Detection of Ultraweak Photon Emission (UPE) from Cells as a Tool for Pathological Studies

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ABSTRACT

Objective: It is well-known that all living cells emit ultra-weak photon emission (UPE), which is due to byproducts of chemical reactions in cell metabolisms. It has been shown that Reactive Oxygen Species (ROS) in the cells enhances the UPE intensity. The magnitude of such UPE is extremely weak (i.e. a few to 10^3 photons/(sec.cm^2)), and the detection of such ultra-weak signals is hardly possible via sensitive instruments like photomultiplier tube (PMT) that can detect single photons.

Materials and Methods: H_2O_2 factor with various concentrations was applied on the HT-29 cells to generate ROS. H_2O_2 concentrations were so low to be nondestructive to the cells. Then, the effect of ROS generation on UPE intensity was investigated. PMT was used to detect UPE from HT-29 cells.

Results: The topical application of H_2O_2 was significantly different ($P < 0.05$) in comparison with HT-29 cells without H_2O_2 at a concentration of 1mM in 5 min detection time. The integrated UPE in the presence of H_2O_2 at concentration of 3mM was significantly higher ($P < 0.05$) than the integrated UPE in other groups at the same detection time. The difference between the concentrations of 3mM and 4mM was not significant ($P > 0.01$) for integrated UPE in the cell groups in the presence of H_2O_2.

Conclusion: The results show that the recorded UPE from HT-29 cells increased with the topical application of exogenous ROS inducer. As a result, UPE can be used as a non-invasive technique for monitoring ROS in cells.

Keywords

Ultra-Weak Photon Emission, HT-29, Reactive Oxygen Species, Hydrogen Peroxide, Photomultiplier Tube (PMT)

Introduction

Information is a principal parameter of life communication. Biological systems (e.g. cells) can interact with each other via many mechanisms and at many levels, depending on the type and complexity of the biological system and the nature of the information transfer. Electrical and chemical mechanisms are the most well-known ways of cell-to-cell transmissions. However, there is this conjecture that cells also may communicate via electromagnetic waves. This latter topic was pioneered by *A.G. Gurwitsch* [1-4], who found out radiation emitted from the living cells could cause other cells to divide. In fact, based on the Gurwitsch's conclusion there should be a photon production mechanism by cells, which are emitted without any external excitation. These biological photons are due to chemical processes, and nowadays are called ultraweak photon emission (UPE). In different literature sources, UPE

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is referred to by different names such as biophotons, ultraweak emission, ultraweak bioluminescence, self-bioluminescent emission, photoluminescence, delayed luminescence, ultraweak luminescence, spontaneous chemiluminescence, ultraweak glow, biochemiluminescence, metabolic chemilumi-nescence, dark photobiochemistry and bioluminescence. It has been clearly demonstrated that all living cells (without external excitation) spontaneously and continuously produce UPE. On the other side, it seems that UPEs are the by-products of metabolism inside cells and therefore they may appear as trivial signals. The intensity of UPE is of the order of a few, up to 10^4 photon/ (cm^2s) (or equivalently 10^{-19} to 10^{-14} W/cm^2). UPEs are produced from diverse naturally occurring oxidative and biochemical reactions, especially free radical reactions and the simple quenching of excited molecules. The study showed that a spectrum of this signal ranges from ultra-violet (UV) to visible and infrared spectra, i.e. in 200-800nm region. UPE happens in all living organisms and it is different with other types of biological light emissions like fluorescence, delayed luminescence and bioluminescence.

Reactive oxygen species (ROS) are considered to have a noticeable harmful effect on cells [5]. It is believed that ROS is responsible for initiating many diseases [6]. An important source of ROS formation in cells is the mitochondrial oxidative phosphorylation. The mitochondrial oxidative metabolism and lipid peroxidation are regarded as the main sources of weak biological UPE. The excited electron of carbonyl species R=O* and singlet oxygen O_2 are responsible for photon emission. The excited electron can emit its energy as a photon in the visible range when it is released to the ground state. The origin of UPE was also frequently discussed from the point of view that, usually, only primary emission emanating from the surface would be measured. Emission occurring on deeper layers may be absorbed and become a part of the transmission of excited states, both dark and light, the latter resulting in secondary radiation from other sources [7, 8]. Cheun et al. in 2007, Madin-Darby canine kidney (MDCK) cells were subjected to H_2O_2 and measured the UPE intensity by using PMT and florescence microscope apparatus [9]. They found out that the enhancement of UPE intensity was associated with the concentration and addition of H_2O_2 to the sample [9]. *Rastogi et al*. detected UPE from root cells by PMT in which the intensity of UPE after adding H_2O_2 was a hundred times higher than the intensity without H_2O_2. However, they have also found that the addition of glucose oxidase to the cells caused the reduction of UPE intensity [10]. *Prasad* and *Pospisil* [12] used charged coupled device (CCD) to measure the UPE from human skin. They demonstrated that UPE increases with the topical application of induced exogenous ROS, and therefore a two-dimensional imaging of UPE from skin can be used as a non-invasive tool for the spatial and temporal monitoring of oxidative stress [11, 12]. *Van Wijk et al*. [13] used CCD to monitor the photon emission both without and with luminol in a Rheumatoid Arthritis (RA) mouse model. They showed that the used imaging technology may be useful for the future study of human RA. *Cervinkova et al*. [14] investigated the response of UPE signals when a biological sample was affected by a certain antioxidant solution. They studied the changes in the UPE intensities of temporal developments of the optical signal. *Nerudova et al*. [15] provided an experimental analysis of the spectral properties of UPE from HL-60 cells and from yeast cells. They have demonstrated a clear difference in the UPE spectra between two organisms using rigorous methodology and error analysis [15]. *Zhengyong et al*. [16] analyzed the current application of UPE in biomedicine, agriculture, environmental science, food detection and other aspects based on discussing the generation mechanism and detection of UPE.

Basically, the intensity of UPE is very weak,

i.e. between a few to several hundred photons s^{-1} cm^{-2} (17, 18), so the detection of UPE was ignored for more than half a century. However, by developing instruments like PMT and CCD, a single photon could be detected. *Konev et al*. [19] were the first to utilize UV-sensitive PMT to detect UPE from the living organisms. They investigated the UPE of more than a hundred different organisms [20]. *Grasso et al*. [21] attempted to detect 25 samples of human tissue using PMT, nine of which were normal and the other were tumor tissue. They observed that the UPE from a tumor tissue was much more than a normal tissue.

As mentioned above, the intensity of UPE can be an indicator of cellular metabolism [22]. The ROS in the cells causes the enhancement of UPE intensity and thus the detection of UPE can be used as a non-invasive method for the diagnosis and optical biopsy.

HT-29 cells are the most common cancer of digestive tract and the fourth cause of cancer death in the world. Therefore, in this paper, the above cells are considered for our study. In this paper, the UPE detection from HT-29 cells is studied by using PMT tool. To generate ROS for the detection of UPE with more intensity, H_2O_2 factor with various concentrations is added to HT-29 cells.

Material and Methods

HT-29 Colon Cell Preparation

HT-29 cells were purchased from Pasteur Institute of Iran. HT-29 cells were cultured in a humidified incubator with 5% Co_2 and 95% air at 37°C temperature. The culture medium was contained 1640 RPMI (Roswell Park Memorial Institute), that was supplemented with 10% FBS (Fetal Bovine Serum 2016) (Gibco), 1% pen-strep (IDZ), 0.5% Amphotericin B. The medium was replaced every 2 days. When the cells reached 80% confluence, there were passages with 0.25% Trypsin and 0.53 mM PBS (Phosphate Buffered Saline) to remove all traces of serum containing trypsin inhibitor. About 10^4 of HT-29 cells were seeded in petri dishes with 3.5 cm diameter and were incubated for 48 h.

Optical Measurement Apparatus

9235B as a 51mm (2”) diameter, end window Photomultiplier (ET Enterprises Limited, United Kingdom) was employed to measure the amount of photons emitted from HT-29 cells. This detector had its maximum response at 350 nm with the quantum efficiency of 30% in detection range of 250 nm to 600 nm. The rise time of the PMT was about 3 ns. The PMT worked at room temperature. Output from the PMT was connected to a counter which was linked to a PC. The dark count at the room temperature was about 4 counts per second (cps) at 820 V.

The PMT, counter and sample were always stored in a dark room to maintain optimum performance, and the rest of electronic setup was located outside the dark room. The atmosphere inside the dark room was set to the same level as ordinary room-air environment. The sample was placed in a 3.5cm in diameter polystyrene petri dish for PMT measurement. We employed H_2O_2 (Ghadir, 34%, Iran) to generate reactive oxygen species for the experiments. The concentrations of the employed H_2O_2 were 0, 1, 2, 3 and 4 mM (millimolar). In order to inject H_2O_2 to the sample, a syringe was used, while dark room condition did not change, and the amount of H_2O_2 we used was 3ml for each petri dish. Schematic dark room of the experimental setup is shown in Figure 1.

Data Analysis

A statistical analysis was performed using SPSS version 21 statistical software (SPSS Inc., Chicago, IL). According to the Kolmogorov-Smirnov normality test, the data distribution was normal. Consequently, the paired sample t-test was used to compare the recorded UPE from HT-29 cells in the absence and presence of H_2O_2 with a confidence level

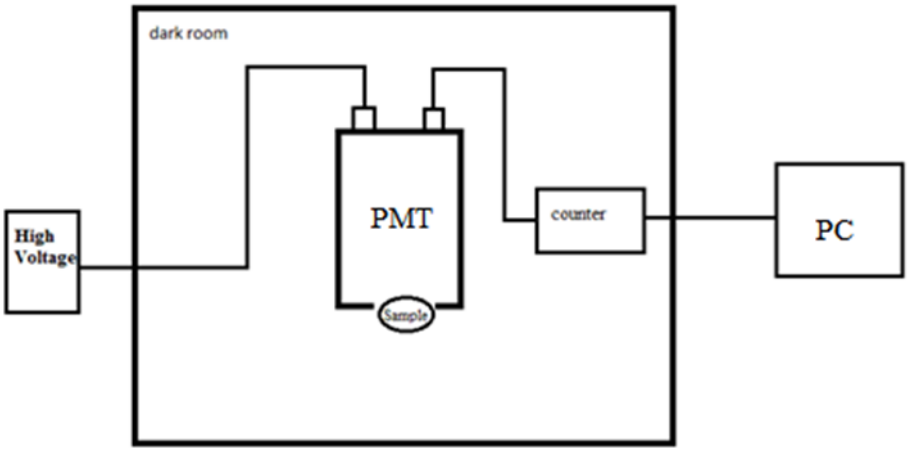

Figure 1: An experimental setup of PMT for measuring UPE

of 95%. The recorded UPE between HT-29 cells at different concentrations of H_2O_2 also was compared using one-way analysis of variance. Data presented as Mean ± SD. $P < 0.05$ were considered as statistically significant.

Results

In all recorded UPE measurements, the level of background noise was measured and subtracted from the recorded UPE.

The recorded UPE from HT-29 cells in the absence and presence of H_2O_2 at a concentration of 1 mM in detection time of 5 min is presented in Figure 2.

Statistical comparison of the results showed that the recorded UPE from HT-29 cells in the presence of H_2O_2 was significantly different in comparison to HT-29 cells without H_2O_2 at a concentration of 1mM in detection time of 5 min ($P < 0.05$).

The integrated UPE in different HT-29 cell groups in the presence of H_2O_2 at concentrations of 0, 1, 2, 3 and 4 mM in detection time of 5 min is presented in Figure 3.

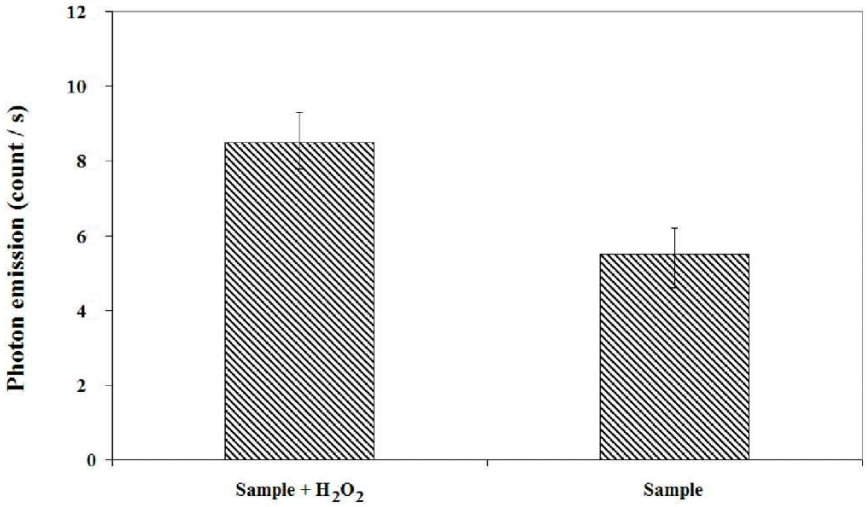

Figure 2: Recorded UPE from HT-29 cells in the absence and presence of H_2O_2 at concentration of 1 mM in detection time of 5 min

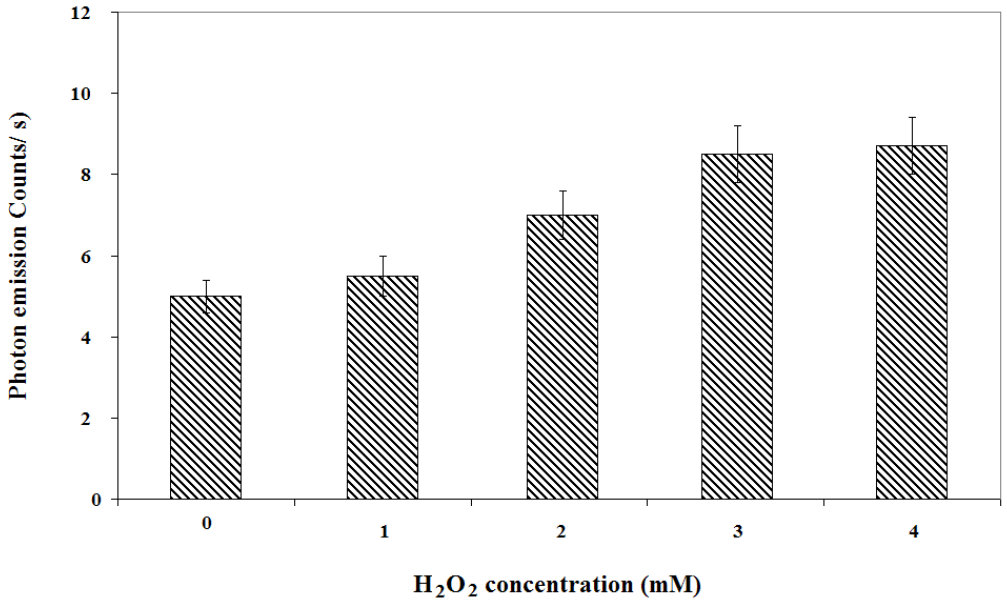

Figure 3: Integrated UPE in different HT-29 cell groups in the presence of H_2O_2 at concentrations of 0, 1, 2, 3 and 4mM in detection time of 5 min

Figure 3 vividly shows that the application of H_2O_2 to the cells causes their death. It is beneficial to point out that photon radiation increased as a result of the reaction between H_2O_2 and cells. Figure 3 indicates that any increase in the concentration of H_2O_2 will be associated with an increase in the integrated UPE. The integrated UPE from HT-29 cell group in the presence of H_2O_2 at concentration of 3mM was significantly higher than the integrated UPE from other groups at the same detection time ($P < 0.05$), but the difference in the integrated UPE from HT-29 cell groups in the presence of H_2O_2 was not significant ($P > 0.01$) between concentrations of 3mM and 4mM.

The integrated UPE from different HT-29 cell groups in the presence of H_2O_2 at concentrations of 0, 1, 2, 3 and 4mM in detection times of 1, 2, 3, 4 and 5 min is presented in Figure 4.

Figure 4 indicates that any increase in detection time will be associated with an increase in the integrated UPE.

As it can be seen in Figure 5, the UPE intensity of HT-29 cell is plotted as a function of time. In the first region, data is obtained before injecting H_2O_2 to the HT-29 cells. In the second region, H_2O_2 affects cells during the measurement. It is observed that UPE continuously increases with more prolonged periods until it reaches the saturation value and then decreases with time to near the moment before exposure with H_2O_2.

Discussion

According to the previous studies, ROS and RNS in different living cells are formed by linking to enzymatic activities in the organelles like mitochondria, chloroplasts and peroxisomes. In mitochondria, the role of cellular respiration is the most important among other organelles, and it is the major source of ROS production in cells [23, 24].

In the presence of metal oxidases (e.g. M^{ox}, like Fe^{2-}, Mn^{2+} and Cu^{+}), H_2O_2 is broken down into a $HO^{\bullet}$ radical and a hydroxide ion OH^{-}. Hydroxyl radical tends to absorb additional electrons from molecular cells and also starts a chain reaction [23, 24]. Consequently, this causes to enhance the amount of UPE. As shown in the results, when the physiological

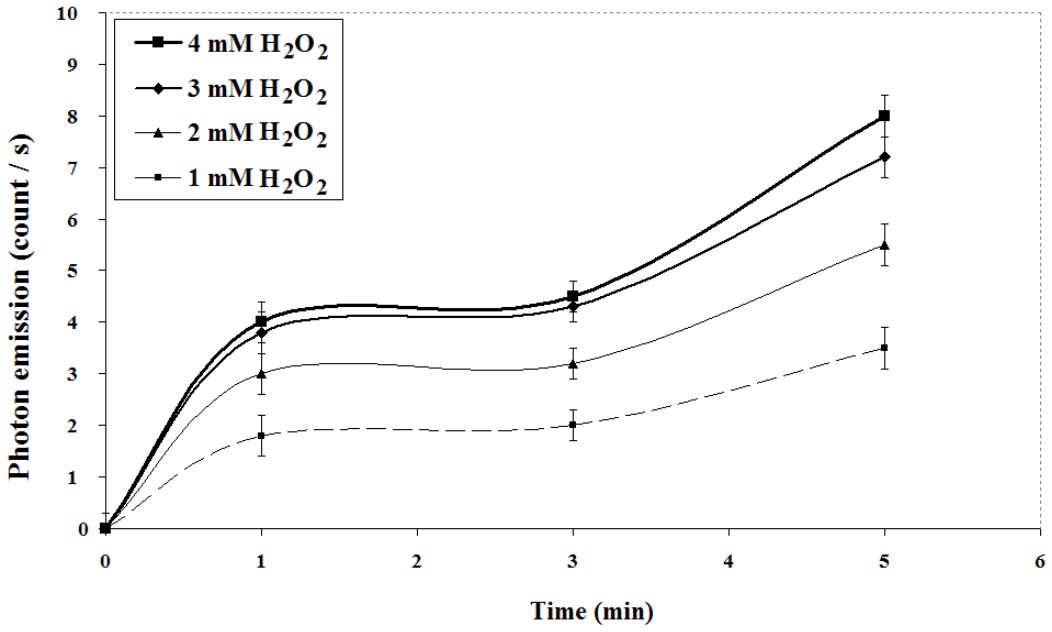

Figure 4: Integrated UPE in different HT-29 cell groups in the presence of H_2O_2 at concentrations of 0, 1, 2, 3 and 4 mM in detection times of 1, 2, 3, 4 and 5

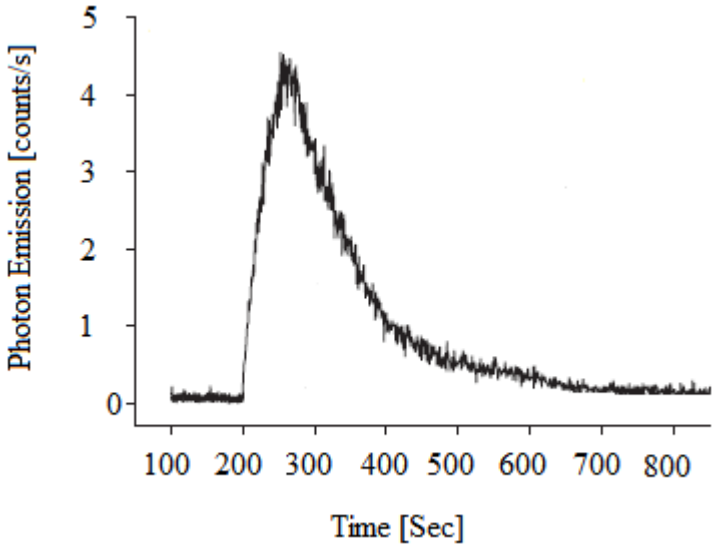

Figure 5: Intensity of UPE versus time from HT-29 cells

concentration of 1mM H_2O_2 was used, UPE enhanced in front of pure cells. H_2O_2 with concentrations of 1mM and 2mM is considered and the amount of UPE from cells is measured. The number of UPE extracted from the concentration of 2mM is higher than 1mM. Higher concentrations of H_2O_2 cause more oxidative stress and further emission of photons, while no significant difference in the UPE among such concentrations was found for 3mM and 4mM concentrations. It means that cells are in the saturated state in which af-

ter that no remarkable change is found in the amount of released photons. Our results indicate that any increase in the detection time will be associated with an increase in the integrated UPE. After adding H_2O_2 in the first 200 seconds, no enhancement in UPE emission was observed. Nevertheless, by increasing the time, the intensity and amount of UPE increased toward the highest peak, and after that the intensity started to decrease to the background emission.

The results indicate that the UPE intensity increases by ROS production. Accordingly, in the areas with the probability of diseases, e.g. cancer, which arises from the ROS production [14], the UPE detection can be a good method to diagnose some diseases.

Suzuki and et al. [25] detected the UPE by a two-dimensional photon counting system from physically injured seedlings of soybean and adzuki bean. According to their study, H_2O_2 inside the cancer cells increased the UPEs, which is an affirmation of the ROS chain reaction theory. Rastogi and Pospisil [11] showed that using H_2O_2 as an excitation factor for ROS production, the UPE from palmar side was twice higher than the dorsal side of the hand. They showed also that the UPE from damaged cells is more than healthy cells confirming our work on HT-29 cells. This demonstrates that the increment of ROS in different parts of body can cause significant enhancement in the amount of UPE. Prasad and Pospisil [12] employed the excitatory factors such as Xanthine, H_2O_2 and Fenton to measure the UPE on the dorsal side of the hand by using CCD, which utilized a two-dimensional imaging technique. They showed that UPE increased by adding the mentioned factors. In this study, HT-29 cells are used, that is the most common cancer of digestive tract and the fourth cause of death associated with cancer in the world. The factor of H_2O_2 was added to HT-29 cells to detect UPE using PMT. The detection using PMT besides other methods such as biopsy can be employed to identify the ROS generated from lipid and protein oxidation. In fact, the PMT is a simple counting device and an inexpensive tool that is available and applicable in the diagnostic centers with acceptable precision.

Unlike PET and CT systems that use invasive methods to detect diseases, UPE detection is utilized as a non-invasive method. Yet, little research on this aspect of human knowledge is conducted. We hope our research can provide some input in developing this part of knowledge for finding better methods of diagnosis.

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Conflict of Interest

None

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