**RESEARCH ARTICLE**

# *In vitro* Experiments of Magnetic Field in Human Exposures of Intermediate Frequency

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**Abstract**

Electromagnetic safety standards are continually updated according to the latest and accumulating scientific pieces of evidence in cell studies, in order to confirm the exact impacts of Electromagnetic Field (EMF) on in-vitro cellular level. Various biological experiments, of both in-vivo and in-vitro, have been conducted for the investigation of the potential effects of EMF, however, these investigations are mostly focused either on the power frequency or microwave EM exposures aiming at the impact on osteocyte or cancer cells. This paper presents an investigation of the bio-electromagnetic impact of the cell viability to HT1080 and UR61 cells and the DNA integrity to the HT1080 cell, under an acute magnetic field of intermediate frequencies (IF) at 100 kHz. IF frequencies are commonly adopted in the wireless power transfer in E-vehicle applications in recent years; this study aims to provide the missing evidence of in-vitro experiments of Magnetic Field in Human Exposures of IF. The impact of the cell viability variation was investigated by MTT assay, while the DNA integrity was investigated by Comet assay. Three identical repeated experiments of prolonging exposure of 6-hours magnetic field of 21A/m, at 100 kHz were carried out for DNA fragmentation; results have indicated that exposure level which is the reference level for the general public of ICNIRP of 21A/m for HT1080, would barely cause any impact on DNA integrity. Three identical repeated experiments of prolonging exposure of 24-hours at the same level of exposure for cell viability investigation, results have indicated that there is a significant statistical difference of cell viability observed in both HT1080 and UR61 cells. The cell viability outcomes were concluded by t-test for a p-value of less than 0.05.

**Keywords:** human exposure; *in vitro*; intermediate frequency

## 1. Introduction

*In vitro* studies are considered for identifying possible cell responses to Electromagnetic Field (EMF) exposures which might relate to adverse health effects. In order to confirm any impacts of EMF on a cellular level, various biological experiments have been conducted previously for investigation of the potential effects of EMF [1]–[3]. However, the researches of *in vitro* experiments outcomes are still limited in providing sufficient evidence for a conclusion, and they are mainly focused on exposures of either power lines or mobile phones.

Electromagnetic safety of biological effects of extremely low frequency and radio frequency have been well studied, however, the intermediate frequencies (IF) range, from 300 Hz to 10 MHz, has been studied far less and is causing concern [4]. IF exposures of Wireless power transmission (WPT) in E-vehicles are typical concerns of health effects. It has been evidenced that the external magnetic field for E- vehicles in the vicinity of their WPT system is large and could exceed the reference levels specified in EM safety guidelines [5, 6]. This paper studies the impact of cells in vitro under the maximum reference level of a magnetic field at 100 kHz of 21A/m for the general public per ICNIRP 2010 guidelines, for the applications of WPT of E-Vehicles.

A coil structure for an in vitro experiment is adopted and fabricated facilitating for the uniformity requirement of the magnetic field inside the coil structure for the cell exposure. This adopted structure also resolves the problem of insufficient experimental space for a multilayer of dishes in an in vitro exposure, and reduces the number of experiments to be carried out enabling a large number of samples for the statistical analysis. The study analyzed the impact of IF magnetic field exposure to HT1080 and UR61 cells. Repeated experiments for our conclusion, which was discussed and recommended in ICNIRP for biological effects of cellular study, have also been carried out in our study [7].

## 2. Exposure System

### 2.1. *IF Field control*

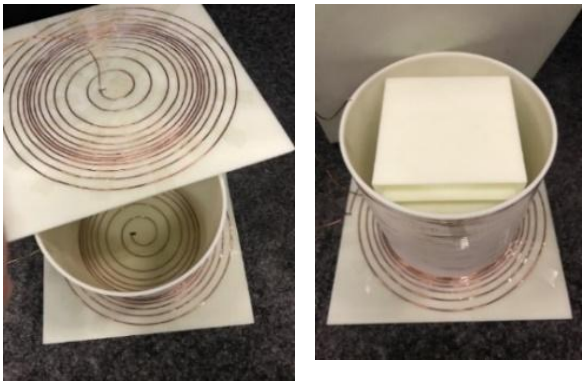
As Figure 1 illustrates, the main part of the exposure system fabricated for the uniform magnetic field is a coil winding structure composed of a finite-length solenoid and terminated by two planar spiral coils at both ends, compensating for the non-uniform magnetic field distribution along the length of a finite length solenoid.

The exposure space inside the coil is 20cm in height, 10cm in radius R, which is matched to the dimension of treated cell plates. The solenoid coil contains 40 turns; the upper and lower practical planar coils, contain 13 turns with radii of 1.5R. The exact radii of all the designated 13 turns of the planar coils were optimized with a discretizing of continuous current density as [8]:

$$\sigma(r') = H_0 \int_0^\infty RJ_1(sR)J_1(sr')ds \quad (1)$$

A good uniformity of the magnetic field inside the coil structure is hence achieved [8], increasing the available experimental space for multilayer dishes of *in vitro* exposure; the standard deviation of the magnetic field was measured to be less than 3% of the mean.



*a* *b*

**Figure 1.** *Practical coil winding structure.*

*a Upper view of the solenoid patched with spiral plates at both ends*

*b Multilayer shelf holding cell plates inside the coil structure*

**2.2.** *Thermal control*

The electrified coil was settled into an incubator in which the experimental environment is constant at 37 ºC in 5% $CO_2$. A thermal control as required of a 0.1˚C variance in cells for *in vitro* experiments is also maintained [9]. The temperature rise of the cells in our experiment due to field exposure is estimated to be less than 0.1˚C based on Heat Equation [10]:

$$\rho C \frac{\partial T}{\partial t} + \nabla \cdot (-K \nabla T) = Q \tag{2}$$

with the dissipation of heat by the cell solution in the dishes and by the ambience, where $\rho$ is the density of the solution, $C$ is the specific heat capacity, $K$ is the thermal conductivity and $Q$ is the heat source for a maximum of SAR. The boundary condition for the estimated temperature is:

$$K \frac{\partial T}{\partial t} = -h(T_1 - T_0) \tag{3}$$

where $h$ is the heat transfer coefficient, $T_1$ is the surface temperature of the cell solution and $T_0$ is the ambient temperature. The Joule heat can be ignored by forced airflow and thermal insulation. The variation of temperature was measured as negligible by a thermal meter after the exposure duration in our experiments.

## 3. *IN VITRO* EXPERIMENTS

HT1080 and UR61 cell lines were cultured and divided into two groups – the treated group to be exposed under 21A/m magnetic field at 100 kHz, and the control group with shame exposure. HT1080 cell is a fibrosarcoma cell line extensively adopted in biomedical research [11], while UR61 is a neuron-like cell commonly adopted for investigation of diseases of the brain [12].

DNA integrity was tested by the Comet Assay to HT1080, and the cell lines were seeded in 90mm petri dishes. The concentration of DNA extracted from the cells was first examined after exposure to ensure a value of more than 100 ng/μl for precise results. The length of DNA fragment can then be estimated by gel electrophoresis. Cell viability was tested by MTT Assay to both HT1080 and UR61 cells; cell lines were seeded in 96-well plates with an ideal cell number of around 8000 per well. Cell cultivation before exposures is a critical

target for reliable results: the cell suspension in the solution medium was maintained to be evenly distributed before cultivating into the wells for a standard error of the starting concentrations between the wells to be within 5%. The cell requirements of occupying 80% of the area of the well for the observation of the response of exposure, and separations from each other to avoid contact inhibition were also maintained. The number of isolated cells in the well was kept close to 8000 in our experiments. The concentration of viable cells colored by MTT solvent was obtained by reading the absorbance optical density (O.D.) for each well after the exposures.

## 4. EXPERIMENTAL RESULTS

### 4.1. *DNA Integrity*

The DNA concentrations of cells were tested as shown in Figure 2. For two experiments, the average DNA concentration of treated cells are 177.8 ng/$\mu$l, 507.66 ng/ $\mu$l, the average DNA concentration of sham-exposed cells are 205.6 ng/$\mu$l, 447.4 ng/ $\mu$l at the wavelength of 260nm, where the concentration differences are 13.5%, 13.46% respectively. Normally, when DNA concentration was more than 100 ng/$\mu$l, Comet assay can be done relatively precisely. By comparing of the two experiments, concentration of treated DNA is larger than untreated one. The hypothetical reason for this concentration difference could be operation error or cell proliferation.

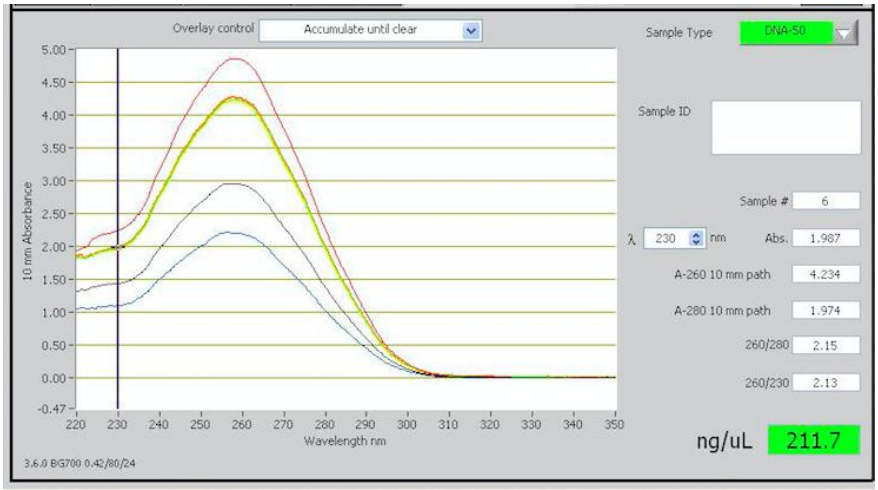



**Figure 2.** *DNA concentration curves*

**Table 1***: DNA concentration*

| **Experiment 1** | Dish 1 | Dish 2 | Dish 3 |
|---|---|---|---|
| treated | 109 ng/$\mu$l | 213.3 ng/$\mu$l | 211 ng/$\mu$l |
| untreated | 146 ng/ $\mu$l | 229.9 ng/$\mu$l | 241 ng/$\mu$l |
| **Experiment 2** | Dish 1 | Dish 2 | Dish 3 |
| treated | 513.98 ng/ $\mu$l | 453.62 ng/ $\mu$l | 555.38 ng/ $\mu$l |
| untreated | 412.42 ng/ $\mu$l | 462.25 ng/ $\mu$l | 467.64 ng/ $\mu$l |

After three treated-control groups for each experiment with two repeated experiments, Figure 3 shows the results of comet assay in HT1080 cells after 6-hours exposure - the 1st lane is the marker, 2nd lane to 4th lane are treated samples and 5th lane to 7th lanes are control samples in each photo, the brighter line represents the higher DNA concentration of specific DNA fragment lengths. Only the samples at the top level of the base pairs length were observed, indicating that the DNA strand breaks did not happen in our experiments.

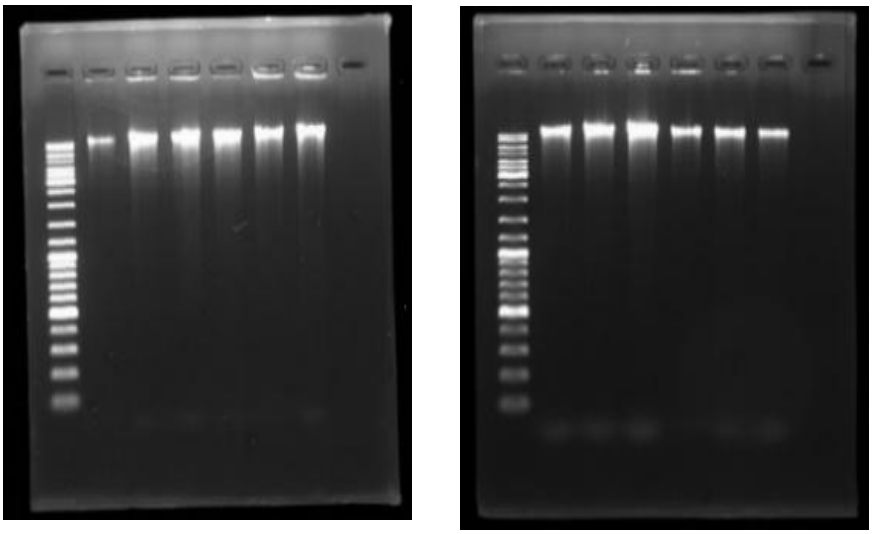


**Figure 3.** *Lengths of DNA fragments, 1st lane is the marker, 2nd to 4th are treated samples and 5th to 7th are control samples in each photo.*

**4.2.** *Cell Viability*

After three repeated experiments with 48 filled wells of 96-well plate for each group in every experiment, the calculated O.D. values were acquired by reading the absorbance of 570 nm and subtracting 650 nm for noise. The percentage of cell viability in each well was as follows:

$$percent.cell.viability = 100\% * \frac{O.D.value}{average.absorbance} \quad (4)$$

The solution in wells at the edges of the plate evaporates fast; the O.D. values were then usually not applicative. Such data were not adopted in our analysis. Histograms in comparison of the average viability rate after 24-hours exposure between the control and the treated cells are summarized and illustrated in Figure 4 and Figure 5.

All experiments for both HT1080 and UR61 cells with treated magnetic field exposure resulted in an increased average rate in cell viability as Figure 3. A highly statistical significant difference ($p<0.001$) in between each treated-control group was also evidenced by t-test, the detailed variation in samples are displayed in the form of box-plots in Figure 6 and Figure 7 - the box of interquartile range heightened along with the median increased for each treated group, while the degree of dispersion and skewness has no obvious trend of change; few identified mild outliers were considered to be caused by experimental errors. The t-test indicated that the exposure induced a significant increase in viable cells in all the experiments.

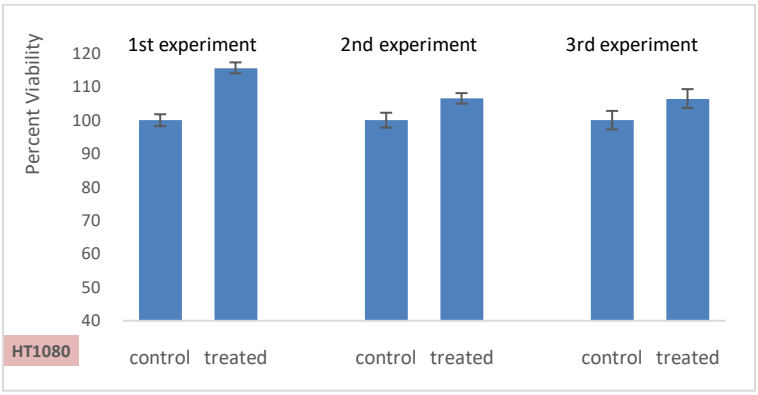



**Figure 4.** *Average viability values of MTT test of three repeated experiments treated with magnetic field compared with control group of HT1080 cells.*

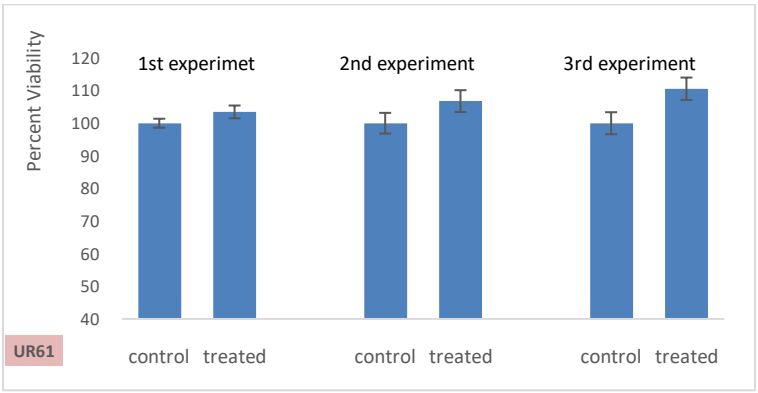



**Figure 5.** *Average viability values of MTT test of three repeated experiments treated with magnetic field compared with control group of UR61 cells.*

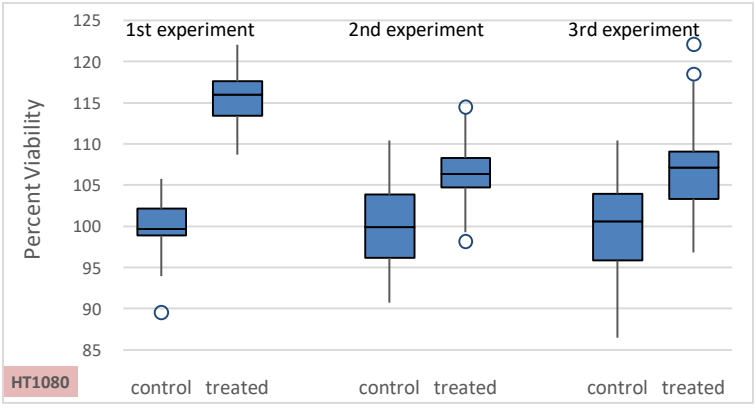



**Figure 6.** *Box-plots of analyzed samples of three repeated experiments treated with magnetic field compared with control group of HT1080 cells.*

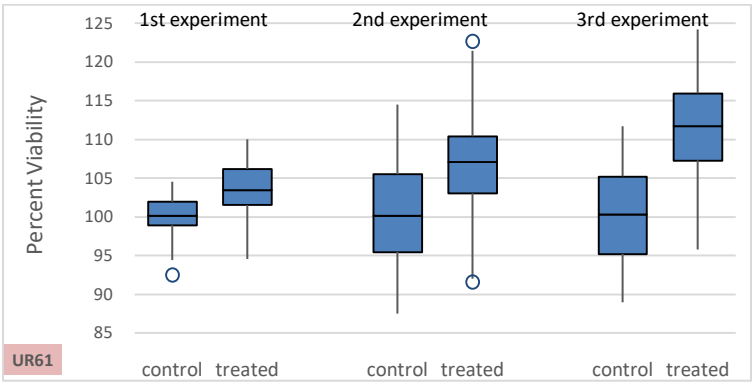



**Figure 7.** *Box-plots of analyzed samples of three repeated experiments treated with magnetic field compared with control group of UR61 cells.*

## 5. Discussion

It was observed that in our repeated in-vitro experiments, a continuous 6-hours exposure to 21A/m magnetic field at 100 kHz has barely caused any impact on DNA integrity in HT1080 cells, while significant difference of cell viability was observed in both HT1080 and UR61 cells for 24-hours exposure at the same field strength and frequency. The cell viability outcomes were concluded by t-test for a p-value of less than 0.05, with an increase of cell viability rate. Consistent results were obtained for all repeated experiments.

This implies that a high level of IF magnetic fields will induce biological changes to the human body. In general, the existing evidences are far less conclusive including this study; this study provided the evidential significance of such potential biological effects of IF exposure in WPT applications in E-vehicles. Existing observations are yet to be substantiated with further parametric analysis and repeated experiments; the reason of the variation of the apoptosis cells remains to be investigated.

## 6. Conclusion

A study of potential risks of cell death and DNA damage in human cells due to IF exposure has been investigated in this paper. The impact of the cell viability variation was investigated by MTT assay, while the DNA integrity was investigated by Comet assay. Three identical repeated experiments of prolonging exposure of 6-hours magnetic field of 21A/m, at 100 kHz were carried out for DNA fragmentation; while three identical repeated experiments of prolonging exposure of 24-hours at the same level of exposure were carried out as well for cell viability investigation. Results have indicated that the exposure level which is the reference level for the general public of ICNIRP of 21A/m, would barely cause any impact on DNA integrity for HT1080 cell lines, but a significant statistical difference of cell viability was observed in both HT1080 and UR61 cells.

## 7. REFERENCES

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