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Development and estimation of the adherent cell culture flask with acoustic window film for ultrasound irradiation to glioblastoma cells

神経膠芽腫細胞のための超音波照射用音響窓付き付着細胞培養フラスコの開発と評価

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1. Introduction

In recent years, the transcranial brain tumor treatment system by HIFU has been studied. [1–3] This treatment has the advantage that it is minimally invasive and it can be applied repeatedly unlike radiotherapy. However, when brain tumor cells are coagulated and are necrotized with this treatment system, the surrounding normal brain tissue may be affected malignantly by the necrosis.

Therefore, we have been considering trial to use apoptosis induction into brain tumor cells by ultrasound exposure for ultrasound brain tumor treatment system.

When the commercial polystyrene cell culture flasks with thickness of about 2 mm were employed as containers of target brain tumor cells exposed to ultrasound, there was a problem that ultrasound energy was reflected at the surface of the flask with different specific acoustic impedance from water outside of the flask and culture medium in the flask. In addition, it is necessary to position the bottom of the flask in the ultrasound wave beam completely.

We published the development and estimation of brain tumor cells culture flask with acoustic window film for ultrasound exposure at Japanese Journal of Applied Physics.[4)]

We will present the handmade flask with the PET film bottom (acoustic window) for adherent cell culture flask and ultrasound irradiation.

2. Methods

In this study, we used YKG-1 glioblastoma cell lines (JCRB ® Responsible BANK) in this study. This cell line is the highest malignant adherent cells Grade IV which should be cultured by adhered on the bottom inner surface of the flask.

The following characteristics are required for the adherent cell culture flask with acoustic window film for ultrasound irradiation to glioblastoma cells. (1) No interference to the propagation of ultrasound wave into the flask, (2) High cell adhesion, (3) Low toxicity to cells. For transmission of ultrasound into the cell culture flask with acoustic window, an acoustic window film that does not interfere with ultrasound propagation should be employed.

From previous research, PET film (Toray Industries, Inc. Lumirror® film, Thickness 25 μm) with the excellent cell adhesion ability was used for cell culture flask for ultrasound irradiation experiment. [4-6)] The PET film and the PET body were welded (Seidensha Electronics Co., Ltd., Sonopet, Σ-620S / Σ-P30S). PET film with a common material with main body of the flask in this situation. The conditions like output power, contact load and welding time of the ultrasound welding machine were examined. The set items are two items, contact load and welding time.

Durability tests were performed for the fabricated cell culture flask with acoustic window film by using our standing wave type ultrasound irradiation system in our laboratory. Block diagram of experimental system is shown in Fig. 1. The fabricated adherent cell culture flask with acoustic window film for ultrasound irradiation to glioblastoma cells for 2 minutes from the planer type ultrasound transducer. The ultrasound exposure conditions are as follows. Continuous waves with applied voltage from 40 mVp-p and sound pressure with 230 kPa (acoustic intensity of 3.0 W/cm^2) at frequency of 1 MHz from a function generator were amplified by using a RF power amplifier with gain of 50 dB. Output amplified signals were applied to the ultrasound transducer. The fabricated culture flask with the acoustic window for the ultrasound irradiation was set at the position of 45 mm height from a surface of plate of the ultrasound transducer.

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We checked the leak of staining solution (red colored liquid) from the fabricated culture flask. Temperature change in the flask was also measured at the same time.

Further, from the numerical simulation of the influence of the adherent cell culture flask with acoustic window film for ultrasound irradiation to glioblastoma cells in the sound field of the ultrasound wave, the optimum value of the diameter of the flask was determined.[7)]

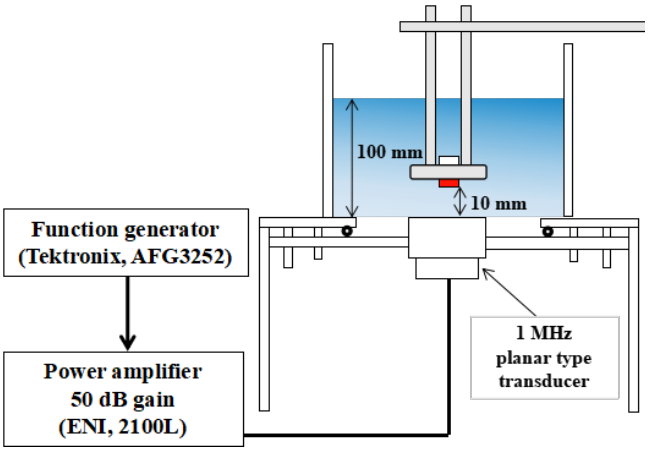

Fig. 1 Block diagram of ultrasound irradiation system for our fabricated cell flask with acoustic window film

4. Conclusions and discussion

As results, it was found that favorable contact load was from 10 to 30 N, favorable welding time 0.09, 0.11, 0.13 s, so these conditions were considered. Experimented results are shown in Fig. 2. In spite of exposure to high intensity ultrasound field with generation of acoustic cavitation, the fabricated cell culture flasks with acoustic window film by ultrasound welding were not broken and not damaged.

Then, the temperature inside the flask before ultrasound irradiation, were measured during and after ultrasound irradiation. Because the flask is small, the culture solution becomes small amount. Therefore, it was found to be influenced by outside temperature and flask body temperature. It can be said that the consideration and establishment of experimented technique and the establishment are also important to confirm the apoptosis.

Furthermore, from the numerical simulation of the influence of the adherent cell culture flask with acoustic window film for ultrasound irradiation to glioblastoma cells on the sound field of the ultrasound wave, the optimum diameter of the flask was determined. As a result, it was found that it should be more than 10 mm for the inner diameter of the flask. When the inner diameter will be less than 10 mm, the ultrasound was cut off in the flask.[7)]

In the future, it is necessary to develop a brain tumor cell culture flask with an acoustic window for ultrasound irradiation which has biological safety and can cultivate cells.

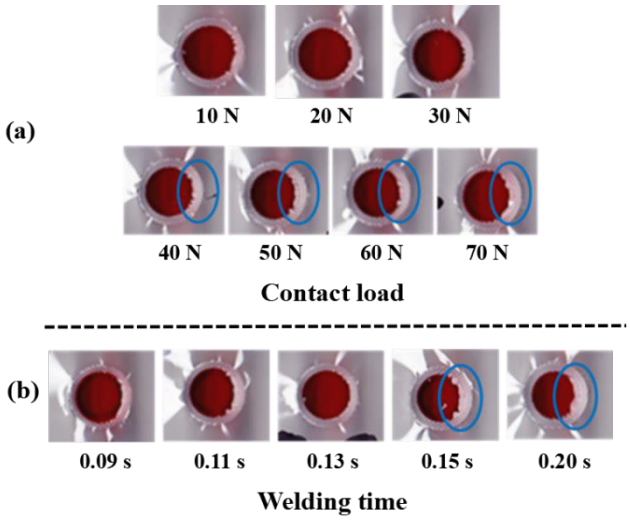

Fig. 2 Observation of the welded state between film and the flask body by ultrasound welding
(a) Relationship between contact load and welded state
(b) Relationship between welding time and welded state

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