Effect of Pulsed Electromagnetic Fields on the Bioactivity of Human Osteoarthritic Chondrocytes

Patrick Sadoghi, MD; Andreas Leithner, MD; Ronald Dorotka, MD; Patrick Vavken, MD, MSc

Abstract

Low-frequency pulsed electromagnetic fields (PEMFs) are used for the treatment of human osteoarthritic cells in vivo without knowledge of underlying principles. The authors evaluated the effect of PEMFs on human chondrocytes of the osteoarthritic knee in vitro. Biopsies of the cut femoral condyles after total knee arthroplasty were kept in a standard cell culture medium consisting of Dulbecco’s modified Eagle’s medium: nutrient mixture F-12, 10% fetal calf serum, PenStrept (Mediatech, Inc, Manassas, Virginia), and ascorbic acid for 4 days and randomly split into an exposed group (PEMF for 4 hours daily for 4 days at 75 Hz and 1.6 mT) and a control group. Both groups were retained for biochemical and polymerase chain reaction analysis (glycosaminoglycan and DNA levels). A P value less than .05 was considered significant.

DNA analysis revealed no differences between groups and no increase in content after exposure (P = .88 and .66, respectively). The increase of glycosaminoglycans was 0.4 ± 1.6 ng (95% confidence interval [CI], 1.4 to 0.5) and −0.5 ± 1.8 ng (95% CI, 0.6 to −1.5) in the exposed and control groups, respectively, with no significant difference (P = .24). A smaller decrease of glycosaminoglycan and DNA levels was observed over 4 days in the exposed group compared with the control group, with no statistical significance. The authors concluded that low-frequency PEMFs do not significantly influence the biosynthetic activity of explantcultures of human osteoarthritic cells in vitro. Nevertheless, they may be suitable as an adjuvant to a larger treatment regimen.

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Low-frequency pulsed electromagnetic fields (PEMFs) for tissue repair are commonly used in orthopedic applications.¹–⁷ The underlying principles of PEMF are electromagnetic fields, which interact with the human electromagnetic field, resulting in lower body electricity.⁸–¹² This electricity influences paramagnetic atoms and dipole molecules of human tissue and human body cells.⁸–¹² Therefore, a possible influence on the biochemical processes is assumed. These effects have been used to stimulate bone healing in fractures, non-unions, and prosthetic loosening.⁵,¹³–¹⁹ The analogic effects of PEMF on human fibrous tissue are discussed in the literature, which reveals that PEMF is applied in cases of enthesisopathies (tennis and golfer’s elbows) and degenerative joint diseases (hip and knee osteoarthritis).¹²,²⁰–²⁴

Although the analogic effects of PEMF in cases of hip and knee osteoarthritis have been proven, it is assumed that most of this effect derives from improved tissue perfusion and oxygenation, whereas the actual influence of electromagnetic fields on biosynthetic activity of osteoarthritic chondrocytes is elusive and has not yet been differentiated from analogic effects.¹²,²⁰,²¹ The goal of this study was to evaluate the effect of PEMFs on human osteoarthritic cells of the osteoarthritic knee in vitro. The authors present the first study in the literature evaluating the effect of PEMFs on chondrocytes from osteoarthritic cartilage of the knee joint. They hypothesized that PEMF influenced the biosynthetic activity of explantcultures of chondrocytes from osteoarthritic knees with respect to the expression of glycosaminoglycan and DNA levels.

**Materials and Methods**

The institutional review board and the local ethics committee approved the study protocol, and it conforms to the Declaration of Helsinki.

**Patients and Samples**

Patients undergoing total knee arthroplasty for degenerative joint disease at the senior author’s (P.V.) institution in December 2006 provided informed consent to be included in this study. Exclusion criteria were systemic diseases, inflammatory arthritis, recent knee injections, and previous knee surgery. According to the sample size estimation, 11 patients were included in each group (22 total patients undergoing total knee arthroplasty). The women:men ratio was 8:3. Average patient age was 71 years (range, 62-80 years). Samples could be obtained in all cases, and no samples were lost during the experiment.

**Procedure**

Cut femoral condyles were obtained intraoperatively and aseptically transferred to the laboratory. Punches with a diameter and depth of 5 mm were used to create equal disks of Outerbridge grade 4 human osteoarthritic cartilage. Biopsies were kept in a regularly changed standard cell culture medium consisting of Dulbecco’s modified Eagle’s medium: nutrient mixture F-12 (Life Technologies, Grand Island, New York), 10% fetal calf serum (PAA Laboratories, Linz, Austria), 100 mg/µL of PenStrept (Mediatech, Inc, Manassas, Virginia), and ascorbic acid and allowed to stabilize for 4 days. After day 4, the samples were randomly allocated with a computer-generated sequence into an exposed group and a control group. The allocation was concealed using alphanumeric codes, and all investigators other than the senior author were blinded to the group allocation during the entire experiment and analysis.

The exposed group was exposed to PEMF for 4 hours daily for 4 days. The explants were cultured in 6-well plates, which were kept on a coil connected to an energy source outside the incubator, creating an electromagnetic field with a sinus mode at 75 Hz and at 1.6 mT; this was based on reports by Chang et al⁸ and Luo et al²⁵ showing that the most effective impact of PEMF is observed at approximately 50 Hz and higher. After 4 days of exposure, the coil was disconnected from the energy source, and the samples were kept in the culture for another 4 days. The control group was kept in the same arrangement for a corresponding period, but the coil was not connected to an energy source at any time. Samples were retained for biochemical and polymerase chain reaction analysis before treatment, immediately after the last treatment session, and 4 days after the last treatment session.

To assess the contents of glycosaminoglycans and DNA, papain digests were used for modified dimethylmethylen blue and Hoechst assays. The samples were enzymatically digested in a buffered papain solution, and DNA and glycosaminoglycan contents were detected fluorometrically and photometrically according to Farndale et al²⁶ and Kim et al.²⁷ These preparations and analyses were performed blinded by all investigators, excluding the senior author, with respect to the involved patient, type of sample, and analysis.

**Statistical Analysis**

With respect to the literature, the sample size calculation was based on expected mean differences in proliferation and biosynthetic activity ranging from 12% to 17%, with a 10% SD.³,⁵ To obtain an α of at least 5% and a β of at least 80%, eleven patients were included. In addition, post hoc power was calculated according to the magnitude of observed differences in outcome between the exposed and control groups according to Hoenig and Heisey.²⁸ All data were double entered and matched in Excel (Microsoft, Inc, Redmond, Washington) spreadsheets. Mean differences between pre- and postexposure values and corresponding controls were calculated. All results are presented as mean±SD with 95% confidence intervals (CIs). These variables revealed a normal distribution and similar parameters of variance. To
test the study’s hypothesis, the data were transferred to SPSS version 11 software (SPSS, Inc, Chicago, Illinois) and compared using Student’s 2-way t test for primary and secondary outcomes. A P value less than .05 was considered significant.

**RESULTS**

**Biochemical Analysis**

All samples in both groups were used for final analysis. DNA analysis revealed no differences between groups (P=.88), and the exposed group showed no increase in DNA content after exposure to PEMF (P=.66) (Figure 1). Mean increase in glycosaminoglycans was 0.4 ± 1.6 ng (95% CI, 1.4 to 0.5) for the exposed group and −0.5 ± 1.8 ng (95% CI, 0.6 to −1.5) for the control group (P=.24) (Figure 2; Table). The glycosaminoglycan production per cell (the glycosaminoglycan/DNA ratio) showed no significant difference between the exposed and control groups (P=.62) (Figure 3). Figure 4 shows the hematoxylin-eosin, azan, and alcian stains 4 days after exposure on 50-µm slices. With the sample size of 22, the magnitude of the differences in DNA and glycosaminoglycan measurements between groups was not large enough to reveal more than 80% power.

**DISCUSSION**

The goal of this study was to evaluate the effect of PEMF on human chondrocyte cells from osteoarthritic osteoarthritic knees in vitro. The authors hypothesized that PEMF influences the biosynthetic activity of explant cultures of osteoarthritic cells with respect to glycosaminoglycan and DNA levels. They also wanted to assess whether the effects of PEMF could only be seen during the exposure period or were longer lasting (ie, whether PEMF could be seen as a treatment in itself or an adjuvant to a larger treatment regimen).

Although stimulation of human osteoarthritic chondrocytes with PEMF for 4 days revealed higher glycosaminoglycan and DNA levels in the exposed group than in the control group, these differences did not reach statistical significance; therefore, the results failed to support the primary study hypothesis. In addition, the study did not adequately address whether PEMF decreased the degeneration of cartilage over time.

Various studies have evaluated the potential of pulsed electromagnetic fields on cartilage repair and chondrocyte bioactivity, but definitive conclusions and recommendations cannot be made due to different cell types, tested species, PEMF characteristics, exposure protocols, cell culture media selection, and serum content of these previous investigations. 27-33 Although Nicolin et al34 and De Mattei et al35 reported that PEMF had a significant effect on increasing chondrocyte proliferation and producing extracellular matrix, other studies demon-

![Figure 1: DNA content (µg/mg) of osteoarthritic chondrocytes stimulated with pulsed electromagnetic fields (pEMF) vs control group before treatment (pre-Tx), immediately after treatment (post-Tx), and 4 days after treatment (4d post-Tx).](image1)

![Figure 2: Glycosaminoglycan content (µg/mg) of osteoarthritic chondrocytes stimulated with pulsed electromagnetic fields (pEMF) vs control group before treatment (pre-Tx), immediately after treatment (post-Tx), and 4 days after treatment (4d post-Tx).](image2)

**Table**

<table>
<thead>
<tr>
<th>Group</th>
<th>Glycosaminoglycan Content, mg/mg</th>
<th>Mean±SD (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Baseline</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposed group</td>
<td>8.3±0.8</td>
<td>(6.5-10.1)</td>
</tr>
<tr>
<td>Control group</td>
<td>8.0±0.6</td>
<td>(6.8-9.3)</td>
</tr>
<tr>
<td>Imm after treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposed group</td>
<td>8.3±0.7</td>
<td>(6.8-9.8)</td>
</tr>
<tr>
<td>Control group</td>
<td>8.5±0.8</td>
<td>(6.8-10.2)</td>
</tr>
<tr>
<td>4 d after treatment</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Exposed group</td>
<td>8±1.4</td>
<td>(4.8-11.1)</td>
</tr>
<tr>
<td>Control group</td>
<td>5.4±0.4</td>
<td>(4.4-6.4)</td>
</tr>
</tbody>
</table>

Abbreviation: CI, confidence interval; Imm, immediately.
strated no effect of PEMF on chondrocyte cell proliferation.29,31,35-40 Sakai et al29 reported that glycosaminoglycan depositions responded differently from growth and articular cartilage cells after exposure to PEMF. Pezzetti et al31 revealed that optimal PEMF exposure time for chondrocyte proliferation is serum concentration dependent and that cartilage chondrocytes responded worse to PEMF than to nasal chondrocytes. This is in line with the findings of Elliot et al33 and De Mattei et al,39 who also showed that electromagnetic fields that were generated by coils and oriented vertically and horizontally had different effects on the proliferation of chondrocytes. They also reported that PEMF had no effect on extracellular matrixes,33,39 which agrees with the findings of Chang et al.8 Sunk et al30 reported that the influence of PEMF on chondrocytes of articular cartilage may be temporary, which is in agreement with Pezzetti et al,31 who showed that PEMF-induced cell proliferation of human chondrocytes could only be reported with added culture media.

The current study’s findings imply that PEMF does not significantly influence the bioactivity of osteoarthritic cells with respect to elevated glycosaminoglycan or DNA levels after 1 or 4 days of treatment in the exposed group. Furthermore, the glycosaminoglycan and DNA levels of the control group decreased over time. This decrease may be delayed using PEMF because after 1 day of exposure, the glycosaminoglycan and DNA levels were higher in the exposed group. Nevertheless, after 4 days of treatment, this difference was not observed because the glycosaminoglycan and DNA levels decreased to the same level in the exposed group. Therefore, the authors feel that PEMFs have no direct influence on the bioactivity of human osteoarthritic cells with respect to glycosaminoglycan and DNA levels, but they may be seen as an adjuvant treatment to a larger therapeutic regimen.8

The authors analyzed the biological activity by glycosaminoglycan measurements because procollagen and glycosaminoglycans are the original product of the cell and therefore reflect cellular metabolism independent from extracellular protein processing.29 However, to evaluate the production per cell, the authors also measured DNA expression and the glycosaminoglycan/DNA ratio according to Wolf et al.41

The current study had several limitations. The authors did not differentiate between osteoarthritic and physiological human chondrocytes, evaluating the influence of PEMF on physiological chondrocytes for comparison. The use of healthy chondrocytes would have been of general scientific interest, and it is to be expected that these cells would have had the potential for a stronger response to treatment than osteoarthritic cells; however, the goal of this study was to translate a clinical scenario into a laboratory setup, rendering the biology of healthy cells meaningless.

**Figure 3:** Glycosaminoglycan (GAG) content (µg/mg) per DNA content of osteoarthritic chondrocytes stimulated with pulsed electromagnetic fields (PEMF) versus control group before stimulation (pre-Tx), directly after (post-Tx), and 4 days after stimulation (4d post-Tx).

**Figure 4:** Hematoxylin-eosin (I), azan (II), and alcian (III) stains of human osteoarthritic chondrocytes 4 days after treatment (original magnification ×100 [left column] and ×200 [right column]) on 50-µm slices.
A longer exposure, both in number of total days and hours per day, could have resulted in a stronger response, but the authors chose 4 days to ensure that all explants were stable and that the results would not be biased by cell culture conditions. The exposure duration of 4 hours for 4 days was chosen because it is representative of a clinical application, whereas longer exposures, such as 24 hours per day, cannot be reproduced in a clinical setting with human patients.

In addition, because this study addressed a complex problem for a limited time, the authors stress that the findings are considered preliminary results and that further investigations and in-depth studies are needed to clarify this issue. This is further underlined by the fact that the intensity used for PEMF differs between studies, and no consensus on an optimal intensity has yet been reached in the literature. Also, although an a priori sample size calculation was performed, a post hoc power analysis did not reveal more than 80% power. However, to the authors’ knowledge, this is the first study in the scientific literature evaluating the effect of PEMF on chondrocytes from osteoarthritic cartilage of the knee joint.

**CONCLUSION**

Low-frequency PEMFs do not significantly influence the biosynthetic activity of explantcultures of human osteoarthritic cells in vitro. Nevertheless, they may be suitable as an adjuvant to a larger treatment regimen.

**REFERENCES**


