Proceedings of Symposium on Ultrasonic Electronics, Vol. 36 (2015)
5-7 November, 2015

High-speed observation of cell−microbubble interaction from frontal and lateral directions

正面と側方からの細胞−気泡間相互作用の高速度観察

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1. Introduction

We have been studying the sonoporation technique that transduces drugs and genes into cells by exposure of cells with attached microbubbles to pulsed ultrasound.[1)] Low transduction efficiency is a major problem of this technique, and high-speed observation of cell-bubble interaction is an important technique to understand the sonoporation mechanisms and also to improve the efficiency. In our previous studies, high-speed observations were carried out using an inverted-type microscope, and a cell with an adjacent bubble floating beneath a coverslip was observed from the bottom. However, observation of cell damage from this direction was difficult, especially in the situation in which a bubble adheres to the top of the cell, because the image of an oscillating bubble interferes with observation of change in the cell.

In this study, a new observation chamber in which the direction of observation was changed from a frontal direction to a lateral direction was designed, and interaction between a bubble and a cell was observed using the conventional and newly developed chambers.

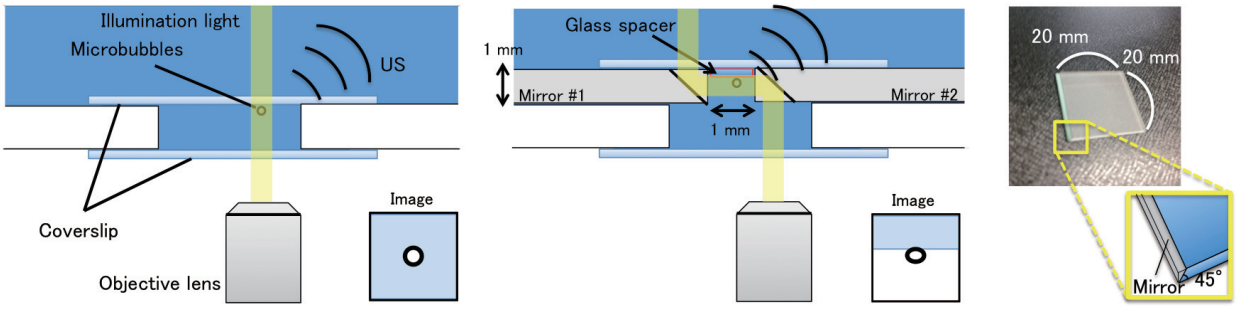
2. Materials and methods

Figure 1 shows cross-sectional views of the conventional and newly developed observation chambers. Chamber **(a)** was used for observation from a frontal direction and chamber **(b)** was used for observation from a lateral direction. A water tank with a hole of 15 mm in diameter in the base plate of 4.0 mm in thickness was placed on the stage of an inverted-type microscope (IX70, Olympus), and the observation chamber was made by covering the top and bottom faces of the hole with coverslips.

As shown in **Fig. 1b**, observation from a lateral direction was achieved by using a pair of small mirrors (Chrovit, Technical, Aomori) inserted between the top surface of the base plate and upper cover slip. The mirror is 1 mm in thickness and has a light-reflecting surface of a 45-degree angle on one side (**Fig. 1c**). Mirror #1 was arranged so that light from a source illuminates a bubble and a cell from a lateral direction, and mirror #2 was arranged so that the lateral image is incident on an object lens of the microscope. Furthermore, a glass spacer of 1 mm in width and 0.1 mm in thickness was placed between the mirrors to visualize a contact region of the spacer and bubble.

Cell-bubble interaction was observed using both observation chambers. Human prostate cancer cells (PC-3 cells) were incubated on a coverslip in the experiment in which chamber **(a)** was used and on the glass spacer in the experiment in which chamber **(b)** was used. The observation chambers were filled with Hanks' balanced salt solution suspended with two types of microbubbles, and the cover slip or spacer was attached to the chamber with the cell face down so that bubbles attach to the cell surface. Albumin-shelled microbubbles of 3.0−4.0 μm in diameter were prepared by agitation of saline supplemented with bovine albumin and were used for frontal observation. Bubbles of a bubble liposome[2)] were used for lateral observation.

A focused transducer of 50 mm in aperture and 70 mm in focal length was used to generate 3-cycle pulses of 1 MHz in center frequency and



(a) Observation from a frontal direction (b) Observation from a lateral direction (c) Chrovit

Fig. 1. Observation chambers for frontal and lateral observations

1.3 MPa in peak negative pressure. High-speed observation was carried out using Ultranac (Nac Image Technology, Tokyo) with a x40 object lens. Twenty-four frames were taken at one time, and bubble behavior under one shot of the pulsed ultrasound was captured for 6 μs at a framing rate of 4 frames in one ultrasound cycle.

3. Results and discussion

Figure 2 shows cell-microbubble interaction observed from a frontal direction. A still image taken before insonation (**Fig. 2a**) indicates the presence of an albumin-shelled microbubble neighboring a spherical cell (arrowhead). Brownian motion of the bubble indicates the bubble floating beneath a coverslip. High-speed photos (**Fig. 2b**), which were selected from a series of high-speed frames, visualizing bubble fragmentation at 1.5 μs and following non-spherical contraction at 2.0 μs. Decrease in cell image contrast was confirmed at 4.5 μs, indicating that cell deformation causes the membrane damage. A still image taken after insonation (**Fig. 2c**) confirms the leak out of the cytoplasm.

Figure 3 also shows the interaction but that observed from a lateral direction. The white horizontal line in the image indicates the surface of the coverslip. A still image taken before ultrasound exposure visualizes a spherical cell with a bubble liposome on its top (**Fig. 3a**, arrowhead). The bubble showed no Brownian motion, indicating its adhesion to the cell membrane. Separation of the bubble from the coverslip surface was approximately 25 μm.

High-speed photos in **Fig. 3b** show decreased amplitude of bubble radial oscillation and no occurrence of bubble fragmentation. This indicates that the adherent bubble showed dumped oscillation because acoustic energy absorbed by the bubble was partially used to deform a viscoelastic cell. A still image taken 0.5 minutes after insonation (**Fig. 3c**) depicted generation of a saclike expansion at the location of bubble adhesion, indicating the ability of dumped oscillation to generate temporal membrane damage. It is well known that reduction of membrane tension promotes resealing of the membrane opening and that inflow of Ca^{2+} during sonoporation initiates a rapid process to reduce membrane tension by fusing vesicles existing inside the cell.[3] Leou et al. also observed occurrence of an expansion during sonoporation and suggested the possibility that expansions work as a buffer compartment to store excess Ca^{2+} that has flowed into cells during sonoporation.[4]

4. Conclusion

An observation chamber that enables lateral observation was newly developed, and cell-bubble interaction was investigated by high-speed observation from frontal and lateral directions. The experimental results indicate the importance of observation from the lateral direction, especially for elucidation of the mechanism of *in vivo* sonoporation, because lateral observation enables visualization of cell change without disturbance of the oscillating bubble image even in a condition in which the bubble adheres to the top of cell.

Reference

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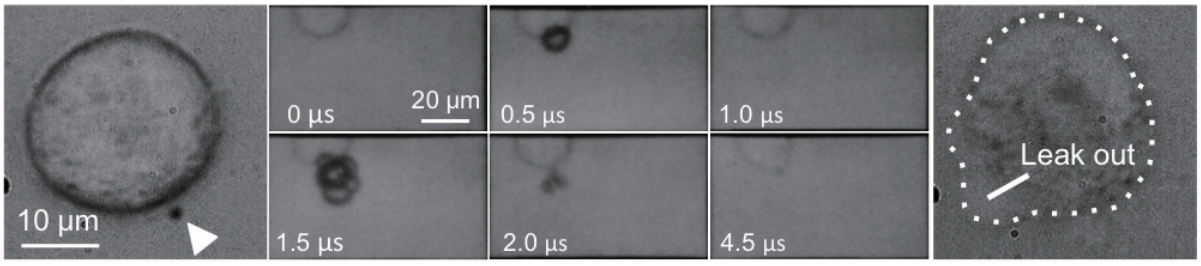
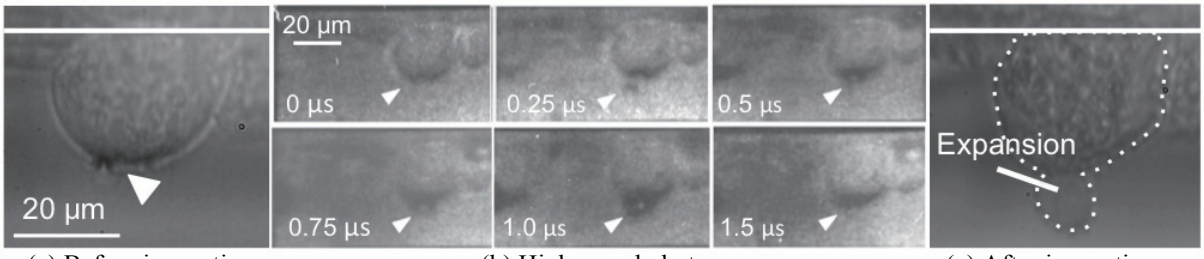


(a) Before insonation (b) High-speed photos (c) After insonation

Fig. 2. Observation from frontal direction



(a) Before insonation (b) High-speed photos (c) After insonation

Fig. 3. Observation from lateral direction