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Analysis of yeast cell death induced by 40kHz ultrasound

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1. Introduction

Treatment of ultrasound to induce cell death has a wide potential and is considered important in medical usage. Recent studies have reported that high frequency ultrasound is effective in inducing cell deaths in many different cell types [1,2]. Furthermore, low intensity focused ultrasound is ready to apply to tumor therapy by inducing cell death [3]. Thus, many research concerning the effect of ultrasound on inducing cell death is being conducted. Death of normal and cancer cells occurs by physiological death pathway which has been well studied. However, the mechanism of cell death induced by ultrasound is not well elucidated yet. In this study, we developed a system in which 40kHz ultrasound is transmitted efficiently into 1.5ml E-tube containing cell culture mixture. This system is easy to use and analyze the ultrasound irradiated samples including cell suspension. Furthermore, we studied the effect of 40kHz ultrasound on the yeast cell death. To determine whether thermal or radical effect take part in ultrasound induced cell death, we analyzed the cell death of ultrasound irradiated cells in the presence or absence of heat shock and hydrogen peroxide as a radical source.

2. Materials and Methods

2. 1. Yeast Cell Culture

Yeast *Saccharomyces cerevisiae* BY4743 was purchased from EUROSCARF (Germany). Yeast strain was incubated in YPD medium at 30°C at shaking incubator of 180 rpm.

2. 2. Ultrasound Irradiation System

A pyrex beaker of 60mm OD was used as an ultrasonic transmission cell. BLT (Bolt-clamped Langevin Transducer) with 40kHz nominal frequency was attached at the bottom of transmission cell. BLT was excited by an ultrasonic generator (50W, Kodo Technical Research Co., Korea). Two vertical height adjustors were placed at 15cm distance. At the middle of the two adjustors, the transmission cell was fixed using a bolt. Distilled water was poured into the beaker, and ultrasound was transmitted using an ultrasound producer.

In order to transmit 40kHz ultrasound into the yeast cell suspension of the 1.5ml E-tube, a stable E-tube fixing system was devised using acrylic. (**Fig 1**) U-shaped acrylic extension was added to the middle of 160mm x 38mm acrylic. In the middle of U-shaped extension, a hole of 10mm was made to fix the E-tube.

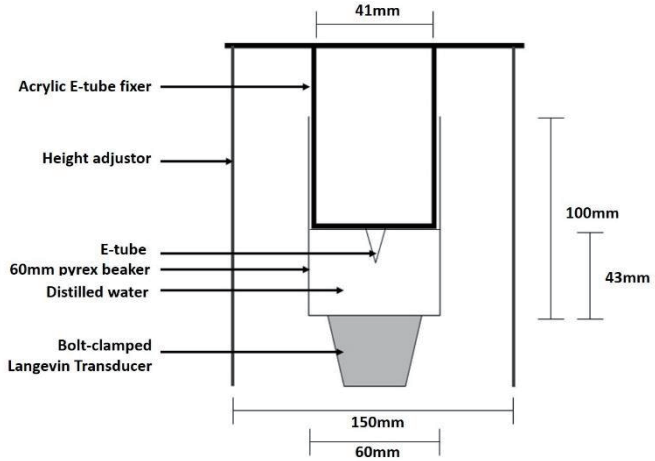

Fig 1. Ultrasound Irradiation System

2.3. Irradiation System Optimization

40kHz ultrasound was transmitted to yeast at different positions. Absorbance of 335nm light in KI solution (0.1M) was used to compare the transmission of ultrasound at different positions. [4] Possible thermal effect of ultrasound on inducing cell death was determined by exposing the cell to 40° C, 50°C, 60°C environment for 1 minute.

2.4 Cell Death Assay

To measure the cell death, yeast was treated with ultrasound for different time, and the colony formation spotting assay was conducted. To determine the effect of ultrasound in radical formation, hydrogen peroxide of 0.1mM, 0.5mM and 1mM was treated to each yeast along with ultrasound.

2.5 ROS Detection

For observing the ROS induced by ultrasound in the cell, ROS Detection Kit (Enzo Life Sciences) was used, and the samples were observed under a Zeiss Axiovert40 Fluorescence microscope. (Zeiss)

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3. Result

To determine the optimal position of E-tube for transmission of 40kHz ultrasound, optical density of KI solution after ultrasound transmission was checked in three trials. The graph illustrates that value is high near 4.3cm, 6.4cm and low near 5.2cm, 7.3cm. (**Fig 2**) Thus, it was verified that when E-tube is placed at 4.3cm height along with the water level, ultrasound is most effectively transmitted.

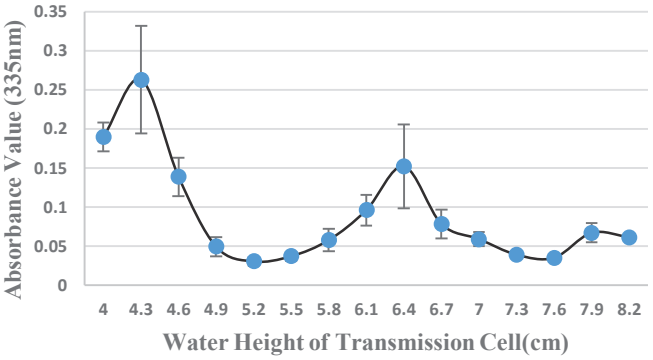

Fig 2. Absorbance value of iodine derived from 0.1M KI solution after ultrasound transmission at different heights

To determine whether non-thermal effect of 40kHz ultrasound induces cell death in the established system, 40kHz ultrasound was transmitted to the yeast with different time interval. (**Fig 3**) Under this system, cell death was examined after the yeast was exposed to the ultrasound for 1 minute.

The temperature of the system increased to 52° C when ultrasound was transmitted, raising doubt to the non-thermal effect of ultrasound in inducing cell death, since heat shock can be another source of cell death. Thus, the yeast was exposed to different temperature for 1 minute to make sure the non-thermal source of ultrasound is the cause of the cell death. Cell death was not observed until the cell was exposed to 60°C environment for 1 minute. (**Fig 4**) These results concluded that the cell death was induced in the established system by the non-thermal effect of ultrasound.

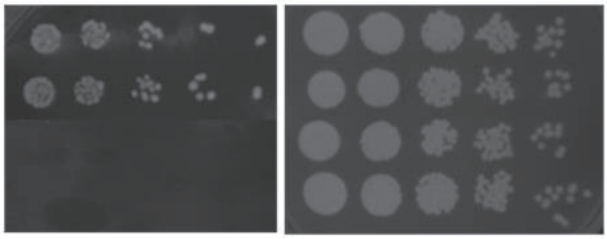

Fig 3. Spotting Assay of Yeast Exposed to US for Different Times

Fig 4. Spotting Assay of Yeast Exposed to Different Temperatures

To test the effect of ultrasound in inducing cell death via radical formation, different concentration (0.1mM, 0.5mM, 1mM) of hydrogen peroxide was co-treated with ultrasound. Co-treatment of ultrasound and hydrogen peroxide induced more cell death when compared to cells treated only with the same concentration of hydrogen peroxide. (**Fig 5**)

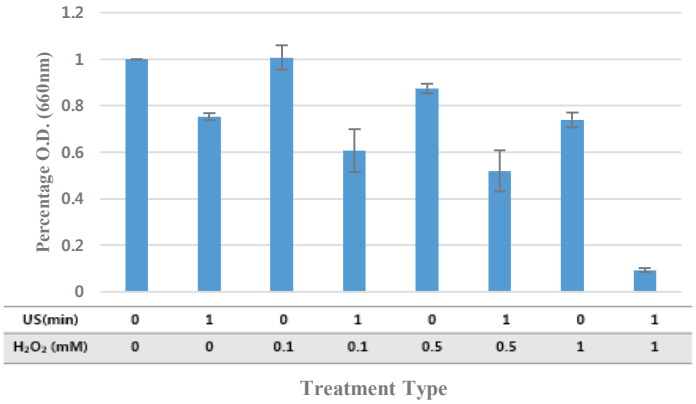

Fig 5. Viability of cell treated with different combinations of ultrasound and hydrogen peroxide

ROS was also observed when the yeast was treated with ultrasound for 5 minutes, stained with ROS dye, and viewed under the microscope.

4. Discussion

In this study, the convenient irradiation system was established. Under this system, yeast cell death induced by ultrasound was observed with non-thermal effect. The co-treatment of ultrasound and hydrogen peroxide synergistically induced the cell death. This suggests that ultrasound may produce the oxygen radical in our system. ROS in the cell was observed (data not shown), and it showed that ultrasound may induce cell death through production of oxygen radical.

5. References

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