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Cell death under different acoustic environments of high intensity ultrasound

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1. Introduction

Ultrasound has infinite potentials for basic and applicable medical treatment including the death cell imaging and cell death inducing system[1]. Recently increasing studies has reported that high powered ultrasound could induce apoptotic cell death in different suspension cell culture condition[2,3]. In aqueous physical condition, the fidelity of ultrasonic transmission may be an important factor for inducibility of death in suspension cell culture. It is reported that ultrasonic transmission depends on the liquid medium composition, e.g., water/polyethylene glycol mixture[4]. Therefore ultrasound-induced cell death in suspension culture may be resolved by the efficiency of transmission in liquid media. However the exact physical and biological mechanism has been still unknown. The purpose of this study is to find the optimal ultrasonic transmission and to test the suspension cell death in different water/polyethylene glycol environment.

2. Materials and methods

2.1 Ultrasonic Irradiation System

Fig. 1 shows schematic diagram of ultrasonic irradiation system. A pyrex beaker of 60 mm OD was used as an ultrasonic irradiation cell. A Bolted Langevin Transducer of 40.9 kHz attached at the bottom of the cell and a generator (50W, Kodo Technical Research Co., Korea) were used for generation of ultrasound. Liquid such as distilled water or aqueous solutions of polyethylene glycol (200, 4000) was filled into the irradiation cell to transmit ultrasound. A flat-bottom pyrex tube of 12 mm ID was used as the container of yeast culture, and located in the irradiation cell. Each pyrex tube was covered with cap to prevent contamination of yeast culture. The heights, h_1 and h_2 in Fig 1, are critical to the efficiency of ultrasound transmission. The height of coupling liquid, h_1, and the location of container, h_2, are determined by the resonance condition of irradiation cell and the concentration of ultrasonic energy, respectively.

2.2 Yeast Cell Culture

Yeast *Saccharomyces cerevisiae* BY4743 (EUROSCARF) were incubated in YPD liquid medium at 30 °C. Each yeast culture was uniformly controlled as optical density of 0.1.

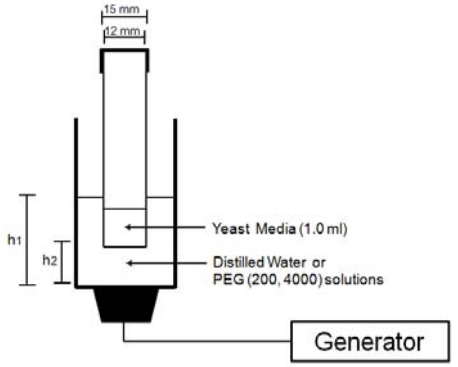

Fig. 1 Schematic diagram of Ultrasound irradiation system

2.3 Laser Doppler Vibrometer (LDV)

The vibrating amplitudes of pyrex tube were measured by LDV (Polytec OFV-534 & OFV-2570), to compare transmitted power into the yeast cell. A small reflector was attached on the inner bottom of pyrex tube for effective reflection of laser beam.

2.4 Cell death assay

Ultrasound of 40.9 kHz was irradiated to 1.0 ml of culture with different time intervals. To measure the effect of ultrasound-induced cell death, colony formation assay and proliferation assay were employed as described previously[5].

3. Result

To optimize condition of coupling liquid height and position of pyrex tube, h_1 and h_2, the vibrating amplitudes of pyrex tube were measured for various h_1 and h_2. Fig. 2 shows the variation of vibrating amplitude for different h_1 for h_2 = 2 cm. It was observed that similar peaks appeared near h_1 = 3 cm for different coupling liquids. Fig. 3 shows the variation of vibration amplitude for different h_2 for h_1 = 6 cm. It was also observed that similar peaks appeared near h_2 = 2.5 cm for different coupling liquids. It is noticeable that the vibration amplitude for PEG 200 is relatively smaller than that for other

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liquids. For the analysis of yeast cell death with 40.9 kHz ultrasound irradiation, we examined cell viability after different ultrasonic exposure. Fig. 4 shows the ultrasonic cell death with different coupling liquid (Distilled Water, PEG Solutions). Viability of cultured yeast cells decreased when aqueous solutions of PEG were used as coupling liquid, compared to distilled water or untreated samples. (Fig. 4)

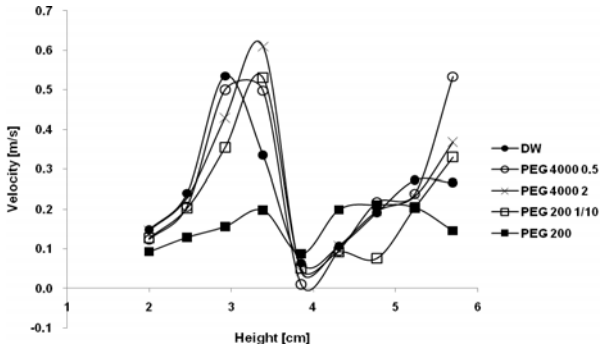

Fig. 2 Peak velocity of pyrex tube versus height of coupling liquid. (h2 = 2 cm)

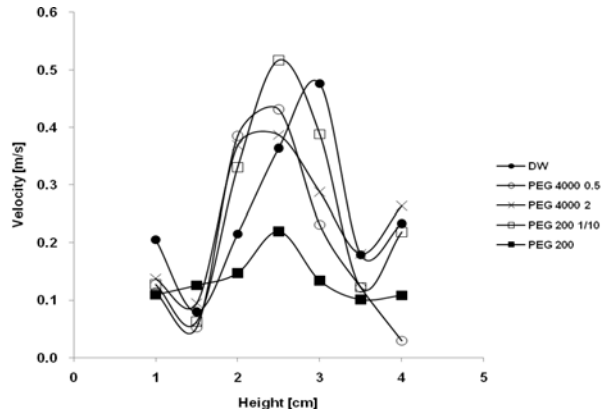

Fig. 3 Peak velocity of pyrex tube versus position of pyrex tube. (h1 = 6 cm)

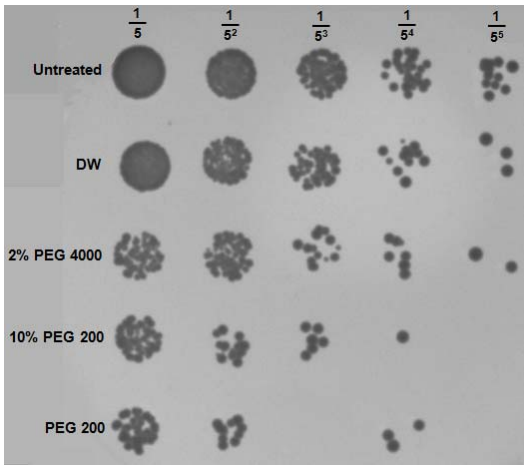

Fig. 4 Yeast cell death with different coupling liquid. (US 10 min treated, h1 = 6 cm, h2 = 2 cm)

4. Discussion

In this study, we studied to find the optimal condition of ultrasound-induced cell death in yeast cell suspension. Although the amplitude of vibrating tube under different coupling liquids was diverse, similar patterns were observed in Fig. 2 and Fig. 3. The transmitted power of ultrasound was maxima for the coupling liquid of 3.5 cm high (Fig. 2), and for pyrex tube located at 2.5 cm to 3.0 cm above from the bottom of cell. (Fig. 3) Since the half wavelength of 40.9 kHz ultrasound in water is 2.0 cm approximately, these results seem relevant. To determine the ultrasound induced cell death in suspension, we observed the colony formation of irradiated yeast cell under various coupling liquid. (Fig. 4) With 10 min irradiation in h_1 = 6 cm, h_2 = 2 cm condition, ultrasound induced cell death. Although PEG solutions were similar of peak velocity with water, the cell death was more potent in PEG solution than in water.

5. Conclusion

In order to find the optimal condition of ultrasound-induced cell death in yeast cell suspension, the vibration of yeast container were measured by LDV with different heights of coupling liquid and positions of yeast container. The optimal condition of ultrasonic transmission was determined for various coupling liquids. PEG solution is relatively effective for ultrasound-induced cell death in suspension. This implies that PEG can be applied as useful coupling medium on cell death experiment. However the cell death is not linear correlated with the velocity in our experiment. The relationship between the ultrasonic intensity and cell death should be studied in detail.

6. References

[1]. Sun SY, Hail N Jr, Lotan R. (2004) Apoptosis as a novel target for cancer chemoprevention. J Natl Cancer Inst; 96: 662-672

[2]. Ashush H, Rozenszajn LA, Blass M, Barda-Saad M, Azimov D, Radnay J, Zipori D, Rosenschein U. (2000) Apoptosis induction of human myeloid leukemic cells by ultrasound exposure. Cancer Res 2000; 60: 1014-1020

[3]. Lagneaux L, de Meulenaer EC, Delforge A, Dejeneffe M, Massy M, Moerman C, Hannecart B, Canivet Y, Lepeltier MF, Bron D. (2002) Ultrasonic low-energy treatment: a novel approach to induce apoptosis in human leukemic cells. Exp Hematol. 30: 1293-1301

[4]. Varada Rajulu A, Mabu Sab, P. (1996) Ultrasonic studies of water/poly(ethylene glycol) mixtures. Eur. Folym. J. Vol. 32: 267-268

[5]. Lee SY, Kwon YM, Kong HJ, Kim YH, Kang KI. (2010) Yeast cell death induced by high intensity ultrasound. Proc. Symp. Ultrasonic Electronics. Vol. 31: 391-392