Effects of Static Magnetic Field and Pulsed Electromagnetic Field on Viability of Human Chondrocytes in *Vitro*

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Running title: Effects of Magnetic Field on Human Chondrocytes

Summary

Effects of electromagnetic fields (EMFs) on human cell lines were described in numerous studies, but still many questions remain unanswered. Our experiment was designed with the aim of studying the effects of EMFs on the metabolic activity of chondrocytes *in vitro*. Human chondrocyte *in vitro* cultures, cultured in medium supplemented with 20 % fetal calf serum, were exposed to static magnetic field (SMF) (intensity of 0.6 T) and pulsed electromagnetic fields (PEMF) (21.2 MHz period of 15 ms, burst duration of 2 ms, amplification 3 dBm (0.1 V) and maximum output of 250 W) continually for 72 h. After the exposure, viability was determined using the MTT test and compared with a non-exposed control culture. As compared to the control sample the exposure to SMF resulted in a statistically significant increase ($p<0.001$) in viability. However, the increase of viability after PEMF exposure was not significant. This could be due to the frequency dependent effect on

human cells. The experiments demonstrated that magnetic fields, using the above parameters, have a positive effect on the viability of human chondrocytes cultured *in vitro*.

Keywords

Chondrocytes • Magnetic field • Osteoarthritis

Introduction

Osteoarthritis (OA) is the most common articulation disorder of the hyaline cartilage and subchondral bone and is often seen in knee joints by the age of 70. Pathological changes of the weight-bearing joints are common at the age of 40. The disease is responsible for substantial direct and indirect socioeconomic cost. The treatment options are unsatisfactory. Treatment of OA is difficult and only few methods are available. Surgical treatment has limited indications and is not sufficient, nonsurgical treatment involves mostly medication with NSA (non-steroid antiphlogistics) or/and SYSDOA (symptomatic slow acting drugs in osteoarthritis) and adjuvant therapy with different electromagnetic fields. So autologous chondrocyte transplantation is used in its treatment of joint lesions to avoid a secondary OA. The European League Against Rheumatology has now rated pulsed electromagnetic field treatment for OA as 1B evidence and it received a B rating for strength of recommendation. The decision was made since no effects were calculable from previous data, poor practicality of delivery to the patient population in most cases and due to economic considerations.

The effects of EMFs on human cells were described in numerous in vitro and in vivo studies (Ciombor *et al.* 2002, Wilmot *et al*. 1993, Dini and Abbro 2005, Yoshizawa *et al*. 2002, Gál *et al*. 2005). The extracellular matrix of the hyaline cartilage is piezoelectric, so

that it is capable of converting electromagnetic oscillations to mechanical vibrations and vice versa (Jacobson *et al*. 2001). Certain authors have described positive effects on growth and on the production of extracellular matrix in an endochondral ossification model (Ciombor *et al.* 2002; Indouraine *et al*. 2001). On the other hand also negative effects on the chondrogenic layer of the articular zone after exposure to PEMF at a the frequency of 75 Hz in rats were described (Wilmot *et al*. 1993). In contrast to those results SMFat an intensity of 6 mT has a negative effect on cell shape in some human cells (Dini and Abbro 2005).

EMFs have positive effects on the metabolism of tissues through of hyperemization. The aim of our study was to analyze the effects of EMFs on human chondrocytes *in vitro* with the exclusion of that effect. Our choice was based on different studies of EMF effects describing mostly effects of pulsed electromagnetic fields (PEMFs). The selection of chondrocytes, as the cellular object of our study, has also been based on studies reporting about autologous transplantation of *in vitro* cultivated chondrocytes used in treatment of articular lesions (Bačenková *et al*. 2001, Rosocha *et al*. 2002). We preferred static magnetic field (SMF) and pulsed EMFs (PEMFs), because many instruments use this kind of EMFs in clinical practice with positive effects after application in therapy of degenerative arthrosis (Hinman *et al*. 2002).

Methods

Isolation and cultivation of chondrocytes

Human adult articular cartilage was obtained from human tissue donors respecting all ethical rules of Slovak law regulations. Cartilage from the lateral region of the lateral femur condyle in an amount of 3–4 mm^3 was procured. The cartilage from donors not later than 12 h after death (4 males, age 25–50 years) was used. Donors had no autoimmune, or metabolic disease, or malignancy in their history. Cartilage after the procurement was transported in a

medium composed of phosphate buffered saline (PBS) with antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B) (Gibco BRL). The separation of chondrocytes started not later than 6 hours after the procurement, cartilage specimen was cut into small pieces and digested with type II collagenase (1 mg/mL) (Gibco BRL) in a Nutrient mix F 12 (NUTmix) medium (Gibco BRL) by incubation for 16–18 h at 37 ºC. The cell suspension was cleared on a cell sieve (pore diameter 40 μm) (Falcon). Chondrocytes after digestion were rinsed in the medium and centrifuged at 100 ***g*** for 15 min. Viability and number of isolated cells were estimated by vital staining using trypan blue. Cells were plated in complete medium on cell culture flasks (Falcon) for adherent cell lines. Chondrocytes were grown in NUTmix supplemented with 20 % fetal calf serum (FCS) (Gibco BRL), insulin-transferrin-selenium A (10 μL/mL) and antibiotics (100 U/mL penicillin, 100 μg/mL streptomycin and 0.25 μg/mL amphotericin B) (Gibco BRL) and incubated at 37 ºC in 5 % CO_2 atmosphere. Cells normally reached confluence after 14–21 days. Cells were consequently isolated from the cell culture flasks by trypsinization – 0.1 % trypsin/EDTA (Gibco). The cell suspension was then rinsed three times in medium and thereafter resuspended in complete cultivation medium and plated into 75 cm^2 cell culture flasks or used in experiment in 96-well tissue culture plates for adherent cell lines (Sarstedt).

Generators of EMF

Static magnetic field (SMF) with an intensity of 0.6 T (Sabo *et al*. 2002) and high-frequency pulsed EMF (PEMF) with a frequency of 21.2 MHz, period of 15 ms, burst duration 2 ms, amplification 3 dBm (0.1 V) and maximum output of 250 W were used for exposure of chondrocytes in the experiments.

Experiment and data evaluation

Microplate with 32 filled wells with 7000 cells per well and 16 blank wells was inserted into EMF at 37 ºC in 5 % CO_2 atmosphere. After 72 h of exposure to EMF we added to every well 10 μl of 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) at a concentration of 5 mg/mL and incubated for 4–5 h; thereafter we added to each well 100 μl SDS and incubated for subsequent 12 h. The absorbance of the reduced MTT was determined spectrophotometricaly (540 nm) in an ELISA reading device MRX Dynatech (Great Britain).

Statistical analysis

The results were evaluated using STATSDIRECT software ver. 2.5.2 by the Student unpaired t-test and the Mann-Whitney U-test.

Results and Discussion

Three experiments with different starting concentrations of chondrocytes without application of EMFs were performed. The starting concentrations of 14×10^3, 7×10^3, 3.5×10^3 and 1.75×10^3 cells per well were used. In those experiments, the highest metabolic activity after 72 h of cultivation was observed in the cultures with the starting concentration of 7×10^3 cells per well, because chondrocytes did not form yet a compact monolayer and therefore were not affected by cell-cell contact inhibition. Low energy PEMFs affect growth of human chondrocytes *in vitro* (Fiovanti *et al*. 2002), regardless of age and sex of donor (Indouraine *et al*. 2001). Stimulation effects are also dependent on the presence of growth factors (Pezzetti *et al*. 1999) and density of chondrocytes in cell culture (De Mattei *et al*. 2001), which might be due to cell–cell contact inhibition of adherent cell lines. The above data correspond to our experiments with different starting cell numbers per well. The most

significant stimulation of chondrocyte growth was observed after 72 h of cultivation when 7×10^3 chondrocytes per well were applied. Using higher concentrations of chondrocytes per well would cause formation of a compact monolayer earlier than in 72 h. Therefore, we could not observe significant changes of chondrocyte growth because of cell-cell contact inhibition at the higher starting concentration of 14×10^3 cells per well.

On the basis of those experiments the starting concentration of 7×10^3 cells per well was estimated as suitable to carry out the following experiments. Subsequently a series of five experiments with a starting concentration of 7000 cells per well in static and pulsed EMFs were performed. Results of the MTT test are shown in Fig. 1 and 2 and in Tables 1 and 2. All experiments with SMF resulted in a highly significant statistical difference ($p < 0.001$) between the control and the exposed samples of chondrocytes. Those data indicate that SMFs induce a significant increase in metabolic activity in all the experiments after 72-h application of SMF. The results correspond with positive effects of EMFs presented in clinical studies (Hinman *et al*. 2002, Trock *et al*. 1993). The data are in diagreement with the results of a similar study performed on leukemic cells HL-60 (Sabo *et al*. 2002), which suggests differences in the influence of EMFs on different cell types.

Experiments with high-frequency PEMFs show positive effects on chondrocyte metabolism, but these results were not achieved in all experiments which differ from earlier published studies in this field (Ciombor *et al*. 2002, Fiovanti *et al*. 2002, Indouraine *et al*. 2001), where significant effects on proliferation, metabolism, maturation and matrix proliferation of chondrocytes were observed. Statistic analysis demonstrated a highly significant difference in the first, second and fifth experiment ($p < 0.001$). In the third and fourth experiment there was no significant difference found ($p > 0.05$). This phenomenon may be due to the parameters of PEMFs that differed from the above papers on the variability of the response of chondrocytes, depending on the age of the tissue donor. Positive effects on

matrix proliferation were present also after stimulation of chondrocytes with high-frequency PEMFs in other laboratories (Mohamed-Ali *et al*. 1995).

In conclusion, we have demonstrated that static magnetic field and pulsed electromagnetic fields stimulate metabolic activity of chondrocytes *in vitro*. The results presented here suggest that a static magnetic field is more appropriate for stimulation of metabolic activity of chondrocytes than pulsed electromagnetic fields of megahertz frequencies. This fact can be utilized for clinical application of static magnetic field during *in vitro* cultivation of chondrocytes for autologous transplantation in the treatment of articular lesions in the line of evidence based medicine.

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Table 1. Results of experiments with static magnetic field compared with control group.

Table 2. Results of experiments with pulsed magnetic field compared with control group.

Fig. 1. Average absorbance values of MTT test of an experiment with static magnetic field compared with control group.

Fig. 2. Average absorbance values of experiments with pulsed magnetic field compared with control group.

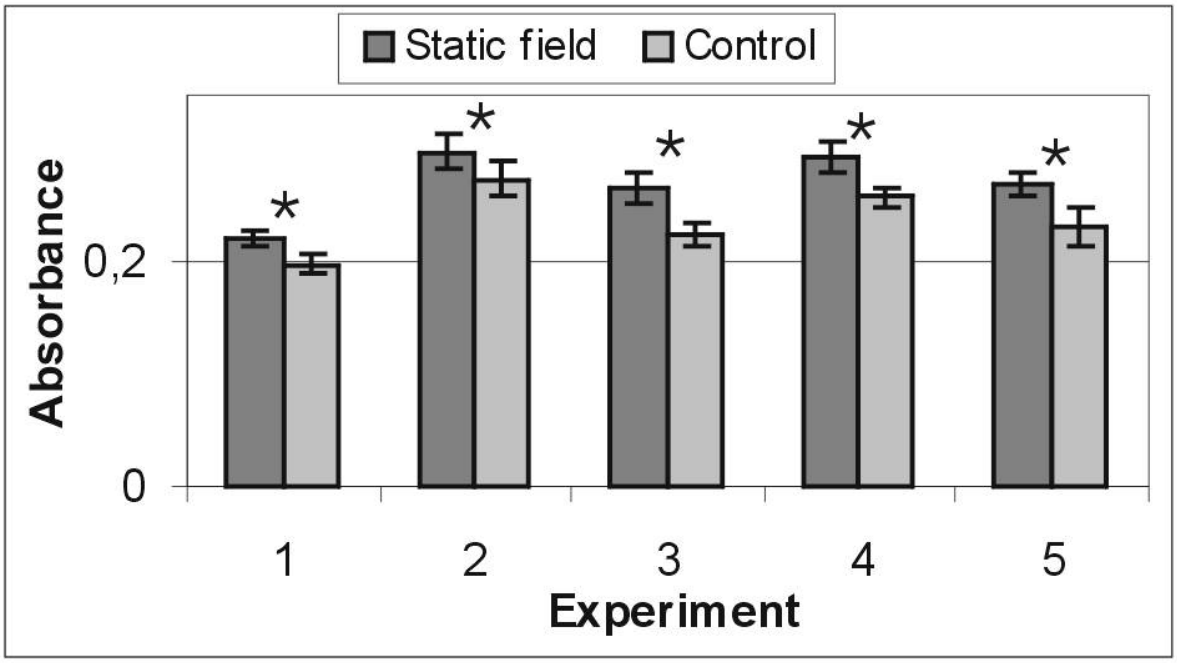

Fig. 1

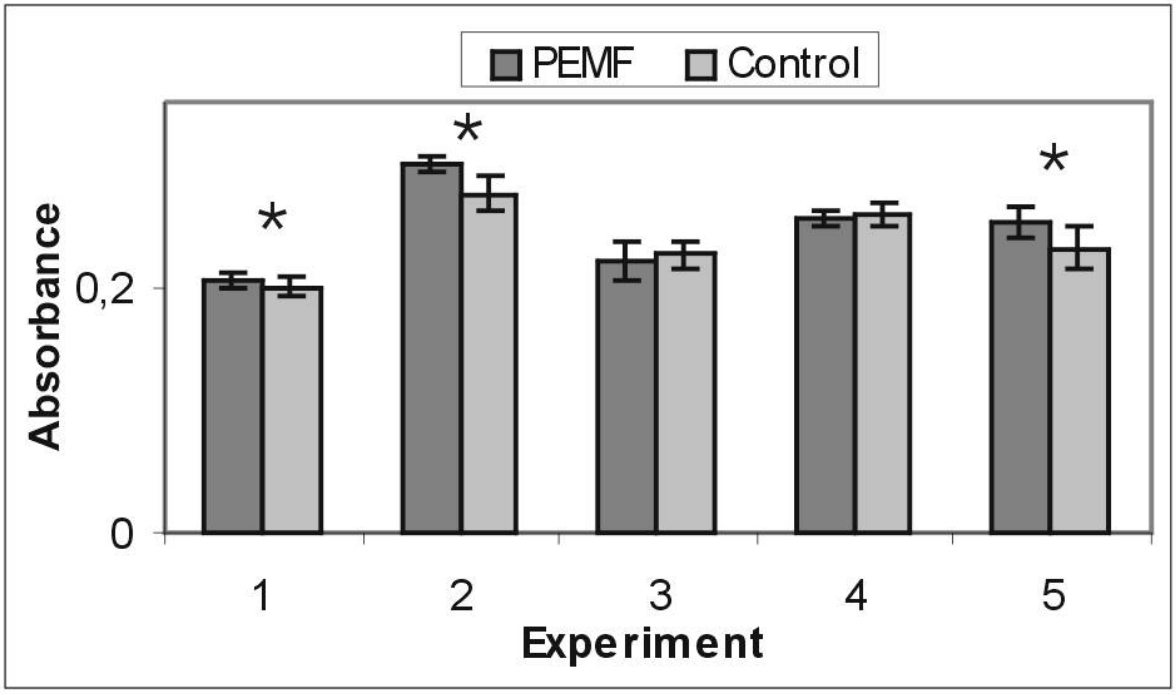

Fig. 2

Table 1.

	1st experiment	2nd experiment	3rd experiment	4th experiment	5th experiment
Difference static field/control	12.40 %	8.91 %	17.99 %	13.76 %	16.34 %
SD	3.01 %	5.27 %	4.87 %	4.48 %	3.86 %

Table 2.

	1st experiment	2nd experiment	3rd experiment	4th experiment	5th experiment
Difference PEMF/control	3.27 %	8.75 %	2.30 %	1.78 %	9.23 %
SD	2.69 %	2.35 %	7.17 %	2.72 %	4.93 %