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Relationship between stability and viscoelastic property of fluorescence microbubbles (14 - 16 points)

蛍光マイクロバブルの粘弾性的性質と寿命の関係性

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1. Introduction

Microbubbles (MBs) with indocyanine green derivative (ICG-C18)[1] has been developed as a prototype of contrast agents for dual imaging of ultrasound and near-infrared (NIR) fluorescence imaging. To enhance the fluorescence intensity from MBs, the lipid shell coating MBs composed of DSPC and egg-yolk lecithin including several different phospholipid and cholesterol[2]. The property of lipid membranes significantly affects the characteristic as ultrasound contrast agents such as backscattering coefficient and stability of MBs. In this report, we discuss whether the viscoelastic property of lipid membrane depend on the presence of lecithin based on the measurements of acoustic attenuation in MBs suspension. In addition, the stability of MBs under no exposure of ultrasound, i.e. life time of MBs, was discussed based on the analysis of radius-time curve for a single MB.

2. Method

2.1 Preparation of microbubbles

Two types of MBs labeled with ICG-C18 were prepared. One was composed of DSPC, DSPE-PEG2000, lecithin and ICG-C18. The other type of ICG-C18 MBs had no lecithin.

2.2 Analysis of viscoelastic properties

Acoustic attenuation in MB suspension were measured to quantify the viscoelastic properties of the lipid shell coating MBs. Ultrasound pulse with a center frequency of 10 MHz and peak negative sound pressure of 9.3 kPa was emitted from a flat transducer. The reflected wave from a reflector was received by the same transducer and its power spectrum was analyzed. By dividing with the power spectrum in absences of MBs (i.e. in water), we evaluated the round-trip attenuation. The mean diameter of MBs was ranged from 1.55 μm to 1.75 μm for MBs with lecithin and 1.4 μm to 1.55 μm for MBs without lecithin. The number density of MBs in the solution at the attenuation measurement was ranged from 1×10^{10} 1/m^3 to 1×10^{11} 1/m^3.

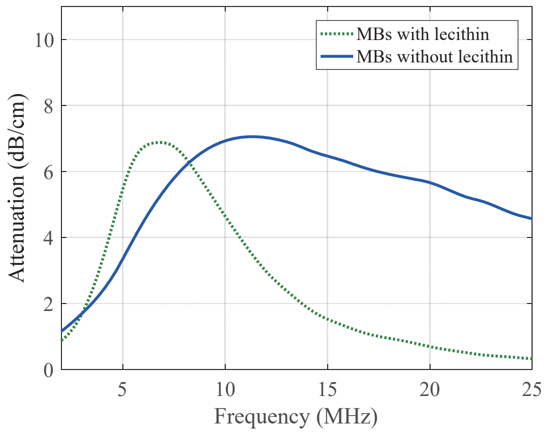

Fig. 1 Typical attenuation characteristic for each types of MBs.

The dilatational elasticity (E^S) and viscosity (κ^S) of lipid membrane was analyzed assuming the linear oscillation of MBs. We obtained the estimates when the difference in attenuation between theoretical calculation and experimental data reached minimum.

2.3 Analysis of stability

To evaluate the stability of MBs, the shrinkage (i.e. dissolution) of MBs were observed by an optical microscope under the static condition with no ultrasound exposure. Radius-time curve of a single MBs with initial radius of 4.5 μm – 5.5 μm was analyzed where the radius (R) was calculated from bubble shadow area (S) assuming of spherical shape i.e. $R = \sqrt{S/\pi}$. The time until the bubble absolutely dissolved into surrounding water was evaluated as the disappearance time.

3. Results

3.1 Viscoelastic properties

Fig. 1 shows the typical frequency-dependent

Table I Viscoelastic properties and stability of ICG-C18 MBs

Type	Viscoelastic properties		Stability
	E^S (N/m)	κ^S (× 10^{-9} Ns/m)	Disappearance time (s)
MBs with lecithin	0.39 ± 0.18 (n=11)	4.2 ± 4.15 (n=11)	13 ± 5.9 (n=5)
MBs without lecithin	2.2 ± 0.74 (n=12)	41.9 ± 4.98 (n=12)	22 ± 5.5 (n=5)

attenuation for two types of MBs. The attenuation characteristic of MBs without lecithin has a broader peak with higher peak frequency than MBs with lecithin. The estimated parameters (E^S and κ^S) were summarized in **Table I**. MBs without lecithin was approximately 5 times larger for E^S and 10 times larger for κ^S than those with lecithin.

3.2 Stability

Fig.2 shows the pictures of MBs and the radius-time curve during the shrinkage of MBs for each type of MBs. Buckling behavior[3] was typically observed in case of MBs without lecithin. Thus, the radius seemed to be discretely decreased. In contrast, MBs with lecithin shrank while keeping the spherical shape. MBs was absolutely dissolved for several ten seconds. The disappearance time for each MBs is summarized in Table I. It was found that the disappearance time for MBs without lecithin was longer than that for MBs with lecithin.

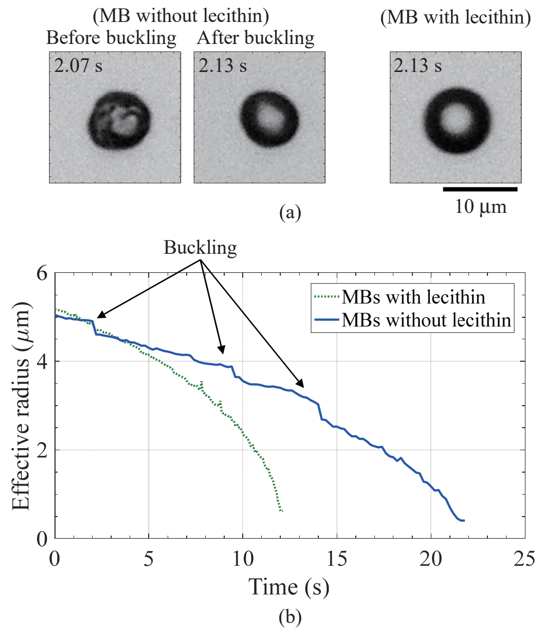

Fig. 2 (a) Pictures of MBs during dissolution and (b) radius-time curve for ICG-MBs with/without lecithin.

4. Discussions

Our results showed that the viscoelastic characteristic (E^S and κ^S) for ICG-C18 MBs became reduced owing to the adding the egg-yolk lecithin into the DSPC-based lipid shell. It is speculated that DOPC and POPC with low phase transition temperature of -17°C and -2°C, which are included in egg-yolk lecithin, force the shell behaving as viscoelastic gel and viscos fluid, resulting in small dilatational elasticity and viscosity of the shell. The fact that buckling behavior is frequently observed only in the case of MBs with lecithin supports the hypothesis.

The decrease in life time of MBs by adding the lecithin is possibly explainable from the standpoint of lipid composition. Previous study showed that fluid-state lipid shell did not play role in the resistance to the gas transportation[3]. If DOPC and POPC change the state of lipid shell to the fluid-state, it is possible that the resistance of ICG-C18 MBs with lecithin is drastically reduced.

5. Conclusions

We evaluated the change in viscoelastic properties and stability of microbubbles caused by incorporating the egg-yolk lecithin into the lipid shell. We confirmed that adding the egg-yolk lecithin reduced the dilatational elasticity to 1/5, dilatational viscosity to 1/10 and the life time (disappearance time) to half.

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