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Effect of exposure to standing wave ultrasound field on proliferation of brain tumor cell

定在波超音波の音場への暴露が脳腫瘍細胞の増殖に及ぼす影響

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1. Introduction

Brain functions should be preserve in brain tumor treatment, because important organs like cerebrum, brainstem etc. exists in the brain and such organs in the brain are essential for sustain of life. Therefore, unlike the most of other tumor therapy, it is difficult to ablate wide area of tissues that include the surrounding normal tissues are thought to be spread and infiltrated by tumor cells. Recently, the high-intensity focused ultrasound (HIFU) surgery is used for brain tumor therapy [1]. The coagulative necrosis is induced on the tumor cells by heat generated from the high-energy of focused ultrasound. This ultrasound therapy has some benefits. For example, it is low invasive therapy due to trans-skull and it can be performed repeatedly unlike the radiation therapy. On the other hand, it is thought that the inflammatory reaction caused by necrosis damages the normal cells surrounding tumor cells. We are studing about ultrasound exposure conditions to induce apoptosis cell death to brain tumor cells. We believe it is possible to suppress the damage to surrounding normal cells, since apoptosis does not induce an inflammatory response. In this study. We will report ultrasound exposure conditions for induction of cell death to human glioblastoma U-87MG cells in this symposium.

2. Material and method

2.1. Standing wave type ultrasound exposure system

The standing wave type ultrasound exposure system was used for exposure of human glioblastoma U-87MG cell (ATCC) to ultrasound field. The stainless steel vibrating plate (diameter of 180 mm) equipped on the bottom of water tank (the size of 140×140×170 mm^3) via an O-ring. At the bottom of water tank has acoustic window (diameter of 120 mm). Standing wave type ultrasound field was formed in water tank using the vibrating plate driven with the Langevin type transducer at the center and on the opposite side of acoustic radiation surface of the vibrating plate.

U-87MG cell was grown as a monolayer on the bottom surface of our fabricated cell culture flask [2]. Our fabricated cell culture flask was filled with Minimum Essential Medium with 10 % fetal bovine serum and 1 % penicillin-streptomycin to a position of 5 mm from the bottom of cell culture surface. The photograph of our fabricated cell culture flask is shown in **Fig. 1.** Our fabricated cell culture flask made of acrylic resin (the area of cell culture surface: 38.5 mm^2) has thin membrane acoustic window. This thin polystyrene membrane acoustic window (OPS®, thickness: 25 μm, Asahi Kasei Chemicals) does not block the ultrasound propagation.

Our fabricated cell culture flask with culture medium MEM and glioblastoma U-87MG cell was set in the water tank of our ultrasound exposure system at distance of 45 mm from stainless steel vibrating plate. The distilled water was filled in water tank to a position of 100 mm from the vibrating plate. Schematic diagram of ultrasound exposure system for induction of cell death to brain tumor cells is shown in **Fig. 2.** The -3 dB beam area of measured acoustic field in water tank of our ultrasound exposure system was 10.2 mm^2.

2.2. Ultrasound exposure to U-87MG cells

U-87MG (cell concentration: approximately 2.3×10^5 cells/mL) in our fabricated cell culture flask were incubated at 37 ℃ in humidified atmosphere with a 5 % CO_2 incubator for 2 days. U-87MG was placed at water tank of our standing wave type ultrasound exposure system (in Fig. 2) and irradiated continuous wave ultrasound with frequency of 150 kHz for 5 minutes. We prepared the negative control sample as reference for comparison with the ultrasound irradiated sample. This sample was kept in water at the same temperature during the same time as ultrasound exposure time. The viability (ratio of the number of survived cells exposed to ultrasound to the number of control cells) of glioblastoma cells were

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measured using Trypan blue dye exclusion test at every 2 hours after ultrasound exposure.

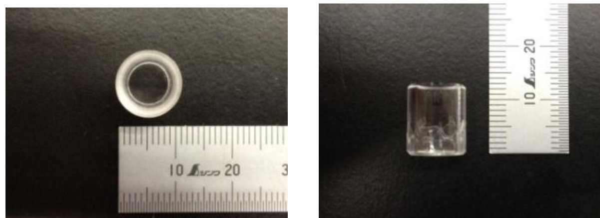

Fig. 1 Our fabricated cell culture flask with acoustic window for ultrasound irradiation

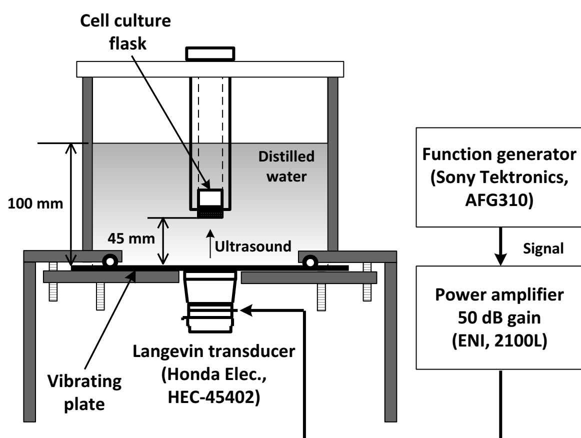

Fig. 2 Schematic diagram of ultrasound exposure system for induction of cell death to brain tumor cells.

3. Results and discussion

Relationship between the viability of U-87MG cell and the time elapsed from the ultrasound exposure is shown in **Fig. 3**. The viability of sonicated cell was more than 80 % until after 2 hours from sonication. After 4 hours from sonication, low level of the cell viability was observed. However, although ultrasound exposure condition was constant with spatial-peak acoustic intensity 720 mW/cm^2, the measurement error of sonicated cell viability was very large. We considered the cause of large measurement error as follows. The state of observed U-87MG cell on bottom surface of cell culture surface was different for every sonication. **Figure 4** shows the change in state of U-87MG on the bottom surface of our fabricated cell culture flask after ultrasound exposure for 5 minute. It can be thought that the effect of 5 minutes sonication on the adhered cells on the bottom surface of the cell culture flask differ from those on the floated cells from the bottom surface.

4. Conclusion and future works

We estimated the effect of exposure to standing wave ultrasound field with frequency of 150 kHz on proliferation of brain tumor cells. After 4 hours from sonication, cell viability was decreased. However measured cell viability after sonication for 5 minutes had large error. We considered that one of the causes of large measurement error is the difference of adhesion state of cells on the bottom cell culture surface of the culture flask. In the future, we would like to fabricate new type cell culture flask. In this flask, we would like to decrease the number of floated cells from the cell culture surface by mechanical vibration of ultrasound irradiation. Then, we believe it is possible to suppress the large error of measured cell viability after sonication by using new cell culture flask.

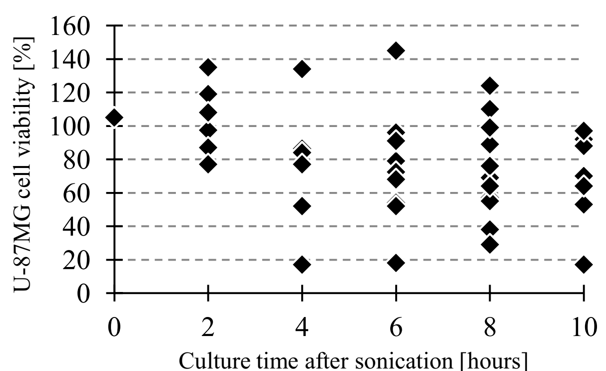

Fig. 3 Relationship between the viability of U-87MG cell and the time elapsed from the ultrasound exposure (spatial-peak acoustic intensity: 720 mW/cm^2)

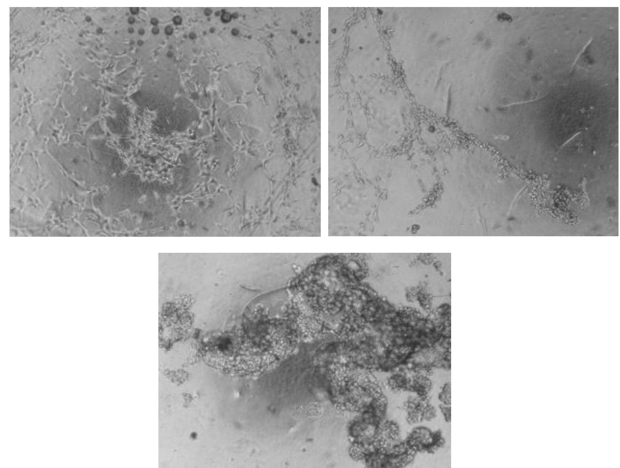

Fig. 4 Various state of U-87MG observed on the bottom surface of our fabricated cell culture flask after ultrasound exposure for 5 minutes

Reference

[1] InSightec: EX Ablate 4000 White paper **1** (1) (2010)

[2] S. Iwashiro, et al., IEICE Tech. Repo. 113 (68) US2013-9, 7 (2013)