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Action of The Static Magnetic Fields on The Antioxidant Activity in The Fibroblasts' Culture

Streszczenie. *W niniejszym artykule przedstawiono ocenę działania antyoksydacyjnego stałego pola magnetycznego (SMF) w hodowli fibroblastów. Do badań użyto komór testowych z indukcją 0,3; 0,4; 0,5; 0,6; 0,7 T, które wcześniej zaprezentowano na międzynarodowej konferencji BEMS w Halifax w 2011 roku. Oceniono aktywność dysmutazy ponadtlenkowej (SOD, EC.1.15.1.1), peroksydazy glutationowej (GPx, EC. 1.11.1.9), reduktazy glutationowej (GR, EC. 1.5.4.2), całkowity potencjał antyoksydacyjny i stężenie dialdehydu malonowego, jako wskaźnika szybkości peroksydacji lipidów. Nie stwierdzono statystycznie istotnych różnic w aktywności enzymów, całkowitego potencjał antyoksydacyjnego oraz stężenia dialdehydu malonowego. Podczas całego eksperymentu mierzono jednorodność stałego pola magnetycznego na badanej powierzchni wszystkich komór i nie stwierdzono różnic. Uzyskane z badań dane wskazują, że stałe pole magnetyczne o powyższych parametrach nie mają szkodliwego wpływu na procesy odnowy tkanek* ***(Działanie stałego pola magnetycznego na aktywność hodowli fibroblastów)****.*

Abstract*. Our goal was to evaluate the antioxidant activity of the static magnetic field (SMF) in the fibroblasts' culture. We used the test chambers with the induction 0,3; 0,4; 0,5; 0,6; 0,7 T, previously presented in BEMS Meeting in Halifax. We evaluate the activity of superoxide dismutase (SOD, EC.1.15.1.1), glutathione peroxidase (GPx, EC. 1.11.1.9), glutathione reductase (GR, EC. 1.5.4.2), total antioxidant potential and malone dialdehyde concentration, as an indicator of lipid peroxidation rate. No statistically significant differences in the enzymes activity, total antioxidant potential and malone dialdehyde concentration were revealed. During the experiment the homogeneity of SMF in all chambers was measured, no differences were detected. Our data suggest that SMF in above parameters have no harmful influence on the regenerating tissues.*

Słowa kluczowe: stałe pole magnetyczne, komora badawcza, hodowla komórkowa, 2D symulacje pola magnetycznego, 3D symulacje pola magnetycznego.
Keywords: static magnetic field, test chamber, cell culture, 2D magnetic field simulation, 3D magnetic field simulation.

Introduction

Living organisms, including people, since the beginnings of the life on the Earth live in the magnetic field of the Earth, therefore they are genetically adapted to this field. The magnetic field of the Earth is a constant field exhibiting the following parameters: intensity Hz = 24 A/m, magnetic induction = 30 μT. In areas where there are large deposits of iron ores and in the vicinity of the poles the values of Hz and Bz are higher, however, they do not doubly exceed the values quoted.

In the 19th and 20th centuries electric engineering came into being and developed and it stimulated the development of the material engineering of permanent magnets[1, 2].

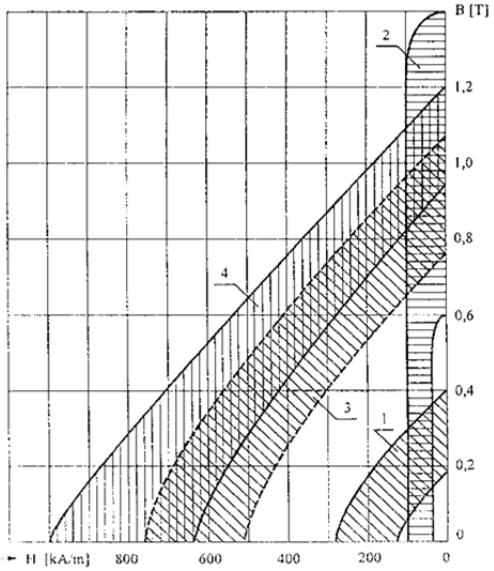

Fig. 1. Ranges comprising the characteristics of the demagnetization of permanent magnets, were: 1 – barium ferrites and strontium ferrites, 2 – alnico, 3 – samara-cobalt, 4-neodymium magnet.

At present the most popular and most broadly used permanent magnets are ferrite magnets (1 – fig. 1) and neodymium magnets (4 – fig. 1). Their more and more extensive application in industry [3, 4, 5] connected with the drop of costs of their production entails the need to extend basic examinations evaluating their impact upon functioning of living organisms [6]. So far only several works cited in the global medical literature have tackled the problem of evaluating the influence of constant magnetic fields on cell cultures[7, 8, 9]. The permanent magnets used in the works do not have any descriptions of their characteristics nor models of the spatial distribution of the intensities of the constant magnetic field, prepared beforehand. Figure 2 presents a concept of magnetic chamber.

Our goal was to make an attempt at optimization of this research. We created computerized simulations of the 3D (fig. 3) distribution of the intensities of the constant magnetic field for several sizes of permanent NdFeB magnets which, as we assumed, can be used for the research into cell cultures. Our next step was constructing models of circuits for several sizes of magnets on the basis of the spatial parameters of a standard plastic container used for cell cultures. We compared the assumptions of the intensities simulation with the real measurements carried out on the models of the real circuits. The final effect was to be a proposal of a model for examining the impact of constant magnetic fields on various factors of in vitro cell cultures.

During annual scientific BEMS meeting in 2011 in Halifax we presented design of chamber used for investigation of constant magnetic field impact on cell cultures. The final results were presented in 2012 BEMS meeting in Brisbane [10, 11]. We conducted computer simulations of 2D and 3D constant magnetic field distribution and verified them experimentally in chambers with magnetic flux densities equal to 0.3, 0.4, 0.5, 0.6 and 0.7 T, respectively. Statistical analysis did not show any statistically significant differences between simulations and measurements. This allowed us to design chambers basing on correct computer models only. Our investigation was founded on the assumption that at present there are no publications assessing constant magnetic field impact on cell metabolism and fibroblast/culture anti-oxidizing response in particular. The known research has not

produced thorough analysis of the subject matter and has been conducted without prior accurate modeling of constant magnetic field intensities. Our research is of pioneering character and is based on correct practical technical preparation of test stands.

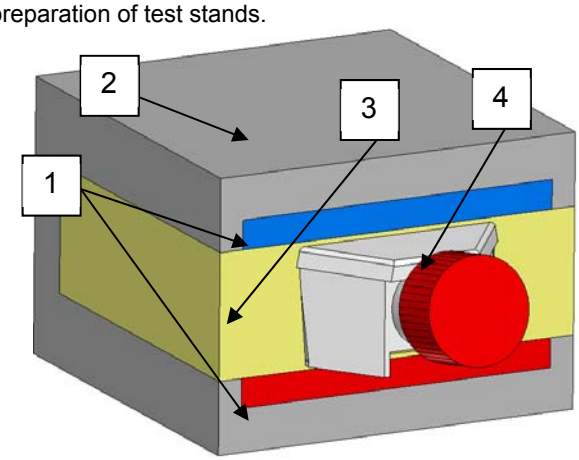

Fig. 2. Concept of magnetic chamber, were:
1 – permanent magnets, 2 – ferromagnetic yoke, 3 – non-magnetic distance plates, 4 – test cob.

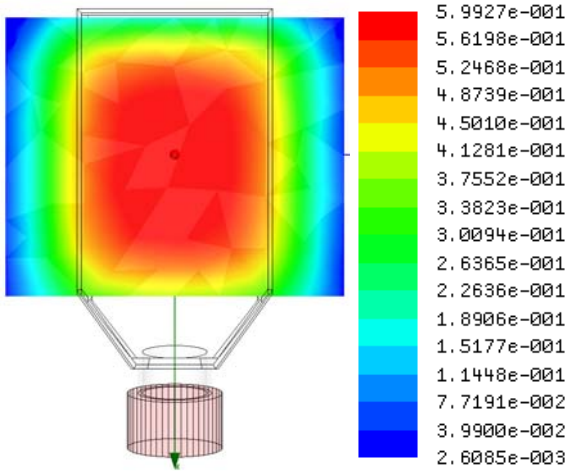

Fig.3. Results of 3D FEA of magnetic flux density, permanent magnets dimensions: 66x15x72mm

Magnetic field distribution in central part of the chamber is uniform. Measured and calculated values of magnetic flux density in this chamber zone are shown in Table 1.

Table 1 Comparison of results of calculations and Lab test

Magnet dimensions	**The maximum flux density in the slot [T]**	
	Calculations	Lab Test
4 mm	0,29	0,30
6 mm	0,40	0,40
8 mm	0,48	0,47
11 mm	0,58	0,58
15 mm	0,68	0,66
20 mm	0,71	0,72

Material and methods

Twelve chambers were built, two for each magnetic flux density value (0.3, 0.4, 0.5, 0.6 and 0.7 T, respectively) as well as two placebo chambers, magnet-free, made for control purposes. Fig.4. presents a photo of test chamber.

Fibroblasts were collected from mice skin explants. Next, the cells were grown on Dulbecco MEM medium in cell culture flasks of 50 ml each and with breeding surface equal to 25 cm2. The flask was filled with 10 ml of medium enriched with inactive calf foetal serum so that final concentration was equal to 10%; antibiotics were also added – 1000 U. of penicillin, 10 mg of streptomycin and 25 µg of amphotericin B per 1 ml medium. At the start of culture, each flask was filled with fibroblast suspension – 500 thousand per 1 ml of medium. After 24 hours from culture initiation the medium was completely replaced, the culture was placed in constant magnetic field and breeding continued for three more days. The incubations of test and control cultures were conducted in presence of air containing 5% (in terms of volume) of CO2, at temperature equal to 37°C. Heraeus incubator was used. All actions related to isolation of fibroblasts were conducted with attention to maintain full aseptic conditions.

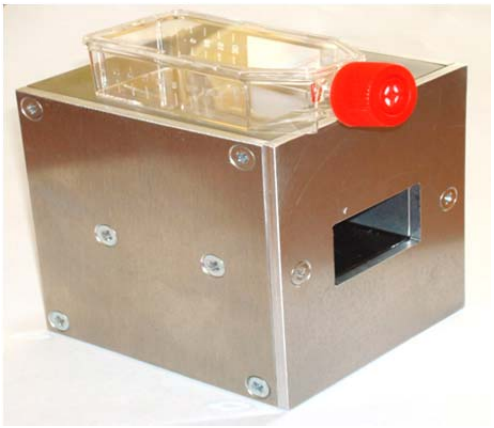

Fig.4. Photo of test bed

The cell breeding lasted for four days and then cultures were terminated. In order to obtain samples for fibroblast counting, the cells were subjected to trypsin. Cells freed from the medium were washed, spinned and obtained residue was suspended in 1 ml of PBS. Next, fibroblasts were counted with the help of automatic counter. Eventually the washed fibroblasts suspended in 1 ml of PBS solution were mechanically homogenised in test tubes placed in cooling (ice) bath. The homogenising time was determined experimentally, by assessing effectiveness of homogenisation with the help of optical microscope. The obtained homogenate was used for biochemical determination of markers.

The following quantities were determined in the samples: number of cells in each culture, superoxide dismutase and glutathione peroxidase.

Discussion and conclusions

Figures 5 to 9 present the distribution of glutathione peroxidase and superoxide dismutase in the particular magnets and placebo for 5 series of the fibroblasts' culture. Figure 10 presents number of fibroblasts in the cultures of the particular series, depending on the type of the magnet.

The statistical analysis of results has not shown statistically significant differences either in number of fibroblasts in different cultures or in glutathione peroxidase activity in different cultures with respect to each other/placebo culture.

The experimental tests conducted so far have not covered the influence of constant magnetic field on anti-oxidizing response of cell cultures and fibroblasts in particular. The ever-widening use of permanent magnets in everyday appliances and in industry affect workers employed at PM appliances production, since exposure time to magnetic fields generated by PMs increases. Since magnetic fields intensities at production sites tend to greatly exceed Earth's magnetic field intensity, the potential hazard of their adverse impact is important [12].

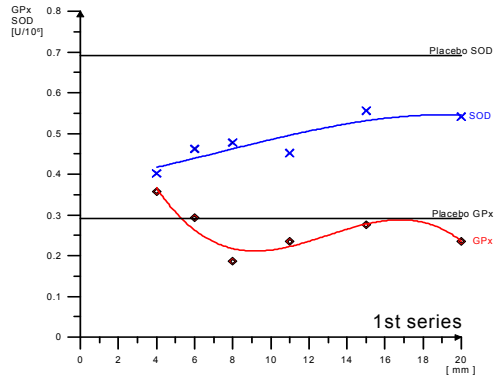

Fig.5. The distribution of glutathione peroxidase and superoxide dismutase in the particular magnets in 1st series and placebo.

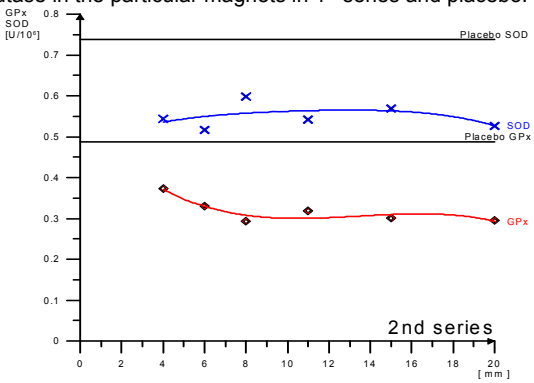

Fig.6. The distribution of glutathione peroxidase and superoxide dismutase in the particular magnets in 2st series and placebo.

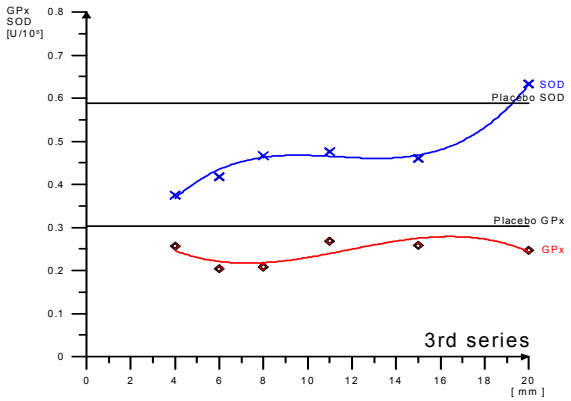

Fig.7. The distribution of glutathione peroxidase and superoxide dismutase in the particular magnets in 3st series and placebo.

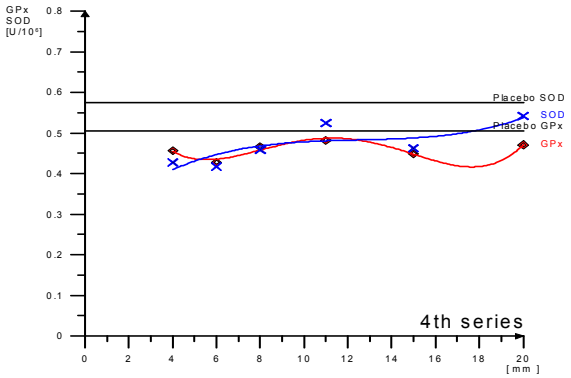

Fig.8. The distribution of glutathione peroxidase and superoxide dismutase in the particular magnets in 4st series and placebo.

Our research makes it possible to assess parameters from the viewpoint of imminent hazards to cell anti-oxidizing response and potential favourable and stimulant impact of constant magnetic fields on this response. The statistical analysis has not shown any statistically significant changes, and hence we may conclude that constant magnetic fields generated by permanent magnets do not exert adverse influence on anti-oxidizing response of cell cultures. This provides a useful starting point for research conducted at tissue and organ level of constant magnetic field impact and aimed at its practical application in therapy [13, 14].

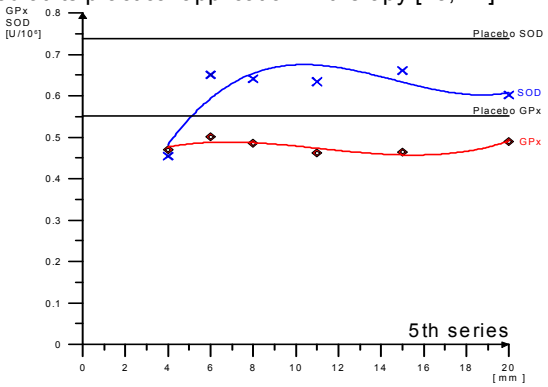

Fig.9. The distribution of glutathione peroxidase and superoxide dismutase in the particular magnets in 5st series and placebo.

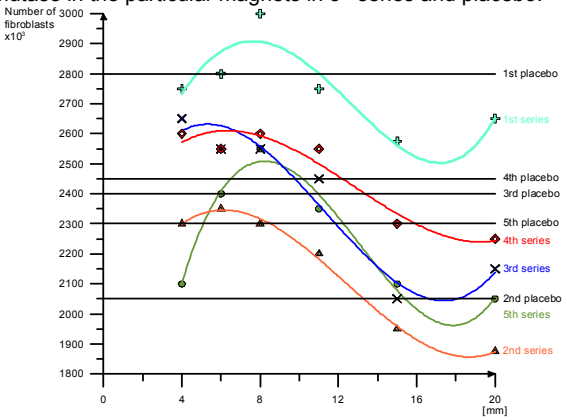

Fig.10. Number of fibroblasts in the cultures of the particular series, depending on the type of the magnet.

Important issue to be considered in the course of research was preservation of constancy and uniformity of constant magnetic field distribution along the culture surface. This condition was fulfilled since measurements of magnetic field intensities in test chambers had been conducted several times before actual experiments took place, before the tests, during the tests and also after tests were completed [15, 16]. All measurement results were identical.

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