2P5-24
Proceedings of Symposium on Ultrasonic Electronics, Vol. 34 (2013) pp. 347-348
20-22 November, 2013

Development and estimation of brain tumor cells culture flask with acoustic window film for ultrasound exposure

超音波照射用音響窓付き脳腫瘍細胞培養フラスコの開発と評価

Sakino Iwasiro[1†], Akiko Watanabe [1], Yoshifumi Onozuka[2], Masatsune Minai[1], Hiroyuki Nishimura[1], and Shinichi Takeuchi[1]
([1] Toin University of Yokohama; [2] Seidensha Electronics Co.)
岩城咲乃 [1†]，渡邉晶子 [1]，小野塚善文 [2]，薬袋正恒 [1]，西村裕之 [1]，竹内真一 [1]([1] 桐蔭横浜大, [2] 精電舎)

1. Introduction

In recent years, the transscanial brain tumor treatment system by High Intensity Focused Ultrasound (HIFU) has been studied.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 by the necrosis. Therefore, we have been considering trying to use apoptosis induction into brain tumor cells by ultrasound exposure for a standing wave type 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 that of water outside of the flask and that of culture medium in the flask, but ultrasound energy did not reach the cells sufficiently.

Therefore, we prepared the original cell culture flasks with acoustic window for ultrasound irradiation experiments and cell culture.

2. Methods

2.1. Fabrication of cell culture flask

We chose PET film (Toray Industries, Inc. Lumirror® film, Thickness 25 μm) and polystyrene film (Asahi Kasei Chemicals Corp., OPS® film, Thickness 25 μm,) as the candidate of acoustic window films, since they showed high cell adhesiveness in the cell adhesion test[1)] and the cytotoxicity tests[1)]. It was found from the cytotoxicity tests that it is desirable not to use an adhesive for bonding between the film for acoustic window and a bottom end of the flask body. Therefore, we have investigated the bonding method by heat welding and by using ultrasound welding which is used for bonding of the commercial cell culture flasks.

A standing wave type ultrasound irradiation system which has -3 dB ultrasound beam width of 10 mm × 15 mm and beam area of 118 mm^2 used in. It was found from the past study that cell culture flask for the adherent cell should have cell culturing area as large as -3 dB beam area of ultrasound waves. The PMMA hollow cylindrical pipe (outer diameter of 10 mm, wall thickness of 1.5 mm, height of 12 mm) and the PET resin hollow cylindrical pipe (outer diameter of 10 mm, wall thickness of 1.25 mm, height of 12 mm) are used for the culture flask body. The photograph of the resin pipes of PMMA and PET for the culture flask body is shown in Fig.1.

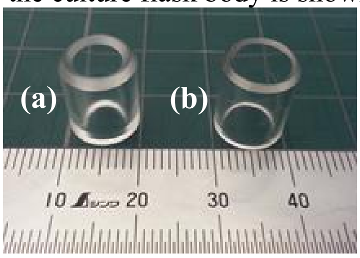


Fig. 1 Photograph of the resin pipes for the culture flask body (a) PMMA, (b) PET

2.2. Durability for ultrasound exposure

Durability experiments of the adhesive cell culture flask with acoustic window fabricated by heat welding or ultrasound welding against the ultrasound exposure were performed by using our standing wave type ultrasound exposure. The block diagram of the experimental system is shown in Fig. 2. The cell culture flask with acoustic window was placed at a height of 45 mm from the stainless steel disk vibrator on the bottom of water tank of the experimental system. Continuous wave output signal with voltage amplitude from 100 mV$_{0\text{-}P}$ to 500 mV$_{0\text{-}P}$ from a function generator (F.G.) was amplified by using an RF power amplifier with gain of 50 dB and was applied to a Langevin type transducer at frequency of 150 kHz. The stainless steel disk vibrator was driven and standing wave ultrasound was irradiated to the under test adherent

---
tm21b01k@ust.toin.ac.jp (S.Iwashiro)

cell culture flask with acoustic window for 20 minutes. The under test flask containing staining solution was exposed to the standing wave ultrasound. The durability of under test flask was estimated by observation of the leakage of staining solution.

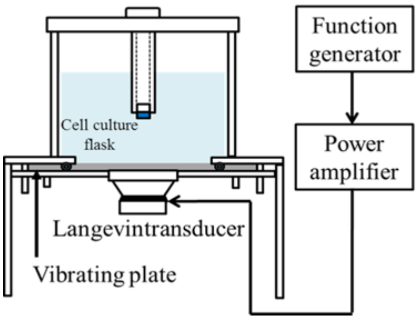


Fig. 2 Block diagram of ultrasound irradiation system for our fabricated cell flask with acoustic window film

3. Experiments

3.1. Adhesiveness between acoustic window film and flask body

When heat is applied, polystyrene film is shrunk, melted and stuck to the ends of both the PET flask body and the PMMA flask body. However, it was found that it was difficult to bond the PET film and the end of both the PET flask body and the PMMA flask body by heat welding. When water was put into the flask, the PET film was peeled off from the bottom end of the flask body immediately.

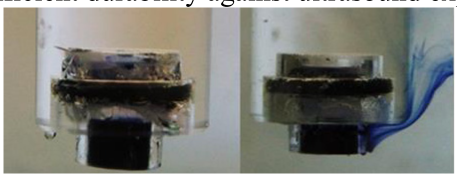
We would like to use a PET film with the highest cell adhesiveness to the cell culture flask for ultrasound irradiation from the results of a cell adhesion tests. Therefore, we tried to perform ultrasound welding between the PET film acoustic window and the PET flask body.

Moreover, we tried to perform ultrasound welding between PET film and a PMMA pipe flask body. This combination of PET film and a PMMA pipe flask body have shown good result in the heat welding. When water was put into the flask, the PET film was not peeled off from the bottom end of the flask.

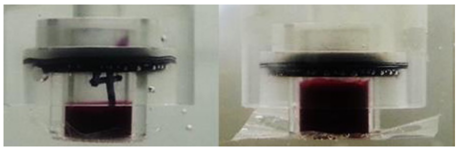
3.2. Durability for ultrasound exposure

Experimented results are shown in Fig. 3. Ultrasound exposure system was driven by using the output signals with voltage from 100mV$_{0\text{-}P}$ to 200 mV$_{0\text{-}P}$ of the function generator for 20 minutes. There was no leakage of the staining solution from the under test flask fabricated by heat welding after 20 minutes ultrasound exposure. However, the leakage of the staining solution from the flask after 10 minutes ultrasound exposure with output voltage of the function generator higher than 300 mV$_{0\text{-}P}$. Therefore, it is easy to damage the flask at high output voltage of the function generator. It was considered that there was a problem in durability. Therefore, the durability test of the flask fabricated with ultrasound welding was performed by using output signal from the function generator with amplitude voltage of 300 mV$_{0\text{-}P}$ for 20 minutes.

The leakage of staining solution from the flask fabricated by ultrasound welding could not observed. Damage of the acoustic window film and entering of water into the flask by peeling of the film were not observed even in high intensity ultrasound acoustic field with generation of acoustic cavitation. Therefore, it can be thought that the flask fabricated by ultrasound welding had sufficient durability against ultrasound exposure.



(a)F.G.100 mV$_{0\text{-}P}$ (b) F.G.300 mV$_{0\text{-}P}$



(c) F.G.300 mV$_{0\text{-}P}$ (d) F.G.300 mV$_{0\text{-}P}$

Fig. 3 Obserration of damage of our fabricated cell flask by ultrasound irradiation (a)(b)fabricated by heat welding(PMMA body and OPS film), (c)fabricated by ultrasound welding(PET body and OPS film), (d) fabricated by ultrasound welding(PET body and Lumirror film)

4. Conclusion and problem

As a result, polystyrene film (OPS® film) could be bonded to the PMMA flask body by heat welding. However, it was difficult to bond PET film (Lumirror® film) to the PMMA flask body by heat welding. PET film (Lumirror® film) could be bonded to the flask body by ultrasound welding. The flask fabricated by using ultrasound welding was not damaged by exposure to high intensity ultrasound acoustic field.

References

1. Sakino Iwashiro, Akiko Watanabe, Masatsune Minai1, Hiroyuki Nishimura1, and Shinichi Takeuchi1 , IEICE.Tech.Rep., US2013-9, 7-12, 2013.
2. T. Takatera, A. Watanabe, T. Sato, H. Nishimura, N. Kawashima, S. Takeuchi: Jpn. J. Appl. Phys. 43 (2003) 3251.
3. A. Watanabe, K. Kawai, T. Sato, H. Nishimura, N. Kawashima, S. Takeuchi: Jpn J. Appl. Phys. 43 (2004) 3245.