In Vitro Physical and Biological Evaluations of a 2.4 GHz Electromagnetic Exposure Setup

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Abstract **─** In this paper, a 2.4 GHz electromagnetic radiation system for cells in vitro was designed from the perspective of optimal energy coupling of cell samples. The validity of the design was verified by FDTD simulation, physical test and biological experiment. The electromagnetic parameters of SAR (Specific Absorption Rate) and temperature rise were obtained by FDTD simulation. The validation of temperature simulation was confirmed by comparing the actual measurement data and the simulation data. The SAR relative uniformity between samples was tested by cell biological experiment, in which ROS (Reactive Oxygen Species), a typical and sensitive biological parameter reacting to electromagnetic radiation in cells, of different sample dishes induced by 2.4 GHz electromagnetic radiation with an incident power of 0.5 W was analyzed. We found that the size of cell dish affects the energy coupling intensity, the polarization characteristics of electromagnetic wave determines the distribution pattern of SAR, and the uniformity of sample energy absorption in this radiation system is good.

I. INTRODUCTION

In the electromagnetic radiation experiments of cells in vitro, the cell samples and their containers (such as test tubes [1], flasks [2] and dishes [3]) are generally placed in the radiation system to observe and test the biological effects of electromagnetic radiation. The cell radiation systems in vitro mainly include: TEM (Transverse Electromagnetic) cell [4], waveguide cavity [5], WPC (Wire Patch Cell) device [6], anechoic chamber [7], etc..

Some achievements have been made in the design and research of the radiation system so far, but there are still difficult points and deficiencies, which are mainly reflected in the following aspects: ① Most radiation devices could not guarantee the basic environment of the cell survival, such as constant temperature, humidity, carbon dioxide, oxygen, sterility, etc., which results in large systematic error of biology experiment results. ② Generally, most of the cells cultured in vitro have the characteristics of adherent growth and growth inhibition, which means that the cells will adhere to the bottom of the container and grow into a monolayer with a thickness of 5-10 μm. The sensitivity of the existing test instruments can hardly obtain the actual data of electric field intensity and SAR value in the cell layer. Even if the data is detected, the error is large. ③ Although the structure of the cell layer and cell samples is simple, it is difficult to obtain uniform radiation field in a cell layer, especially the uniformity of SAR between multiple samples in one radiation system. There is a lack of biological verification study on the influence of non-uniformity of SAR on biological results. ④ Lacking of standards for radiation environment, radiation mode, excitation source, spatial location relationship and size and sample coupling direction, results in poor repeatability and reliability of the biological conclusions. Hence, it is necessary to design a 2.4 GHz electromagnetic radiation system for cells in vitro, and comprehensively investigate the rationality and effectiveness of the design combined with biological and physical verification methods.

In daily life, the electromagnetic waves we exposed to are mostly 2.4 GHz uniform plane waves, and the polarization modes of the uniform plane waves are mainly linear polarization and circular polarization. The industrial microwave heating equipment and medical physiotherapy equipment in ISM (Industrial Scientific Medical) frequency band also produce Gaussian pulse waveform. Furthermore, it is found that the size of biological samples also affects the energy absorption of electromagnetic waves [8]. Considering that the characteristics of electromagnetic waves and sample size might influence the energy coupling in cell samples, the polarization and waveform characteristics of 2.4 GHz electromagnetic waves and the diameter of cell dishes were set as variable quantities in FDTD simulation of this study to get a comprehensive understanding of the essential factors that determine the interaction between

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electromagnetic waves and biological samples.

II. CELL DISH MODEL

The basic requirements of the sample containers for cell radiation experiment are large bottom area with enough cells, low center of gravity and easy to set into the radiation system. Among the numerous containers for in vitro cell culture, the bottom of a test tube is hemispherical, and a special scaffold is needed to hold in place. The height of a culture flask is large and not easy to be placed into the radiation system. The cell dishes are low in height, large in bottom area and stable in placement. Hence, cell dishes are ideal containers for cell radiation experiment. The common cell dish types are 35-mm and 60-mm.

As shown in Fig. 1, the 60-mm cell dish with no lid was modeled as a hollow cylinder with an outer radius of 30 mm, an inner radius of 28.5 mm, a wall thickness of 1.5 mm and a height of 15 mm. The culture medium was modeled as a solid cylinder with a height of 1.6 mm. The radius of the culture medium cylinder was the same as the inner radius of the cell dish. The volume of the culture medium was 4 mL (a conventional amount in 60-mm cell dish). The electrical properties and thermal parameters are given in Table 1.

Table 1: Electrical properties and thermal parameters of materials at 37°C 2.4 GHz [7,9]

	ε_r	σ (Sm^{-1})	C (kJ ($kg°C)^{-1}$)	K (W ($km)^{-1}$)	ρ (kgm^{-3})
Medium	71	2.5	4.2	0.6	1000
Petri dish	2.5	0.001	0.12	L2	1100

The modeling of the 35-mm cell dish was similar to that of the 60-mm cell dish model. The outer diameter of the 35-mm cell dish was 35 mm, and the inner diameter was 32 mm. The wall thickness was 1.5 mm, and the height of the cell dish was 10 mm. The volume of the culture medium was 2 mL (a conventional amount in 35-mm cell dish).

There is an adherent cell monolayer between the cell dish and the culture medium. The thickness of the cell monolayer is the cell diameter, generally 5-10 μm. For such a thin cell layer, there are two methods of modeling. The first one is non-uniform grid modeling: set the grid height at 10 μm for the cell layer, then gradually increase the grid size from the cell layer to outwards. But the starting base of the grid size is too small, the design and calculation of the grid are very cumbersome. The second method is to design a uniform grid. If the grid is set according to the actual thickness of the cell layer of 10 μm, there will be too many calculations even for computers. Zhao [10] confirmed that for highly dissipative materials, the spatial step size around 0.1 mm would be small enough to reduce the finite differential error to an acceptable level. Considering the storage capacity and computing time of the computer, the space step size should be properly selected. Consequently, in this paper, uniform space step of 0.1 mm was used to model the cell dish, cell layer, culture medium and surrounding space, and a layer of grid above the inner bottom wall of the cell dish was set as the cell layer (Fig. 1 (b)).

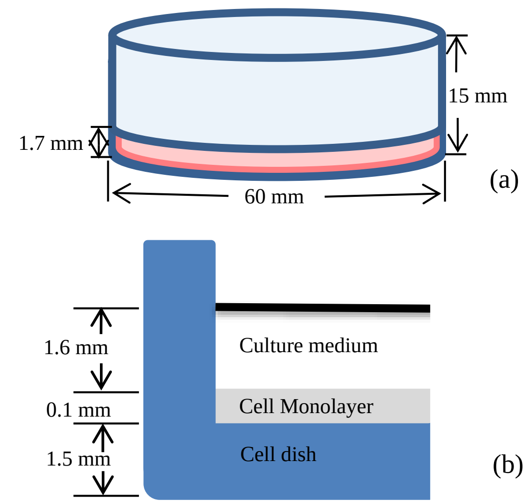

Fig. 1. 60-mm cell dish model: (a) cell dish size, and (b) local size of the cell dish.

MC3T3-E1 cells of mouse osteoblastic cell line were chosen as the cell samples for this electromagnetic radiation experiment. MC3T3-E1 cells in the induction culture have the characteristics of proliferation, differentiation and mineralization, consequently, the dielectric constant of the cell layer is changing in different periods of cell radiation experiments. The dielectric constant of osteoblasts could not be accurately calculated, nor could the dielectric constant of other cells or bone tissue be directly used for simulation. Considering that the energy transfer and heat production of the cell layer could be ignored [11], and the composition of the culture medium is very close to that of most cells in vitro, the dielectric constant of the cell layer was set the same to the dielectric constant of the culture medium.

III. EFFECT OF CELL DISH SIZE ON SAR DISTRIBUTION

The 35-mm and 60-mm cell dish models were respectively radiated by 2.4 GHz linearly polarized plane wave. The wave source was located right below the cell dish, and the PML boundary condition was used to simulate the distribution of SAR values in the cell layer of a single cell dish model in an infinite space.

The distribution of SAR values in one single cell dish were simulated by FDTD solutions. As shown in Fig. 2 and Fig. 3, the trend of SAR distribution in the cell layer of 35-mm cell dish and 60-mm cell dish is similar. However, the size of the cell dish can affect the aggregation intensity of electromagnetic waves in the cell layer. The relatively high SAR values in the cell layer of the 60-mm cell dish account for more proportion than that of the 35-mm cell dish, which means the energy coupling effect of the cell layer in 60-mm cell dish is higher than that in 35-mm cell dish. Therefore, it is more likely to start the bioelectromagnetic effect of cells in 60-mm dish at 2.4 GHz.

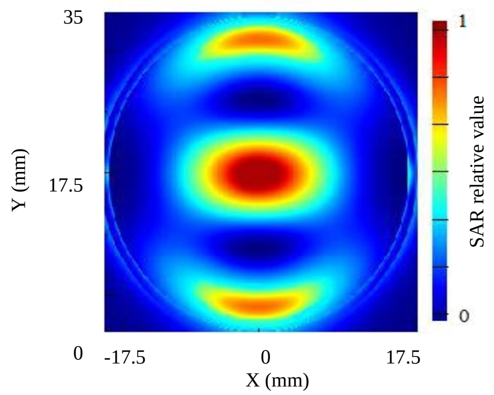

Fig. 2. SAR distribution in the cell layer of 35-mm dish at XOY plane with 2.4 GHz linear polarization plane wave.

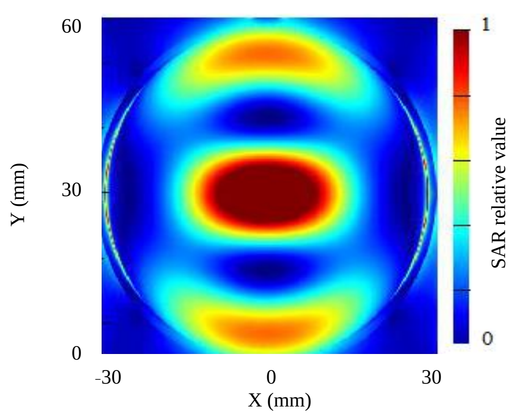

Fig. 3. SAR distribution in the cell layer of 60-mm dish at XOY plane with 2.4 GHz linear polarization plane wave.

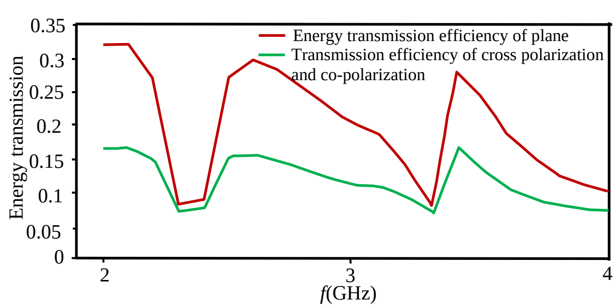

Fig. 4. The energy transmission efficiency of 2 GHz~4 GHz linear polarization plane wave through 60-mm dish sample model.

It can be seen from Fig. 4 that the overall energy transmission efficiency of 2 GHz~4 GHz linear polarization plane wave is basically below 30%, which means that the residual energy is less than 30% of the original incident energy after passing through the 60-mm cell dish, cell layer and culture medium. In the frequency range of 2 GHz~4 GHz, the transmission efficiency of co-polarization and cross polarization waves is the same. At 2.4 GHz, the overall energy transmission efficiency curve of linear polarization plane wave reaches the lowest point (less than 10%). Consequently, the 60-mm sample model has a significant energy absorption of linear polarization plane wave at 2.4 GHz, and more than 90% of the energy is absorbed by the sample dish model.

IV. EFFECT OF WAVEFORM ON SAR DISTRIBUTION

The SAR distribution in the cell layer of a single 60-mm dish model with 2.4 GHz linear polarization Gaussian wave is shown in Fig. 5. From the comparison of SAR distribution in Fig. 5 and Fig. 3, it can be concluded that wave forms have mild effect on the distribution pattern of SAR in the cell layer of the 60-mm cell dish model.

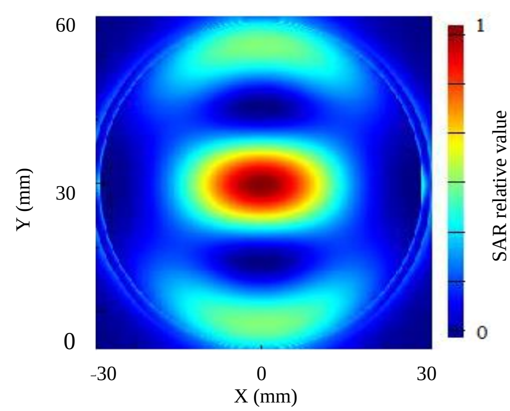

Fig. 5. SAR distribution in the cell of 60-mm dish at XOY plane with 2.4 GHz linear polarization Gaussian wave.

V. EFFECT OF POLARIZATION MODE ON SAR DISTRIBUTION

The cell layer SAR distribution of a single 60-mm dish model with 2.4 GHz circular polarization plane wave and circular polarization Gaussian wave is shown in Fig. 6 and Fig. 7.

As shown in Fig. 3, Fig. 5, Fig. 6 and Fig. 7, the polarization characteristics of 2.4 GHz electromagnetic waves are the main factors to the distribution pattern of SAR, while the waveforms have mild effect on the distribution pattern of SAR.

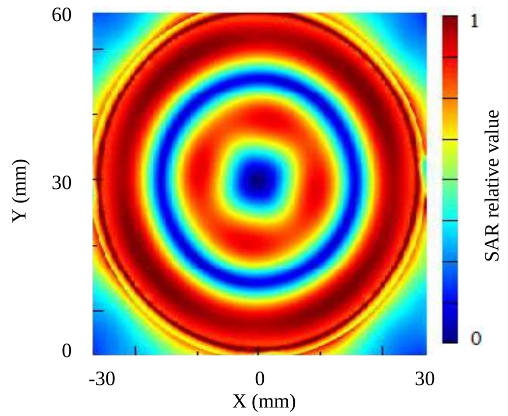

Fig. 6. SAR distribution in the cell layer of 60-mm dish model at XOY plane with 2.4 GHz circular polarization plane wave.

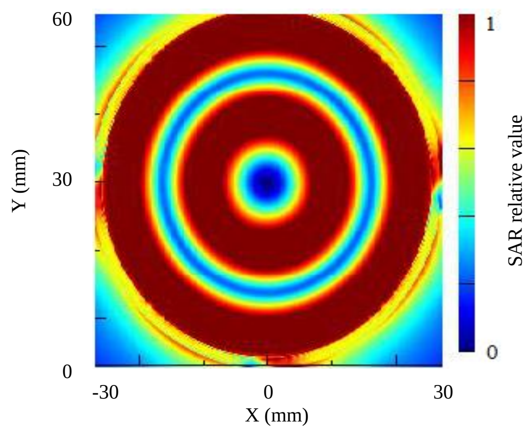

Fig. 7. SAR distribution in the cell layer of 60-mm dish model at XOY plane with 2.4 GHz circular polarization Gaussian wave.

VI. CELL RADIATION MODELING SYSTEM

The basic requirements of an ideal biological sample radiation system are: ① A stable air environment with constant temperature, humidity, carbon dioxide and oxygen and free from germs to ensure the basic living conditions for cells in vitro. ② A stable radiation environment with relatively uniform SAR values between samples and no interference of external radiation signal. ③ Multiple sample containers can be accommodated in the radiation system to provide an adequate amount of samples. ④ Maximize energy coupling of the samples can be achieved, so that the biological effects can be started easily.

It has been found that when the ratio between the sample size and wavelength is 0.4:1, the energy coupling effect is the best [8]. The wave length of 2.4 GHz electromagnetic wave is 12.24 cm, therefore the optimal size of sample is about 5 cm. The 60-mm cell dish with an inner diameter of 5.7 cm is the best cell container close to the optimal size. In part III of this study, it has been verified that the energy coupling effect of the cell layer in 60-mm cell dish is better than that in 35-mm cell dish. In order to increase the total amount of samples, six 60-mm cell dishes were set in the radiation system. The culture medium is right above the monolayer of adherent cells in the cell dish. The frequency of 2.4 GHz is 1/10 of the resonance frequency of water molecules. Water in the culture medium will strongly absorb the 2.4 GHz electromagnetic radiation. Therefore, the signal transmitting antenna was placed 3 cm below the cell dish to reduce the radiation loss and distance attenuation and to ensure a convective space. In order to achieve the maximum energy absorption and radiation uniformity, the signal transmitting antenna was placed perpendicular to the cell layer [13], as shown in Fig. 8.

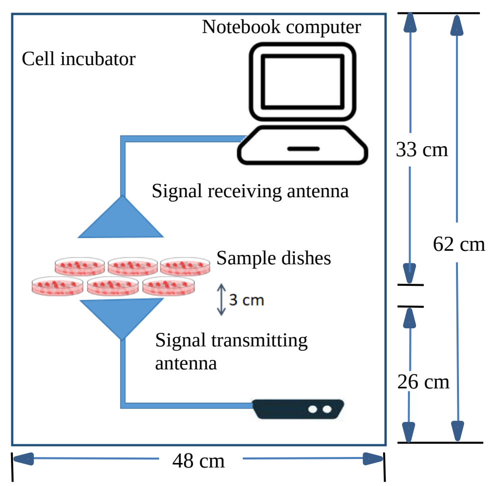

Fig. 8. Sketch map of sample placement in the cell incubator.

The environment of constant temperature, humidity, carbon dioxide and oxygen concentration and free from germs inside the cell incubator is the most ideal environment for cell culture. The double-layer metal shell of the cell incubator can also shield the interference of external electromagnetic waves, which meets the requirements of the radiation environment for the bioelectromagnetic experiments. The samples and the radiation equipment were placed in the cell incubator for cell radiation experiments, and the whole system could be considered as a TEM chamber. According to the requirements for the experimental space of the TEM chamber, the sample size should be less than 1/8 of the chamber size. Therefore, the length of the cell incubator used for cell radiation should not be less than 48 cm. According to the size of the incubator and the cell samples and the position of the radiation system components in the cell incubator, the 1:1 modeling was built up (Fig. 8).

The temperature rise of cell layer was obtained by MATLAB software combined with the electric field strength simulated by FDTD solutions and biological heat conduction equation which has been deduced in our previous research [12]. 2.4 GHz plane wave was used in simulation, since all electromagnetic waves could be expanded by plane wave, which is similar to the sine component of Fourier expansion. The power intensity was set to 0.1 W and 0.5 W, and average SAR values of the cell layers are 0.17 W/Kg and 0.84 W/Kg respectively (Table 2).

Table 2: The SAR average and standard deviation of the cell layers in the six dishes

		1	2	3	4	5	6	Total
0.1W	Mean (W/Kg)	0.2519	0.2519	0.1387	0.1387	0.1107	0.1107	0.1671
	Deviation	0.1041	0.1041	0.0547	0.0847	0.0433	0.0433	0.0674
0.5W	Mean (W/Kg)	1.2594	1.2595	0.6936	0.6936	0.5537	0.5537	0.8356
	Deviation	0.5203	0.5203	0.2733	0.2733	0.2164	0.2194	0.3367

The general temperature trend of the cell monolayers of six cell dishes within 180 minutes is shown in Fig. 9. The temperature trend of the maximum temperature point of the cell monolayers within 180 minutes is shown in Fig. 10. The heat balance point is around 90 min. The maximum temperature rise point of cell layer is 0.2°C of 0.1 W 2.4 GHz plane wave, and 1.1°C of 0.5 W 2.4 GHz plane wave.

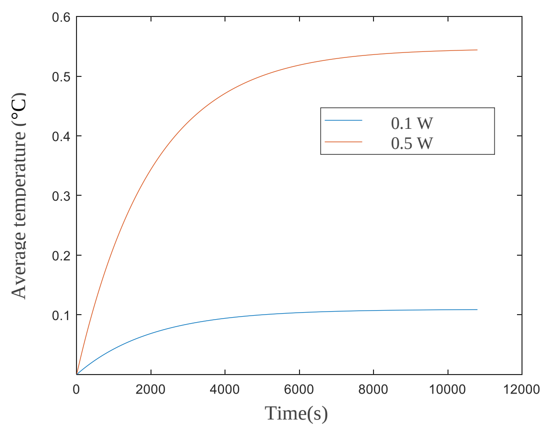

Fig. 9. General temperature trend of the cell monolayers of six cell dishes within 180 minutes.

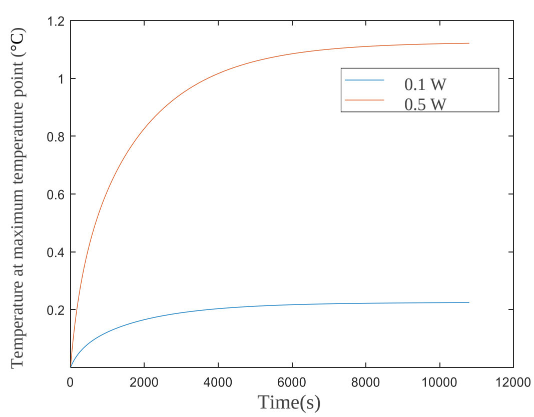

Fig. 10. Temperature trend of the maximum temperature point within 180 minutes.

VII. PHYSICAL VALIDATION

In an open space, 2.4 GHz plane wave was vertically radiated from the bottom of a single 60-mm sample dish. The power density values on the culture medium surface were measured at three random points, and the average value of the three random points was recorded. The energy transmission efficiency calculated from one average value of the power density was considered as one effective data. The energy transmission efficiency data were calculated and recorded for three times, then compared with the theoretical value of 2.4 GHz plane wave energy transmission efficiency obtained by FDTD simulation. It can be seen from Fig. 11 that the measured values of the energy transmission efficiency of 2.4 GHz plane wave are close to the theoretical value, and the simulated and measured energy absorption efficiency of samples are both more than 90%.

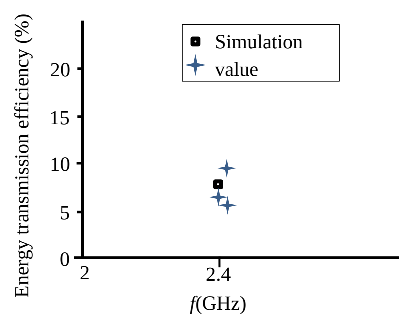

Fig. 11. Comparison between simulated theoretical value and measured value of the energy transmission efficiency.

In the cell radiation system, the temperature rise values of the cell layers in six dishes were measured, and the average values were recorded every 10 minutes, then compared with the simulated temperature rise. As shown in Fig. 12, the theoretical temperature rise trend is in good agreement with the measured temperature rise trend.

VIII. BIOLOGICAL VALIDATION

As shown in Table 2, the SAR values between cell dishes are not identical. The thermal effect of 2.4 GHz electromagnetic radiation increases the temperature of the maximum temperature point of cell layer about 1°C with 0.5 W incident power (Fig. 10). The influence of the inhomogeneity of SAR between samples and the temperature rise in cell layers caused by the thermal effect of 2.4 GHz electromagnetic radiation on the biological experiment results need to be analyzed by cell biological experiments.

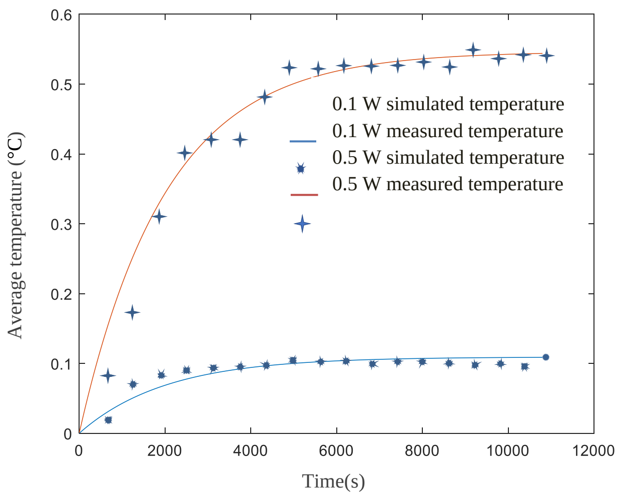

Fig. 12. Comparison between simulated theoretical temperature rise value and measured value.

ROS is a typical and sensitive biological parameter reacting to electromagnetic radiation [14]. Therefore, ROS was used to investigate the response of cells in six dishes to 2.4 GHz plane wave with an incident power of 0.5 W.

MC3T3-E1 cells induced by conditional culture were irradiated with 0.5 W 2.4 GHz plane wave for 90 minutes in a 37°C incubator. ROS was detected immediately after the 90-min irradiation. The amounts of ROS in the six cell dishes were recorded as 1#, 2#, 3#, 4#, 5# and 6#, respectively. Labeling sequence of the cell dishes was the same as Table 2. In the control group, the cell incubator temperature was 37°C, the radiation equipment was not turned on, and the ROS was measured. In the sham group, the cell incubator temperature was 37°C, the radiation equipment was turned on, but with no signal transmission, and the ROS was measured after the 90-min irradiation. In the T+1°C group, the radiation equipment was not turned on, but the temperature of the cell incubator increased to 38°C, and the ROS was measured after 90 minutes. The statistical results of experimental data are shown in Fig. 13.

As shown in Fig. 13, there is no significant difference between the T+1°C group and the control group, so the change of ROS is caused by the "non-thermal effect" of 2.4 GHz 0.5W plane wave irradiation, rather than the "thermal effect". There is no significant difference between the control group and the sham group, therefore the influence of heating from the radiation equipment and antenna on the biological experimental results can be excluded. Although the ROS levels of 1#, 2#, 3#, 4#, 5# and 6# cell dishes are statistically different from those of sham group, control group and T+1°C group, there is no significant difference among 1# to 6# cell dishes, which is equivalent to using a biological experiment method to verify the uniformity of sample energy absorption in the radiation system.

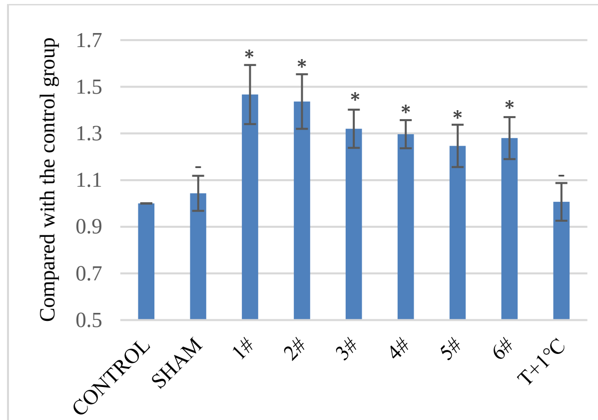

Fig. 13. ROS relative level in cells after 90-min 2.4 GHz 0.5W plane wave irradiation (*p*≥0.05 -, *p* <0.05 *).

IX. CONCLUSION

A 2.4 GHz electromagnetic radiation system for cells in vitro was designed from the perspective of energy coupling of cell samples, and the parameters were optimized. This design could not only guarantee the basic environment of the cell survival but also the relatively consistent of radiation absorption between the samples on biological results. In the future, the different frequency bands and antennas and the signal processing method can be used for the experiments and data acquisition [15-21].

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