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Experimental analysis of behavior in nanobubbles using echograms under various conditions of ultrasound exposure
超音波照射条件に対するナノバブルの超音波断層像を用いた挙動解析

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1. Introduction

Recently therapeutical 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 the artificial blood vessels [1,2]. However, microbubbles we used were not appropriate for *in vivo* applications because of the limitation of metabolism. Thus we have adopted nanobubbles (NBs) [3], which were intended to be sealed perfluoropropane gas into the lipid bilayer with a drug-retaining function as the feature. Meanwhile, there were two major problems in NBs, which are easily destructed by an external force, and are invisible using a conventional optical observation due to an average diameter of 0.5 µm. Thus it is necessary to investigate their behavior under various conditions of ultrasound exposure. In this study, we observed the behavior of NBs in a circulation path using echograms (ultrasound images), where concentration of NBs is referred to the brightness in echograms.

2. Experimental methods

Fig.1 shows the experimental setup to observe the behavior of NBs under ultrasound exposure, with the total length of the path of 1000 mm and inner diameter of the path of 1 mm. The ultrasound transducer, which has a diameter of 2 mm with the central frequency of 5 MHz, was set with the angles of θ = 60 deg and φ = 30 deg, and the distance from the path of d = 60 mm, which are the same orientation of conventional method of active induction of microbubbles. The ultrasound probe (11L with Logiq7, GE Healthcare) was set to observe cross section of the path with the distance between the transducer and the probe of l = 80 mm. As shown in Fig.2, when NBs suspension was filled in the path, the brightness in the path shows the maximum brightness. Then the suspension was circulated to observe variation in echograms under ultrasound exposure. According to the circulation time and the mechanical stress, NBs are destructed to show lower brightness in the echograms. In addition, we prepared two kinds of NBs (A and B) as shown in Table 1, where composition of lipids are different, to compare these features.

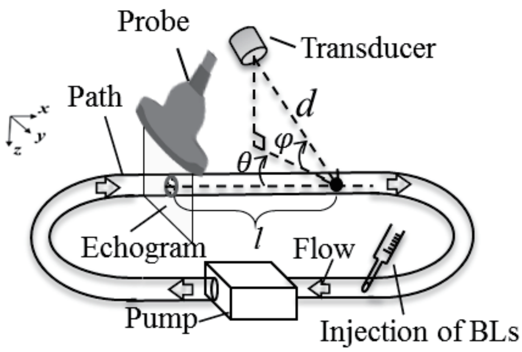

Fig.1 Experimental setup.

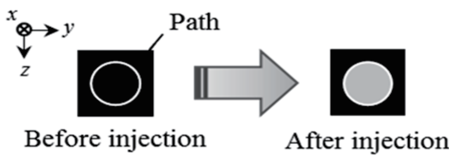

Fig.2 Variation in brightness on echogram according to the existence of NBs.

Table 1 Compositions of the lipids in NBs.

	Composition of the lipids
Bubble A	DSPC:DSPE-PEG
Bubble B	DSPC:DSPE:DSPE-PEG2k

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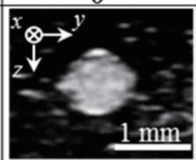
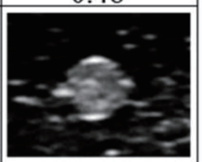
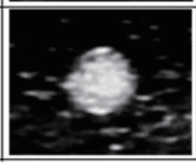
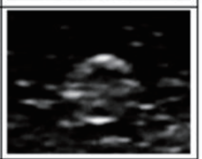
3. Results

We have set flow velocity of 30 mm/s and the condition of the transducer with the sound pressure of 100, 200 and 300 kPa-pp, where a NBs are exposed by ultrasound 0.12 sec per cycle. Also the conditions of echograms were set with a mechanical index of 0.27, dynamic range of 30 dB, and observation time of 5 sec per cycle. Table 2 shows the representative results in echograms, which include the sound pressure of 0 and 300 kPa-pp, and total time of exposure of 0 and 0.48 sec. Fig.3 shows the time variation of the normalized brightness with comparison of two kinds of NBs. In the both NBs, the brightness decreased according to the time of exposure and the sound pressure, which indicates that NBs were destructed by time and accelerated by the sound pressure of ultrasound exposure. Comparing two NBs, brightness attenuation in the bubble B was less than A.

Table 2 Representative results in echograms with the conditions of sound pressures and total times of ultrasound exposure (Bubble A).

Bubble A		Total time of ultrasound exposure [sec]	
		0	0.48
Sound Pressure [kPa-pp]	0		
	300		

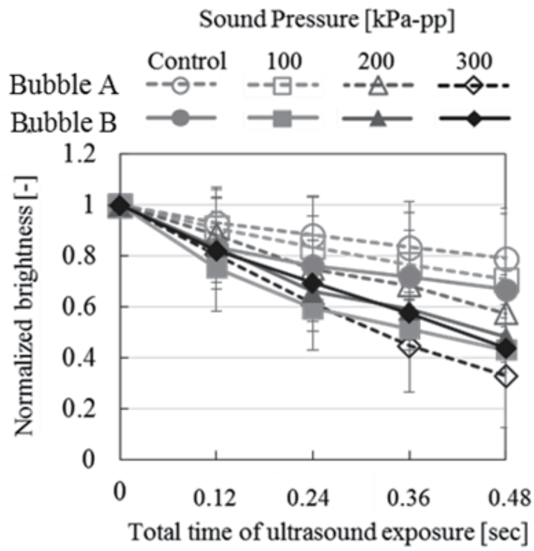


Fig.3 Time variation of brightness in the echograms and comparison of two kinds of NBs.

Fig.4 shows the average decrease in brightness per cycle, where the endurance of bubble B versus sound pressure was confirmed. Furthermore, in the bubble B, there was not significant difference in average decrease between 100 and 300 kPa-pp. From these results, the bubble B is more suitable for our purpose than A because several 100 kPa-pp was necessary for active control of NBs to propel and to form aggregations.

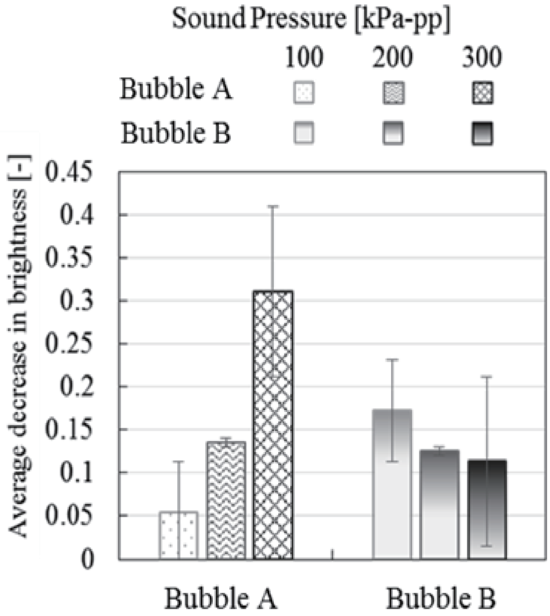


Fig.4 Comoparison of average decrease in brightness per cycle between bubble A and B.

4. Conclusions

We have examined and observed the behavior of two kinds of nanobubbles under ultrasound exposure using brightness variation on echograms. There was a difference of endurance versus sound pressure in two bubbles. Because the nanobubbles should not destruct under sound pressure of several 100 kPa-pp for active control, we found bubble B was more appropriate than A. We are going to optimize the ultrasound conditions to use bubble B for further experiments.

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