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Exposure of Weak Time-Invariant Electromagnetic Fields to B16-BL6 Cell Cultures Alter Biophoton Emission Profile as a Function of Distance

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Abstract

Biophoton emission is produced by all living systems; this emission pattern has been shown to be altered by the presence of an electromagnetic field (EMF). Cultures of B16-BL6 cells were exposed to a weak EMF produced by a specially constructed EM generator, called the "Resonator", for one hour. This EM generator incorporates multiple geometric ratios in its design, including the golden ratio (phi), pi, root 2, root 3, and root 5. It has been used previously to purify water of toxins. There was a significant decrease in mean photon counts from B16-BL6 cells exposed at a distance of 1 m compared to those exposed at 0 m. Alterations in the spectral power density variability were also observed in the 8 - 10 Hz range. The EM generator may have an impact on the viability of the exposed cell cultures, but only at specific distances.

Keywords

Electromagnetic Fields, B16-BL6 Cells, Spectral Power Density, Biophoton Emission

1. Introduction

All living biological systems emit low levels of electromagnetic radiation, referred to as biophoton emission. It has been proposed that metabolic activity is the primary source of biophoton emission, specifically the oxidation of free radicals. This is supported by experimental evidence including the addition of hydrogen peroxides to tissue, or a reduction of tissue antioxidants [1]. This pro-

posed mechanism is the same for both types of biophoton emission: spontaneous and induced. Spontaneous biophoton emission is produced by the natural metabolic activity of the organism. Induced biophoton emission is observed after an organism has been exposed to one or more of several factors, which can be natural or artificial. These factors include bacterial or viral infection, stress, external temperature, and ionizing radiation, to name a few [2]. Electromagnetic fields (EMFs) have also been previously demonstrated to induce biophoton emission in combination with specific wavelengths of light [3].

The examination of the effect of exposure to an EMF alone on biophoton emission has not been done. In an experiment by Karbowski and colleagues, three complex, time-varying EMF patterns were used [3]. The central effect observed was the increase in mean photon counts when B16-BL6 cells were exposed to a combination of both an EMF and 450 nm light. In this experiment, the aim was to focus on the characteristics of a single EMF. Additionally, previous experiments by Persinger and colleagues [4] have demonstrated an inverse relationship between photon flux densities and nanotesla magnetic fields over cell aggregates. This experiment illustrated a conservation of energy effect between the measured geomagnetic field and the added—or subtracted—changes in photon emission intensity from nearby cells.

The EMF used in this experiment, was generated by a novel device invented by Dr. K. Shallcross. This EM generator, referred to as the "Resonator", uses a series of metallic roller magnets arranged at specific angles to create a pattern derived from a sacred geometry. Sacred geometry refers to numerical values derived from simple geometric relations, such as π, √2, √5, and [5]. When the device is on, the roller assemblies rotate, which generates a weak EMF, in the range of 1 μT to 10 μT. When the device is off, no EMF is generated.

Previous experiments with the "Resonator" have shown differential effects on the growth rate of bacterial cultures [6]. Three of the examined bacteria species were shown to have an increased growth rate after exposure, while one species had a decreased growth rate. This suggests that the complexity of the "Resonator" field is such that it can have opposite effects on different species. This is most likely mediated via different effects on cellular metabolic pathways.

In addition, exposure of the "Resonator" EMF to B16-BL6 cells for three hours has been shown to significantly decrease cell viability [7]. Takeda and colleagues have demonstrated that biophoton emission in cancerous cells was related to the population size [8]. Thus, if exposure to the "Resonator" EMF is correlated with decreased cell viability, and biophoton emission is correlated with cell population, there should be an observable decrease in the biophoton emission of exposed cell cultures.

2. Methods

B16-BL6 cell cultures were grown in DMEM supplemented with 10% fetal bovine serum and 1% antibiotics on 10 mm Petri dishes and allowed to reach con-

fluence in a water-jacket copper-lined incubator at 37˚C (95% air, 5% CO_2). Cell cultures were grown on Petri dishes in groups of 16. Once confluent, the plates were removed from the incubator and placed into one of four conditions: Field 1 m, Field 0 m, Sham 1 m, Sham 0 m. Each condition contained four cell plates. These conditions refer to the state of the "Resonator", either with an active magnetic field in the Field conditions, or with the device turned on but with no movement of the coils; these were the Sham conditions. The cell plates were placed on a table at either 1m or 0 m from the "Resonator". Vertically, the cell plates were separated from the "Resonator" by a distance of approximately 10 - 15 cm. The distance of vertical separation was constant between the 1m and 0m conditions. The plates were arranged side by side, such that they formed an approximate square on the surface of the table. This table did not make physical contact with the "Resonator" to remove any vibrational effects from the spinning of the "Resonator" coils. The "Resonator" was then turned on for the Field conditions. Cell plates were left on the table for no less than one hour.

Once the exposure was complete, the biophoton output of the cell plates was immediately measured. This measurement was performed in the experimental chamber, a specially constructed space measuring 1.32 m long, 1.27 m wide, and 1.70 m in height. Before the experimental chamber was opened, a baseline measurement was taken. The cell plate was placed on a small polystyrene box, which is located on a chair in the centre of the experimental chamber. The sample was measured with four PMTs, which were located on three walls and the ceiling of the experimental chamber. Each PMT was separated from the sample by a distance of 15 cm. A one-minute delay between the closure of the experimental chamber and the start of the measurement ensured minimal light contamination of the cell plate measurement. Each cell plate was measured for two minutes. Including the one-minute delay, each plate was in the experimental chamber for three minutes. After each plate had been measured, a post baseline measurement was performed. This procedure was identical to the cell plate measurement, including the one-minute delay. Field and sham measurements for plates exposed at any given distance were recorded on the same day to minimize daily variations.

Data from the measurement sessions was exported into IBM SPSS for statistical and spectral analyses.

3. Results

All of the analyses below were performed with the data from the PMT located at the Head position. Only the second minute of the measurement was used for analyses. A total of 14 cases from two different days were removed due to outlier effects. This gave a total N of 41.

First, a comparison was made between the baseline condition and all cell conditions. A Student's t test for independent samples revealed a significant difference between the baseline and the cell conditions ($t = -6.260$, $p < 0.001$, $\eta^2 =$

0.445). The cell condition had significantly higher mean photon counts than the baseline condition. This is shown in Figure 1.

To expand on the B16-BL6 conditions, a pair of Student's t tests for independent samples were used to compare mean photon counts between field condition and distance. This is shown in Figure 2. There was no significant difference in mean photon counts between the Sham 0m condition and the Sham 1 m condition ($t_{(14)} = 0.163$, $p = 0.873$). The mean photon counts of the Field 1 m condition were significantly lower than the Field 0 m condition ($t_{(18)} = 2.120$, $p = 0.048$, $\eta^2 = 0.200$).

A series of one-way ANOVAs were performed to compare the spectral power density variability by condition membership. The spectral power density variability is the standard deviation of the spectral power density, per case. For this procedure, the spectral power density variability was averaged into multiple

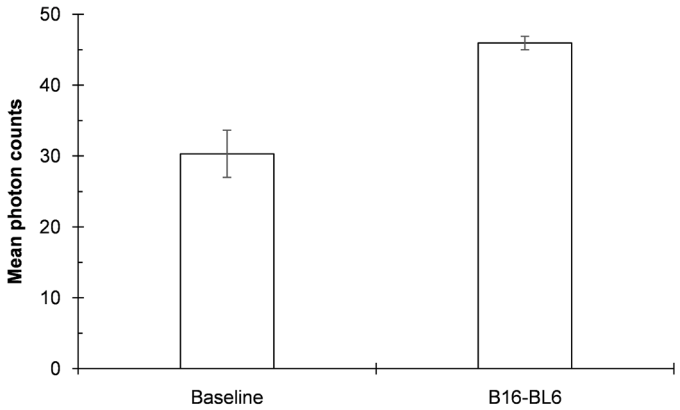

Figure 1. Comparison of the mean photon counts between the baseline condition and all B16-BL6 conditions. The B16-BL6 condition was significantly higher than the baseline condition. Error bars represent standard error of the mean; $n = 5$ for baseline, $n = 36$ for B16-BL6.

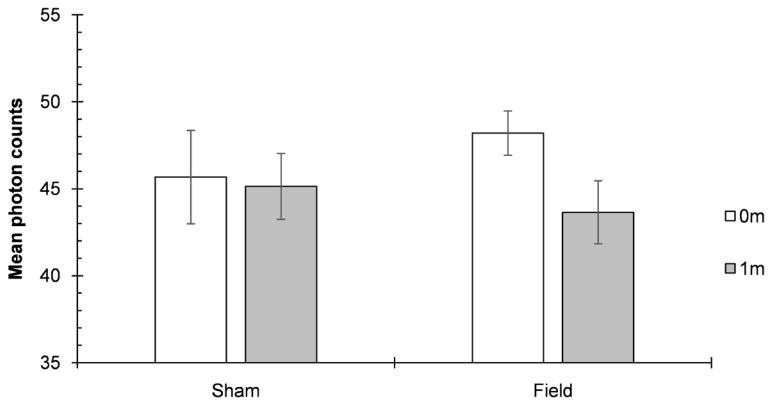

Figure 2. Comparison of the mean photon counts between Sham and Field conditions at 0 m and 1 m. There was no significant difference between the Sham 0 m and Sham 1 m conditions; the Field 1 m condition was significantly lower than the Field 0 m condition. Error bars represent standard error of the mean; the n for each condition was 8, 8, 12, and 8, respectively.

frequency bins, each with a width of 1 - 4 Hz. The only bin that produced a significant main effect was the low alpha (8 - 10 Hz) frequency bin ($F_{(3,32)} = 4.047$, $p = 0.015$, $\eta^2 = 0.255$). Post-hoc tests revealed the effect was being driven by the difference between the Sham 1m and Field 1 m conditions ($p < 0.05$). This is shown in Figure 3.

4. Discussion

The mean photon counts from B16-BL6 cell cultures were significantly higher than the baseline, regardless of experimental conditions. It is evident that the presence of a biological system increases the mean photon count as measured by the PMT. This is consistent with previous findings.

A comparison of mean photon counts between the experimental conditions revealed novel results. The sham conditions were not significantly different from one another, which would be expected. The proximity to the static EMF produced by the "Resonator", while inactive, did not seem to impact the mean photon counts.

The field conditions exhibit distance effects. The Field 1 m condition had significantly lower mean photon counts compared to the Field 0 m condition. There are three possible interpretations: one, the induced EMF from the "Resonator" at 0 m led to increased mean photon counts in the Field 0 m condition; two, the induced EMF from the "Resonator" at 1 m led to decreased mean photon counts in the Field 1 m condition; three, a combination of effects one and two. Another factor to be considered is the impact of vibrational effects. Vibrational effects have been implicated in a possible mechanism of "Resonator"-cell interaction [7]. Thus, vibrational effects may interpose themselves with the EMF

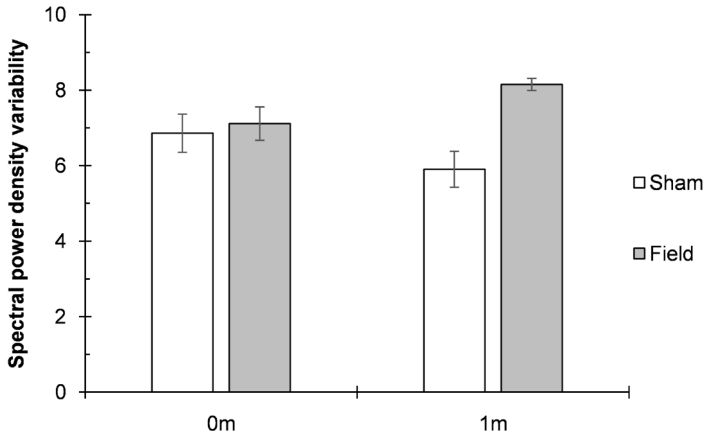

Figure 3. Comparison of the spectral power density variability in the low alpha frequency bin. There was a significant difference between the four conditions; post-hoc tests revealed the significance was being driven by the Sham 1 m and Field 1 m conditions. The Field 1 m condition spectral power density variability was significantly higher than the Sham 1 m condition. Both Sham and Field 0 m conditions were not significantly different from any other conditions. Error bars represent standard error of the mean; the *n* for each condition was 8, 8, 12, and 8, respectively.

effects while the "Resonator" is active; however, the plates were not placed in direct contact with the "Resonator" to minimize the effects of vibration.

The spectral power density of the four conditions was compared with a series of one-way ANOVAs; no significant differences were found. The variability of the spectral power density was also compared in a similar fashion; a significant effect was found in the low alpha frequency bin. This frequency bin was an average of the spectral power density variability scores in the 8 - 10 Hz frequency range. Post-hoc Tukey HSD revealed that the effect was driven by the Sham 1 m and Field 1 m conditions. The spectral power density variability was significantly higher in the Field 1 m condition compared to the Sham 1 m condition.

The implications of these two effects, mean photon counts and spectral power density variability, are centred on the Field 1 m condition. This condition had significantly lower mean photon counts than the Field 0 m condition, and significantly higher spectral power density variability than the Sham 1 m condition. This implies fewer photons are being released and the rhythmicity of photon emission in the 8 - 10 Hz range is less consistent. If the B16-BL6 cells' metabolic activity is altered by the generated EMF, this may explain why the counts were lowered in the Field 1 m condition. The EMF that is generated is an extremely low-frequency field which does not change as a function of position (relative to the "Resonator"), although the intensity decreases with distance. This is a significant quality, since the signal pattern and frequency remain consistent over distance, the only EMF factor that would change between the Field 0 m and Field 1 m conditions is the intensity of the field.

It is important to mention that the spectral power density variability of the Sham conditions was not significantly different from the spectral power density variability of the Field conditions. This may be a function of low sample size. High variability is also a common issue when biophoton emission is involved. This is often why spectral analyses are performed. The cell cultures were removed from the incubator for exposure and for measurement. Ideally, both of these processes should be carried out in a temperature-controlled environment.

This experiment was meant to act as a brief observation of the biophoton emission patterns produced by the B16-BL6 cell cultures. As such, no cellular observations were made. Future experiments should include cell counts and viability. Comparisons could then be made directly between cellular variables and measured photon activity.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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