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Study of induction method of nanobubbles using frequency response analysis of ultrasound to verify with florescent observation

周波数応答解析を応用したナノバブルの誘導制御法の検討と蛍光観察による検証

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1. Introduction

Recently therapeutically applications using ultrasound and microbubbles have been reported. A drug delivery system, for example, is expected to treat diseased area by delivering microbubbles with a high concentration in blood flow. We have been successfully reported our achievements to control microbubbles in artificial blood vessels [1,2]. However, because microbubbles we used were not appropriate for *in vivo* applications due to the limitation of metabolism, we adopted to use nanobubbles (NBs) [3] included in bubble liposomes [4], which were originally developed for *in vivo* applications. Since NBs are invisible with a conventional optical observation, we have examined to find the destruction condition of NBs with echograms (ultrasound images) [5]. However, observation using echograms has a problem because there is no method to evaluate destruction effect of NBs by echogram itself. Therefore, we used fluorescently dyed NBs to observe with a fluorescent microscope to measure behavior of NBs directly. By using the fluorescent observation, we could visualize the behavior of NBs under various ultrasound conditions including central frequency and sound pressure of applied ultrasound. In this presentation, we present the results of fluorescent observation to apply for active induction of NBs.

2. Experimental methods

We used NBs composed of DSPC, DSPG, and DSPE-PEG2000, which have an average size of 500 nm with a single lipid layer [3]. NBs were formed by homogenization in an atmosphere of perfluoropropane (PFP) before mixing with sucrose solution and freeze dried. Upon the experiment NBs were reconstituted by adding Milli-Q water and lightly shaking the vial. NBs were dyed with DiO, which is a fluorescent lipophilic cationic indocarbocyanine dye that causes them to emit a distinct fluorescence with a wavelength of 501 nm under excitation light with a wavelength of 484 nm.

Before the fluorescent observation we have measured the attenuation property of NBs according to the impulse response, which might be related to the resonant frequency of NBs. Fig.1 shows the experimental setup to measure the attenuation through NBs suspension confined in a thin channel with w_1 = 10 mm and d_1 = 2 mm. The ultrasound transducer has a diameter of 2 mm with the frequency band of 3-7 MHz. The hydrophone (HNR-1000, Onda) has a frequency range from 0.25 to 10 MHz. The distances in Fig.1 were set with $l_1 = l_2$ = 30 mm.

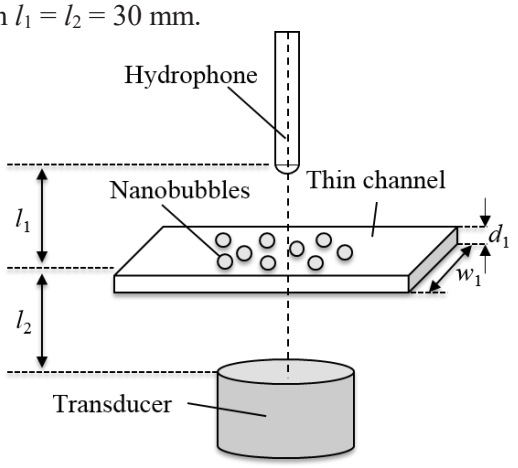

Fig.1 Experimental setup to measure ultrasound attenuation of NBs suspension.

Fig.2 shows another experimental setup to observe behavior of NBs behavior in flow under ultrasound exposure. The size of the thin channel is w_2 = 2 mm and d_2 = 2 mm. The ultrasound transducer to propel NBs and the fluorescent microscope (Olympus BXFM) were set

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perpendicular to the flow direction. The distance between the sound axis of the transducer and the center of observation area of the microscope was set with a = 3 mm. We also established two areas, which sizes are w_x = 2 mm and w_y = 1 mm, in the observations area to evaluate the effect of induction by ultrasound emission. The fluorescent images were recorded with maximum 30 frames/sec.

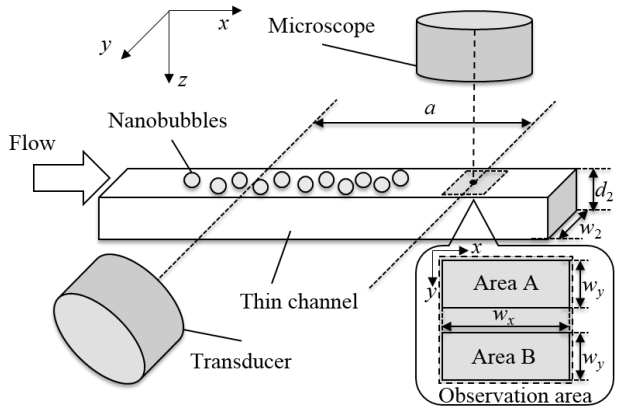

Fig.2 Experimental setup to observe behavior of NBs.

3. Results

In the experimental setup of Fig.1, we have emitted a pulse wave, which has central frequency of 5 MHz, duration time of 1.0 μs, and the maximum sound pressure of 100 kPa-pp. Fig.3 shows the calculated distribution of ultrasound attenuation versus frequency with FFT analysis including three trials. We have found the obvious concave property of attenuation around 2 MHz, which we consider that NBs oscillated with the frequency near 1 MHz and 5 MHz, whereas NBs did not respond with 2 MHz.

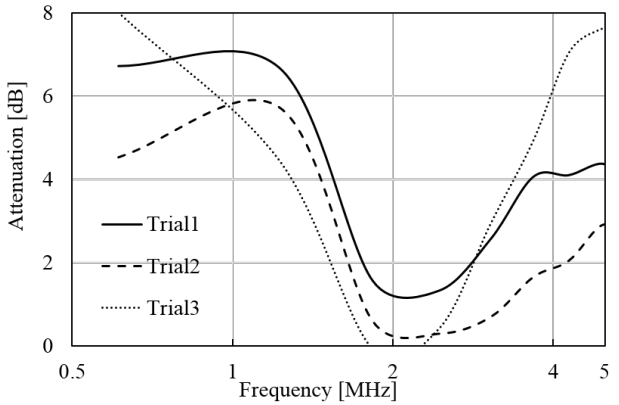

Fig.3 The ultrasound attenuation property though NBs suspension calculated with FFT analysis.

In the next experiment shown in Fig.2, we have observed the behavior of NBs under ultrasound exposure. The transducer emitted a sinusoidal wave with single frequency selected from 1, 2 and 5 MHz, where the maximum sound pressure was fixed to be 200 kPa-pp. Fig.4 shows the brightness difference between the area A and B, where the difference is positive when more NBs were found in the area A, versus flow velocity from 10 to 40 mm/s. With the frequency of 5 MHz, we confirmed a distinct decrease of the brightness difference in proportion to flow velocity, which indicates that the active induction becomes difficult according to flow velocity, and shows the same tendency to our experience using microbubbles [1,2]. On the other hand, with frequency of 1 and 2 MHz, we observed slight brightness differences, which indicates a low controllability of NBs. Considering with the results with Fig.3, the result of controllability with 2 MHz is reasonable, whereas further discussion is necessary with 1 MHz.

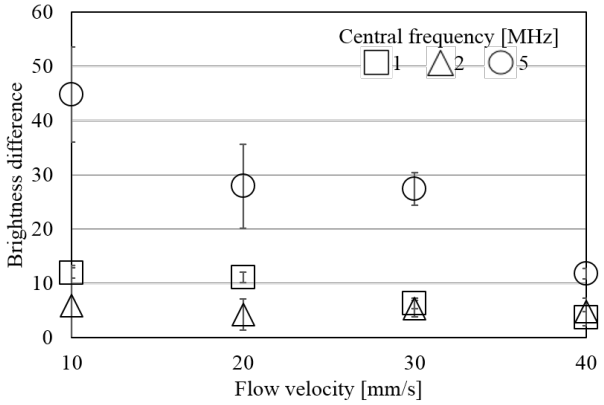

Fig.4 The brightness difference between the area A and B versus flow velocity of NBs suspension.

4. Conclusions

In this paper, we observed the behavior of NBs using florescent microscope under ultrasound exposure, which includes three different frequencies of sinusoidal waves, after examining the ultrasound attenuation through NBs suspension. With the frequencies of 2 and 5 MHz, the behavior of NBs was reasonable, which predicts that there would be an optimal frequency or their combination to induce NBs. We are going to apply the results for active induction of NBs in *in vivo* blood vessels.

References

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