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Chapter 12. EFFECT OF A WEEK-LONG EXPOSURE OF INTERMITTED LOW ELECTROMAGNETIC FIELD ON PROLIFERATION POTENTIAL OF HUMAN CELLS

12.1. Introduction

The rapid development of technologies present in everyday life in the last 30 years has resulted in the appearance of many electronic devices in households and workplaces. As a consequence, current-operated devices generate an electromagnetic field (EMF) which has become one of the environmental factors affecting living organisms and has also become subject of environmental monitoring. Workers who spend most of their time in close proximity to wiring are exposed to the constant presence of extremely low-frequency electromagnetic field (ELF-EMF) of approximately 50–60 Hz due to the frequency in electrical sockets [1]. Non-ionizing electromagnetic field interactions with biological objects at various levels have lately become a fast-growing research area but it has been the subject of public concern for many years [2]. Questions regarding its possible health risks are even more frequent and this particular area remains unclear, especially regarding long-term radiation. A couple of studies confirmed that ELF-EMF

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may be a potentially genotoxic and cytotoxic factor [3, 4], thus the main goal of this project was to verify if a week-long exposure has influence on human cells. This experiment models a typical week of workers' exposure to ELF-EMF with a frequency of 50 Hz with consideration of a two-day weekend break.

12.1.1. Physics of EMF

According to the classical electrodynamic theory an electromagnetic field is combination of electrical and magnetic field, which propagates in a certain medium. Properties of electromagnetic fields depend on many factors like their source, frequency, propagation medium etc. The method of electromagnetic fields generation depends on their properties and applications. Generally, any current flow through the conductive wire generates electromagnetic field. In this case, it is necessary to obtain higher magnetic field than electrical field by coil. According to Faraday's law of induction – a current flowing through the coil's wires generates magnetic flux proportional to the flowing current, the proportional coefficient is coil's induction. In general assumption, a coil is linear electrical element, so the output magnetic flux is similar to the input. Magnetic flux divided by surface represents magnetic flux density which is also named the B-field (1). It is described by following formula [5]:

$$B = \frac{LI}{S} \tag{1}$$

Where:
L – value of coil inductance [H]
I – value of current flowing through the coil [A]
S – surface area crossed by magnetic flux [m^2]
B – value of magnetic flux density (B-field) [T]

12.1.2. Influence of ELF-EMF on human cells

Last thirty years of research provided ambiguous claims about EMF impact on cells condition [5]. Depending on frequency, exposure time or type of electromagnetic wave used in experiment as well as type of cells tested, carcinogenic, proliferative and antiproliferative effects were distinguished. Several studies established a connection between ELF magnetic field exposure and leukemia and brain tumors [6] while others discarded such influence [7]. The possible carcinogenic influence caused by increase in

the oxidative stress in cells exposed to ELF-EMF was also shown in studies [8]. Contrarily another study showed possible anti-carcinogenic effect of ELF-EMF exposure on melanoma cells caused by downregulation of human endogenous retroviruses (HERVs) genes therefore neutralizing them [9].

In contrast to possible negative influence on human cells, electromagnetic field is commonly used in orthopedic treatments as a tissue's regeneration stimulating factor [10]. In the case of osteoblast-like cells, the proliferation-enhancing effect of ELF-EMF is confirmed [11]. The influence of the electromagnetic field on the efficiency of wound healing has been studied, but also in this regard the results of various research groups are contradictory. More recent studies focused on reducing inflammation after skin exposure to ELF-EMF and confirmed the possible alleviation of the irradiated area by contributing to the reduction of pro-inflammatory cytokines [12].

Clinical trial conducted in the Department of Biochemistry, University of Cambridge based on ELF-EMF classification as a possible harmful factor didn't provide significant evidence of such influence, but highlighted the fact that small changes in genes expression can exert significant effects on health [13]. Meta-analysis that focused on damage of mammalian cells exposed to electromagnetic field clarified the importance of including various genotoxicity endpoints in the assessment of DNA damage in the future studies [5]. Depending on the examined tissue and the type of cells exposed to ELF-EMF, the effect on their condition differs, which is why it is important to analyze a variety of cell lines under different irradiation conditions.

12.2. Materials and methods

12.2.1. Cell culture and cultivation

To determine the proliferation efficiency a clonogenic assay was performed with 5 samples each for control (CTRL), sham (SHAM), i.e. samples subjected to the same conditions as treated ones but with the turned off coil and treated groups (EMF) of HeLa GFP H2B cell line. Cells were seeded onto cell dishes (⌀ 3.5 cm) in the final concentration of 1700 cells per milliliter and incubated in a final volume of 2 ml Dulbecco's Modified Eagle Medium (DMEM/Ham's F12), supplemented with 7.5% Fetal Bovine Serum (FBS) in 5% CO_2 at 37°C. Control and sham samples were incubated under normal conditions, with SHAM (according to common practice an

inactive procedure to mimic experimental conditions) samples incubated inside unpowered coil. Test samples were additionally irradiated (frequency: 50 Hz, magnetic flux density: 85 μT) for 6 hours per day for 5 days and 2 days without irradiation afterwards as to model a worker's week of exposure to EMF irradiation.

12.2.2. Coil, irradiation system

The irradiation system was set up so that it would enable us to perform CTRL, SHAM and EMF tests at the same time (Figure 1). The set up for SHAM experiments consisted of an unconnected coil (L_{Sham}). The set up for EMF samples consisted of a generator connected to coil (L_{EMF}) via ammeter. The generator allowed us to choose the voltage of 3.6 mV, sinusoidal wave and the frequency of 50 Hz as an output signal. The ammeter connected between generator and coil shows value of current flowing through the coil. The coils were located inside incubator so that coil generating electromagnetic filed would not affect SHAM and CTRL samples.

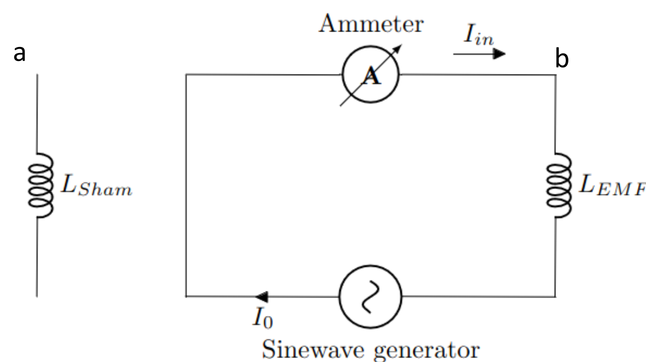

Fig. 1. Schemes of sham system (a) and irradiation system (b)
Rys. 1. Schemat układu dla próby pozornej (a) i układu dla próby badawczej (b) wpływ promieniowania

12.2.3. Clonogenic assay

After appropriate incubation of all samples (CTRL in normal conditions for 7 days, SHAM in unpowered coil in normal conditions for 7 days and EMF in treated conditions for 5 days and normal conditions of 2 days) were fixed with 96% EtOH, then washed with ddH2O. To enhance the visibility of formed colonies, samples were incubated in 0.2% crystal violet solution to obtain an intensive colour. After, dishes were twice quickly washed with ddH2O. All images of culture dishes with colonies were taken with

the use of G-BOX XT4 from SynGene and its software. Procurement of images after staining has been performed with visible light and white background. Colonies were then counted manually excluding abortive and merged ones.

To determine the performance of cells proliferation after ELF-EMF treatment both Proliferation Efficiency (PE, as a number of full-sizes colonies and seeded cell ratio) and Survival Fraction (SF, as a non-treated and treated samples PE ratio) factors have been used.

12.3. Statistical analysis

To determine the occurrence of differences in proliferation efficiency, non-parametric Kruskal-Wallis and Wilcoxon tests have been used. Analysis was prepared for 0.05 alpha significant levels.

12.4. Results and discussion

12.4.1. Clonogenic results

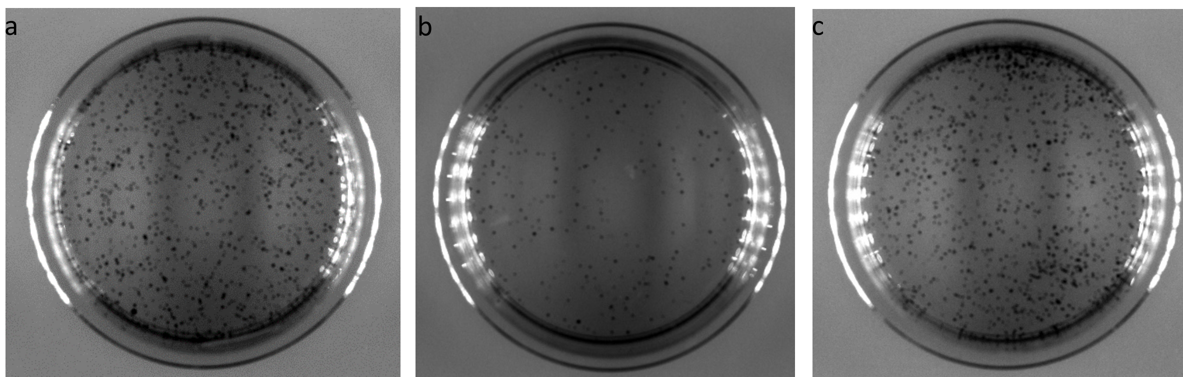

Fig. 2a. SHAM 2b. EMF 2c. CTRL plates with stained colonies
Rys. 2a. Płytka SHAM 2b. Płytka EMF 2c. Płytka CTRL z wybarwionymi koloniami

Table 1

Colonies counted for each experimental set

SET	SHAM	EMF	CTRL
1	382	279	348
2	598	222	603
3	325	392	405
4	532	369	396
5	591	567	699

Although data suggests (Table 1) fewer colonies in treated samples (EMF) (Figure 2b) compared to non-treated ones (SHAM and CTRL) (Figure 2a and Figure 2c) the results of Kruskal-Wallis' test show no significant differences in proliferation efficiency exist between any of the analyzed groups (Figure 3). When treated samples were compared to control and sham groups individually, Wilcoxon test resulted in p-values equal to 0.15 and 0.22 respectively. Moreover, there were no differences between ratio of PE from treated samples to PE of either control or sham samples (Figure 4). However, it is observed that nearly for all analyzed samples PE values for EMF samples are less than in the control and sham groups.

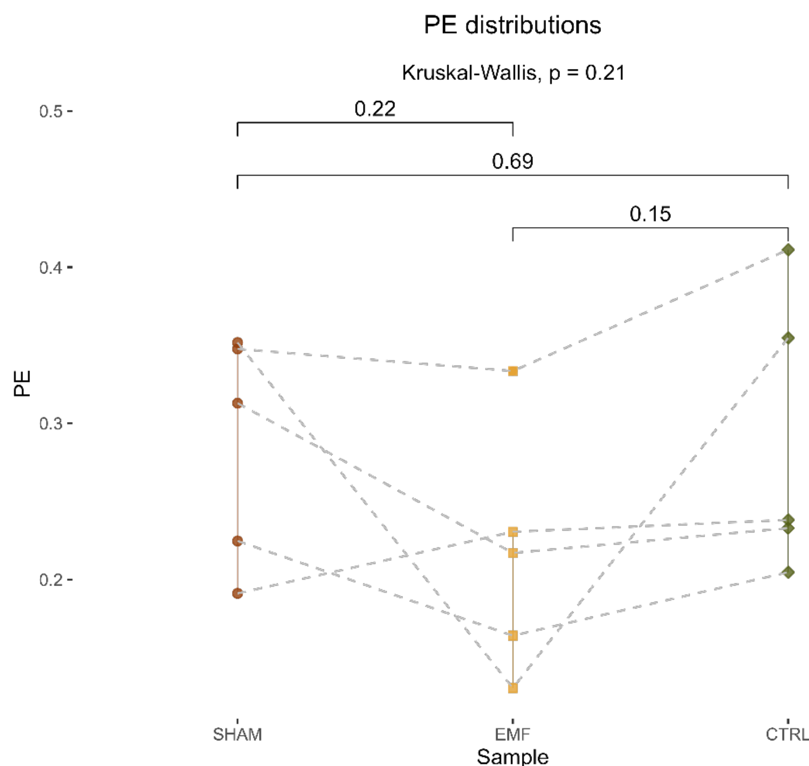

Fig. 3. Comparison of PE values between sham, treated with EMF and control samples. Results of Kruskal-Wallis and Wilcoxon tests. Dashed lines connect the linked samples. The Upper and lower quartiles are marked with the vertical lines

Rys. 3. Porównanie wartości PE pomiędzy próbami pozorną, badaną i kontrolną. Wyniki testów Kruskala-Wallisa i Wilcoxona. Przerywanymi liniami zaznaczono odpowiadające sobie próby. Górne i dolne kwartyle oznaczono za pomocą pionowych linii

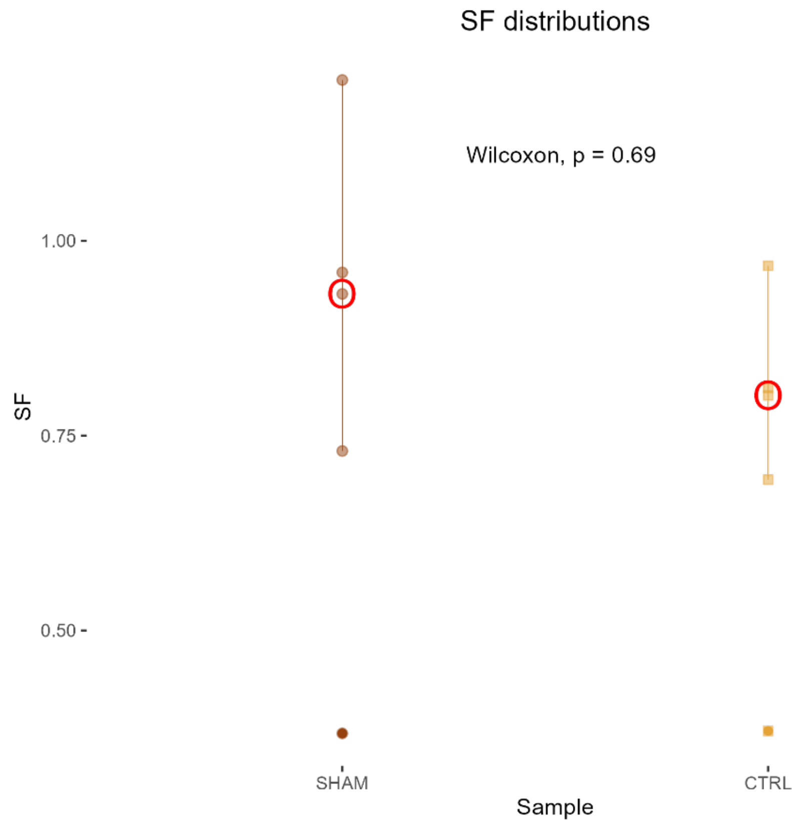

Fig. 4. Comparison of SF values between sham and control as a non-treated sample. Results of Wilcoxon test. The Upper and lower quartiles are marked with the vertical lines. Red circles represent each group medians.
Rys. 4. Porównanie wartości SF pomiędzy próbami kontrolnymi i pozornymi. Wyniki testu Wilcoxona. Pionowymi liniami zaznaczone zostały górne i dolne kwartyle. Czerwonymi okręgami oznaczono mediany.

12.5. Conclusions

The main results of this work show that one week-long exposure of ELF-EMF has no statistically significant impact on HeLa cells proliferation. However, it has been shown that samples treated with EMF irradiation are characterized with lower PE ratio values, thus those results might be correlated with conclusion provided by Kirschenlohr et al. [14] that non-significant changes in cell function caused by tested factor, yet difficult to observe, may carry health consequences in the future. Therefore, it can be supposed that the impact of ELF-EMF may be a potential risk factor for cells condition. Moreover, there were statistical differences between proliferation efficiency between control samples and those which were incubated in sham conditions, which was also verified by statistical analysis of surviving fractions. Future studies should take into

account the conclusions received so far and not only monitor the impact of ELF-EMF with different parameters at different times on different cell lines, but also check multiple endpoints that may be more sensitive to those slight changes.

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Bibliography

1. Commission Regulation (EU) 2017/1485 of 2 August 2017 establishing a guideline on electricity transmission system operation (OJ L 220, 25.8.2017, pp. 1–120).
2. W.K. Stevens, Scientists Debate Health Hazards of Electromagnetic Fields, *New York Times* (1989) **07.11(C):**1
3. C.T. Mihai, P. Rotinberg, F. Brinza, et al., Extremely low-frequency electromagnetic fields cause DNA strand breaks in normal cells., J Environ Health Sci Engineer (2014) **12**:15.
4. S. Ivancsits, E. Diem, A. Pilger, H.W. Rüdiger, O. Jahn, Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts. *Mutation research* (2002) , **519(1–2):**1–13.
5. P. Vijayalaxmi, T.J. Prihoda, Genetic damage in mammalian somatic cells exposed to extremely low frequency electro-magnetic fields: A meta-analysis of data from 87 publications (1990–2007), *International Journal of Radiation Biology* (2009), **85(3)**:196–213.
6. E.M. Purcell, D.J. Morin. Electricity and Magnetism. *Cambridge Univ. Press*, p. 364.

7. C.D’Angelo, E. Costantini, M.A. Kamal, M. Reale, Experimental model for ELF-EMF exposure: Concern for human health, *Saudi Journal of Biological Sciences* (2015), **22(1)**:75–84.
8. N. Leitgeb, Comparative health risk assessment of electromagnetic fields, *Wien Med Wochenschr* (2011), **161**:251–262.
9. R. J. Bułdak, R. Polaniak, Ł. Bułdak, K. Żwirska-Korczala, M. Skonieczna, A. Monsiol, E. Birkner, Short-term exposure to 50 Hz ELF-EMF alters the cisplatin-induced oxidative response in AT478 murine squamous cell carcinoma cells, *Bioelectromagnetics* (2012), **33(8)**:641–651.
10. Y. Wei, H. Xiaolin, S. Tao, Effects of Extremely Low-Frequency-Pulsed Electromagnetic Field on Different-Derived Osteoblast-Like Cells, *Electromagnetic Biology and Medicine* (2008), **27(3)**:298–311.
11. C.L. Ross, M. Siriwardane, G. Almeida-Porada, C.D. Porada, P. Brink, G. J. Christ, B.S. Harrison, The effect of low-frequency electromagnetic field on human bone marrow stem/progenitor cell differentiation, *Stem Cell Research* (2015), **15(1):** 96–108.
12. M. Pesce, A. Patruno, L. Speranza, M. Reale, Extremely low frequency electromagnetic field and wound healing: implication of cytokines as biological mediators, *European Cytokine Network* (2013), **24(1**):1–10
13. A. Karimi, F. Ghadiri-Moghaddam, M. Valipour, Y. Yahyavi, Effects of prolonged exposure to ELF-EMF on HERVs expression in human melanoma cells, *Mol Biol Res Commun*. (2022), **11(2)**:67–71.
14. H. Kirschenlohr, P. Ellis, R. Hesketh, J. Metcalfe, Gene Expression Profiles in White Blood Cells of Volunteers Exposed to a 50 Hz Electromagnetic Field, *Radiation Research* (2012), **178(3)**:138–149.

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Abstract

Extremely low frequency electromagnetic field (ELF-EMF) is generated among others by power lines and electrical devices; therefore, most people are exposed to its effects each day. This experiment models a typical week of workers' exposure to ELF-EMF with consideration of a two-day weekend break and analyzes its effect on human cells. Determination of proliferation potential was performed on HeLa cells with intermittent ELF-EMF of 50 Hz generated by a coil particularly measured for this experiment. Cytotoxicity was examined by a clonogenic assay that permits to inspect the ability of formation clonogenic colonies by tested cells. For this purpose, cells were cultivated in three groups: control one (cultivated in normal conditions), sham one (cultivated in an unwired coil in normal conditions) and exposed one (cultivated for 5 days in conditions of intermittent irradiation followed by two days of relaxation). To analyze the experiment's results appropriate devices and statistical tools have been used.

Keywords: electromagnetic field, HeLa cells, cancer, proliferation, cytotoxicity