1P5-14

Fluorescent observation of microbubble behavior according to time division emission of multiple focal points considering blood vessel shape

血管形状を考慮した複数焦点の時分割照射に対する微小気泡の挙動の蛍光観測

Kiyonobu Nozaki[1†], Asuka Furutani[1], Kosuke Watanabe[1], Takuya Katai[1], Kohji Masuda[1], Johan Unga[2], Ryo Suzuki[2], and Kazuo Maruyama[2] ([1]Graduate School of Bio-Applications and Systems Engineering, Tokyo University of Agriculture and Technology, [2] Faculty of Pharma-Sciences, Teikyo University)

野崎浄信 [1†]，古谷飛鳥 [1]，渡邉晃介 [1]，片井拓也 [1]，桝田晃司 [1]，Johan Unga[2]，鈴木亮 [2]，丸山一雄 [2] ([1] 東京農工大学大学院生物システム応用科学府, [2] 帝京大学薬学部)

1. Introduction

Drug delivery system is expected to treat diseased area by using microbubbles containing specified drug with a high concentration in blood flow. Although we have successfully reported to control microbubbles (MBs) under observation through visible light [1,2], there was a limitation in quantitative evaluation of MBs concentration because the size distribution of MBs includes smaller diameter than the optical limitation (500 nm). To address this issue, we have ever examined to evaluate MBs concentration with echograms (ultrasound images) [3]. However, there was a fundamental problem that observation of MBs using echogram accelerates the destruction of MBs. Therefore, we recently adopted to observe fluorescently dyed MBs to refer direct behavior of MBs with a fluorescent microscope. Furthermore, our conventional approach discussed mainly using single focal point of ultrasound emission, which has a limitation in induction performance considering various three-dimensional shape of blood vessel. In this paper, we present the results of fluorescent MBs observation for active induction in an artificial blood vessel using time division emission of multiple focal points.

2. Experimental methods

We used MBs ready for *in vivo* applications, which have an average size of 2 μm with a single lipid layer of DSPC, DSPG, and DSPE-PEG2000 [4]. They were dyed with DiI, which is a fluorescent lipophilic cationic indocarbocyanine dye that causes them to emit a distinct fluorescence with a wavelength of 565 nm under excitation light with a wavelength of 550 nm.

Fig.1 shows experimental setup to observe behavior of MBs in flow under ultrasound exposure. An artificial blood vessel with a Y-shape bifurcation, which was made of dimethylpolysiloxane (PDMS), was installed in a water tank filled with degassed water. The ultrasound transducer, which has 64 elements with a frequency of 3 MHz on the surface two-dimensionally, was fixed in the water tank to guarantee the distance l = 60 mm and the angle φ = 45 degrees to observe the MBs behavior using a fluorescent microscope (Olympus BXFM).

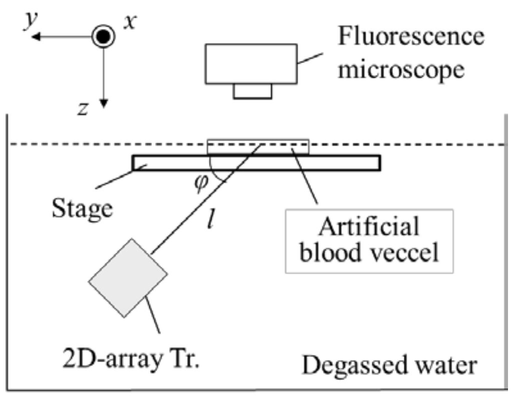

Fig.1 Experimental setup to observe behavior of MBs.

Fig.2 shows a schematic view of the artificial blood vessel in the horizontal plane, where the bifurcation point was defined as (x, y) = (0, 0). Each path has a cross section of square shape, where one sides in upstream and downstream are 1.4 mm and 1.0 mm, respectively. We produced two focal points alternatively, which were the upper point F_u (d, 0) and the bifurcation point F_b (0,0) by time division emission, with the parameters of the distance d between two points and the emission duration τ for each point. Then, transition rate μ between the two points was defined as $\mu = d/\tau$. As shown in Fig.2, two regions of interest (Area A and B) were established in lower paths to evaluate the degree of induction using the concentration of MBs

ultrason@cc.tuat.ac.jp

in two areas, which were converted from the fluorescent magnitude with the correlation coefficient measured in advance, as similar to our preceding research [3].

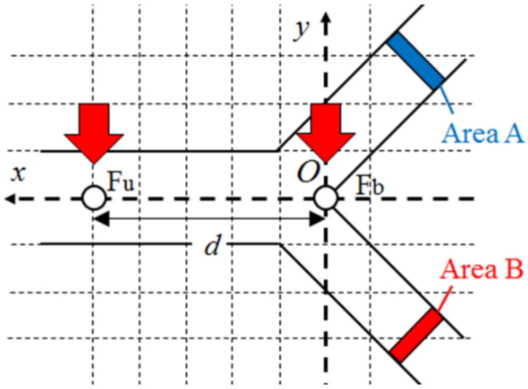

Fig.2 The schematic view of the artificial blood vessel and definition of two focal points and evaluation areas.

3. Results

We have produced two focal points, each of which has a maximum sound pressure of 400 kPa-pp and duty ratio of 25%, with the parameters of d = 0, 5, and 10 mm by fixing μ = 100 mm/s. Note that a single focal point is produced with duty ratio of 50% when d = 0. According to the actual measurement, the beam width of the focal point was 2.7 mm.

We injected 0.5 mL of a MBs suspension with a concentration of 31.3×10^6 /ml at a flow velocity of 10 mm/s. Fig.3 shows the representative microscopic images around the bifurcation point when a MBs suspension was appeared under ultrasound exposure with various condition of the distance d. The induction performance with d = 5 mm seemed to be more effective than that with d = 0 or 10 mm. Fig.4 shows time variation of the MBs concentration difference between two areas according to the distance d, where the pulsatile waves indicate the oscillation caused by roller pump. The tendency of induction performance seen in Fig.3 was quantitatively confirmed. From these results, because of the beam width of 2.7 mm, two focal points adjoining each other along the flow direction was more effective to induce MBs than the single focal point (d = 0), even though the acoustic energy was distributed. On the other hand, two focal points, which were completely separated (d = 10 mm), the induction performance was less than the single focal point since the cooperation between two points did not work efficiently.

4. Conclusions

In this paper, we experimented the induction performance of a MBs suspension with two focal points using time division emission. According to the fluorescent observation, there is an appropriate position of the focal points, which should be taken the relationship between beam width and blood vessel shape into account.

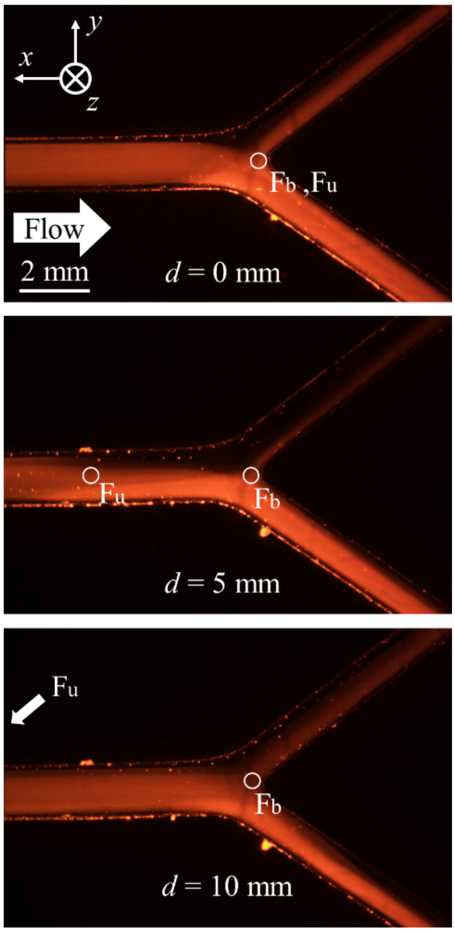

Fig.3 Microscopic images with a MBs suspension flow under ultrasound exposure.

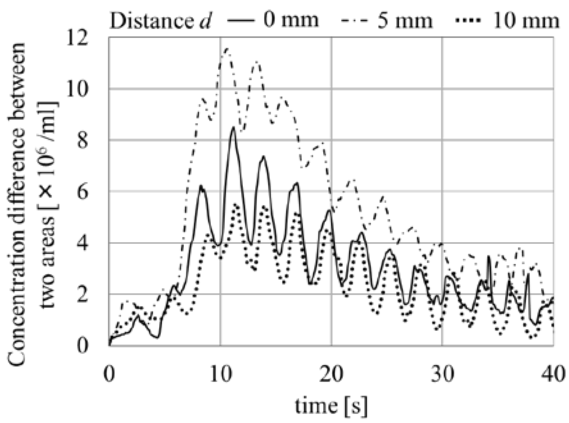

Fig.4 Time variation of concentration differences between two areas with the parameter of distance d.

References

1. K.Masuda, et al: Jpn. J. Appl. Phys. 49, 2010.
2. R.Koda, et al: Jpn. J. Appl. Phys. 52, 2013.
3. H.Wada, et al: Jpn. J. Appl. Phys. 55, 2016.
4. J.Unga, M.Hashida: Adv. Drug Deliv. Rev., 72, 2014.