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Enhancement of ultraweak photon emission with 3 MHz ultrasonic irradiation on transplanted tumor tissues of mice

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Abstract

We investigated photon emissions of various bio-samples which were induced by ultrasonic stimulation. It has been reported that ultrasonic stimulations induced the thermal excitation of the bio-tissues. After ultrasonic stimulation, any measurement of photon radiation in the visible spectral range has not been carried out yet. The instruments consisted of electronic devices for an ultrasonic generator of the frequency 3 MHz and a photomultiplier tube (PMT) system counting photons from bio-tissues. The transplanted tumor tissues of mice were prepared for the experiments and their liver and spleen tissues were also used for the controls. It was found that the continuous ultrasonic stimulations with the electrical power 2300 mW induced ultraweak photon emissions from the tumor tissues. The number of induced photon was dependent of the type of the tissues and the stimulation time intervals. The level of photon emission was increased from the mouse tumor exposed to the ultrasonic stimulations, and the changes were discriminated from those of the spleens and livers.

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Keywords: Ultraweak photon; Ultrasonic stimulation; Tumor tissue; Mouse

1. Introduction

With modernized technologies in medicine, new instruments and analyses for examining the human body have been developed. Techniques for detection, or localization, of tumors include the use of endoscopes for hollow organs of the body and X-rays, magnetic resonance imaging, or ultrasound for less accessible parts of the body [1,2]. As a biomedical diagnostic tool, ultrasound has long been used in medicine practically. For example, ultrasound for breast imaging has evolved since its original description in the early 1950s from the ability to differentiate cystic from solid lesions [3,4]. It could also aid the differential diagnosis of benign from malignant breast lesions. There has been an increasing interest and awareness of the use of ultrasound for therapeutic applications in medicine [5,6]. The effects of ultrasound at various energy levels on bio-samples have been explored in vitro studies. The excised sciatic nerves from the bullfrog were stimulated by 3.5 MHz continuous wave ultrasound at various energy levels for specific time interval [7]. Comparative studies were done on the efficacy of the topical application of therapeutic ultrasound on the tissue repair process in rat tendons [8], and on the effects of low-intensity ultrasound on peripheral nerve regeneration in the conduits seeded with Schwann cells [9]. It was known that ultrasonic stimulation may affect the growth and proliferation of the cells [10] and it also accelerated fibrinolysis in vitro and in vivo, primarily through non-thermal mechanisms including cavitations [11]. The effect of the diagnostic ultrasound

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stimulation on maternal and fetal chromosomes was so mild that there was no increase in the number of chromosome aberrations in blood cultures [12].

The combined researches which include both optic light and ultrasound have been carried out. Especially, as an intriguing phenomenon, weak light emission associated with the acoustic cavitation activity, sonoluminescence, was still poorly understood and intensively investigated [13]. For example, researchers looked for sonoluminescence in vivo in the human cheek [14]. The oscillatory behavior of the minute gas bubbles in liquids exposed to ultrasound was investigated with physical and mathematical models. Unstable oscillations sometimes lead to transient the cavitation in which a bubble violently collapses during a single acoustic half-cycle producing high temperatures and pressures. They suggested that the sonoluminescence provides some non-chemical evidence for the formation of free radicals. The chemical effects of the cavitation in organic liquids remained relatively unexplored and were actively investigated [15].

It was known that all biological systems continuously emit photons with very weak intensity of about 10^{-16} W/cm^2 in the spectral range from ultraviolet to near infrared without any external stimulation. This phenomenon was referred to as ultraweak photon emission or 'biophoton' emission differing from the usual bioluminescence [16,17]. These ultraweak photon emissions of various biological systems from mammalian cells to human bodies were detected by very sensitive photon-detecting systems using photomultiplier tubes. Many results have revealed some associations between the ultraweak photon emissions and the physiological states of living systems, including the oxidative metabolism, germination of seedlings, embryology, morphogenesis, injury and healing [18]. In the previous works we have measured spontaneous photon emission from various cancer tissues, and found that the intensity of spontaneous photon emissions from the cancer tissues was mostly discriminated from those of the normal tissues [19,20]. A scanning system was also designed for the detection of spontaneous ultraweak photon emission from the nude mice with the transplanted tumors. The profiles of the intensities of photon emissions from the tumor mice were measured and compared with those obtained from the control mice [21].

Recently a new technique called near infrared emission spectroscopy induced by ultrasonic irradiation has been developed. They demonstrated that ultrasonic excitation with a 20 kHz 130 W ultrasonic processor really induced electromagnetic waves in the spectral range of near infrared from 1500 nm to 3000 nm [22]. In this study we investigated on photon emissions from tissues of the transplanted tumor mice on which the ultrasonic stimulations were applied. We tried to detect that the ultrasonic excitation with a 3 MHz piezoelectric generator which induces visible lights in the spectral range from 350 nm to 650 nm. As control samples, the bio-samples such as liver and spleen tissues of the mice were also prepared. Because a photomultiplier tube (PMT) was used for the detection of photon emission, a precise spectral distribution could not be obtained. The PMT system, however, was designed to detect even extremely weak intensity of photon emissions.

2. Materials and methods

2.1. Bio-tissue preparation

Six 2-week-old male C3H mice were purchased from the Animal Center (Jung-Ang Lab. Animal Inc., Seoul, Republic of Korea), and the cell line of the fibroblast sarcoma, NCTC-2472 (Korean Cell Line Bank, Seoul, Republic of Korea) was injected to the back of mice. The transplanted tumor tissues of the mice were growing for five weeks after the injections. Procedures and care involving the animal conformed to institutional guidelines, which were in full compliance with current international laws and policies (Guide for the Care and Use of Laboratory Animals, National Academy Press, 1996). All mice were anesthetized with ketamine 0.2 ml intraperitoneally and the surgical procedures were performed under general anesthesia. The pieces of tumor tissues were prepared by cutting the round-shape tumor of the size around 1.5 cm in diameter and 3 mm in depth from the mice, and whole livers and spleens were also extracted. The fresh samples within several hours were used for the experiment.

2.2. Photon-detecting system

We used the photon-detection system as described in Refs. [19,20]. The numbers of emitted photons from the samples were measured by the PMT (R331-05 selected, Hamamatsu, Japan) and associated data acquisition systems. The PMT was operated at room temperature (20 °C) and had a spectral response in the range from 350 to 650 nm. The wavelength of the maximum response was 420 nm. The proper housing and magnetic shield case for the PMT were used to ensure complete light-shielding and magnetic-shielding. The distance between the PMT and the sample was 5 cm, and actual photon-detections were made within a dark chamber in a dark room. In the dark chamber, a sample was put in the sample holder on which the ultrasonic vibrator was positioned as shown in Fig. 1a. The gate time for gathering the photon signal from the PMT was 1 s.

2.3. System for ultrasonic generator

An electric circuit was made for the generation of the ultrasonic signal. A commercial piezoelectric ultrasonic vibrator was used for the ultrasound generator. Equivalent circuit for the generation of the ultrasonic signal was shown in Fig. 1b. The signal waveform was sinusoidal with the frequency of 3 MHz. The wave signals were also modulated on-and-off by a square type of waveform with the variable stimulating time interval, T_s, from 0.1 to 1 s in