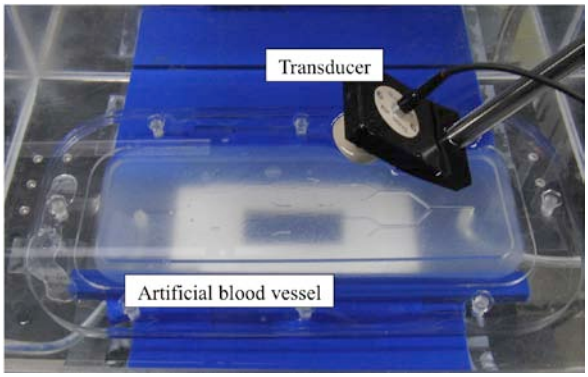

Figure 3. Configuration between transducer and the artificial blood vessel.

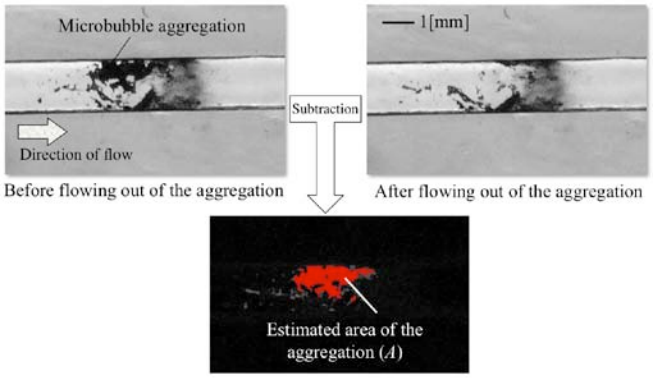

Figure 4. Procedure to calculate the size of flown aggregations in the middle of the straight path model.

To evaluate the size of flown aggregations quantitatively, we used the artificial blood vessel of straight path model. Figure 4 shows the procedure of the image processing to

calculate the size of flown aggregations in the middle of the path, where the flow velocity and the maximum sound pressure were 20 mm/s in this case. Because the timing of fly of aggregations cannot be predicted, the microscopic images were continuously recorded as a video file from the beginning of the experiment. Then the successive two frames just before and after the fly of the aggregations were found in the video file to obtain a subtraction image between them. The subtraction image was converted to a gray scale image for application to unsharp masking for edge enhancement of the aggregations. Finally calculating the binary image by discriminant analysis method [16], the size of flowed aggregations is obtained.

III. Results and Discussion

A. The size of flown aggregation

We examined the experiment, which was explained in the previous section, with the parameters of flow velocity and the maximum sound pressure. The size of microbubble aggregation increases according to the exposure time of ultrasound emission, where the several small aggregations were gradually coalesced. The aggregations have flown to downstream between 30 and 60 s after the beginning of ultrasound emission. Figure 5 shows the calculated size of flown aggregation versus flow velocity and maximum sound pressure. Though we have tried sound pressure less than 100 kPa, no aggregation was optically observed. In our previous research [14] significant aggregations were confirmed when sound pressure was 50 and 100 kPa, where the diameter of the path was 2 mm. Thus there might be a relationship between the size of aggregation and the diameter of the path. These results show that the size of aggregation increased in proportion to sound pressure and inversely to flow velocity. Comparing the sizes of flown aggregation with the section areas of the capillary model shown in Table 1, in all of the conditions in the experiment, the size of flown aggregation was greater than the section area of path E in Fig. 2.

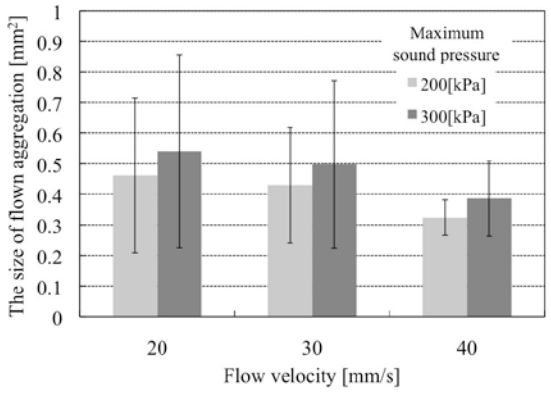

Figure 5. The size of flown aggregation versus maximum sound pressure and flow velocity, at central frequency of 3 MHz of ultrasound emission.

Thus by forming aggregation in upstream of the narrowest path, we have anticipated that there is a possibility to affect to a regular parallel flow in the capillary model. Because the capillary model has 16 narrowest paths in parallel, the entire flow from inflow to outflow of the model is not severely affected if one of narrowest paths is blocked.

B. Flow variations in the artificial blood vessel of capillary model

Figure 6 shows the successive microscope images of the observation area after ultrasound emission with injection of the suspension, at a flow velocity of 20 mm/s. Adjusting the center of the focal area of sound field corresponds in the center line of the path C, ultrasound was emitted at a central frequency of 3 MHz and maximum sound pressures of 300 kPa. In 40 s after the injection of the suspension, microbubbles aggregation was observed in the focal area. In 46.1 s the aggregation flaked off the vessel wall and flew to downstream. In the next moment the flown aggregation was caught at a bifurcation between the paths of D and E. Because the location of the flown aggregation had not changed for more than 10 s, finally we have injected colored water with the suspension of microbubbles. As shown in the last image, it was clearly confirmed that the aggregation blocked a path, where colored water could not penetrate to downstream.

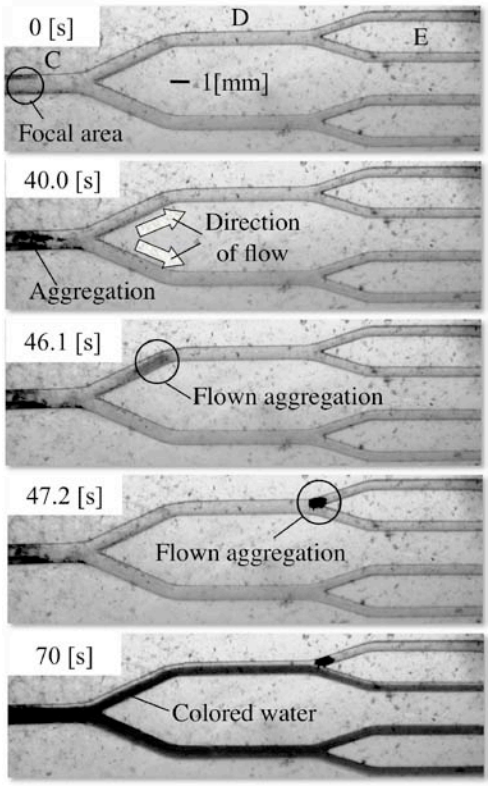

Figure 6. Microscope images of the observation area in the capillary model after injection of the microbubble suspension under continuous ultrasound emission at central frequencies of 3 MHz (maximum sound pressure, 300 kPa; flow velocity, 20 mm/s).

We verified the same experiment under similar parameters with Fig.5, where duration of ultrasound emission was maximum 70 s. Figure 7 shows the probability of path block, which was calculated by dividing the success number of path block by the number of attempts (20 times). In this experiment we did not find more than 2 flown aggregations in one attempt. The locations where the path block was found were random, but they were only in the paths of E, which indicates that the probability of path block might be higher if the diameter of a path is narrower than 0.5 mm.

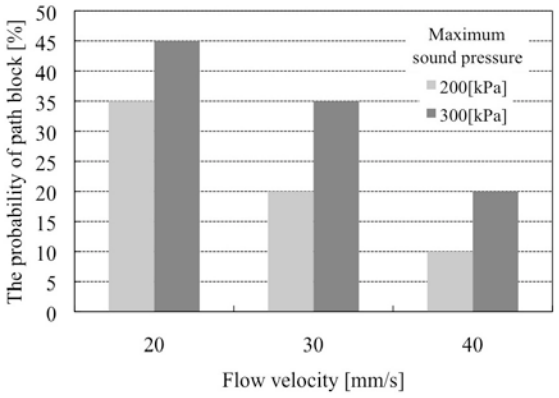

Figure 7. The probability of path block in the capillary model versus maximum sound pressure and flow velocity, at central frequency of 3 MHz of ultrasound emission.

C. Discussion

In this chapter we revealed the possibility that the aggregations can block a path of the diameter of 0.5 mm in an artificial capillary. This result indicates that commercially available microbubbles, which is used as contrast agent for diagnosis, can be applied for a novel therapy of artificial embolization. Since the microbubble aggregations were produced under ultrasound exposure of continuous wave more than 30 s, there is no anxious of embolization in normal diagnostic use of contrast echography. The flown aggregations were found when the maximum sound pressure was 200 and 300 kPa. In this condition we did not observe the destruction of microbubbles because the size of aggregation increased. However, there is a possibility of thermal destabilization in microbubble aggregations. We are going to investigate the behavior in microbubbles with higher magnification to observe individual microbubble. Also we are going to elucidate the vessel diameter effect on the aggregation size, accounting as well for the surface forces effect on microbubbles.

Because not only the size of aggregation but also the probability of path block increased inversely proportional to flow velocity in these experiments, more significant effect to flow will be expected in actual capillary in living organs. However, the location of blocked path in lower course cannot be designated in the experiment. Thus to apply to *in vivo* application, we consider that the following techniques are necessary to enhance the possibility of artificial embolization; 1) recognition technique of precise shape of blood vessel including the target area for treatment, 2) adjustment method of the focal area of sound field on the desired blood vessel by mechanically of electrically, 3) construction of three-dimensional sound field with multiple sound source to constrain the course of flown aggregation. As our previous attempt of active selection of microbubbles, an aggregation produced by secondary Bjerknes force might be induced to the desired target area by making use of primary Bjerknes force.

IV. Conclusion

In this study, we have examined the possibility of artificial embolization using two types of artificial blood vessels. First we estimated the sizes of flown aggregation, which increased in proportion to sound pressure and inversely to flow velocity, with straight path model. Second we investigated the probability of path block in the capillary model, where the sizes of flown aggregation were expected greater than the section area of the minimum path. As the results we confirmed the probability increased in proportion to sound pressure and inversely to flow velocity, as well as the size of aggregation. Because we showed very preliminary results in this paper, we are going to investigate with more kinds of parameters to enhance the possibility of artificial embolization.

References

[1] E. Stride and N. Saffari: “The potential for thermal damage posed by microbubble ultrasound contrast agents,” Ultrasonics, Vol.42, Issues 1-9, pp.907-913, 2004.

[2] G.J.Liu, F.Moriyasu, T.Hirokawa, et al. "Expression of heat shock protein 70 in rabbit liver after contrast-enhanced ultrasound and radiofrequency ablation," Ultrason. Med. Biol., Vol.36, pp.78-85, 2010.

[3] K. Osawa, Y. Okubo, K. Nakao, N. Koyama, and K. Bessho: “Osteoinduction by microbubble-enhanced transcutaneous sonoporation of human bone morphogenetic protein-2,” J. Gene Med. Vol.11, No.7, pp.633-41, 2009.

[4] N.Kudo, K.Okada, K.Yamamoto, "Sonoporation by single-shot pulsed ultrasound with microbubbles adjacent to cells," J. Biophys, Vol.96, No.12, pp.4866-4876, 2009.

[5] L. J. M. Juffermans, A. van Dijk, C. A. M. Jongenelen, B. Drukarch, A. Reijerkerk, H. E. de Vries, O. Kamp, and R. J. P. Musters: “Ultrasound and Microbubble-Induced Intra- and Intercellular Bioeffects in Primary Endothelial Cells ,” Ultrason. Med. Biol. Vol.35, No.11, pp.1917-1927, 2009.

[6] Y. Yamakoshi and T. Miwa: “Effect of Ultrasonic Wave Irradiation Sequence in Microhollow Production Produced by Bubble Cavitation,” Jpn. J. Appl. Phys. Vol.50, 07HF01, 2011.

[7] V. Garbin, M. Overvelde, B. Dollet, N. de Jong, D. Lohse, and M.Versluis: “Unbinding of targeted ultrasound contrast agent microbubbles by secondary acoustic forces,” Physics in Medicine and Biology Vol.56, pp.6161-6177, 2011.

[8] S.Kotopoulis, M.Psotema, "Microfoam formation in a capillary," Ultrasonics, Vol.50, pp.260-268, 2010.

[9] Y. Yamakoshi and T. Miwa: “Microbubble Self-Trapping to Surface of Target,” Jpn. J. Appl. Phys. Vol.47, pp.4127-4131, 2008.

[10] Y. Matsumoto and S. Yoshizawa: "Behaviour of a bubble cluster in an ultrasound field," Int. J. Numer. Meth. Fluids, Vol.47, pp.591-601, 2005.

[11] N. A. Pelekasis, A. Gaki, A. Donikov, and J.A. Tsamopoulos: “Secondary Bjerknes forces and the phenomenon of acoustic streamers,” J. Fluid Mechanics Vol.500, pp.313-347, 2004.

[12] K. Masuda, Y. Muramatsu, S. Ueda, R. Nakamoto, Y. Nakayashiki, and K. Ishihara: "Active Path Selection of Fluid Microcapsules in Artificial Blood Vessel by Acoustic Radiation Force," Jpn. J. Appl. Phys. Vol.48, 07GK03, 2009.

[13] K.Masuda, N.Watarai, R.Nakamoto, et al, "Production of local acoustic radiation force to constrain direction of microcapsules in flow," Jap. J. Applied Physics, Vol.49, 07HF11, 2010.

[14] K.Masuda, R.Nakamoto, N.Watarai, et al, "Effect of existence of red blood cell in trapping performance of microbubbles by acoustic radiation force," Jap. J. Applied Physics, Vol.50, 07HF11, 2011.

[15] T. Nakano, K. Yoshida, S. Ikeda, H. Oura, T. Fukuda, T. Matsuda, M. Negoro, and F. Arai: “Multi-scale transparent arteriole and capillary vessel models for circulation type blood vessel simulator,” Proc. IEEE IROS, pp.75-80, 2009.

[16] B. Raytchev, O. Hasegawa, and N. Otsu: “User-Independent Gesture Recognition by Relative-Motion Extraction and Discriminant Analysis,” New Generation Comput. Vol.18, No.2, pp.117-126, 2000.