# The Effect of Wi-Fi Radiation on the Mineralization and Oxidative Stress of Osteoblasts

**Mengxi Wang [1], Guohui Yang [2], Yu Li [3], Qun Wu [2,*], and Yingsong Li [4]**

[1] Fourth Affiliated Hospital of Harbin Medical University, Harbin 150001, China

[2] School of Electronic and Information Engineering, Harbin Institute of Technology, Harbin 150001, China

[3] Department of Life Science and Engineering, Harbin Institute of Technology, Harbin 150001, China

[4] College of Information and Communication Engineering, Harbin Engineering University, Harbin 150001, China
*qwu@hit.edu.cn

***Abstract*** — Some articles reported that Wi-Fi radiation induced oxidative stress (OS) in cells and vital organs. However, the possible effects of Wi-Fi electromagnetic fields (EMFs) on bone cells have not yet been investigated. MC3T3-E1 cells were cultured in cell incubators during induced differentiation and placed 3 cm from Wi-Fi antenna. A 2.45-GHz Wi-Fi signal, transmitted between a Wi-Fi router and a laptop Wi-Fi antenna, radiated on cells for 30 min/day over a 7-day period. The two modes of the Wi-Fi irradiation were 100 mW and 500 mW. The specific absorption rates (SARs) in the cell layer by 100 mW and 500 mW Wi-Fi were 0.1671 W/kg and 0.8356 W/kg, respectively, represented as SARa and SARb, and the cell layer temperature increased by 0.065°C and 0.32°C, respectively, after 30 min of irradiation by finite difference-time domain (FDTD) simulation. The cell oxidative stress indexes were measured by a microplate reader, and the calcified nodules were examined by alizarin red S staining. At a 3-cm close range, 2.45-GHz Wi-Fi radiation increased Reactive oxygen species (ROS) and glutathione (GSH) levels in osteoblasts with the increase of irradiation time, and the quantity of mineralization slightly depended on the radiation intensity.

***Index Terms*** — FDTD, GSH, in vitro, MC3T3-E1 cell, ROS, SAR, temperature.

## I. INTRODUCTION

The extensive presence of Wi-Fi in domestic premises and public spaces has aroused concern among the general public, especially after 2011, when the World Health Organization (WHO) and the International Association of Research on Cancer (IARC) classified radio-frequency radiation as "possibly carcinogenic to humans" (Group 2B; limited evidence of carcinogenicity in humans and less than sufficient evidence of carcinogenicity in experimental animals) based on epidemiological evidence of a connection between brain tumors and mobile phone use [1].

The study of biological electromagnetic effect needs to reveal the electromagnetic mechanism as well as the biological mechanism. Therefore, a large number of biological experiments are required to verify the theoretical mechanism between biological response and electromagnetic parameters. For in vitro bio-electromagnetic experiments with microwave (MW), cells are usually in plastic culture containers, such as tubes [2], culture flasks [3] and Petri dishes [4], filled with a culture medium and set close to a wave source. Due to the culture medium above the cells and the high-water content of biological cells, the attenuation of MW is rapid, presenting a challenge for temperature measurements and calculations in a thin adherent cell layer at the bottom of a cell container.

Kunt [5] confirmed that long-term exposure to electromagnetic fields could affect bone metabolism and increase OS by increasing the total oxidant status and decreasing the antioxidant status. OS induced by Wi-Fi irradiation on organs in vivo, such as the uterus [6] and brain [7], and on cells in vitro [2] has also been affirmed. OS could disturb the bone remodeling process and lead to skeletal system disorders. It is reasonable to speculate that bone, as the largest organ of the body, might also be affected by Wi-Fi EMFs. Osteoblasts, the primary bone-forming cells, control the synthesis, secretion and mineralization of bone matrix and maintain the metabolism of mature bone. MC3T3-E1 cells, preosteoblastic cells derived from mice, were employed in this study, which have been used in researches of EMFs biological effects on osteoblasts [8]. This study was designed to analysis the effects of 2.45-GHz Wi-Fi on OS indexes and mineralization of osteoblasts in vitro.

*Submitted On: May 19, 2020*
*Accepted On: March 6, 2021*

https://doi.org/10.47037/2020.ACES.J.360515

## II. MATERIALS AND METHODS

### A. Exposure system and experimental design

MC3T3-E1 cells (iCell Bioscience Inc., Shanghai, China) of the clone-14 preosteoblastic murine cell line were induced in a Forma Series II 3110 water jacketed $CO_2$ incubator (Thermo Fisher Scientific Inc., Waltham, Massachusetts, USA) with 5% $CO_2$ at 37°C. Four milliliters of α-minimal essential medium (α-MEM; Gibco Invitrogen, Grand Island, New York, USA) containing 100 IU/mL penicillin, 100 μg/mL streptomycin, 10% fetal calf serum (α-MEM-FCS), 10 mmol/L β-phosphoglycerol, $10^{-8}$ mol/L dexamethasone and 50 μg/mL ascorbic acid was changed every 48 h. A Wi-Fi router, six 60-mm cell culture dishes, and a notebook with an antenna were placed in a cell incubator during the experiment (Fig. 1). The distance between the router antenna and the cell dishes was 3 cm. Both the wireless access point and the wireless network card of the notebook were connected by standard 3 dB gain antenna. The front end of the wireless access point was driven by a gain-adjustable power amplifier which transmits signals according to the IEEE 802.11b protocol, and the signals were received by the notebook computer. After the Wi-Fi connection established, Internet Control Message Protocol (ICMP) packet was sent by the notebook test program to ensure that the power of Wi-Fi was maintained at a certain power value. In the control groups, the Wi-Fi router and computer were placed in the cell incubator without data transmission.

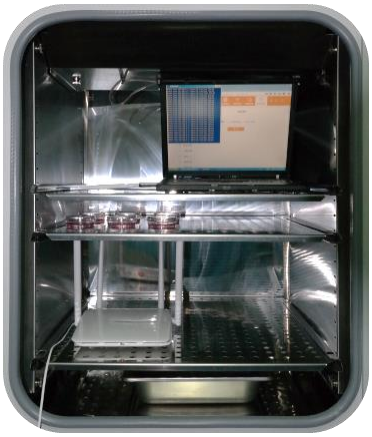


Fig. 1. Photo of the exposure system.

The metallic incubator will lead to resonances, but it could also shield the external electromagnetic interference, equivalent to the transverse electro-magnetic cells (TEM) model, which meets the requirements of the radiation environment standard of biological electromagnetic experiment.

### B. FDTD simulation

The computing method of SAR and temperature rise in the cell monolayers of this model has been described in our previous article [9].

The SAR and electric field distributions in the cell layers at the bottom of 60-mm diameter Petri dishes were illustrated in Fig. 2. The mean SAR for the six dishes was 0.1671 W/kg for 100 mW Wi-Fi and 0.8356 W/kg for 500 mW Wi-Fi (Table 1). The temperature variation in the cell layers in 30 min of irradiation was shown in Fig. 3. The average temperature of the cell layer in each dish after 30 min of irradiation was shown in Table 2.

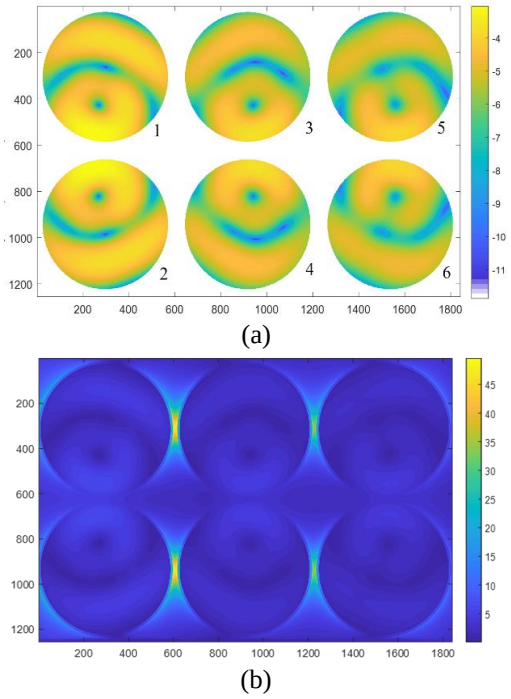


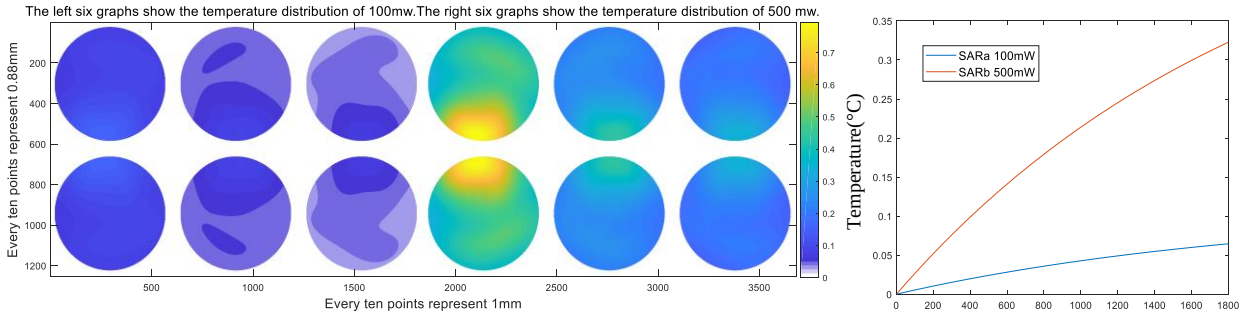

Fig. 2. SAR and electric field distributions of the cell layer plane. (a) SAR distribution and (b) electric field distribution.

Table 1: SAR of the cell monolayers

| Incident Power | | SAR in the Cell Layer of a Single Dish | | | | | | Mean SAR of 6 Dishes |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | |
| 0.1 W | Average (W/kg) | 0.2519 | 0.2519 | 0.1387 | 0.1387 | 0.1107 | 0.1107 | 0.1671 |
| | Standard deviation | 0.2081 | 0.2081 | 0.1093 | 0.1093 | 0.0866 | 0.0866 | 0.0669 |
| 0.5 W | Average (W/kg) | 1.2594 | 1.2595 | 0.6936 | 0.6936 | 0.5537 | 0.5537 | 0.8356 |
| | Standard deviation | 1.0405 | 1.0405 | 0.5465 | 0.5465 | 0.4328 | 0.4328 | 0.334 |

Table 2: Temperature of the cell monolayers after 30 min of irradiation

| Incident Power | | Temperature in the Cell Layer of a Single Dish | | | | | | Mean Temperature of 6 Dishes |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 5 | 6 | |
| 0.1W | Average (°C) | 0.0973 | 0.0973 | 0.0535 | 0.0535 | 0.0427 | 0.0428 | 0.0645 |
| | Standard deviation | 0.0209 | 0.0209 | 0.0118 | 0.0118 | 0.0091 | 0.0091 | 0.0279 |
| 0.5W | Average (°C) | 0.4866 | 0.4867 | 0.2674 | 0.2674 | 0.2137 | 0.2138 | 0.3226 |
| | deviation | 0.1045 | 0.1044 | 0.0591 | 0.0590 | 0.0453 | 0.0453 | 0.1394 |




(a) (b)

Fig. 3. Temperature in the cell layers. (a) Temperature distribution of the cell layer plane in each dish after 30 min. (b) Average temperature changes in cell monolayers of 6 dishes in 30 min.

### C. ROS

After 72 h of induction in osteogenic medium (50 μg/mL ascorbic acid, $10^{-8}$ mol/L dexamethasone, and 10 mmol/L β-glycerophosphate), the cells were digested with 0.25% Trypsin-0.53 mM EDTA and then seeded into a 96-well plate at a density of $5\times10^3$ cells/mL in 100 μL of osteogenic medium, which was changed every 48 h. 2',7'-Dichlorofluorescein diacetate (KeyGen Biotech Co., NanJing, Jiangsu, China) was added to each well according to the manufacturer's instructions. After 45 min of incubation in the dark, the cells were exposed to Wi-Fi radiation. The ROS levels were then determined by measuring the fluorescence intensity at 518-nm excitation and 605-nm emission using a spectrophotometer plate reader (Spectra Max M3, Molecular Devices, California, USA).

### D. GSH

Cells were seeded on 60-mm culture dishes at a density of $5\times10^4$ cells/mL in 4 mL of osteogenic medium, which was changed every 48 h. After 72 h, the cells were exposed to Wi-Fi radiation. Then, the cells were digested with 0.25% Trypsin-0.53 mM EDTA. After centrifugation (3500 g, 10 min), the cell supernatant was added to a 96-well culture plate. Cellular GSH levels were determined using a GSH assay kit (KeyGen Biotech Co.) according to the manufacturer's instructions. The optical absorbance values were measured by a microplate reader at 405 nm (SpectraMax M3).

### E. Alizarin red S staining

MC3T3-E1 cells were seeded at $3\times10^3$ cells/mL in 60-mm culture dishes and cultured in 4 mL of osteogenic medium in incubators. After 72 h, the cells were irradiated with Wi-Fi for 30 min/day for 7 days. On day 21, the cells were fixed with 4% paraformaldehyde for 20 min at room temperature. After being washed with 1× PBS (pH 7.2, without calcium or magnesium), the samples were covered with alizarin red S staining solution (pH 8.3, KeyGen Biotech Co.) without light exposure for 90 min at 37 °C and then rinsed with 1× PBS (pH 7.2, without calcium or magnesium). Subsequently, images were acquired by an inverted fluorescence microscope (OLYMPUS TH4-200, Tokyo, Japan).

### F. Statistical analysis

The data were expressed as the mean ± standard deviation (SD) of three or more independent experiments. Significant differences were determined using factorial analysis of variance (ANOVA). Statistical analysis was performed using SPSS 13.0 software (SPSS Inc., Chicago, Illinois, USA), and a value of $P<0.05$ was considered as statistically significant.

## III. RESULTS

### A. Alizarin red S staining on day 21 (Fig. 4)

The calcification of the SARb group was slightly greater than that of the sham and SARa groups. However, the SARa group displayed no significant difference from

the sham group.

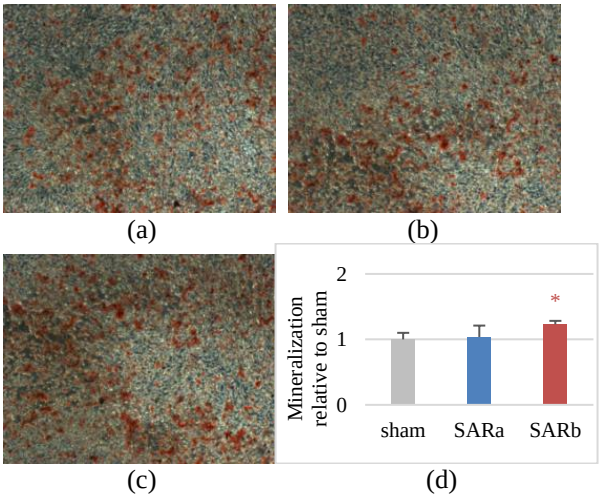



Fig. 4. Mineralized nodule analysis: (a) Sham; (b) SARa; (c) SARb; (d) mineralization relative to sham (P<0.05 *).

### B. GSH and ROS (Fig. 5)

The ROS levels were elevated on day 3 and were even higher on day 6. The ROS level on the 3rd day in the SARb group was higher than that in the SARa group, but the ROS level in the SARb group on the 6th day had no significant difference with that in the SARa group. Despite the similar trends like ROS, the GSH level of the SARb group was higher than that of the SARa group on day 6, and the GSH levels of the SARa group showed no significant difference with that in the SARb group on day 3. Neither the ROS nor the GSH levels showed obvious changes on the 1st day after 30 min of Wi-Fi radiation.

## IV. DISCUSSION

The cell layer temperature increases in vitro caused by the 2.45-GHz Wi-Fi EMFs generally does not exceed 1°C by simulation or actual observation [10-12]. Collin A. [11] calculated temperature rises using FDTD, and the maximum temperature rise was approximately 1°C at 2.45 GHz for a SAR of 16 W/kg. Paffi [12] calculated the maximum temperature changes for plane wave exposures (100 V/m incident electric field at 0.9, 1.8, or 2.5 GHz) by CST simulation and concluded that the temperature increased by approximately 1°C at thermal equilibrium. In our experiment, because the irradiation time was only 30 minutes, the temperature changes were small, which were 0.065°C for 100 mW incident power and 0.32°C for 500 mW incident power respectively by FDTD simulation. Consequently, the effects on OS and mineralization were more likely initiated by the non-thermal effects of Wi-Fi. Furthermore, other studies have reported adverse effects of Wi-Fi radiation within the safety threshold on biological tissue [13]. Some studies revealed that Wi-Fi radiation could impact the reproductive system [14], laryngotracheal mucosa [15], liver [16], brain [7], and fetal tissue [6], partly due to OS [15] and DNA damage [17].

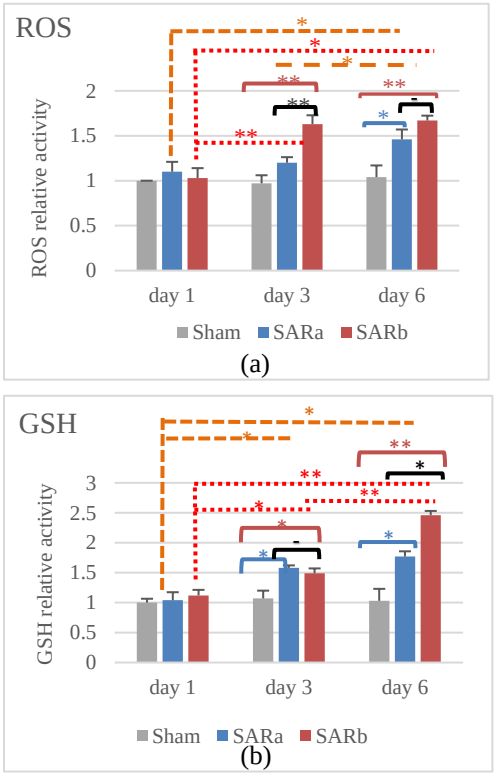



Fig. 5. ROS and GSH levels in MC3T3-E1 cells on days 1/3/6 after 30-min Wi-Fi exposure. (a) Relative OD of ROS. (b) Relative OD of GSH (P≥0.05 -, P<0.05 *, P<0.01 **).

Among all the biological effects of Wi-Fi irradiation, OS mechanisms have received the most attention. The primary cause of OS is the overproduction of ROS. ROS is the general name of a class of molecules or ions with high chemical reactivity and high oxidation activities [18]. In certain unfavorable conditions, such as hunger, nutritional deficits, drug stimulation, severe environmental changes, bacterial infections and various diseases, the ROS levels in cells would increase significantly. Elevated ROS levels could damage proteins, lipids and DNA, and eventually trigger apoptosis and lead to cell death [19]. In an in vitro experiment, Ghazizadeh [20] isolated hippocampal and dorsal root ganglion (DRG) neurons from rats and exposed the neurons to a 2.45-GHz Wi-Fi (12 μW/cm$^2$ SAR: 0.52±0.05 mW/kg) for 1 hr. It was found that Wi-Fi caused $Ca^{2+}$ influx and OS-induced hippocampal and DRG death. Subsequently, to elucidate the electromagnetic effect of radiation from mobile phones (900 and 1800 MHz) and 2.45-GHz Wi-Fi on cells and the relationship between the biological

effects and distance, Çiğ B. [2] set 6 tubes of MCF-7 breast cancer cells at different distances (0 cm, 1 cm, 5 cm, 10 cm, 20 cm and 25 cm) from the radiant (the same appliance as used by Ghazizadeh V.) for 1 hr. Çiğ B. found that cytosolic ROS production, $Ca^{2+}$ concentrations, apoptosis and caspase-3 and caspase-9 values were higher in 900 and 1800 MHz mobile phone groups as well as in 2.45-GHz Wi-Fi groups at distances less than 10 cm. In our study, the ROS levels increased in both the 100 mW and 500 mW groups after 3-6 days of Wi-Fi exposure at a distance of 3 cm. Hence, Wi-Fi radiation might be an exogenous OS stimulus to osteoblasts.

Multiple studies have shown that EMFs could rapidly induce ROS, and some even showed the time-dependent and SAR-dependent manners [21]. Similarly, we found that the ROS level increased on day 3 in the 500 mW group and rose higher on day 6 in both the 100 mW and 500 mW groups, presenting a time-dependent manner. Moreover, the ROS level of the 500 mW group was higher than that of the 100 mW group, showing a SAR-dependent trend.

Özorak [14] reported that 2.45-GHz Wi-Fi and 900- and 1800-MHz mobile phone exposure of 1 hr/day for 120-180 days induced OS in the kidneys of rats during pregnancy by reducing GSH and GSH-Px levels. In contrast, Fahmy [22] confirmed that 2.45-GHz Wi-Fi (SAR 0.01 W/kg, 24 hr/day for 40 days) emitted from an indoor Wi-Fi device increased GSH levels in kidney tissues. Similar to Fahmy, the increased GSH levels were also observed in our study, along with increased ROS levels. It was previously confirmed that EMFs could promote MC3T3-E1 cell mineralization [23]. Our results showed that the calcification of the 500 mW group was greater than that of the sham and 100 mW groups. A high level of ROS could induce apoptosis and reduce osteoblast activity, differentiation, mineralization and osteogenesis, however, antioxidants could activate the differentiation and mineralization of osteoblasts either directly or by counteracting the action of oxidants [18]. Thus, it is possible that the drastic GSH increase in the 500 mW group in the later period of this experiment might contribute to calcification, since GSH could serve as a direct ROS scavenger to restore the dynamic balance between ROS generation and elimination in cells [21].

The obvious change in ROS and GSH levels presented on day 3 and day 6, but not on day 1, which might be related to the "cumulative effects" [24] or "window effects" [25].

## V. CONCLUSIONS

ROS, GSH and mineralization changes in osteoblasts were caused by non-thermal effect of 2.45-GHz Wi-Fi, and displayed a SAR-dependent trend. The cumulative effect of repeated exposure could aggravate the degree of cellular reaction. Long-time, high-intensity, close-range contact with Wi-Fi signal radiation sources and equipment should be avoided. In the future, the experiment can be predicted using adaptive algorithms under MIMO environments [26-30].

## ACKNOWLEDGMENTS

This work was supported by National Natural Science Foundation of China, grant number 61571155 and the Fundamental Research Funds for the Central Universities (3072021CF0804) and Funding of Key Laboratory of Advanced Marine Communication and Information Technology, MIIT, China (AMCIT2101-03).

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