

The Cellular Response of the Reaction to the Electromagnetic Field Frequency at 2.4 G Hertz

Hsien-Chiao Teng

Department of Electrical Engineering, Chinese Military Academy, Fengshan, Kaohsiung, Taiwan 830, ROC.

scteng@cc.cma.edu.tw

Abstract: Electromagnetic field (EMF) initiates the cellular response of being stimulated frequency at 2.4 GHz. By using signal-to-noise ratio spectrum analysis of the fluctuating process and measuring the magnetic fluctuation at the distance 10^{-1} mm perpendicular to the cells layer, the rat liver epithelial cellular responding extremely low frequency (ELF) 14 Hz was observed when the cells layer cultured into the substrate with patch radiator resonated at 2.4 GHz. Twenty percent inhabitation of the gap junction intracellular communication (GJIC) from the analysis of Lucifer yellow fluorescence microscopic image confirmed the biological effect while comparing with the control. [The Journal of American Science, 2005;1(2):9-13].

Key words: ELF, EMF, radiator, power gain, gap junctional intracellular communication

Introduction

There have been of considerable discussions about the biological response to the exposure of electromagnetic field (EMF). Public hazard and health effect is one of the major concerns. We go further to answer if biological system reacts to cellular phone operated at 2.4 GHz. In this article, we investigated if any intrinsic signal can be initiated from the rat liver epithelial cells monolayer. The cells layer can function as a thin film substrate for patch resonator operated at 2.4 GHz. Near magnetic field fluctuation can be measured and transformed to the oscilloscope voltage $V = \{V_1, V_2, \dots, V_{N-1}, V_N\}$. Computing the autocorrelation of sequence V , we get

$$R_q = \left(\frac{1}{N}\right) \sum_{p=1}^N V_p V_{p+q} \quad (1)$$

$$S_k = \sum_{q=1}^N R_q e^{\frac{i2\pi kq}{N}} \quad (2)$$

S_k is the Fourier exponent expansion of R_q and also is the power density component of the system at frequency $\omega_k = \frac{2\pi}{N}k$ (fundamental frequencies).

The unit of S_k is watt per frequency for each V_p and $i = \sqrt{-1}$. Relying on cells layer surface electrical current distribution, gap junctional intracellular communication (GJIC) modulation can be used to express the cell's response under the exposure of EMF. Since GJIC is affiliated with many endpoints [Trosko, 2001], GJIC modulation is a good scale factor to evaluate if the ELF signal being existed for the cell system. Scrape loading dye transfer of Lucifer yellow is the technique used to measure GJIC modulation [Upham, 1998].

Materials and Methods

By using IE3D simulator, we simulated the design to confirm the operation frequency at 2.4 GHz before fabrication. Figure 1 depicted the patch design with a single rat liver epithelial cells layer cover on substrate. Radiation pattern was measured at 2.4GHz frequency. The glass substrate was etched concavely at 0.15 mm in depth where the cells would be cultured into it. The aluminum metal layer (two radiators) was deposited 0.05 mm thick by evaporation before etching the substrate. The thickness of the cell layer was about 1 μ m. Therefore, the depth between the metal and cell was about 0.2 mm. The thickness of the substrate was 0.4 mm. The length of the substrate is 80 mm and the width of the substrate is 10mm. The smaller patch radiator is 8 mm wide and 20 mm in length. The bigger patch radiator is 50 mm long and 8 mm wide. The gap between two patch radiators is 2 mm wide. The total design met 50 Ω (ohm) impedance matching. The feeding was designed three dimensional n-type transmission line under Bluetooth IEEE 802.11/a/b protocol [Wong, 2003].



Figure 1a. Biological cell patch (cell layer is deposited on the substrate)

In addition, Figure 1b showed the magnetic fluctuation very near the cell layer on the patch substrate.

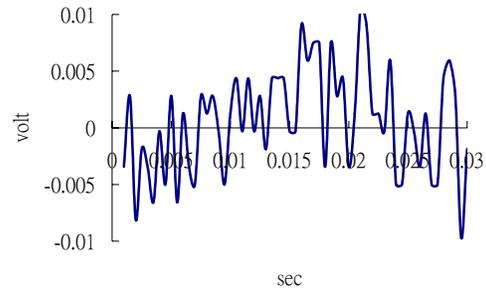


Figure 1b. The near geo-magnetic field fluctuation (10^{-4} m to the rat liver epithelial cell layer) of the patch resonated at 2.4 GHz

Cell Culture

The rat liver epithelial cell line was obtained from the Fisher Scientific (WB344). It was derived from normal liver and maintained in D-medium (Formula 78-5470EF, GIBCO, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (GIBCO) and 50 μ g/ml gentamicin (Quality Biological, Inc., NY, USA). The cells were incubated at 37°C in a humidified atmosphere containing 5% CO₂ and 95% air and were fed or trypsinized every two to three days.

Power Spectrum

Matlab and Fortran computer languages were used for power density spectrum analysis of sequence V. The sample rate was taken 2000 times in a second. Trial signal, $A \times \sin(\omega t)$, was created by Matlab. We took the maximum peak of sequence V and add trial signal with amplitude $A = V_{\max}$ to V and refer the result to be V_{test} at frequency ω . We performed Power density spectrum (PDS) calculation by taking autocorrelation and Fourier transform of V_{test} . Then, using with different amplitudes, for instance $A = 0.7 \times V_{\max}$, $A = 0.4 \times V_{\max}$, and $A = 0.03 \times V_{\max}$ for a given test signal at frequency ω , we refer results to be $S_{0.7}(\omega)$, $S_{0.4}(\omega)$ and $S_{0.03}(\omega)$ respectively. The spectrum

curve is then defined as $pS^2 + qS + r = 0$ and shown in Figure 2. By using square curve fitting, we can solve the equations to get p, q and r-values respectively. Intrinsic ELF would have its own specific power depicted as a small signal buried in background. The threshold value of r is the condition of recognizing the cellular response frequency and highly related to thermal motion. Accordingly, intrinsic ELF have to be reconfirmed by bioassay of GJIC.

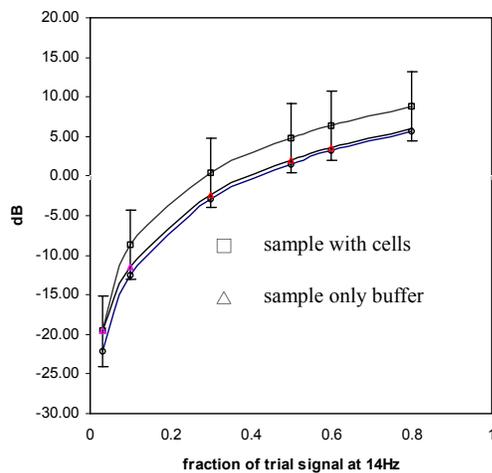


Figure 2. In comparison with the trial signal at 14Hz SNR spectrums of the magnetic field fluctuation at the distance 10^{-4} m to the rat liver epithelial cell layer and the controls

Bioassay of GJIC

The scrape load/dye transfer (SL/DT) technique was used to measure the GJIC within cells. After exposure to ELF at intrinsic frequency, the cells were rinsed with phosphate buffered saline (PBS), and a PBS solution containing 4% concentration lucifer yellow fluorescence dye is injected into the cells by a scrape using a scalpel blade. Afterwards the cells were incubated for 3 min and extra cellular dye was rinsed off and fixed with 5% formalin. We then measured the

area of the dye migrated from the scrape line using digital images taken by an epifluorescent microscope and quantitated with Nucleotech image analysis software [Upham, 1998] for the GJIC images.

Results

Figure 2 illustrated the fitting curves of trial signal at 14Hz in different conditions. Analysis allows us to validate the observation that ELF at 14 Hz may modulate GJIC. GJIC modulation can then be an index to find the buried ELF biological signal. Figure 3a and Figure 3b depicted the images of the GJIC under different condition.



Figure 3a. The fluorescent image of the gap junctional intracellular communication before exposure of 2.4 GHz EMF

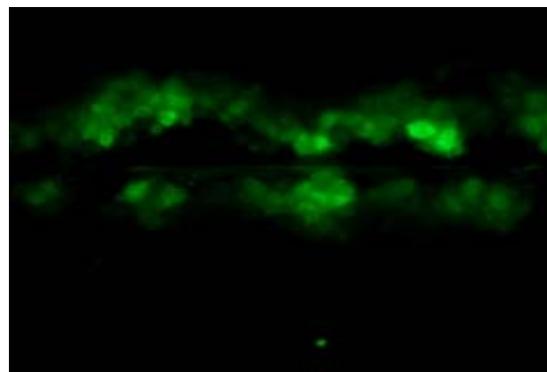


Figure 3b. Rat liver epithelial cells GJIC image at 2.4 GHz (20% inhibition fluorescent than control)

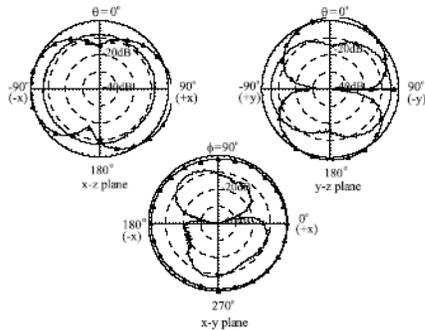


Figure 4. Radiation pattern with cells' thin layer on substrate (rat liver epithelial cells mono-layer cultured on glass plate)

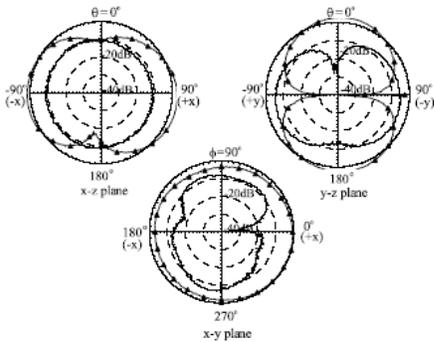


Figure 5. Radiation pattern with no cells layer on the substrate (only substrate glass, medium and patch radiator)

In Figure 4 and Figure 5, we can see the radiation pattern affected by the cell layer. The gain of the system is 2.6 dBi at 2.4 GHz before cultured the cell layer. However, after cultured the cell layer, the gain reduced to 2.0 which matched the evidence of GJIC modulation. We believed the power was absorbed by cell layer system and caused the biological effect.

Discussion

The cell layer, which may function as part of a

patch substrate, affects the induced geo-magnetic field, which fluctuates from 20 m Gauss to 200 m Gauss. The calculated electrical current with confluent cells is about 10^{-9} Amp [Hart, 1996] under the magnetic fluctuation. The cellular reaction to the 2.4 GHz EMF can cause the GJIC modulation. However, the relation among the EMF, cellular response and the GJIC modulation is not linearly correlated. The cellular responding ELF depends upon the mechanism of stochastic resonance. Exposing cultured rat liver epithelial cells into a constant extremely low frequency (ELF) alternating current (AC) magnetic field 150 mG at 7 Hz for 60 minutes, 20% promotion of the gap junction intracellular communication (GJIC) was observed from Lucifer yellow fluorescence microscopic image while comparing with the control. Cellular response is experimentally found to stochastic resonated at 7 Hz [Teng, 2005]. The analysis result of the existence of ELF 14 Hz inhibition of GJIC still confuses us. The possible mechanism is that the power of electromagnetic wave influences the signal transduction pathway [Carafoli, 1991]. Even through the intrinsic ELF 14 Hz for rat liver epithelial cells was the calculation result, we still must emphasis that the GJIC modulation should be caused by 14 Hz instead of 2.4 GHz for the reason of observant same result if exposure rat liver epithelial cells into 14 Hz and 0.15 Gauss environment.

Conclusion

We conclude that the Rat liver epithelial cells layer induced responding extremely low frequency (ELF) in 2.4GHz buried in near magnetic fluctuation field $V(t)$. The intrinsic ELF modulated the GJIC within cells. In addition, this report demonstrated the calculated intracellular signal power curves for cell system. The major concern for this report, not only was the 2.4 GHz influence on cell line system, but also challenged whether cellular phone may cause the

health effect though environment. Our study shows that we can recognize the biological effects of the cells under the reaction of EMF. The investigation revealed clearly the possible damage of the health of the electromagnetic wave environment. The EMF protection must be concerned since wireless antenna always emits 2.4 GHz EMF for application in wireless local area network (WLAN) design [Chen, 2003].

Correspondence to:

Hsien-Chiao Teng

Department of Electrical Engineering

Chinese Military Academy

Fengshan, Kaohsiung, Taiwan 830

Republic of China

Email: scteng@cc.cma.edu.tw

Telephone: 011886-7747-9510 ext 134

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