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Controlled release of particles included in giant cluster vesicles by exposure of ultrasound
超音波を用いた巨大ベシクル凝集体内包微粒子の放出制御

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1. Introduction

Intraoperative localization of main tumors as well as estimation of metastatic status of the regional lymph nodes, especially sentinel lymph nodes (SLNs; the first draining lymph nodes from the main tumor), are essential for surgical treatments of malignant diseases. A multifunctional contrast agent, which has two contradictory properties i.e. retention in tissue for lesion-marking and drift for SLN identification, has been proposed. The agent named Giant Cluster Vesicles (GCVs) can stay in the injected area for a long time and includes liposomally formulated indocyanine green (ICG) derivatives as a tracer for SLN identification. The encapsulated liposomally formulated ICG derivatives can be released by the destruction of GCVs caused by physical stimulus e.g. ultrasound irradiation. To control the destruction of GCVs and the release of liposomally formulated ICG derivatives, we proposed a method that utilizes the interaction of ultrasound contrast agents (UCAs) and ultrasound. In this report, it was investigated whether the interaction enhanced the release of the tracer particles.

2. Materials and methods

2.1 Preparation of Giant Cluster Vesicle

The structure of GCV is shown in **Figure 1**. The GCV, which is an aggregate of vesicles with a particle size of larger than 1 μm, is composed of a lipid bilayer with polyglycerol polyricinoleate (PGPR) as the membrane. We encapsulated LP-ICG-C18 in GCV as liposomally formulated ICG derivatives. LP-ICG-C18 incorporates ICG-C18 into the liposome membrane composed of 1,2-dioleoyl-3-sn-glycero-phosphocholine (DOPC) as the main component and *N*-(methylpolyoxyethylene oxycarbonyl)-1,2-distearoyl-sn-glycero-3-phosphoethanolamine sodium salt (DSPE-PEG5000) and cholesterol. ICG-C18 is an ICG derivative synthesized by substitution of one sulfonate groups of the ICG with an 18-carbon alkyl chain [1][2].

To examine the effect of UCAs, two types of solutions were exposed to ultrasound: one was a mixture of GCVs suspension and 1 mol/L sucrose solution at a 1:1 volume ratio, and the other was a mixture of GCVs suspension and UCAs suspension (Sonazoid®, a clinical UCAs, dispersed in 1 mol/L sucrose solution) at a 1:1 volume ratio. The particle size of UCAs was 2.28 ± 0.75 μm and the number density was 1.06×10^{14} 1/m^3.

2.2 Procedure of GCV destruction

A silicon tube with an inner diameter of 2 mm was placed in an experimental cell filled with degassed water, and 1 mL of sample solution was sent at 0.35 mL/min using a syringe pump (Hygion Touch 200, ISIS Corporation). The sample solution in the tube was irradiated with ultrasound from above using the share wave elastography mode of the ultrasonic equipment (LOGIQ S8, GE Healthcare) and linear probe (9L, GE Healthcare). The center frequency of the transmitted ultrasound was 4.1 MHz. The lateral direction of the probe and the long axis direction of the tube were made orthogonal. The focus point of the ultrasound beam was positioned at the center of the tube. The experiment was conducted three times at the same condition for ultrasound transmitting.

The ultrasonic equipment for this study was adjusted for basic experiments and the settings for ultrasound irradiation differed from those used in

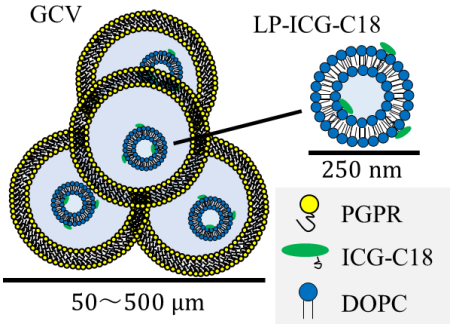

Fig. 1 Structure of GCV. The structures of DSPE-PEG5000 and cholesterol are not shown.

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clinical. In this paper, the results for ultrasound irradiation (negative-peak pressure 3.5 MPa) without UCAs were labeled with (UCA(-), US(+)). The labeling (UCA(+), US(+)) indicated the result in the presence of UCAs under the ultrasound irradiation. The results without ultrasound irradiation and UCAs were labelled with (UCA(-), US(-)).

2.3 Quantification of the amount of released LP-ICG-C18

The sample solution (0.75 mL) collected after the ultrasound irradiation was centrifuged and sedimented at 6,000 rpm for 30 min using a small centrifuge (Puchi Maru 8 Model 2320, Wakenbeatech Co.). The supernatant liquid was collected to recover LP-ICG-C18 released from GCVs. The supernatant solution was diluted two-fold and the absorbance was measured using a UV-visible spectrophotometer (UV-1800, SHIMADZU). The release rate of LP-ICG-C18 was calculated from the concentration of ICG-C18 in the supernatant solution (c_r), the dilution factor (n), and the concentration assuming that all ICG-C18 encapsulated in the GCVs was dispersed in the sample solution (c_{org}).

$$Release\ rate = \frac{n \cdot c_r}{c_{org}} \quad (1)$$

The concentration of ICG-C18 was calculated from the preliminarily-obtained calibration curve of absorbance and ICG-C18 concentration.

3. Results

Figure 2 shows the typical absorption spectra from the three experimental conditions. The maximum absorbance wavelengths of LP-ICG-C18 were in the range of 800 to 820 nm [3]. In Fig. 2, the maximum absorption wavelengths of the spectra were ranged in the same wavelength. Without UCAs, the absorbance at the peak wavelength was found to be less than 0.4. In contrast, the absorption spectrum with ultrasound irradiation in the presence of UCAs (UCA(+), US(+)) was clearly larger than the others.

Figure 3 shows the release rate of LP-ICG-C18 in each condition. Error bars mean standard deviation. The reason for the release rate of approximately 10% at 0 MPa, i.e. without ultrasound irradiation, might be that a small amount of GCVs was destroyed in the process of introducing the sample solution or remained GCVs after centrifuging contaminated the supernatant solution. The release rate of LP-ICG-C18 was low in the absence of UCAs (UCA(-)), regardless of ultrasound irradiation. On the other hand, the release rate of LP-ICG-C18 was clearly high when ultrasound was irradiated in the presence of UCAs (UCA(+)).

The quantitative evaluation suggested that the interaction of ultrasound and UCAs enhanced the amount of released LP-ICG-C18. Currently, we examine the sound pressure dependence of the amount of released LP-ICG-C18 in detail and investigate the practical ultrasound irradiation conditions for safety use.

4. Conclusions

It was reported that ultrasound irradiation in the presence of UCAs could enhance the GCV destruction and the release of included LP-ICG-C18.

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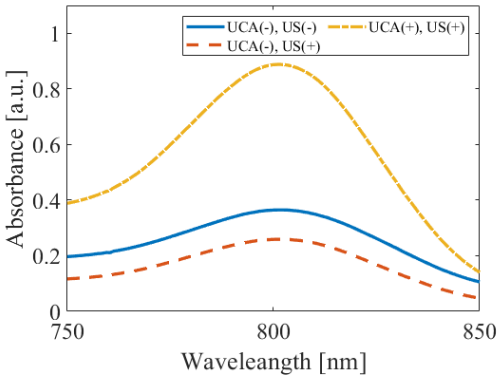

Fig. 2 Absorbance spectrum of supernatant solutions after centrifuging.

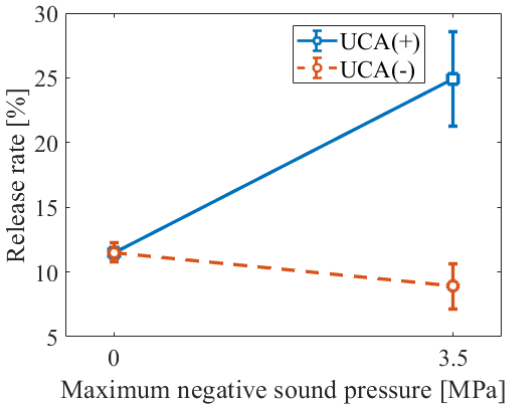

Fig. 3 Release rate of LP-ICG-C18 as a function of sound pressure.