A novel magnetic field generating machine with a wide range of tunable parameters to study in vitro cells

Maryam sadat Nezamtaheri
University of Tehran Institute of Biochemistry and Biophysics
Seyed Peyman Shariatpanahi (pshariatpanahi@ut.ac.ir)
University of Tehran Institute of Biochemistry and Biophysics https://orcid.org/0000-0002-7545-554X
Bahram Goliaei
University of Tehran Institute of Biochemistry and Biophysics
Alireza Madjid Ansari
Academic Center for Education Culture and Research

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A novel magnetic field generating machine with a wide range of tunable parameters to study in vitro cells

Maryam Sadat Nezamtaheri[a] Seyed Peyman Shariatpanahi[b*] Bahram Goliaei[c] Alireza Madjid Ansari[d]

[a] Laboratory of Biophysics and Molecular Biology, Departments of Biophysics, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran. email: ms_nezamtaheri@ut.ac.ir

[b] Laboratory of Biophysics and Molecular Biology, Departments of Biophysics, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran. email: pshariatpanahi@ut.ac.ir

[c] Laboratory of Biophysics and Molecular Biology, Departments of Biophysics, Institute of Biochemistry and Biophysics, University of Tehran, Tehran, Iran. email: goliaei@ut.ac.ir

[d] Integrative Oncology Department, Breast Cancer Research Center, Motamed Cancer Institute, ACECR, Tehran, Iran. email: majidansari@acecr.ac.ir

* Corresponding author: Laboratory of Biophysics and Molecular Biology, Department of Biophysics, Institute of Biochemistry and Biophysics, University of Tehran, 16th Azar St., Enghelab Sq., P. O. Box: 13145-1384, Tehran, Iran. email: pshariatpanahi@ut.ac.ir.

Tel: +98-21-6111-3450

Abstract

Background

Extremely low-frequency magnetic field (ELF-MF) significantly induces apoptosis in cancer cells. To study the biological effects of the ELF-MF on tumor cells, we have designed and constructed a new exposure system that provides a uniform magnetic field with negligible temperature fluctuations during the exposure. Additionally, it provides ideal physiological conditions for live cells inside the incubator. This ELF-MF exposure system eliminates several limitations and disadvantages of other low frequency magnetic field systems; it generates a magnetic field with a frequency of 0 to 70 Hz with a maximum magnetic flux density of 150 mT.

Methods

The capabilities of the setup were examined using a 1 Hz, 100 mT magnetic field, at various exposure times, to induce apoptosis-mediated cell death in the MC4-L2 cell line. After exposure, apoptosis was assessed by flow cytometry.

Results

A biphasic response was observed in cells exposed to ELF-MF: at first a decreasing apoptotic rate during 2-12 hours exposure time was detected, after which apoptosis gradually increased during 24-120 hours of exposure.

Conclusions

We show that ELF-MF exposure with a frequency of 1 Hz and intensity of 100 mT induces apoptosis in MC4-L2 cancer cells in a time-dependent manner. These results show the significance of the long term studies of the ELF exposure effects.

Keywords

Extremely low-frequency magnetic field (ELF-MF); Apoptosis; Cancer cells

Background

An enormous increase in electronic devices usage has raised a growing concern about the hazardous effects of extremely low-frequency magnetic fields (ELF-MFs) on human health. ELF-MFs with frequencies ranging between 0 to 300 Hz are frequently generated by man-made sources including electrical appliances and equipment (1–4). According to the previous studies on childhood leukemia risk due to residential ELF-MF exposure (5,6), ELF-MFs have been classified into group 2B (potentially/ carcinogenic to humans) (7–9). At the same time, many studies have reported the positive effects and therapeutic uses of ELF-MF e.g. in wound repair (10,11), bone repair, pain management (11) and Alzheimer's disease (AD) (12). Recent in vitro and in vivo studies have shown that exposure to ELF-MF directly influences human cells and induces various effects; for instance altering reactive oxygen species (ROS) levels (13), increasing intracellular Ca^{2+} (14,15), changing gene expression (16–18) and enhancing proliferation (15,19,20). Also, ELF-MF has shown antitumor potency in many types of cancers by inducing increased sensitivity to apoptosis. Table 1 shows the growth inhibition and apoptosis effects of ELF-MF on various cancer cells.

Table1: Apoptosis effects of ELF-MF on cancer cells

Effects of ELF-MF exposure on different tissues are very sensitive to the frequency, the amplitude of the field, and the exposure time (31). For instance, Patruno et al. suggested that ELF-MF exposure (50 Hz, 1 mT for 1 hour) induced proliferation in human HaCaT keratinocyte by increasing the mTOR pathway (PI3K/Akt) and activation of ERK signaling pathways (32), while another study by Huang and Chao-Ying et al. showed that ELF-MF exposure in the same cell line (60 Hz ,1.5 mT for 144 h) inhibited cell growth and activated the ATM-Chk2-p21 pathway, resulting in cell cycle arrest at the G1 phase (33). Kim et al. showed that repetitive

exposure to ELF MF (60 Hz 6 mT for 30 min every 24 h for 3 days) induced DNA double-strand breaks (DSBs) and apoptosis mediated by p38 activation in IMR90 (human lung fibroblast) primary cells and HeLa (human cervical carcinoma) cells (27). The same group in another study demonstrated that exposure to ELF-MF (60 Hz 7 mT for 10–60 min) induced DNA DSBs without apoptosis and subsequently activated the DNA damage checkpoint pathway in both non-cancerous and cancerous cells (IMR90 primary cells and HeLa cells) without inducing intracellular ROS production (34). Benassi et al. found that exposure of Human Neuroblastoma Cells (SH-SY5Y) to ELF-MF (50 Hz 1 mT for 24/48/72 h) significantly increased ROS level (35). On the other hand, exposure of SH-SY5Y cells to pulsed electromagnetic field (intensity: 2 ± 0.2 mT; frequency: 75 ± 2 Hz for 10 min, 4 times/week) decreased H_2O_2-induced ROS (36). Zhou et al, showed window effect (frequency-dependent) with proliferation responses. Exposure of 6B 1 hybridoma cells to ELF-MF (30 Hz 0.8 mT for 1 h) significantly inhibited proliferation; although at either lower or higher frequencies, this restriction was highly variable: decreased to zero or even changed to positive values (37).

Interestingly, Grinland et al. showed the window effect (intensity-dependent) on the kinetics of cell cycle progression. Normal human fibroblasts exposed to ELF-MF (60 Hz 20 and 200 μT for various times up to 30 h) showed a significant increase in the length of the G1 phase but no significant effect was observed at higher flux densities 2 and 20 mT (31). These results accompanied by many others indicate that comprehensive studies to investigate wide range effects of different parameters of magnetic field-cells interaction are necessary (12,38). Unfortunately, these types of studies are very rare in the literature; one of the reasons is the limitations of the field generating equipment. Interest in the potential therapeutic effects of ELF-MF on cancer cells has led to the construction of various devices with different capabilities. The

conditions and parameters for ELF-MF exposure setup include (i) amplitude (μT - T), and frequency ranges (subHz - kHz); (ii) uniform field exposure; (iii) similar environmental parameters for exposed and sham cells; (iv) continuous checking of all biological and physical parameters during the course of the experiment in order to detect any misfunctions; and (v) isolation between exposure and sham (39–41). There are different types of ELF-MF generation systems: 1) Helmholtz coils and solenoids that generate homogenous magnetic fields in a wide range of frequencies while the amplitude range is usually less than few milli Teslas (38,42), (fig 1 A). 2) Magnets (43) that can generate up to hundreds of milli Tesla amplitude static magnetic fields (fig 1 B). Changing the amplitudes while using magnets is only possible by changing the distance between the magnets which may reduce the field homogeneity. Furthermore changing the frequency is possible by using rotating magnet setups which impose many limitations on the range of the frequency and the field homogeneity.

Figure 1: Magnetic field systems, A: Helmholts B: Magnet

3) To have better control of the amplitude, in a modified version, electrical coils with magnetic cores can be used. This type of setup has already been used in DC or very limited single frequencies (like 50Hz).

In addition to the frequency and amplitude ranges covered by different ELF-MF generation systems, the ability of long time exposures in an optimal culture condition is very significant. Therefore, many researchers have tried to use the magnetic field generation equipment in incubators. However, the setups have their own limitations to be used in incubators. Helmholtz coil has relatively large amount of magnetic field escape which results in the field interaction with the metallic structure of the incubators and in the homogeneity of the field in the exposure

volume. On the other hand, coils with magnetic cores might be too heavy and large while providing large field intensities in an acceptable volume size of exposure for cell culture studies.

The current study reports a new setup that generates a magnetic field in a wide range of amplitudes (0-150 mT) and frequency (0-70 Hz). Moreover, considering the significance of studying the biological effects of an extremely low-frequency magnetic field in an optimal cell culture conditions, the setup provides a uniform field within the exposure area with negligible field escape which facilitates its ability to be placed within an incubator with least field interaction with the incubator metallic structure.

Materials and Methods

Cells and apoptosis assay

Cells used in these experiments were derived from a murine tumor progestin-dependent C4- HD MC4-L2 (Iranian Biological Resource Center, Tehran, Iran) and were kept in a CO_2 Incubator (Heraeus, D6450 Hanau) at 37 °C and 5% CO_2. Cells were seeded in 35mm cell culture Petri dishes (Nunc, Germany) and allowed to attach. After 24h, cells were exposed to ELF-MF (1 Hz, 100 mT at varying times). Cells were trypsinized with %25 trypsin EDTA, washed twice with cold phosphate-buffered saline (PBS). To do fluorescent labeling and detecting apoptotic and necrotic cells, binding buffer and Annexin V-FITC (IQ products, Netherlands) were added to the cells (20 and 5 µl, respectively). The sample was mixed by gently vortexing and then incubated for 15 min at room temperature in the dark. Subsequently, 2 µl of PI (1 mg/mL) was added to each sample and analyzed on a BD FACSCalibur flow cytometer (BD Biosciences, San Jose, CA, USA). The apoptotic ratio was calculated by dividing the percentage of apoptosis in ELF-MF exposed cancer cells by unexposed cells (control) per time point.

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM) from three or more independent experiments. Flow cytometry data were analyzed in Microsoft Excel and Prism version 8.0.2 (263) software (GraphPad). Statistical analysis for comparisons between groups was performed using GraphPad Prism. $P < 0.01$ was considered to indicate statistical significance.

System structure

Coil and magnet core

Figure 2 shows a schematic diagram of a coil consisting of 2 connected coils in series (with wires of 1.5 mm diameter), with a total number of 1000 turns. The magnetic core consists of laminated cores of stacks of thin sheets of silicon steel coated with an insulating layer. The weight of the core is approximately 100 kg. The coil and the magnetic core form a magnetic circuit which produces a uniform magnetic field in the 2cm gap. The coils and the magnetic coil are covered with Teflon sheets to inhibit iron corrosion in the incubator. The Teflon box is sealed so that humidity diffusion is prevented.

Petri dishes at different positions relative to the magnetic core receive various amounts of field intensity. To tackle this issue and exposing all dishes to a uniform magnetic field and to obtain the appropriate location of the Petri dish, we measured magnetic field intensity at different distances from the center of the magnetic core by a Gauss meter (Repco) (Fig 3). The measurement shows that the variations of the field intensity within a square of 8x8 cm are less

than %5. This result is in agreement with the simulations results handled by COMSOL software to find the magnetic field intensity within the gap (Fig 4).

Figure 2: Internal schematic diagram of ELF-MF exposure system

Figure 3: Intensity of the magnetic field at various distance from the core

Figure 4: Simulation of the magnetic field intensity within the air gap intersection square with the size of 10x10 cm^2 using COMSOL software.

Capacitor bank

In order to provide a wide range of frequencies, considering the large 2H inductance of the coil-core system, a capacitor bank is designed to resonate with the inductor in different frequencies in an LC resonator circuit. The bank includes a wide range of capacitors from milli Farad to few Farads. Different combinations of the capacitor could provide different frequencies with no missing gap in the range 0-70 Hz.

Cooling unit

To remove the generated heat in the core and the coils (resistive and eddy current dissipations), a cold air current is directed into the Teflon box through two silicon hoses, so that the hot atmosphere in the box is removed from the incubator. The cold air current is produced by blowing air current through a radiator cooled with circulating $5C^0$ circulating water. The temperature in the exposure volume is kept 37 C^0 for the optimal cell culture condition.

Results

ELF-MF exposure induce apoptosis

To address the effects of ELF-MF on cancer cells, we performed apoptosis assay under exposure of ELF-MF in exponentially growing MC4-L2 cell cultures. Apoptosis was estimated by the Annexin-V FITC /PI kit and the percentage of dead cells, early apoptotic, late apoptotic and live cells were quantified by flow cytometry. We observed that ELF-MF- exposed cultures (2 h after exposure) showed a small increase (around 13%) in apoptotic cells. A moderate decrease in apoptosis percentage was apparent until 12h and then the apoptosis increased gradually during the reminder of the time course reached to the highest level at 120 h (~%40) (Fig 5, 6).

Figure 5: Induction of apoptosis in MC4-L2 cells exposed to 1 Hz and 100 mT ELF-MF at varying times. Bars indicate means ± SEM obtained from three or more independent experiments. Statistical significance between control and test groups was evaluated by an unpaired t-test. A significant statistical difference was observed between 120h-exposed cells and all time points except 24h-exposed cells. $P < 0.01$ was considered to indicate statistical significance.

Figure 6: Apoptosis induction in MC4-L2 cells exposed to 1 Hz and 100 mT ELF-MF at varying times is represented as exposed: control ratios.

Discussion

Apoptosis is essential to maintain the homeostatic balance and any failure in this process might increase the risk of tumor development. Activation of apoptosis signaling pathways is one of the most effective therapeutic strategies in cancer treatment (44). Reported evidence correlating the

effects of ELF-MF on apoptosis are contradictory. While a growing body of evidence suggests the increased apoptosis responses after exposure to ELF-MFs (21–30), some studies reported the decreased susceptibility to apoptosis (45,46). So far, evaluating the effects of ELF-MF and assessing the importance of intensity, frequency and time on cancer cells has been limited due to the absence of a device that could adjust important parameters including physical factors, heterogeneity of exposure and cell culture conditions. Considering the significant roles of these factors on biological effects, we have designed and developed new instrumentation that can provide a uniform field within the exposure area and can be placed in an incubator.

This study provides a novel approach to verify the effects of short and long times of ELF-MF exposure on cells in an extremely low frequency of 1 Hz. These results indicated that ELF-MF induced apoptosis in the MC4-L2 cell line in a time-dependent manner. Our observations are in line with the previous findings suggesting that the influence of ELF-MF on biological systems might be modulated at particular combinations of frequency, amplitude, and time exposure, phenomena called “window effect” (31,47–49). The molecular mechanism of the window effect is poorly understood. Although some studies have proposed the increased free radicals generated through the radical pair mechanism (RPM) as a possible explanation (50).

Few studies have reported the time-dependent properties of ELF-MF (50). In both primary IMR-90 fibroblasts and cancer HeLa cells; ELF-MF increased cell proliferation with treatment time duration and strength of the magnetic field. It has been shown that one of the main causes of increased proliferation in these cells is the time-dependent reduction of intracellular ROS level (38). In human monocytes, ROS production was time-dependent after 1.0 mT ELF-MF exposure (51). It has been reported that the activation of the ERK1/2 signaling pathway after exposure to ELF-MF at field strengths of less than 1 μT is time-dependent (52). Our results indicated that a

short time exposure to 100 mT and 1 Hz ELF MF first induced and then decreased apoptosis. The decreased apoptosis might be due to the cell adaptation caused by the prolonged exposure of these cells to the culture conditions. Our results coincide with the literature shown that in the brain, ELF-MF exposure of 30 min/day for a period 10 days can affect free radical production and increased exposure time to 60/min/day effected adaptation to this field (53). Our data indicate that continuous exposure to ELF MF from 24 to 120 h induces apoptosis and this emphasizes the significance of the setup capability of long time exposure times.

Abbreviations

ELF-MF: Extremely low-frequency magnetic field

AD: Alzheimer's disease

ROS: reactive oxygen species

DSBs: double-strand breaks

PBS: phosphate-buffered saline

SEM: standard error of the mean

RPM: radical pair mechanism

Declarations

- Ethics approval and consent to participate

  Not applicable

- Consent for publication

  Not applicable

- Availability of data and materials

  All data generated or analysed during this study are included in this published article

- Competing interests

  The authors declare that they have no competing interests

- Funding

  No funding was received

- Authors' contributions

  All of the authors have contributed in the relevant research and writing/editing the manuscript.

- Acknowledgements

  Not applicable

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Figures

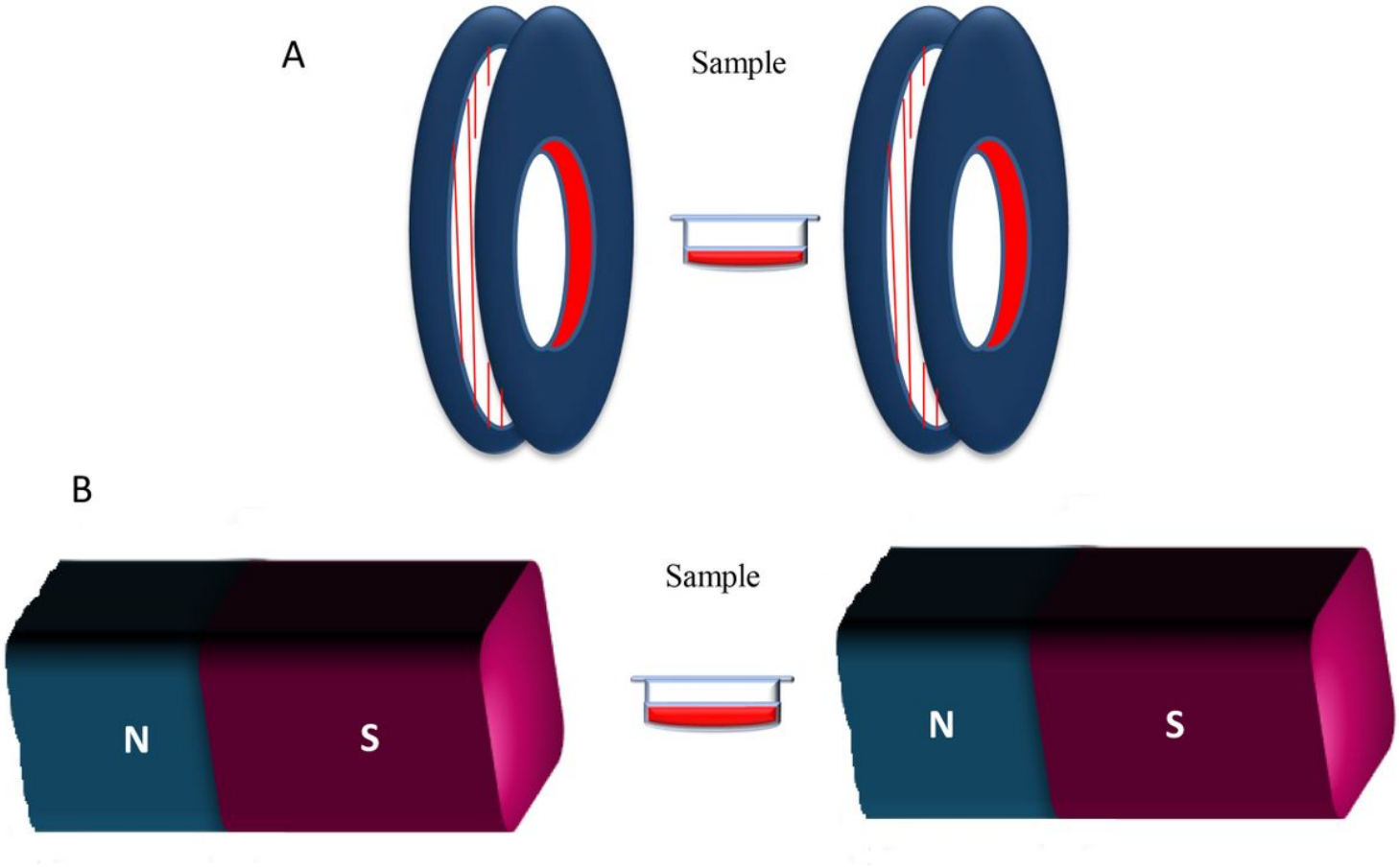


Figure 1

Magnetic field systems, A: Helmholts B: Magnet

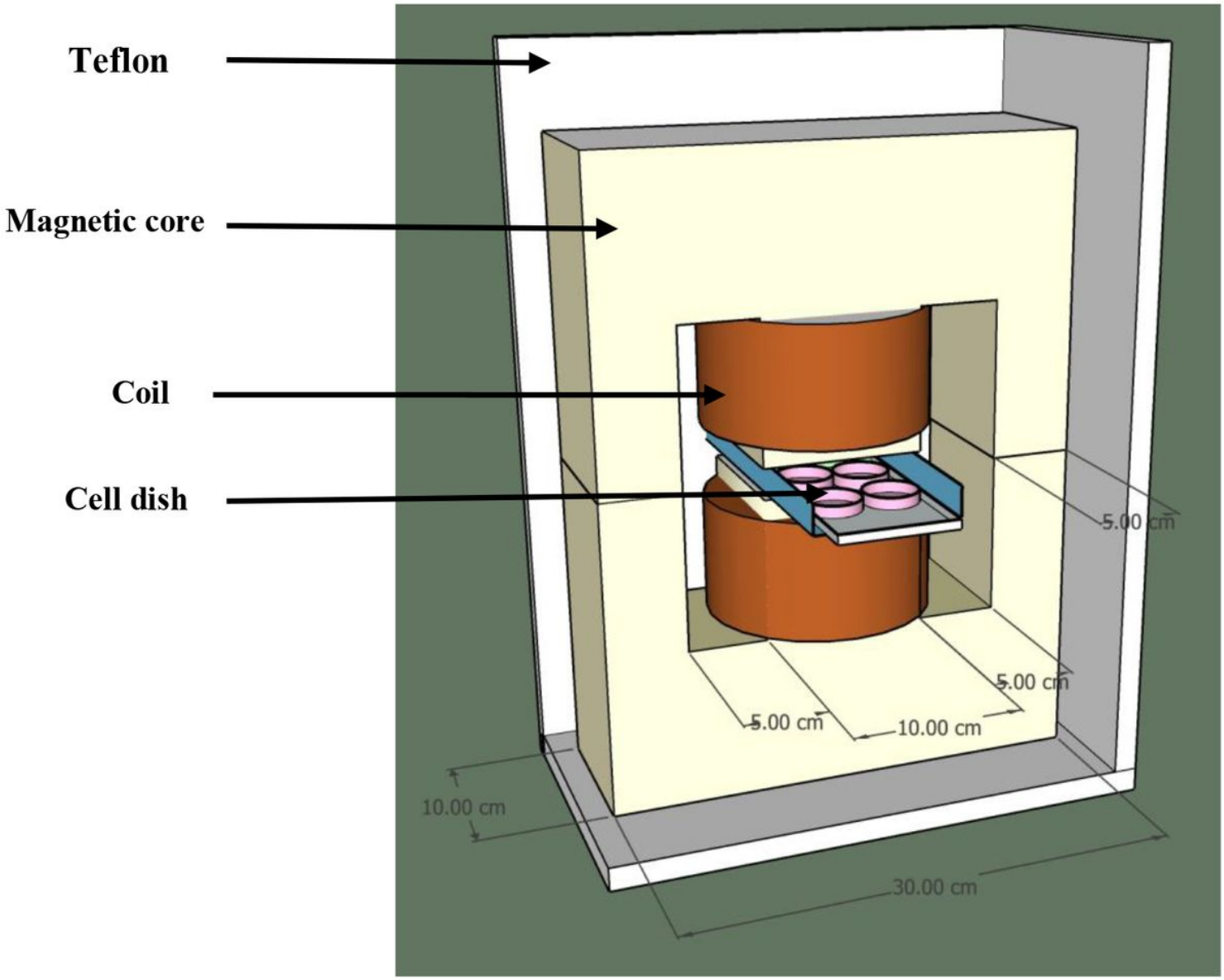
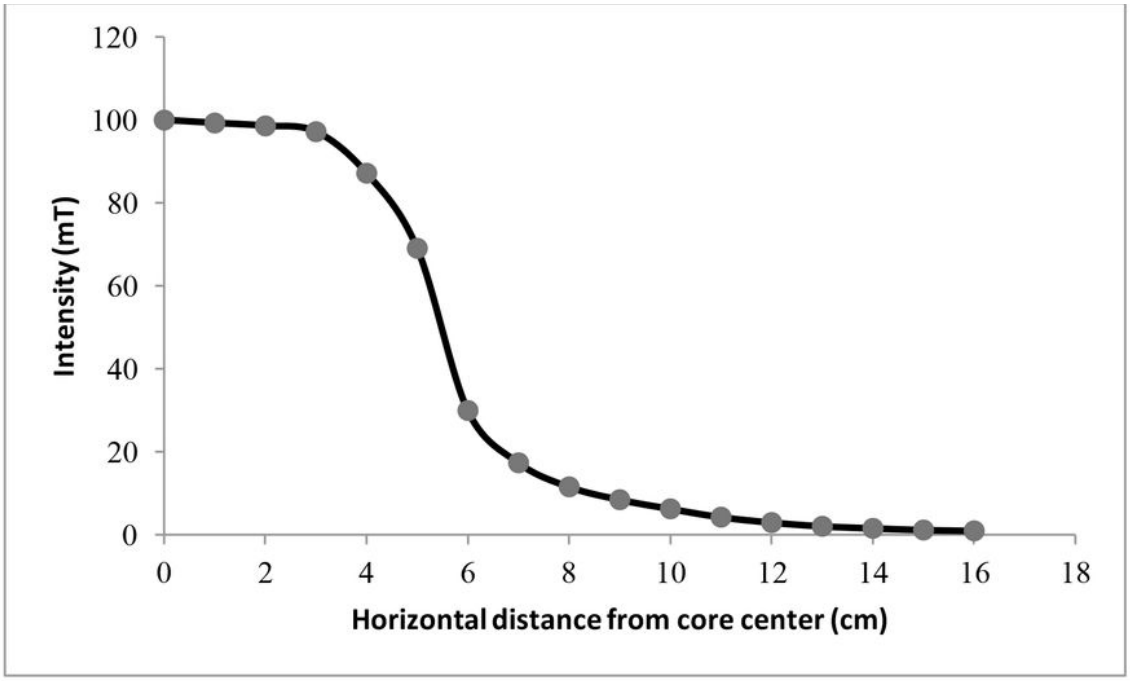


Figure 2

Internal schematic diagram of ELF-MF exposure system



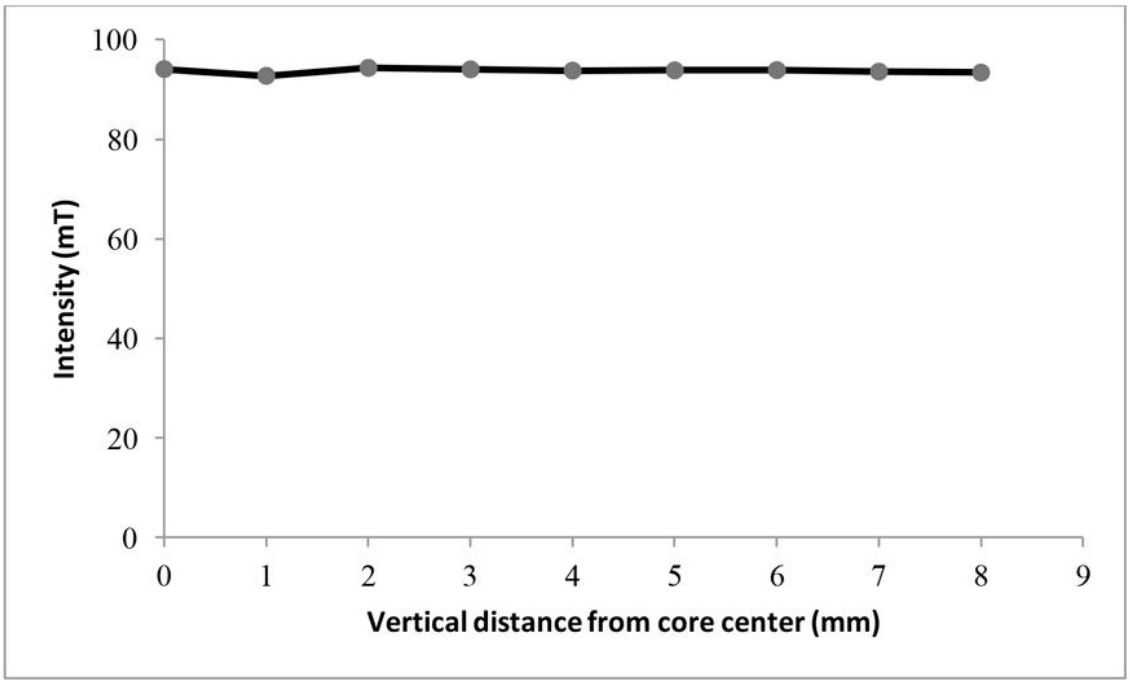


Figure 3

Intensity of the magnetic field at various distance from the core

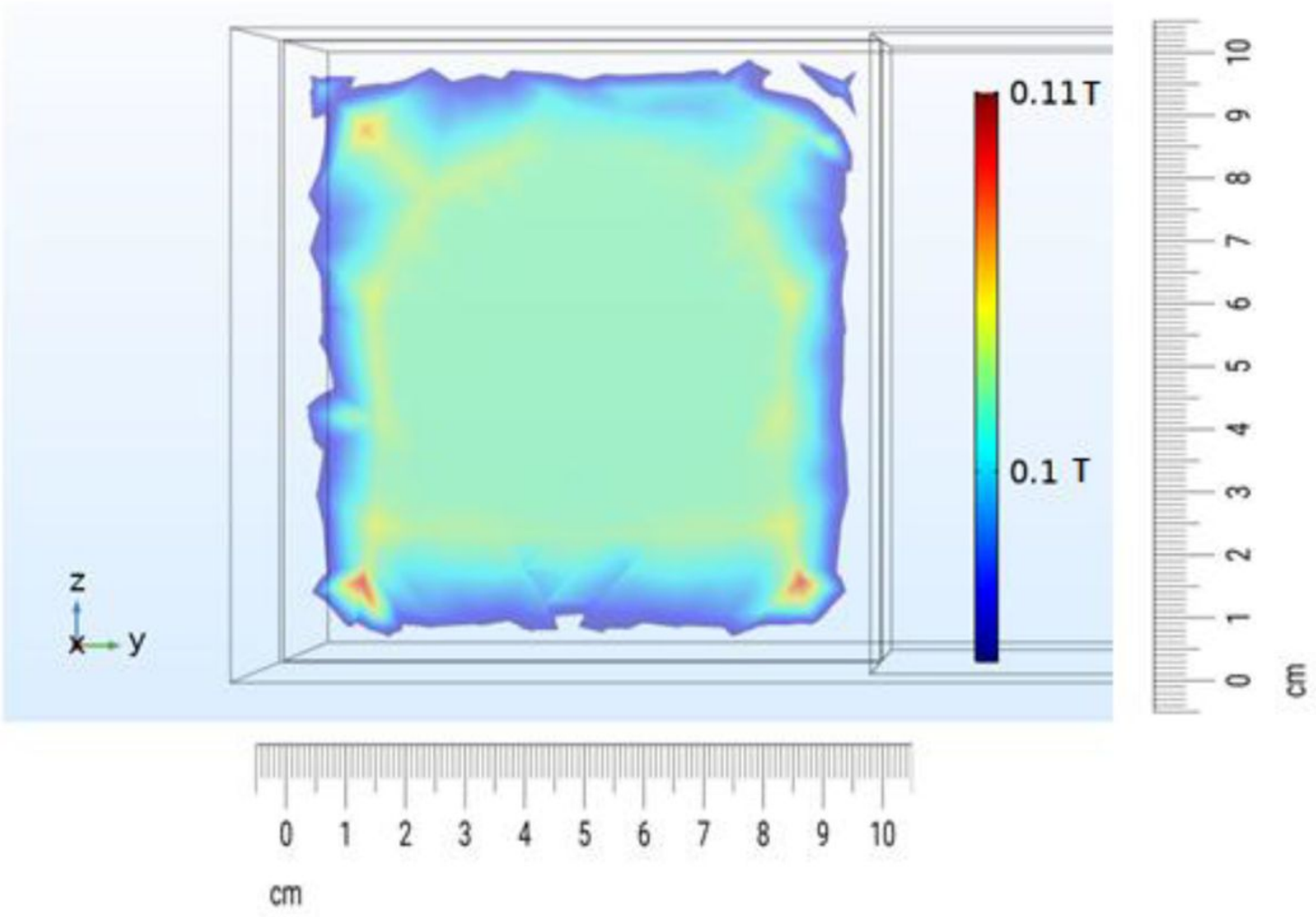


Figure 4

Simulation of the magnetic field intensity within the air gap intersection square with the size of 10x10 cm2 using COMSOL software.

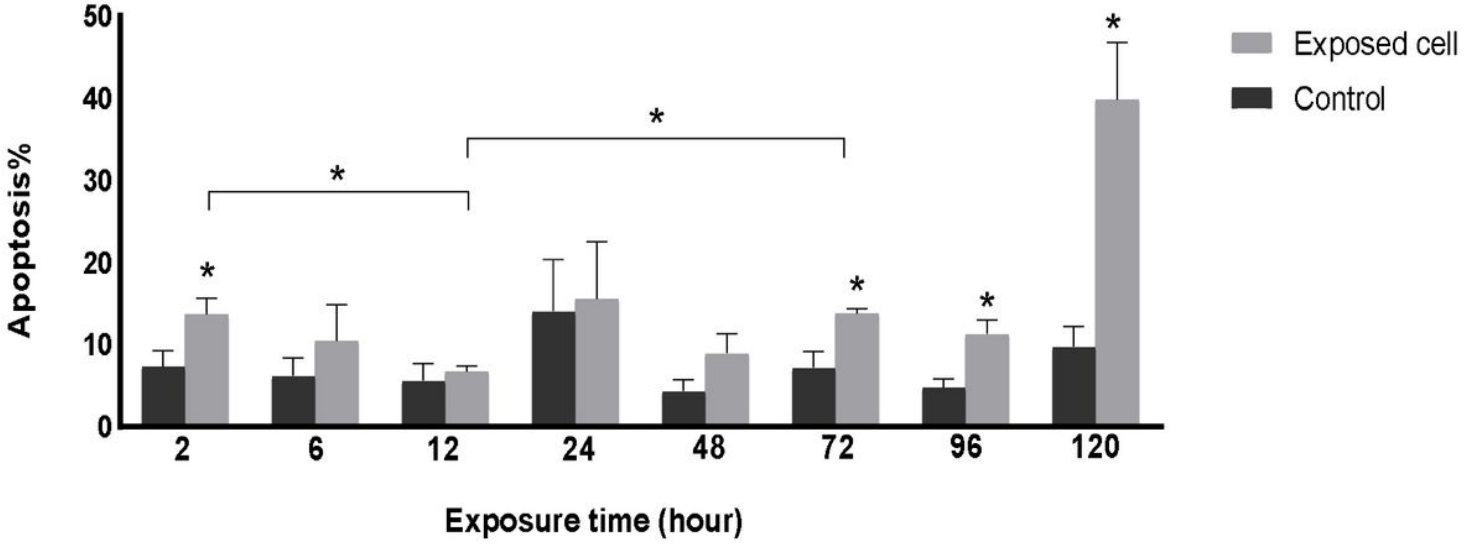


Figure 5

Induction of apoptosis in MC4-L2 cells exposed to 1 Hz and 100 mT ELF-MF at varying times. Bars indicate means ± SEM obtained from three or more independent experiments. Statistical significance between control and test groups was evaluated by an unpaired t-test. A significant statistical difference was observed between 120h-exposed cells and all time points except 24h- exposed cells. $P < 0.01$ was considered to indicate statistical significance.

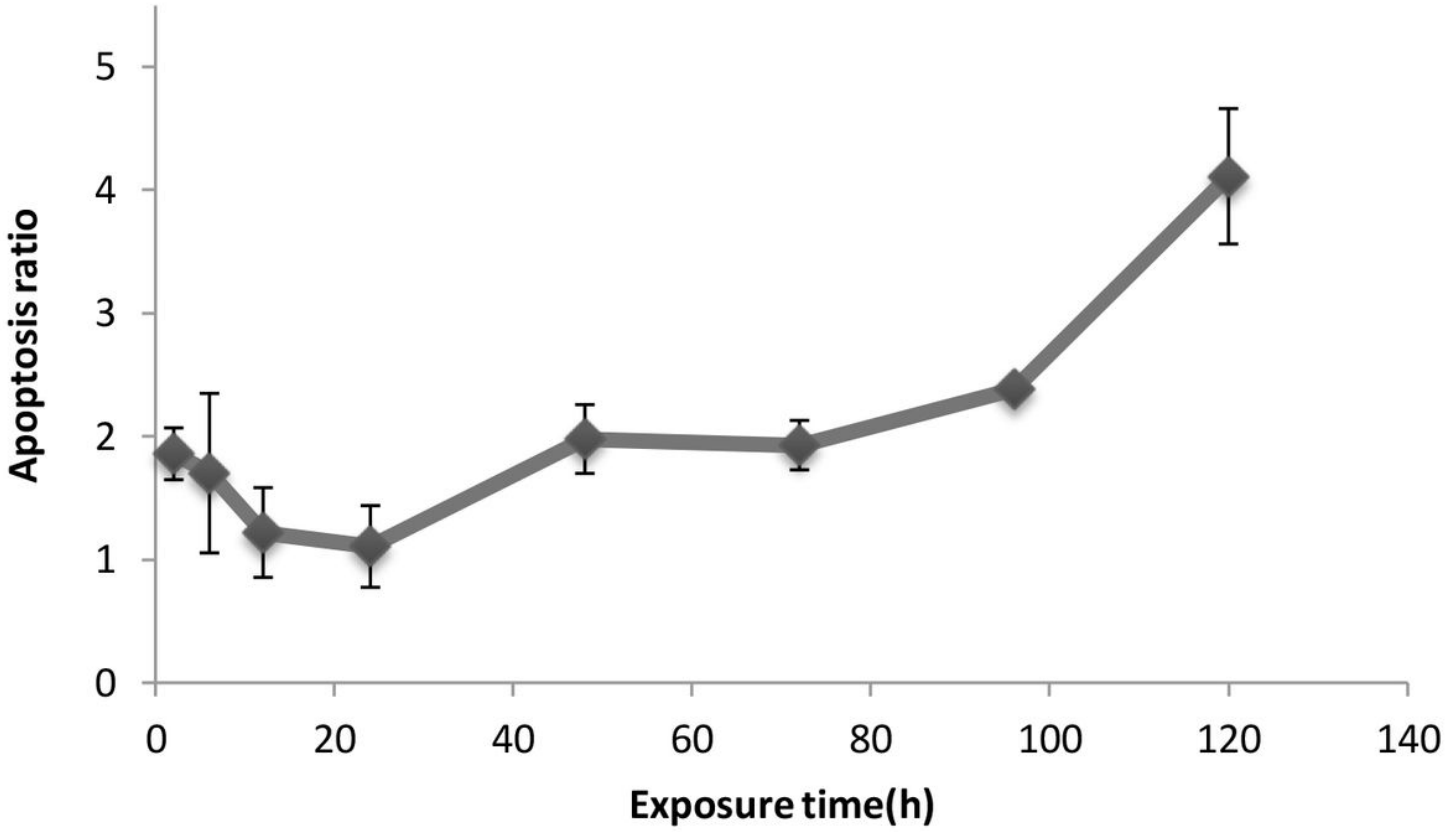


Figure 6

Apoptosis induction in MC4-L2 cells exposed to 1 Hz and 100 mT ELF-MF at varying times is represented as exposed: control ratios.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.pdf