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Influences of low frequency ultrasound to cells cultured on gel: Mechanical effects of bubble vibrations

低周波超音波がゲル上培養細胞に与える影響について
～気泡振動による機械的作用～

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1. Introduction

Ultrasound technologies have been applied to medical diagnosis and therapy because of their noninvasive nature and convenience. However, some problems remain in the safety of ultrasound, especially influences of cavitation. Generally, Mechanical Index (MI) is often used for the safety indicator evaluating the cavitation influence [1]. However, we can not evaluate the response of cell to cavitation, because MI is only based on cavitation occurrence. Thus, it is necessary to investigate the safety of ultrasound in terms of biological and cytological aspects in additional to cavitation dynamics.

In previous studies, many people have been investigated the effects of bubble behaviors to living cells cultured on “rigid” material [2]. It is known that bubble behaviors are affected by physical characteristics of wall to which bubble attaches. Because living tissue is very “soft” comparing with “rigid” material, we should investigate the effect of a bubble on “soft” material. Thus, we need to investigate the safety of ultrasound in condition which bubbles attach on walls like real body tissue. In this study, we examined how a bubble affects to the cells cultured on “soft” gel, based on optical observations of bubble behaviors and cell viability test using fluorescence microscope.

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2. Experimental method

2.1 Cell preparation

We prepared bovine gelatine on acrylic plate with thickness of 2 mm and seeded MDCK cells, canine kidney epithelial cells. We cultured it over four days in CO_2 incubator. In following experiments, we used gelatin gel samples of which surface were completely covered with cells.

2.2 Observation of bubble behavior

Using high-speed video camera (Shimadzu, HPV-1), we observed bubble behaviors in acrylic chamber filled with saline. (see **Fig.1**) The transducer was driven at center frequency of 27 kHz and the acoustic standing wave was formed in the chamber. The initial bubble diameter was about 180 μm. The detailed observation system is described in Ref [3].

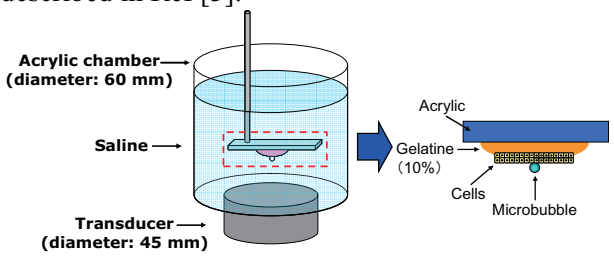

Fig.1 Setup of ultrasound irradiation

2.3 Experimental procedure

We conducted following procedures to investigate effects of bubble behaviors on cells. 1. Observed initial condition of gelatin surface and cultured cells using optical microscope, 2. Observed both behaviors of bubble and gelatin surface using the high-speed video camera, 3. Viability test of cells cultured on gelatin using fluorescence microscope.

3. Result

Damages of cells on the gel surface, which are related to the bubble behaviors, were confirmed. In the observed results using the high-speed video camera (see **Fig.2**), we can find that the vibrating bubble pushes the gel surface during the expansion and pulls during the contraction. This result clearly shows that the force derived from bubble vibration acts on the gel surface.

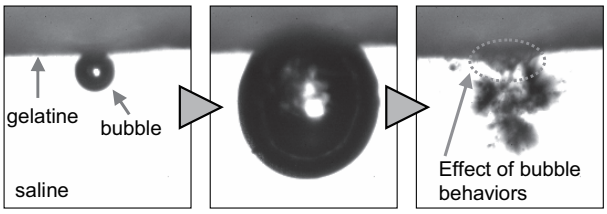

Fig.2 Effect of bubble behaviors

Cell viability test clearly demonstrate the damage of cells on gel surface. **Fig.3** shows microscopic images after ultrasound irradiation. Image (a) and (b) are captured using transmitted light and fluorescence, respectively. The observed regions in images (a-1) and (a-2) were same with that of (b-1) and (b-2), respectively. For cell viability test, we used PI (Propidium Iodide), which is fluorescence dye staining dead cells.

In image (a-1), we can see the large region where cells were desquamated from the gel surface. Additionally, the fluorescence observation showed that the cells were dead around this region [see image (b-1)]. On the other hand, we can find no significant damage of cell in image (a-2). However, convergence of dead cells can be clearly confirmed in image (b-2). Focusing on the damaged area, interesting fact is demonstrated. The width of damaged area is about 500 μm. Looking at high-speed video camera images, the width of area affected by bubble behaviors is comparable in size to that of damaged area.

4. Discussion

This study demonstrated that 1. The surface of gelatine was forced by bubble behaviors with high-speed video camera, 2. Some of cells desquamated or died after ultrasound irradiation, 3. The desquamation and death of cells did not occur in trials without bubbles. In addition, The area which cells were damaged by bubble behaviors was almost same with that of cells desquamated or dead. For these reasons we supposed that bubbles driven by ultrasound injured cells on gelatine.

However, there were differences in exfoliated state between each sample even if the radiation conditions of ultrasound were same. Therefore, we need to find out the relationship between damages to cells and bubble behaviors in the future.

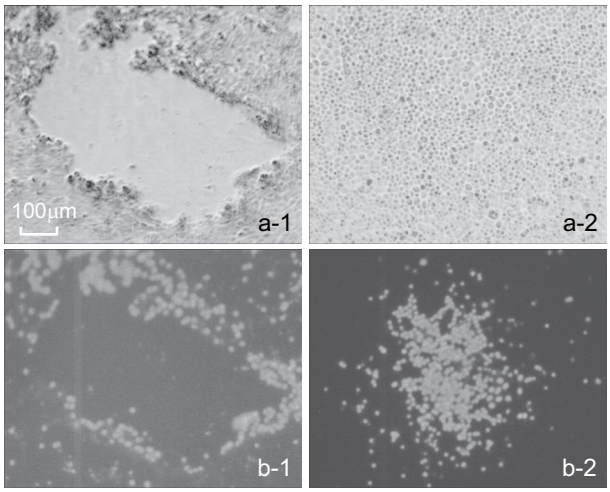

Fig.3 Microscope images

5. Summary

In this study, we researched the safety of ultrasound to living tissue in terms of biological and cytological aspects in additional to cavitation dynamics. As a result, it is suggested that if cavitation occurs near living tissue, surrounding tissue will be injured such as cell desquamation and cell killing.

References

1. T.G. Leighton: The Acoustic Bubble. (1997) 329.
2. A.V. Wamel, A. Bouakaz, M. Verslus, N. Jong: US. Ultrasound Med Biol. **30** (2004) 1255.
3. K. Yoshida, S. Nakatani, A. Tsukamoto, T. Ushida and Y. Watanabe: Jpn. J. Appl. Phys. **47** (2008) 2400.