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Morphological observation of the HT-22 cells in a culture well exposed to ultrasound

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1. Introduction

Ultrasound has potential for the clinical treatment of nerve regeneration in both the central and peripheral nervous system [1, 2]. Brain cells normally don't reproduce themselves, when they become damaged or die. Non-invasive physical stimulation by ultrasound can promote cell viability, proliferation, and change size of the cell body, axon and dendrites (or neurite). Previous studies have shown that ultrasound enhances neurite elongation in rat cortical neurons [1] and ultrasonic stimulation induced the alteration of the functions of cultured Schwann cells as demonstrated by promoted cell proliferation [2]. Ultrasound can modulate neuronal development in vitro through triggering temporary neurite retraction and inducing cell body shrinkage [3].

HT-22, an immortalized mouse hippocampal cell line, has been widely used in vitro model for studying the neurodegenerative disease. The present study aims to observe the morphological responses of HT-22 cells in a well to ultrasound.

2. Materials and methods

An experimental setup used in this study is shown in Fig.1.

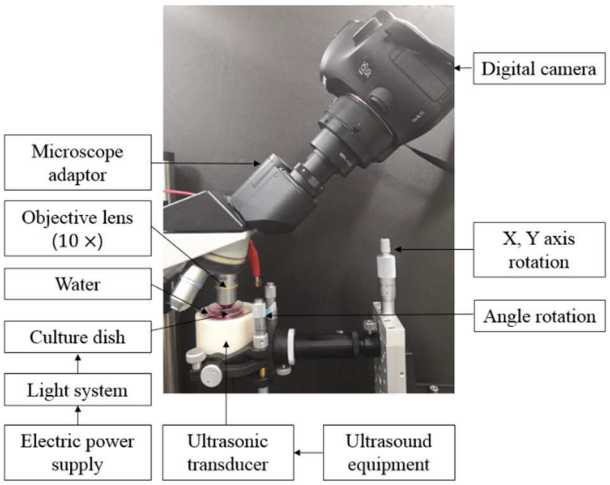

Fig 1. Experimental setup.

Cell culture

HT-22 cell lines were cultured in Dulbecco's Modified Eagle Medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin, and incubated at 37 ℃ humidified air with 5% CO_2. Cell density was 1×10^4 cells/well in a culture dish with 35 mm in diameter.

Ultrasound condition

An ultrasound production system (UTIMS-300, KORUST, Korea) was employed to drive a 1MHz ultrasonic disc transducer. Ultrasound was transmitted from 2.54 mm diameter ultrasonic transducer to the bottom of the 35 mm culture dish through a coupling medium of water. HT-22 cells were exposed to an intensity (I_{TASA}) of 50mW/cm^2 with a duty factor (DF) 25% for dish I, and DF 50% for dish II, pulsed at the repetition frequency of 1Hz. The total exposure time was 30 min. Ultrasound was applied to the cell after 24 hours in culture.

Microscopic imaging

HT-22 cells were monitored by a digital camera (EOS 5D Mark III, Canon Inc., Japan) equipped with a microscope adaptor carrying an objective lens (10×). The digital camera recorded cell behaviors as an AVI movie at 25 frames/sec during the whole experimental period. Light system was fabricated for imaging cells, which consisted of an ULTEM membrane and an LED (t=0.6mm, 1mm x 2mm). The light was placed between the transducer and the bottom of the culture dish. Light was transmitted through cells in the culture well.

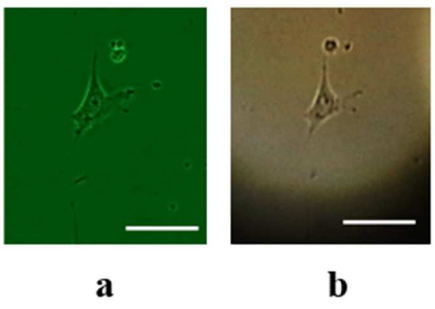

Fig 2. Typical images of the HT-22 cell, (a) obtained using a conventional microscope and (b) taken from the present microscope system equipped with the ultrasonic transducer underneath the culture well. (scale bar=50μm)

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3. Results and Discussion

Typical images of the HT-22 cell are shown in Fig 2, contrasting the images obtained using a conventional microscope and taken from the present microscope equipped with the ultrasonic transducer underneath the culture well. The present microscope has its own adjustable and controllable light source which gives the background color different from the conventional image. As seen in Fig 2, the image with the present microscope (Fig 2b) is virtually characteristically the same as that of the conventional microscope (Fig 2b). This confirms the present microscope can be used for the real time morphological observation of the cell under ultrasonic irradiation.

Condensed experimental results are presented in Fig 3 where the cells were monitored with time for 30 min from 2 min before the ultrasonic irradiation. Fig 3a shows a cell in the control culture dish, whereas Fig 3b and 3c show respectively the cell in the experimental dish I and II. Note that the dish I and II were exposed to ultrasound (50mW/cm^2) at a DF of 25% and 50%, respectively. The cell culture conditions such as the density of cell and the volume of culture medium are the same for all the culture dishes.

As shown in Fig 3, the control cell began to shrink at 4 min and died at 7 min (Fig 3a), In contrast, as shown in the morphological changes of the cell of Fig 3b and c, the ultrasound exposure protected the cell from death, and what was more, induced the cell growth. The cell in the dish I at the low duty factor 25% (Fig 3b), began to shrink at 5 min but started to increase the elongation of cell neurite at 23 min. Meanwhile the cell in the dish II at the high duty factor 50% (Fig 3c), reacted relatively quickly at 1 min and became lager in its body size. The neurite of the cell was significantly elongated or stretched around 5 min after the ultrasound exposure.

In the present experiment, we have found that the low intensity ultrasonic exposure (50mW/cm^2) protects the cells from death and bring about the morphological changes of cell growth. The degree and patterns of the morphological response are found to depend on the duty factor, i.e., ultrasonic power. Studies are underway to optimize the ultrasonic exposure for the morphological responses, based on stochastic quantified analysis on the optical images of cells.

4. Conclusions

We observed that the low intensity ultrasound induced the morphological growth in the HT-22 cell line, including the elongation of-neurite and the increase of cell body size. The morphological responses are found to depend on ultrasonic power. These findings support that an optimized ultrasonic stimulation will be potential in treating nerve regeneration.

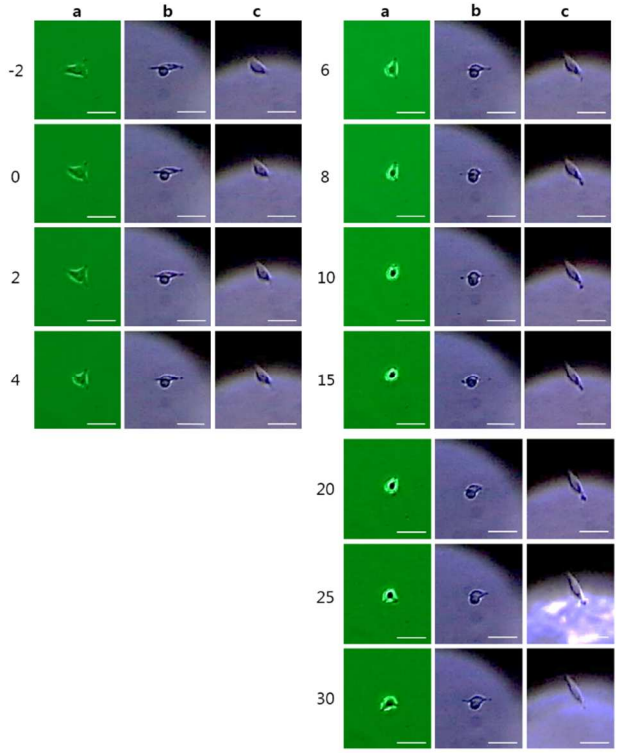

Fig 3. Morphological changes of the cell with time recorded for 30 min from 2 min before ultrasonic irradiation: (a) control culture dish, (b) dish I (DF=25%), (c) dish II (DF=50%). (scale bar=50μm)

Acknowledgment

References

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