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Effects of high-intensity ultrasound on the viability of Streptococcus mutans

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1. Abstract

High powered ultrasound has the potential to induce apoptotic cell death in various suspension cell culture conditions which has been observed in many microorganisms. In this research, sonication of streptococcus mutans is carried out in order to study the effects of ultrasound on its viability and the ability to form biofilms. The viability of the cells decreased significantly after 10 minutes of exposure. This implies that ultrasound could be applied in the field of medical treatments.

2. Introduction

High intensity ultrasound is known to create cavitation bubbles when applied to liquid mediums. Intense heat, shock waves, and radicals are formed when these cavitation bubbles collapse and thus creates both physical and chemical effects.[1] High frequency ultrasound is effective in the area of sterilization for ultrasound can be transmitted via various medium. Many research was performed applying this characteristics of ultrasound to manipulate microorganisms. Recently studies have reported that high frequency ultrasound is effective in inducing apoptotic cell deaths in many different cell culture conditions.[2, 3, 4] In this experiment, ultrasound of 40.9KHz and 208KHz were used to induce cell death among the S.mutans population. Streptococcus mutans is a species of microorganism that resides in the oral cavity of humans and is believed to be one of the main causes for oral cavity formation.[5] By forming a biofilm around the tooth, it secrets lactic acid and causes the pH of the surrounding environment to drop.[6] The purpose of this research is to develop a novel method to prevent oral cavity by the sterilization of S.mutans. The S.mutans samples were exposed to ultrasounds of different frequency with varying exposure times. The death susceptibility was measured through colony formation assay and proliferation assay.
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3. Materials & Methods

A. Ultrasonic irradiation system

Fig. 1 shows schematic diagram of the ultrasonic irradiation system used. A pyrex beaker of 60 mm diameter was used as an ultrasonic irradiation cell. A Bolted Langevin Transducer of 40.9 kHz was attached to the bottom of the irradiation cell and a generator (50W, Kodo Technical Research Co., Korea) was used for the generation of ultrasound. Distilled water of 30℃ was filled into the irradiation cell to transmit ultrasound. A flat-bottom pyrex tube with a inner diameter of 12 mm was installed in the irradiation cell and was used as a container of the cell culture. All pyrex tubes were covered with a cap to prevent any contamination. The distance between the sample and the transducer was set to 2cm.

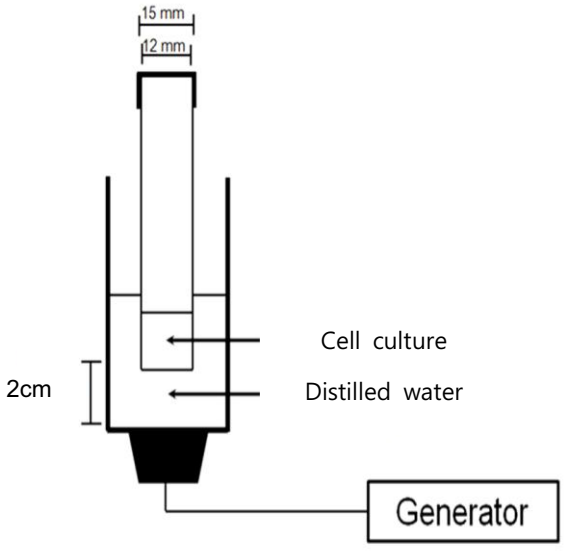

Figure 1 – Experiment setup of the ultrasonic irradiation system.

B. Streptococcus mutans cell culture

Streptococcus mutans (strain 3065, KCTC) were incubated in liquid Brain Heart infusion broth medium (Becton, Dickson) at 37°C. Each cell culture was uniformly controlled to an optical density of 0.1 at 660nm by a spectrophotometer. (Ultrospec 2100 pro, Amersham Biosciences)

C. Prliferation Assay

Ultrasound of 40.9 kHzand 208KHz were irradiated to 2.0 ml of culture with different time intervals. Proliferation assay was carried by incubating the exposed S.mutans samples in liquid Brain Heart infusion broth at 37℃. 200 μL of each sample was placed in a Nunclon Surface 96well plate(NUNC) and its O.D was measured at 660nm by a spectrophotometer (GENios Pro, TECAN) every 30 minutes.

4. Results and Discussion

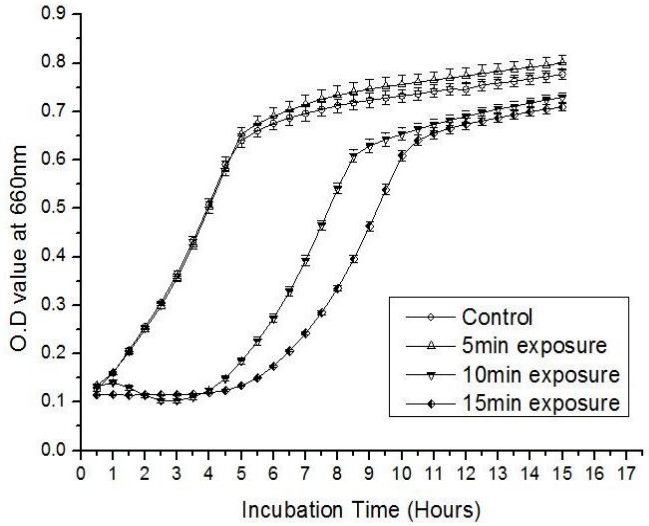

Figure 2 – Proliferation graph of S.mutans with different exposure time to 40.9KHz ultrasound

Figure 2 shows a proliferation graph of the exposed S.mutans samples. Ultrasound exposure time of 5 minutes does not seem to affect the survival rate of S.mutans cells significantly and shows little difference compared to the control group. On the contrary, the viability of S.mutans droped drastically after 10 minutes of exposure to sonification. The third sample with 15minutes of sonification showed the slowest of growths, pointing to the conclusion of lowest cell viability. It is suggested that there is a critical point in sonification time between 5minutes and 10minutes of exposure. Whether the deaths of the S.mutans cells were apoptotic or other by factors such as shock wave or heat is still a subject of future research.

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