Response of MC3T3-E1 Cell Line to the RF Exposure at 2.4GHz

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Abstract: The response analysis of MC3T3-E1 cell line to the radio frequency (RF) at 2.4GHz can be recognized by the observation of gap junctional intracellular communication (GJIC) modulation. Meanwhile, fuzzifier of the local affected near magnetic field fluctuations at specific time range in a special fuzzy inference engine that we developed to contrast with the experimental results of GJIC assay is found to be reasonably agreed. The measurement of local near magnetic field fluctuation can therefore be related to the GJIC so that to express the biological effect of the response of MC3T3-E1 cell line to the RF exposure at 2.4GHz.
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Key words: Gap Junctional Intracellular Communication (GJIC); Fuzzy Inference Engine, Cell Line

1. Introduction

The osteoblastic cell line MC3T3-E1 is established from a C57BL/6 mouse calvaria and selected on the basis of high alkaline phosphatase (ALP) activity in the resting state. Cells basically can differentiate into osteoblasts and osteocytes and have been demonstrated to form calcified bone tissue in vitro **[1]**. There have been of considerable discussion concerning the response of the cell line responding to the RF exposure **[2,3]**. No clinical evidence has shown any human health effect and no mechanism can clearly explain every observed biological effect **[3]**. This report only describes our study of the Osteoblast cellular response to the reaction of external RF exposure at 2.4GHz.

Gap junctional intracellular communication (GJIC) within the cells can induce the physical signals from varying surface current **[4,5]** on the cells. In a cell, six connexin 43 subunits oligomerze in the Golgi apparatus into a connexon, called hemi channel and be transported to plasma membrane of the cell. Before pairing process, hemi channels are closed to avoid leakage of cellular contents and entry of extra-cellular materials. During the pairing of connexons and aggregation into plaques at the plasma membrane, connexin 43 is phosphorylated at least twice and connexons are attracted to those located on the adjacent cells. Two connexons join in an end-to-end manner to form a complete channel. The channel aggregate into large gap junction plaques open to connect two cells for cell-to-cell communication and is called gap junctional intracellular communication (GJIC), which can be modulated by environmental factors, such as low power RF signals.

From theoretical point of view, four different types of interference in environment may obstruct the cellular responded signals being detected. Those types of interference include degrading of the signals, increasing signal bandwidth, coupling of signal to the noise and signal overlapping. Basically, most of the cellular responding signals to the RF exposure should be deterministic signal which is the one whose values in the future can be predicted if enough information about its past is known. However, the stochastic cellular signal can also be one of the possibilities created by the cell lines in vitro under the exposure of RF. Other possibilities include cellular fractal signal that have the property being referred as scale-invariance and chaotic signal to be as deterministic signal with sensitive dependence on some conditions that cannot be predicted exactly in the future **[6]**.

Since the function of the GJIC, cultured cells coupled together in vitro except the stem cells and cancer cells, we can observe the GJIC modulation from the diffusion of the fluorescence (dye). In this article, we will present a novel method to recognize the non-stationary near magnetic field fluctuation caused by the cellular response of the reaction to the RF exposure. The near magnetic field fluctuations created by the induced GJIC surface current of the osteoblast cell system can be analyzed by fuzzy inference engine to connect the GJIC modulation to identify if the cellular response is existed. The varied diffuse range of Lucifer yellow fluorescence expresses the cellular response under the exposure of RF at 2.4GHz may be affiliated with many pathological endpoints **[7]**.

2. Materials and Methods

Data Acquisition

A sensitive probe of Gauss-meter, attached to the cells in the culture dish was used to measure the cell induced near magnetic filed fluctuations. The Gauss-meter was manufactured by F.W. Bell Company (series of 9550) in Florida. The design and set up is shown in Figure 1. The measured near field fluctuation was transformed to electrical voltages shown to the oscilloscope. The oscilloscope was manufactured by Agilent Company (54621A). By using HP Benchlink, we were able to collect the data from the oscilloscope and transform to Microsoft Excel as text files. By placing only medium in culture dish without cell cultured, medium induced fluctuation was also measured. In contrast, control group was the local geomagnetic fluctuation measured. Matlab and Fortran computer languages were used for data analysis.

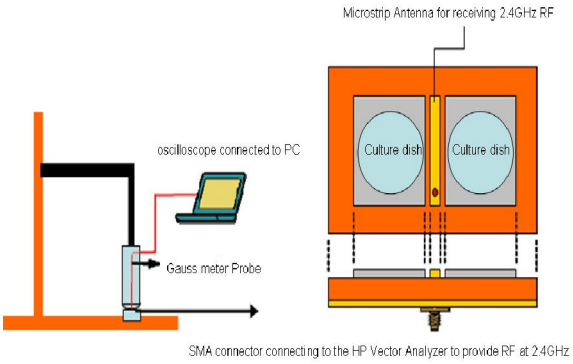


Figure 1. Set up the measurements of near magnetic field fluctuation

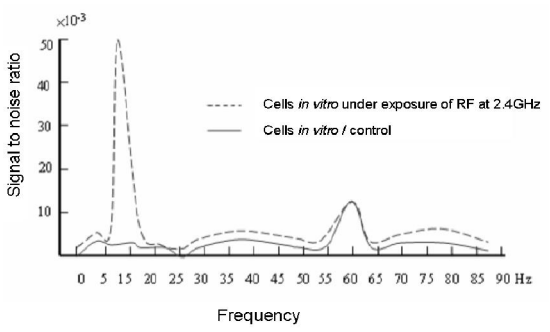


Figure 2. The signal ratio of the responding signal that may caused by MC3T3-E1cells exposed upon RF at 2.4GHz

SNR Analysis

By using RS232, transferred the probe measured data from gauss-meter to the oscilloscope, the output oscilloscopic voltage was restored to Microsoft Excel as text files at the recording rate of 2000 times in a second. We can calculate the autocorrelation and perform Fourier transform to compute the corresponding Power Density Spectrum (PDS) to determine the power of signal, power of noise, and complete the corresponding SNR in data through the formula, $\text{SNR} = \frac{\text{Signal Power}}{\text{Noise Power}}$ (signal to noise ratio) at different frequencies. Upon different order polynomials for curve fitting in contrast to linear, we are able to determine the relationship between the corresponding intrinsic frequency vs. the noise of the signal in data. If the intrinsic ELF signal was not at the frequency of the test signal in the data file, SNR should be zero or negative when SNR is zero. Performing the same procedures, as controls, we computed the SNR at a specific frequency and depicted in Figure 2. It can be accurate to the order of 10^{-6}.

Fuzzy Analysis

Based upon the measurements of near magnetic field fluctuation, we adjusted the distribution of the fluctuations as eight different discrete values. Using If-Then type fuzzy rules converts the fuzzy input to the fuzzy output. Fuzzifier the input (measurement), we make its output values to be only three possibilities. The first possibility is that the cell is responded to the microwave. The second one is that cell did not respond to the microwave. The third one is that the cells response can not be determined either being responded or not. Fuzzy membership functions and the rules are defined. We used of Matlab toolbox as the base to establish an inference engine with three output by using of different membership functions (trapmf) to create 9 rules to performance the antecedents and consequences.

Cell Culture

The osteoblast cell line in vitro was obtained from D.T. Yamaguchi, Research Service and Geriatrics Research, Education, and Clinical Center, VAMC, West Los Angeles, California, USA It was maintained in D-medium (Formula 78-5470EF, GIBCO, Grand Island, NY, USA), supplemented with 10% fetal bovine serum (GIBCO) and 50 µg/ml gentamicin (Quality Biological, Inc., Gaithersburg MD, USA). The cells were incubated at 37˚C in a humidified atmosphere containing 5% CO_2 and 95% air and were fed or trypsinized every two to three days.

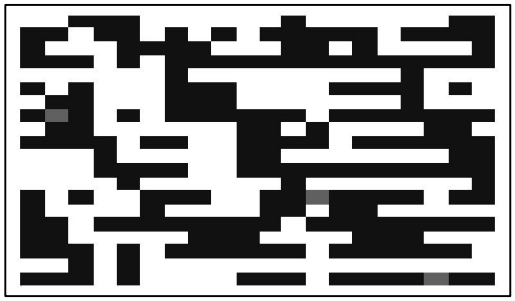
Bioassay of GJIC

The scrape load/dye transfer (SL/DT) technique

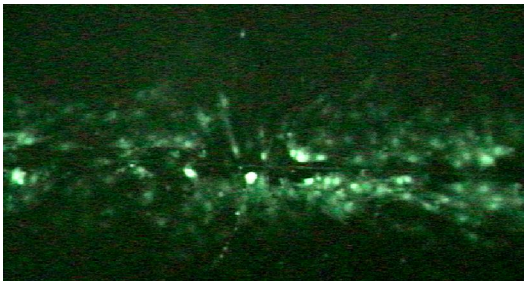
was used to measure the GJIC within cells. After exposure to ELF at intrinsic frequency, the cells were rinsed with phosphate buffered saline (PBS), and a PBS solution containing 4% concentration Lucifer yellow fluorescence dye is injected into the cells by a scrape using a scalpel blade. Afterwards 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 **[5]** for the GJIC images. Since GJIC is affiliated with many pathological endpoints, we use GJIC as a scale factor to evaluate the ELF reaction for cell system. Scrape loading dye transfer of Lucifer yellow is used to measure gap junction intracellular communication (GJIC) modulation under the exposure of RF at 2.4GHz **[8]**. The intrinsic resonance detected in SNR spectrum of the mouse osteoblast cells system is very likely to be a chaotic signal, which is not fully predictable.

3. Results

Figure 3 and Figure 4 depicted the comparisons of the output distribution of the possibilities through fuzzy inference engine and the GJIC. The GJIC of cells was quantified with the measurement of the average distance of dye migration.

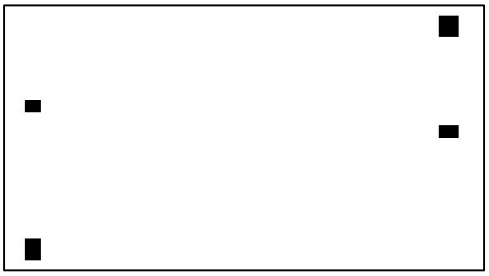


(a)

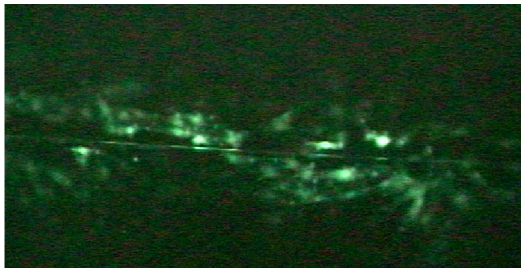


(b)

Figure 3. (a) output distribution of the possibilities through fuzzy inference engine (b) GJIC dye diffusion for the cells *in vitro* exposed RF at 2.4GHz



(a)



(b)

Figure 4. (a) output distribution of the possibilities through fuzzy inference engine (b) GJIC dye diffusion for the cells *in vitro* without RF exposure

In Figure 3 (a), three grey levels are shown, deep-black, light gray and white. In our Fuzzy Inference Engine Design, white level means nothing happen of the cell exposed to the RF at 2.4GHz and deep-black means that it is not for sure if the cell is responded to the RF exposure. The light-gray means the cell is responded to the RF at 2.4GHz. In Figure 4 (a), the experimental result has shown that there is no light-grey can be found in control and the RF exposure increased the numbers of deep-black in Figure 3(a). Meanwhile, we can see clearly the different diffusion of the dye for GJICs from Figure 3(b) and Figure 4(b) that was observed as the evidence for the cell reaction of the RF at 2.4GHz

4. Discussion

Experimental result relates the near magnetic field fluctuation to the GJIC within cells. A strong signal to noise ratio at 14Hz is depicted in Figure 2. It may present that the 14Hz is the responding frequency of the cell to the exposure of RF at 2.4GHz [9]. Graphically, it has shown a frequency band at 14 Hz in the time interval between 0.05 seconds to 0.5 seconds. However, since 14Hz is in the lower frequency band, the time range may need to be relocated. Fortunately, GJIC assay can support the result of the existence of the intrinsic frequency.

5. Conclusion

The main feature of our research is that the cellular response may relate to the change of GJIC.

Additionally, the near magnetic fluctuation expression for cell induced GJIC can be identified at the same time by going through fuzzy inference engine and observing GJIC modulation at 20% in variance.

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