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doi:10.15199/48.2018.12.46

Biological effects of the EMF influence on animal cells and tissues in *in vitro* cultures – a summary of own research

Abstract. *The effects of the electromagnetic field on cells and tissue in in vitro cultures are the subject of the research presented in this work.. Choosing different research object - cancerous and non-cancerous cells, male and female reproductive tissues, and various physical parameters of the EMF, such as the frequency of time-varying fields, magnetic induction value, field shape and time of exposure, there were tested effects (negative or positive) of impact, measured by modern methods of molecular biology through biological parameters in in vitro cultures.*

Streszczenie*. Pole elektromagnetyczne, jako czynnik środowiskowy wpływający na komórki i tkanki hodowane in vitro jest tematem badań przedstawionych w niniejszej pracy. Wybierając różne obiekty badawcze - komórki nowotworowe i nienowotworowe, tkanki męskiego i żeńskiego układu rozrodczego oraz różne parametry fizyczne pola elektromagnetycznego, takie jak częstotliwość pól zmiennych w czasie, wartość indukcji magnetycznej, kształt pola i czas ekspozycji, badano efekty (negatywne lub pozytywne) oddziaływania pola, mierzone nowoczesnymi metodami biologii molekularnej w kulturach in vitro.* ***(Biologiczne efekty oddziaływania PEM jako czynnika środowiskowego na komórki i tkanki zwierzęce w hodowlach in vitro – podsumowanie badań własnych)***

Keywords: electromagnetic field, proliferation, viability, cell cultures, tissue cultures
Słowa kluczowe: pole elektromagnetyczne, proliferacja, przeżywalność, hodowle komórkowe, hodowle tkankowe

Introduction

The electromagnetic field (EMF) is a specific type of energy, composed of two inseparably connected components - electric field and magnetic field. The electromagnetic field is distinguished by the continuity of decomposition in space, the ability to propagate in a vacuum and the and the impact force on the particles of matter loaded with an electric charge [1]. Particularly interesting, due to their universality, are artificial sources of 50 Hz EMF, mainly electrical devices. The specificity of the EMF produced by such devices makes it possible to consider electric and magnetic components separately in his case [7]. The magnetic field accompanies each current flow, and the electric field occurs wherever there is an electric voltage. The generation of EMF by devices in the household, in industry and by transmission lines or transformer stations is *a side effect* of their operation and it is an undesirable effect. However, in medicine in diagnostics and therapy, these fields are produced for therapeutic purposes. Among such devices there can be mentioned the apparatus for magnetotherapy, where the frequency ranges from 1 to 120 Hz, and magnetic induction from 1-30 mT [12], magnetostimulation devices with a frequency of several to 3000 Hz and magnetic induction in the range from 1pT up to 100 μT, nuclear magnetic resonance with constant frequency and magnetic induction from 0.15 to 1.5 T, or fixed frequency prosthesis where magnetic induction is 100 mT [10]. EMF generators used in medicine give the possibility of changing the shape of the field (sinusoid, triangle, rectangle), field modulation, frequency selection, or time of interaction.

Living organisms are the source of the magnetic field. They generate ionic currents which generate a magnetic field. Partial magnetic fields of ions and structures in tissues add up and are also a source of magnetic fields.

Exposure of living organisms to EMF can cause various biological effects depending on the intensity, frequency, dose and time of exposure to EMF. It has been demonstrated that EMF in some short-term exposure conditions may affect the biological properties of the cell, such as proliferation and apoptosis, which proves that the electromagnetic field can be a potential tool for the manipulation of behavior and cells viability [11].

During the exposure to EMF both mechanisms - intracellular and extracellular are exposed to its impact. The mechanisms whereby under the influence of the EMF information are exchanged between cells, and how this biomechanical signaling is transformed, have been studied for many years. It has been demonstrated that the EMF can penetrate both the cellular and nuclear cellular membranes, stimulating or inhibiting various types of cells and tissues.

In the modern papers there are many results of research on the impact of electromagnetic fields (EMF) on living organisms, including humans, but their results often contradict each other. There are many research models - different types of cells or tissues treated with fields of various physical parameters - frequency, shape and time of impact. Epidemiological studies, to verify whether exposure to the EMF may be a potential risk factor for health, have led to conflicting opinions.

Due to ambiguity of results, the impact of the EMF on cell and tissue cultures has become the leading topic of research studies presented in this paper. Choosing different research objects - cancerous and non-cancerous cells, male and female reproductive tissues and various physical parameters of the EMF, such as the frequency of time-varying fields, magnetic induction value, field shape and time of exposure, there were tested effects (negative or positive) of impact, measured by modern methods of molecular biology through biological parameters in *in vitro* cultures.

The main goal of this research is exploring the possibilities of the use of non-invasive EMF therapy in modern medicine and non-pharmacological support of existing therapies.

Materials

To perform the experiments showing the effect of EMF influence there were used the commercial cell lines and tissues of male and female reproductive tract of domestic pig (*Sus scrofa domesticus*) and European roe deer (*Capreolus capreolus*).

The cell cultures were performed according to procedures, in culture vessels, in an incubator at temperature of 37°C, 95% of humidity and 5% of CO_2 in DMEM medium supplemented with 10% fetal bovine serum and antibiotic. One group was subjected to EMF, the

second one was cultured in the same time and conditions, except the exposure to EMF.

The domestic pig tissues used in the studies were collected in slaughter house and European roe deer tissues during planned selected hunts. The samples of tissues were transported to laboratory as soon as possible (approximately 20 min.) in temperature 4 C. The reproductive tract tissue cultures were performed in 24 wells plates in shaking water bath in an atmosphere of 95% O_2 and 5% CO_2 in a special culture medium supplemented with 0.1% bovine serum albumin (BSA) fraction V and 1% commercial antibiotic-antimycotic solution. First, the slices were pre-incubated, than one group incubated under the influence of EMF, the second group cultured in the same time and conditions, without EMF influence. The scheme of the EMF exposure on cell and tissue cultures is shown at figure 1.

Depending on cell line and tissues the results of EMF influence were measured by different methods of molecular biology.

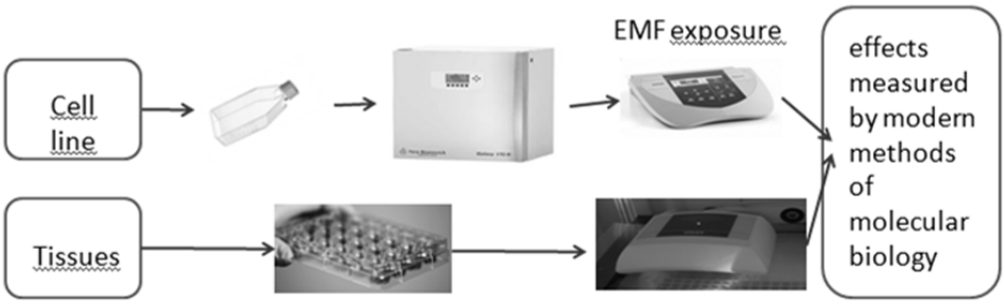

Fig. 1 The scheme of the EMF exposure on cell and tissue cultures

Methods used in the studies

All cell treatments were performed under sterile conditions under the chamber with laminar ventilation, using sterile disposable equipment.

1. Evaluation of cells viability was made using trypan blue staining.
   All procedure was made in according to protocol which describes how to perform a Trypan Blue staining which can be used to differentiate between viable and non-viable cells. The reagent stains dead cells in blue, but does not penetrate into viable cells, so they remain unstained because the cell membrane remains a barrier. This method has been described in the paper [3].
2. Evaluation of cell morphology was performed using the Giemsa reagent staining method.
   The Gram stain method is used to differentiate cells. It is possible based on the evaluation of the cell color after the process of its staining. The method also permits clear cell differentiation in terms of shape, size and structural details. In our case the cells were stained with Giemsa solution. Than stained cells were photographed using a light microscope.
3. Cells migration was observed using a scratch adhesion test.
   The evaluation of the effect of EMF on cell migration was performed using the test based on the observation of "overgrowth" by space cells between two edges, made with a sterile tip after EMF interaction in respect of the control group. Taking pictures under the microscope, there is observed a speed of wound overgrowth by cells and there can be evaluated the speed of their migration to the empty space.
   There was used a program for automatic microscopic images analysis – Tscratch, to calculate the wound size.
4. Real-Time PCR was used to study the genes expression.
   It is a sensitive analytical method of molecular biology. PCR is a polymerase chain reaction that allows amplification of a specific nucleic acid fragments. This method found application in various fields of science. An American biochemist Kary Mullis developed the technique in the 80s of the twentieth century [8]. Real Time PCR using fluorescence techniques allows the monitoring of the amount of reaction product during its duration. Monitoring the increase in the number of copies of the tested sequence in real time is possible by labeling primers, oligonucleotide probes or amplification products with fluorescent molecules. The device in which the reaction is carried out measures changes in fluorescence of the samples tested during each amplification cycle.
   The development of polymerase chain reaction has contributed to significant progress in the field of diagnostics. Thanks to the PCR reaction, it has become possible to synthesize billions of copies of any genomic DNA sequence within a few hours. This enabled acceleration and improvement of the broadly understood diagnostics. In turn, the development of Real-Time PCR has contributed to the improvement of diagnostic analysis in terms of quality and quantity. Real-Time PCR is one of the most commonly used methods from medicine to forensics and toxicology.
5. Western blot was used to detect proteins.
   This method is used in molecular biology for the detection of specific proteins. The samples must be prepared with a mixture of proteins, next the electrophoresis should be carry out (to separate DNA molecules by size, by moving negatively charged nucleic acid molecules through an agarose matrix with an electric field), the separated proteins have to be transfer from the gel to the membrane, incubate with the appropriate antibodies and the desired protein are detected. The scheme of the method is shown at fig. 2.

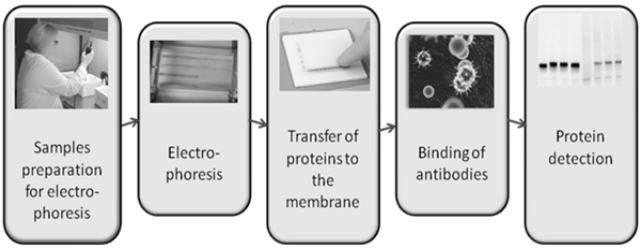

Fig. 2 The scheme of protein detection by Western blot

6. Infrared spectroscopy FTIR and FT-Raman spectroscopy.

Infrared (FTIR) and Raman spectroscopy are used as complementary techniques. Analysis with use FTIR spectroscopy let identify the chemical functional groups in

the sample. In our case the methods let find the differences in spectra of tissues influenced by EMF compared to control group.

EMF Exposure System

The EMF was generated by the Magneris generator with two-piece flat applicator (Astar, Poland). The device let for set the frequency in the range from 2 to 120 Hz, sinusoidal, triangular or square shape, continuous or modulated signal and different times of action. The system was described in our previous papers [4, 5].

The performed studies

The initial studies have focused on the influence of the EMF with the frequency of 2-60 Hz, sinusoidal, rectangular and triangular shapes, and magnetic induction in the 2-6 mT range on cancer and non-cancer cell lines. Human glioblastoma cells (U-87 MG), human bone osteosarcoma cells (143b), human skin fibroblasts (BJ) and human embryonic kidney 293 cells (HEK-293) were examined [5].

The results demonstrated different effects of the EMF on cell viability depending on the type of the line (cancerous and non-cancerous) and physical parameters of the field. Both tumor cell lines (U87_MG and 143B) were characterized by a different sensitivity to the cytotoxic effects of EMF. Significant decrease in viability of both cancerous cell lines were observed for fields of 50 and 60 Hz and sinusoidal shape. An exemplary effect of EMF influence on 143b cell line for the 2-hour exposition, frequency of 50 Hz and a triangle shape is shown in Figure 3. Statistically different viability occurs in the case of a magnetic induction with a value of 3, 4 and 6 mT. The effect of the EMF on the examined cell lines is different and statistically significantly depends on the type of the line - cancerous compared to non-cancerous one, and also depends on the field value, shape and the time of exposure. This study showed that the EMF can be used as a potential therapeutic tool to manipulate cell viability and opens up a new field of research useful for the future clinical application of tissue effects

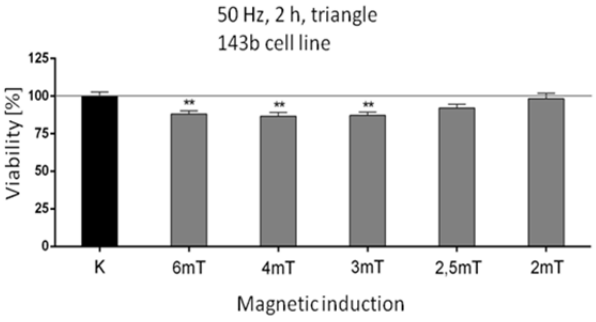

Fig. 3 The viability of 143b cell line after 2 hours exposition on EMF with the frequency of 50 Hz and triangle shape, mean ± S.E.M.; **P ≤ 0.01;

Subsequent studies were conducted on the primary pineal cell line. EMF with the frequency of 50 Hz recognized as an environmental factor, affects the viability of pineal cells in vitro. The effect of the field was checked in two study groups in short-term EMF impact (1, 2 or 3 hours) and long-term impact (3 hours for 2 or 3 days). It was shown that compared to controls, the exposure of cells to EMF induced a significant increase in cell viability at 2 and 3 hours of exposure. At the same time, it was shown that after three days of a three-hour exposure there was a significant decrease in cell viability compared to the control group [2].

The results of these studies suggest that EMF may have a significant biological effect on the pineal cells depending on the time of interaction, and thus affect the functioning of the daily biological clock in humans and animals.

In order to check the influence of EMF on other frequencies on BJ (human fibroblast) line cells, the viability and percentage of cells in different phases of the cell cycle (G1 / G0, S, G2 / M) were evaluated after 2 hours of exposure to sine continuous and pulsed EMF at 5 Hz , 60 Hz and 120 Hz and 2.5 mT magnetic induction. The viability of BJ cells was assessed in two groups - immediately after exposure and after 24 hours. Analysis of the metabolic activity of the cells showed significant differences in the effectiveness of EMF interaction, depending on its character (continuous or pulsed). Exposure of cells to pulsed electromagnetic fields caused a decrease in their lifetime for each of the analyzed frequencies. The reduced metabolic activity was maintained 24 hours after the end of exposure of cells to pulsing EMF. Continuous sinusoidal EMF reduces the viability of BJ cells only at 120 Hz, and this effect lasted for the next 24 hours. There was no significant effect on cell viability immediately after exposure to continuous EMF at 5 Hz, but a significant increase was observed 24 hours after incubation [4].

Exposure of cells to pulsed EMF caused a decrease in their viability for each of the analyzed frequencies.

Based on these results, it can be concluded that EMF with frequencies of 5, 60 and 120 Hz, with sinusoidal continuous or pulsating waveforms, affects the cell cycle and the viability of the cells of the human fibroblast cell line BJ.

The electromagnetic field has an effect on the female and male reproductive systems [9]. It affects the function of ion channels, protein structure and is probably a pathomechanism affecting the embryo and intrauterine environment [13]. There were investigated studies on the impact of EMF with the frequency of 50 Hz and 2.5 mT magnetic induction on morphology, viability and proliferation as well as on the change of the biochemical profile of GC-1 spg cells (mouse type B spermatozoa) *in vitro*. FTIR infrared spectroscopy was used to analyze changes in the biochemical profile of cells. On the basis of the conducted studies, the influence of EMF on the parameters examined was shown depending on the time of exposure.

In order to check how the electromagnetic field affects the female reproductive system, an analysis of the impact on the synthesis and secretion of estradiol-17β (E2) in the uterus of a domestic pig has been performed. Estradiol is the primary hormone that initiates the proliferation of endometrial cells for blastocyst implantation. In the experiment, two EMF frequencies - 50 and 120 Hz were selected. EMF at 50 Hz is the most frequent in the environment, hence it was considered the most suitable for studying the effects of EMF on living organisms. In the conversion of testosterone to estradiol, there is involved the enzyme aromatase P450, Its presence is the effect of expression of the CYP19A3 mRNA gene. Exposure of tested tissues to EMF at 50 Hz did not change the expression of the CYP19A3 mRNA gene in slides. The obtained results allowed to draw the following conclusions: EMF induces changes in the synthesis and release of estradiol E2 in the examined tissues of the uterus depending on the frequency and time of exposure. We suspect that the observed phenomenon may lead to changes in estrogen levels in the intrauterine environment. Exposure of uterine tissues to EMF induces changes in steroidogenic endometrial and myometrial activity, expressed by the variability of CYP19A3 mRNA expression and aromatase P450 activity in the study area. Progesterone acts as a potentially protective factor against the effects of EMF [6].

All performed studies shown the effect of EMF interaction. There were studied different frequencies, different magnetic induction values and different time of interaction. The response of the examined cells was checked immediately after the interaction, as well as 24 hours later. All tests have shown the effect of the electromagnetic field. It could stimulate cell viability, in some cases it inhibited it. It can be concluded that EMF is a factor that can be used as an environmental factor affecting the living organism.

Discussion

The tissues of organisms are mainly diamagnetics, however, many structures have paramagnetic properties. The impact of the external magnetic field, changes the energy state of tissues, which has an impact on their biological functions. Scientists have proven that magnetic fields exist in all our cells and that they work through ions. Research in the field of biophysics is of interest to biologists, physicists, mathematicians and people involved in bioelectromagnetism. The results of contemporary research on issues in the field of bioelectromagnetism enable the enrichment of pharmacological methods of treatment with non-invasive methods of therapy. These therapeutic systems are not widely disseminated, due to the proportionately small amount of research carried out in scientific laboratories. It seems necessary time to separate a new discipline or specialty called bioelectromagnetism, which will combine joint research efforts of electrical engineers, biotechnologists, biologists, medical doctors and pharmacists in achieving the main goal which is better and less invasive therapy of humans and animals.

The presented research results are a summary of the research cycle on the effect of the EMF on cancerous and non-cancerous cell lines as well as on animal tissues of reproductive tract. Different effects of EMF influence shown depending on the physiological status of the cells (cancerous and non-cancerous) as well as kind of tissues and physical parameters of the field.

These results suggest that the use of EMF in the future will have a good therapeutic effect on pathologically changed tissues. Based on the conducted research studies, it was demonstrated the effect of EMF, especially with the frequency of 50 Hz, on cells and tissues. The magnetic induction values accepted for testing are values that occur in the environment in specific situations and organisms, and in particular people are subjected to the influence of such fields for a short time. During the tests, the time of affecting tissues was taken 2 or 4 hours, the time of interaction on the cells was also expressed in minutes. Despite such a short time of interaction in each case, the influence of the EMF on cells physiology was noticed.

Acknowledgements

I would like to thank the staff of the Kolbuszowa Forest District Inspectorate, head PhD. Bartłomiej Peret and forest inspector MSc. Adam Orlewski for help in obtaining the biological material for research studies.

The study was carried out with the use of equipment granted by the project "Centre of Applied Biotechnology and Basic Sciences" supported by the Operational Programme "Development of Eastern Poland 2007-2013". No. POPW.01.03.00-18-018/09,

Part of the research was carried out with funding from the Municipal Office of Kolbuszowa, Poland.

Declaration of interest

The author declare no conflict of interest.

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REFERENCES

1. Gajšek P, Ravazzani P, Grellier J, Samaras T, Bakos J, Thuróczy G: Review of Studies Concerning Electromagnetic Field (EMF) Exposure Assessment in Europe: Low Frequency Fields (50 Hz–100 kHz), Int J Environ Res Public Health. 2016 Sep; 13(9): 875.
2. Koziorowska A., Pasiud E., Fila M., Romerowicz-Misielak M. The impact of EMF at a frequency of 50 Hz and a magnetic induction of 2.5 mT on viability of pineal cells in vitro, Journal of Biological Regulators and Homeostatic Agents, 2016 Oct-Dec;30(4) pp. 1067-1072
3. Koziorowska A., Sołek P., Majchrowicz L., Romerowicz – Misielak M.. The impact of electromagnetic fields with frequency of 50 Hz on metabolic activity of cells in vitro. Przegląd Elektrotechniczny (2017), 1: 161 – 164
4. Koziorowska A, Romerowicz-Misielak M, Filipek A, Koziorowski M: Electromagnetic fields with frequencies of 5, 60 and 120 Hz affect the cell cycle and viability of human fibroblast BJ in vitro, Journal of Biological Regulators and Homeostatic Agents 31(3) (2017), pp. 725-730
5. Koziorowska A., Romerowicz-Misielak M., Sołek P., Koziorowski M,. "Extremely low frequency variable electromagnetic fields affect cancer and noncancerous cells in vitro differently: Preliminary study", Electromagn Biol Med. (2018), No. 37, 35-42.
6. Koziorowska A, Waszkiewicz EM, Romerowicz-Misielak M, Zglejc K, Franczak A Extremely low-frequency electromagnetic field (EMF) generates alterations in the synthesis and secretion of oestradiol-17β (E2) in uterine tissues: An in vitro study, Theriogenology 110 (2018) 86-95,
7. Krawczyk A, Korzeniewska E, Łada-Tondyra E: Magnetofosfeny – historia i współczesne implikacje, Przegląd Elektrotechniczny, R. 94 NR 1/2018
8. Mullis K.B. The unusual origin of the polymerase chain reaction. Sci. Am. (1990), 262, 56–61, 64–65
9. Myung Chan Gye, Chan Jin Park Effect of electromagnetic field exposure on the reproductive system, Clin Exp Reprod Med 2012;39(1):1-9,
10. Pole magnetyczne i światło w medycynie i fizjoterapii, red. Sieroń A. i Cieślar G, α-medica Press, 2013
11. Saliev T, , Mustapova Z, Kulsharova G, Bulanin D, Mikhalovsky S: Therapeutic potential of electromagnetic fields for tissue engineering and wound healing, (2014) Cell Prolif., 47, 485–493
12. Sieroń A, Mucha R, Pasek J: Magnetoterapia, Rehabilitacja w praktyce, (2006), 3/2006, str. 29-32
13. Wdowiak A, Mazurek P. A, Wdowiak A, Bojar I: Effect of electromagnetic waves on human reproduction, Annals of Agricultural and Environmental Medicine 2017, Vol 24, No 1, 13–18