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IN VITRO EFFECTS OF DIRECT AND ALTERNATE ELECTRIC FIELDS ON SAOS-2 CELL LINE

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Abstract – *Objective:* *The exposure of electric fields in daily life is greatly increased through the use of electronic devices, new transportation technologies and various other devices. Alternate current (AC) and direct current (DC) are the types of current flow in a circuit. However, their impacts at the cellular and tissue level, especially in bones, are not well known. Therefore, in the present study, our aim was to investigate the* in vitro *effects and potential differences of both 50 Hz AC and DC electric fields on osteosarcoma cell lines. We hypothesized that exposure of AC and DC electric fields increased the cell numbers in Saos-2 cell line.*

Patients and Methods: *The cells were exposed to 50 Hz AC electric field at different levels (0, 2, 3, 4 and 5 kV/cm) and the cell numbers were determined after 24 hours of exposure. Likewise, the impact of 50 Hz AC electric field on cells was investigated 48 h after the exposure at the same levels. Moreover, cells were also exposed to DC electric field at different levels (0, 0.5, 1, 1.5 and 2.3 kV/cm).*

Results: *Cell numbers in 4 kV/cm and 2 kV/cm AC electric field doses were increased after 24 h and 48 h of exposure, respectively, compared to controls. Likewise, the number of cells in 0.5 and 2.3 kV/cm exposure groups was increased 24 h after exposure to DC electric field.*

Conclusions: *The results show the potential adverse effects of 50 Hz AC and DC electric fields by increasing the number of cells in osteosarcoma cell lines. However, since investigations were performed on a tumoral cell line, these results cannot indicate how electric fields would impact the transformation of normal cells to malignant cells.*

KEYWORDS: *Osteosarcoma, Electrical field, Alternating current, Direct current, Cell proliferation.*

INTRODUCTION

Exposure to electric and magnetic fields impose a great risk on the population's health. The exposure occurs residentially or occupationally via close proximity to electrical equipment, distribution power lines or use of appliances[1]. The association between different types of cancer and exposure to electric fields has been demonstrated previously, especially in children and young adults, including leukemia[2], lymphoma[3], and mammary tumors[4]. However, there is controversy in the literature regarding the adverse effects of exposure to electric fields on human health[5-8]. Osteosarcoma is a skeletal malignancy that constitutes approximately 20% of the bone cancers[9]. On average, a five year survival rate has been reported to be 80% without metastases[10]. The impact of electric field exposure on bone regeneration and homeostasis has been demonstrated previously, including acceleration of osteoblastic cell differentiation in response to 1 kV and 160 μA of an alternating current[11] (AC),

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reduced cAMP synthesis following 2.62 mV/cm for 2.5-30 min electric field treatment in fetal rat bone cells[12], and significant increase in cell proliferation of fetal rat bone cells in response to 0.1 mV/cm of electric field treatment for six hours[13].

In the literature, there is a gap in the knowledge regarding the impact of electric fields on osteosarcoma cells. In one study, an *in vitro* model of human osteosarcoma cell line TE-85 exhibited increased cell proliferation through IGF-II mRNA accumulation following treatment with low amplitude (10-7 V/cm) and low-frequency (10 and 16 hertz) electric field treatment[14]. In another study, a sinusoidal magnetic field of 50 Hz magnitude damaged the surface morphology and growth of MG-63 osteosarcoma spheroids as well as changed lactate dehydrogenase release and diminished glutathione amount. However, this treatment greatly increased the invasive abilities of MG-63 spheroids[15]. On the other hand, exposure of osteosarcoma cells to 100 Hz direct current (DC) electric field with a magnitude of 625 mv/cm decreased both cell adherence and proliferation[16].

However, there is limited and inconsistent data regarding the direct impact of electric fields on the increased incidence of cancer. Therefore, in the present study, we hypothesized that acute exposure of alternate or direct current increases the proliferation of osteosarcoma cell lines.

MATERIALS AND METHODS

Cell Culture

In the present study, a Saos-2 human osteosarcoma cell line was used. The cell line was purchased from ATCC (Manassas, VA, USA). Next, the cells were proliferated from this colony in cell culture. Cell culture protocol was performed as described in previously published studies[17-19]. In brief, cells were thawed quickly in a water bath at 37°C and then centrifuged at 3000 rpm for 4 min. Following the centrifugation, all cells were grown in Dulbecco's Modified Eagle Medium (DMEM)/F-12 medium (Sigma-Aldrich, St. Louis, MO, USA) supplemented with 10% fetal bovine serum (FBS) and a working concentration of 100 IU/ml penicillin and 100 µg/ml streptomycin (Sigma Aldrich, St. Louis, MO, USA). A day later, the growth medium was changed in order to eliminate any remaining dimethyl sulfoxide (DMSO) that might be present in the freezing medium. Cells were maintained in monolayers on cell culture plates in a humidified atmosphere at 37°C and 5% CO_2. Cells were kept in log phase and supplemented with fresh media every 3-4 days. When the cells reached 70-80% confluency, they were passaged via detaching with 0.25% Trypsin-EDTA (Invitrogen, Carlsbad, CA, USA).

Electric Field Treatment

A custom – made treatment chamber was designed, realized and tested in the High Voltage Laboratory of Department of Electrical and Electronics Engineering at Bolu Abant Izzet Baysal University. Treatment chamber includes sensors, heater and custom – made aluminum parallel plates (electrodes). Sensors measure and control chamber parameters were temperature, humidity and pressure. The chamber was equipped with a heater in order to stabilize the temperature at 37°C. Electrodes corners were rounded for minimizing edge effect. Plates with 314 cm^2 (r=10 cm) surface area were positioned parallel to each other. Also the distance between the plates could be adjusted. The electric field treatment chamber used is illustrated in Figure 1. Finite element method was used to provide a description of the electric field profile between the parallel plate electrodes. Electric field intensity and direction were calculated and examined via FEMM 4.2 software (David Meeker, USA). Also, it was observed that uniform electric field was obtained as a result of electrostatic analysis of the treatment chamber in FEMM 4.2 software.

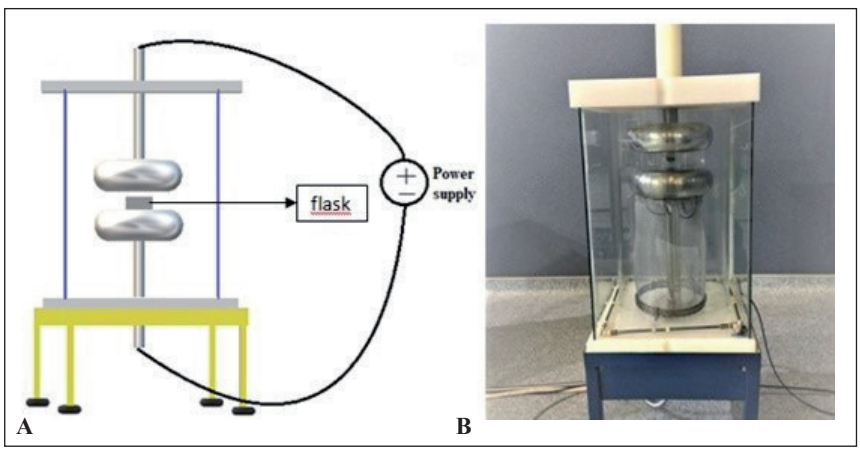

Fig. 1. A custom-made electric field treatment chamber. ***A***, Technical scheme of treatment chamber. ***B***, Full version of treatment chamber. At suitable temperature, cell line is placed between two electrodes and electric field application can be performed.

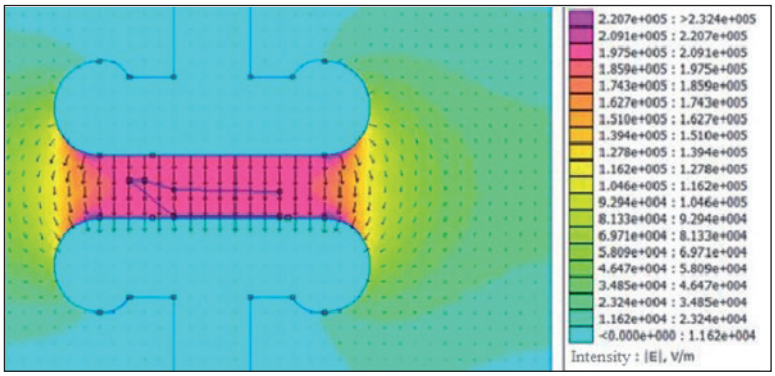

Fig. 2. Example of FEMM 4.2 analysis result for 2 kV/ cm. Colors represent the electric field intensity (V/m) and also arrows represent the electric field direction.

50 Hz AC Treatment: A step – up transformer rated 220 V_{rms} / 50000 V_{rms} was used. Input (primary) voltage of the transformer was controlled with variac (auto transformer) in order to obtain the desired voltage level from the output (secondary) of the transformer.

For 50 Hz AC treatment, 4 cm was the distance used between the electrodes. Electric field intensity was manually calculated according to the equation , where V is the electrical potential, d is the distance, E is the electric field intensity in kV/cm between the electrodes. After this calculation, a deep analysis was realized using FEMM 4.2 software. In Figure 2, the electric field vector and level are shown for 2 kV/cm.

Five different electric field strength levels, 0, 2, 3, 4 and 5 kV/cm, were applied for 10 min (n=8 flask per exposure). Two flasks were placed into treatment chamber for each electric field intensity level. The cells were divided into 2 groups (n= 4 flasks per group). In the first group, cells were analyzed 24 hours following 50 Hz AC treatment. The cells in the second group were analyzed 48 hours following the treatment.

DC Treatment: Step – up transformer and voltage rectifier were used as a DC power supply. The output voltage was controlled with the same variac. For this part, the distance between the electrodes was set at 3.5 cm. After these operations were performed, the same calculation procedure was executed. The cells were treated with 0, 0.5, 1, 1.5 and 2.3 kV/cm electric field for 10 min (n= 4 flasks). The cell numbers were counted 24 hours following the DC treatment.

Statistical Analysis

The differences in cell number were analyzed using one-way ANOVA with Proc GLM in SAS (version 9.2, SAS Institute, Cary, NC, USA). When there was a significant difference, Dunett's multiple comparison tests were applied to determine the differences between AC or DC treated cells *vs.* control cells. $p<0.05$ was considered statistically significant. All data are presented as mean ± standard error of the mean (SEM).

RESULTS

24 h after AC treatment

The number of cells in the 4 kV/cm 50 Hz AC treatment group significantly increased compared to the controls 24 h after treatment ($p<0.05$; Figure 3). However, the number of cells in other treatment groups was similar to the controls ($p>0.05$).

48 h after AC treatment

The number of cells in 2 kV/cm 50 Hz AC treatment group significantly increased compared to the controls 48 h after treatment ($p<0.05$; Figure 4). However, the number of cells in other treatment groups was similar to the controls ($p>0.05$).

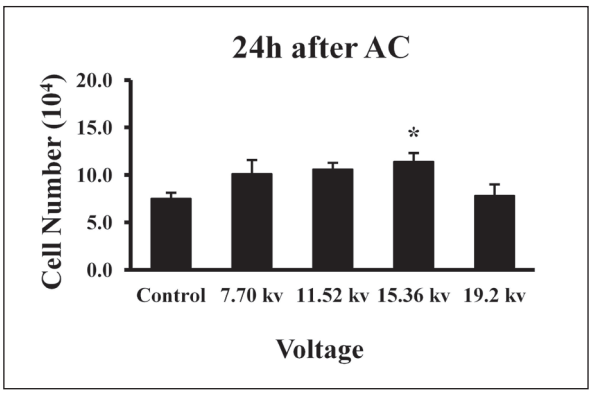

Fig. 3. Effects of AC electric field in osteosarcoma cells 24 h after exposure. X-axis represents different AC voltages. Y-axis represents cell number (expressed as cell number x 104). Data are presented as mean ± SEM (*$p<0.05$ compared with controls).

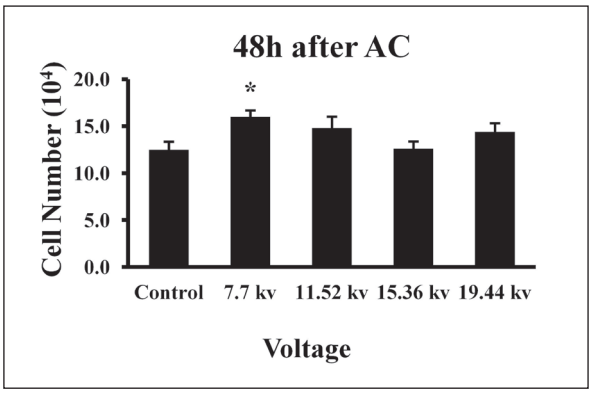

Fig. 4. Effects of AC electric field in osteosarcoma cells 48 h after exposure. X-axis represents different AC voltages. Y-axis represent the cell number (expressed as cell number x 104). Data are presented as mean ± SEM (*p<0.05 compared with controls).

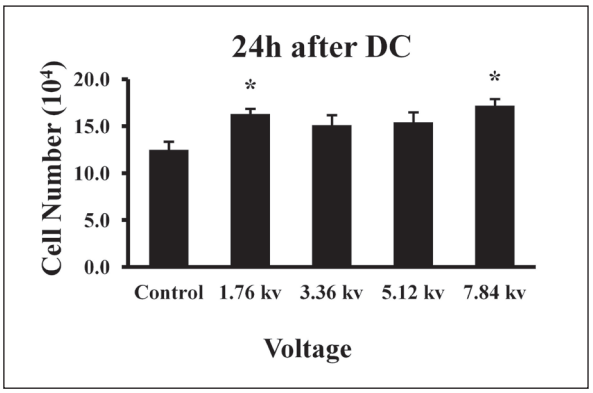

Fig. 5. Effects of DC electric field in osteosarcoma cells 24 h after exposure. X-axis represents different DC voltages. Y-axis represents cell number (expressed as cell number x 104). Data are presented as mean ± SEM (* p<0.05 compared with controls).

24 h after DC treatment

The number of cells in 0.5 kV/cm and 2.3 kV/cm DC treatment group significantly increased compared to the controls 24 h after treatment (p<0.05; Figure 5). However, the number of cells in intermediate dose groups was similar to the controls (p>0.05).

DISCUSSION

In the present study, we aimed at evaluating the impact of 50 Hz AC and DC electric fields on the proliferation of cancer cells. The study's results indicated that there were an increased number of osteosarcoma cells after exposure to an electric field. To our knowledge, this is the first study in the literature evaluating different electric intensities of alternate and direct currents on osteosarcoma cell lines.

In our modern era, society lives in a stream of electric fields from various sources, which can include subways, cell phones, electric medical devices. However, less attention has been paid to the impact of the electric field on cells, especially in the case of pathological conditions. Therefore, in the present study, we chose the osteosarcoma cell lines as our study model, which usually affects young individuals. Previously published studies demonstrated inconsistent results on the impact of the electric field on osteosarcoma cell lines.

Our results indicated an increased cell proliferation 24 h after AC exposure in the highest electric field group. Furthermore, 48 h after AC exposure, the lowest electric field group exhibited an increased cell proliferation. On the contrary, the number of cells at the highest AC group was similar to the controls 48 h after exposure. One possible reason for such a difference might be caused by the delayed cytotoxicity of the highest electric field exposure. However, the lowest AC electric field could be enough to alter the normal cellular and enzymatic activities without any cytotoxic effects. Alternatively, gap junctions are required for mediating the cellular signals to the adjacent cell[20] and such a cell-to-cell coupling might be affected in different ways in response to different AC electric field levels.

The number of cells only increased in the lowest and highest DC electric field groups. However, the intermediate voltages in DC did not cause any effects on cell number. This phenomenon could be explained through the activation of different cellular mechanisms at various DC electric field levels. Moreover, such a pattern in DC is also different than observed in AC groups. Different effects of electric and magnetic fields in cells have been shown previously. While the electric field shows its effects through gap junctional coupling, cell growth was affected in magnetic field through gap junction independent mechanisms[21]. In the present study, a similar mechanism might exist between AC and DC exposed osteosarcoma cells.

CONCLUSIONS

The results of the present studies indicated that both AC and DC electric fields can potentially increase cell numbers, at least in cancerous bone tumors. Further studies are required to demonstrate the cellular and molecular mechanism leading to the increased cell number in malignant cells. Moreover, the mechanistic difference between AC and DC electric fields as well as the response of healthy cells to various electric field exposures needs to be investigated.

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Conflict of Interest

The Authors declare that they have no conflict of interests.

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