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*Original Article*

# Responses of human normal osteoblast cells and osteoblast-like cell line, MG-63 cells, to pulse electromagnetic field (PEMF)

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**Abstract**

The objective of this *in vitro* study is to investigate the effect of pulsed electromagnetic field (PEMF) on cellular proliferation and osteocalcin production of osteoblast-like cell line, MG-63 cells, and human normal osteoblast cells (NHOC) obtained from surgical bone specimens. The cells were placed in 24-well culture plates in the amount of $3x10^4$ cell/wells with 2 ml αMEM media supplemented with 10% FBS. The experimental plates were placed between a pair of Helmoltz coils powered by a pulse generator (PEMF, 50 Hz, 1.5 mV/cm) in the upper compartment of a dual incubator (Forma). The control plates were placed in the lower compartment of the incubator without Helmotz coils. After three days, the cell proliferation was measured by the method modified from Mossman (J. Immunol Methods 1983; 65: 55-63). Other sets of plates were used for osteocalcin production assessment. Media from these sets were collected after 6 days and assessed for osteocalcin production using ELISA kits. The data were analyzed using a one-way analysis of variance (ANOVA). The results showed that MG-63 cells from the experimental group proliferated significantly more than those from the control group (20% increase, $p<0.05$). No significant difference in osteocalcin production was detected between the two groups. On the other hand, NHOC from the experimental group produced larger amount of osteocalcin (25% increase, $p<0.05$) and proliferated significantly more than those from the control group (100% increase, $p<0.05$). In conclusion, PEMF effect on osteoblasts might depend on their cell type of origin. For osteoblast-like cell line, MG-63 cells, PEMF increased proliferation rate but not osteocalcin production of the cells. However, PEMF stimulation effect on human normal osteoblast cells was most likely associated with enhancement of both osteocalcin production and cell proliferation.

**Key words**: osteoblast, MG-63, osteocalcin, proliferation, pulse electromagnetic field

## 1. Introduction

Although pulse electromagnetic field stimulation has been used to promote the healing of chronic nonunion and fractures with delayed healing, relatively little is known about its effects on osteogenic cells or the mechanisms involved. The purpose of this study was to examine the response of osteoblast cells to a PEMF stimulus, mimicking that of a clinically available device we have at Prince of Songkla University Hospital with pulse generator at 50 Hz and 1.5 mV/cm. This wave form has been shown to be clinically beneficial to our patients, but its mechanism of action remains unclear. In this study, primary osteoblasts were cultivated

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from bone marrow and the effect of PEMF on these cells was evaluated and compared to that on human MG-63 osteosarcoma cell line.

In 1981, Jackson and co-worker demonstrated the successful autograft from tibia to alveolopalatal defect in cleft palate children. Burwell, in 1985, reported that there were two types of osteogenic precursor cells. The first type, determined osteogenic precursor cells (DOPC), was derived from bone marrow. The second type, inducible osteogenic precursor cells (IOPC), was derived from connective tissue and blood cells. In 1989, Beresford noted that precursor cells in bone marrow could initiate bone formation. Connolly and colleagues (1989) reported their studies on the development of an osteogenic preparation. They found that whole marrow could initiate bone healing within 5 weeks after bone graft. In addition, if there was a bone marrow preparation by Ficoll-paque and these cells were cultured in a concentration of 1,075 cells/cm$^2$, calcium deposit would behigher. Gallager and co-authors (1996) explained the method of isolation and cultivation of osteoblasts from human bone. First they washed the bone marrow, minced to small pieces and cultured in media containing serum, L-ascorbic acid, vitamin K and calcitriol. After the cells grew confluently, they were trypsinized and tested for osteoblast properties including alkaline phosphatase and osteocalcin synthesis.

The stimulation effect of PEMF on bone formation is still inconclusive. Pezzetti and co-workers (1999) studied effect of PEMF on human chondrocytes. They applied 0.02 mV/cm of PEMF to the cells. The measurement of incorporation of [$^3$H]-thymidine was then carried out to evaluate DNA synthesis. Their results showed that PEMF increased DNA synthesis in osteoblasts resulting in stimulation of bone formation. Recently, Diniz *et al.* (2002) reported that the stimulatory effect of PEMF on osteoblasts was most likely associated with enhancement of the cellular differentiation, but not with the increase in the number of cells.

Therefore, this study was carried out to determine whether the PEMF stimulatory effect on bone tissue-like formation was associated with an increase in the number of cells and/or with an enhancement of the cellular differentiation.

## 2. Material and Methods

### 2.1 Cultivation of primary osteoblasts

Excised bone was collected from ten patients undergoing orthopedic surgery. This project was approved by PSU Ethics Committee and consents were obtained from all ten patients before the surgeries were carried out. The bone was washed several times, then minced into 2-mm fragments and placed in 60-mm plastic Petri dishes. The culture media was αMEM supplemented with 10% FBS, 50 μg/ml penicillin G and 50 ηg/ml amphotericin B. The dishes were then placed in an incubator setting at 37°C in a humidified atmosphere containing 5% $CO_2$.

The medium was changed twice weekly. When the cells growing out from the explants reach confluence, they were trypsinized with 0.08% trypsin and 1.4% EDTA in PBS for secondary culture. The cell monolayers were sub cultured every 2 weeks. All the experiments were performed using osteoblast cells between the 3$^{rd}$ and 10$^{th}$ passage.

### 2.2 Evaluate the effect of PEMF on human primary osteoblasts and osteoblast-like cell line MG 63

The human primary osteoblasts were obtained as described above whereas osteoblast-like cell line MG 63 was purchased from ATCC (The American Type Culture Collection). Each type of osteoblasts was placed in multiple-well plates and set in a dual tissue culture incubator (Forma). The experimental plates were placed between a pair of Helmoltz coils powered by a pulse generator at 50 Hz and 1.5 mV/cm in the upper compartment. The control plates were placed in the lower compartment of the incubator without Helmotz coils. Then the effect on the cells was investigated in terms of MTT assay and osteocalcin production.

### 2.3 MTT (tetrazolium) colorimetric assay

The cell proliferation assay was performed using a method modified from the original MTT colorimetric assay described by Mossman (8). Each type of osteoblasts was seeded on 24-well culture plates (NUNC) at 3x10$^4$ cells/well in 2 ml αMEM medium as described above. After 3 days MTT assay was performed. Briefly, the medium was removed and replaced with 300 μl/well αMEM (without phenol red) medium. Fifty ml of MTT (3-[4, 5-dimethylthiazol-2-yl]-2, 5-diphenyltetrazolium bromide, Sigma) at a concentration of 5mg/ml in PBS was added to each well. The plates were then incubated at 37°C in 5% $CO_2$ for 4 h. After incubation, the medium was removed and 1 ml of dimethyl sulfoxide (Sigma) was added to each well in order to solubilize the blue formazan. After solubilization, absorbance was read immediately using a spectrophotometer at 570 nm.

### 2.4 Osteocalcin Production

Osteocalcin secreted by both types of osteoblasts into culture supernatants was measured by ELISA. Each type of osteoblasts (3x10$^4$) was seeded into each well of 24-well tissue culture plates in complete medium. After overnight adherence the cells at 37°C, the medium was removed, the cells were washed with PBS, and then 2 ml of αMEM containing 100 μg/ml gentamicin was added. After 6 days incubation at 37°C, the supernatants were collected for assay using Osteocalcin ELISA kit (Biomedical Tech, Stoughton, MA.). Absorbency was read using ELISA reader at 450 nm.

### 2.5 Data analysis

The data were analyzed using a one-way analysis of

variance (ANOVA).

## 3. Results

### 3.1 Effect of PEMF on cell proliferation

In order to investigate the effect of pulse electromagnetic fields on cell proliferation, osteosarcoma MG 63 and human osteoblast like cells were seeded into 24-well tissue culture plates at a cell density of approximately $3x10^4$ per well for 3 days under PEMF. MTT colorimetric assay was performed as described. The MG63 human osteoblast-like cells from the experimental group showed significant higher rate of proliferation than those from the control group (20% increase, $p<0.05$) as can be seen from Figure 1. Figure 2 demonstrates that the amount of human primary osteoblasts from the experimental group is significant higher than control (100% increase, $p<0.05$).

### 3.2 Effect of PEMF on Osteocalcin Production

In order to investigate the effect of pulse electromagnetic fields on osteocalcin production, osteosarcoma MG 63 and human osteoblast like cells were seeded into 24-well tissue culture plates at a cell density of approximately $3x10^4$ per well for 6 days under PEMF. No significant difference in osteocalcin production of MG-63 human osteoblast-like cells was detected between the experiment (PEMF) groups and the control (no PEMF) groups as shown in Figure 3. On the other hand, human normal osteoblast cells from the experimental group produced larger amounts of osteocalcin (25% increase, $p<0.05$) than those from the control group as demonstrated in Figure 4.

## 4. Discussion

We have exposed cultured bone cells to a pulsed electromagnetic field (PEMF). We used two different types of osteoblasts, human MG 63 osteosarcoma cell line, and human normal osteoblast cell (NHOC) obtained from surgical bone specimens. The cells were placed in 24-well culture plates and set in a tissue culture incubator between a pair of Helmoltz coils powered by a pulse generator (50 Hz, 1.5 mV/cm) for different periods of time. MTT assay was used to evaluate cell proliferation and osteocalcin production was measured to assess cell differentiation. We found that osteosarcoma MG 63 and human osteoblast like cells responded differently to PEMF exposure. PEMF exposed MG 63 cells increased their proliferation rate but not osteocalcin production. On the other hand, human osteoblast like cells proliferated more and produced higher amount of osteocalcin when exposed to PEMF.

Our results of MG-63 are similar to those from Chang and co-workers reported in 2004. They found no effect on the extracellular matrix synthesis of neonatal mouse calvaria bone cells, while the osteoprotegerin (OPG) mRNA expression was up-regulated and the receptor activator of NF-kappaB ligand (RANKL) mRNA expression was down-regulated, compared to the control. They concluded that the effect of PEMF stimulation on the bone tissue formation was most likely associated with the increase in the number of cells, but not with the enhancement of the osteoblast differentiation. However, these results were contradicted with

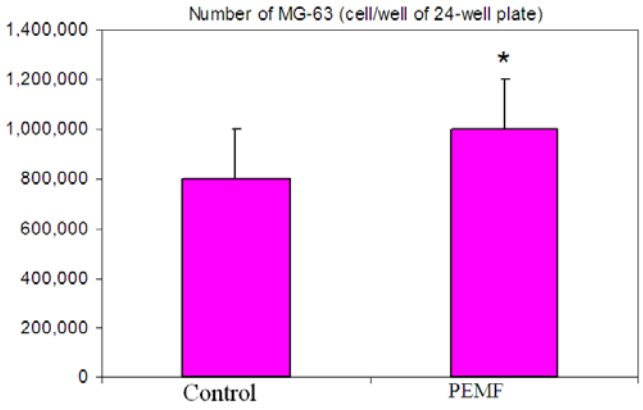


**Figure 1. Effect of PEMF to cell proliferation of MG-63 human osteoblast-like cells. The amount of MG-63 human osteoblast-like cells set in the upper compartment of the incubator between a pair of Helmoltz coils powered by a pulse generator (50 Hz, 1.5 mV/cm) showed higher rate of proliferation than those from the control group (20% increase, $p<0.05$). The control plates were placed in the lower compartment of the incubator without Helmoltz coils. This figure represents 3 experiments.**

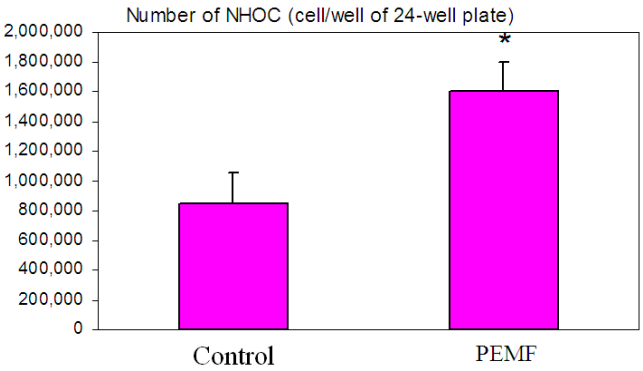


**Figure 2. Effect of PEMF to cell proliferation of human normal osteoblast cell (NHOC). The amount of NHOC set in the upper compartment of the incubator between a pair of Helmoltz coils powered by a pulse generator (50 Hz, 1.5 mV/cm) showed higher rate of proliferation than those from the control group (100% increase, $p<0.05$). The control plates were placed in the lower compartment of the incubator without Helmotz coils. This figure represents 3 experiments.**

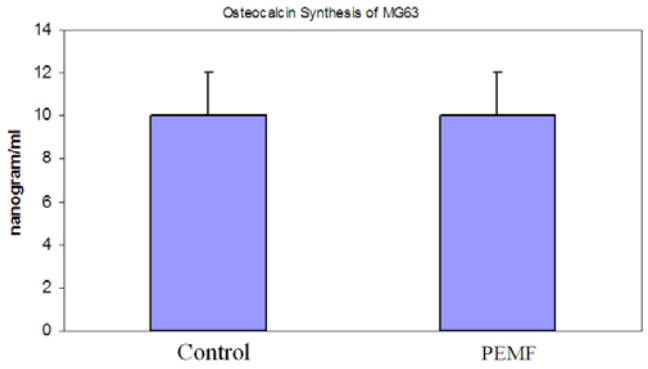


**Figure 3. Effect of PEMF on osteocalcin production of MG-63 human osteoblast-like cells. The amount of osteocalcin production secreted from MG-63 human osteoblast-like cells set in the upper compartment of the incubator between a pair of Helmoltz coils powered by a pulse generator (50 Hz, 1.5 mV/cm) is not different from that of MG-63 human osteoblast-like cells placed in the lower compartment of the incubator without Helmotz coils. The results are expressed as mean ± SD of 3 experiments.**

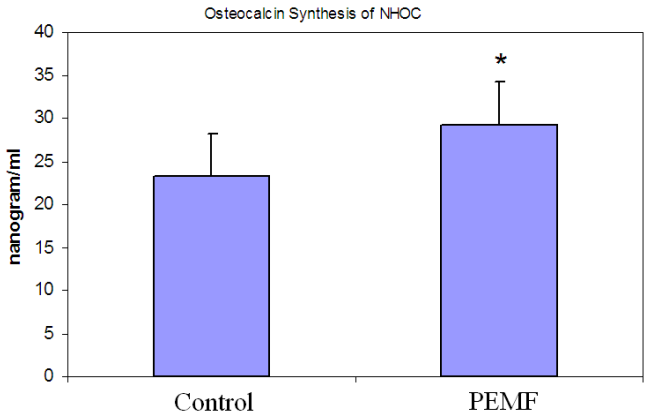


**Figure 4. Effect of PEMF on osteocalcin production of human normal osteoblast cell (NHOC). The amount of osteocalcin secreted from NHOC set in the upper compartment of the incubator between a pair of Helmoltz coils powered by a pulse generator (50 Hz, 1.5 mV/cm) is higher than those secreted from the controls (25% increase, *p<0.05).* The control plates of NHOC were placed in the lower compartment of the incubator without Helmotz coils. The results are expressed as mean ± SD of 3 experiments.**

MC3T3-E1 cell line recently reported by Diniz and co-workers in 2002. They showed that the stimulatory effect of PEMF was most likely associated with enhancement of the cellular differentiation, but not with an increase in the number of cells. This conclusion was based on their finding that PEMF treatment of osteoblasts in the active proliferation stage accelerated cellular proliferation, enhanced cellular differentiation, and increased bone tissue-like formation. In addition, PEMF treatment of osteoblasts in the differentiation stage enhanced cellular differentiation and increased bone tissue-like formation. However, PEMF treatment of osteoblasts in the mineralization stage decreased bone tissue-like formation. Therefore, they concluded that PEMF had a stimulatory effect on the osteoblasts in the early stages of culture, which increased bone tissue-like formation. In the year 2000, Lohmann *et al.* also reported that the pulsed electromagnetic signal caused an enhanced differentiation of MG63 osteoblast-like cells as evidenced by decreased proliferation and increased alkaline phosphatase-specific activity, osteocalcin synthesis, and collagen production.

In our studies, PEMF enhanced cell proliferation of both MG 63 and NHOC. However, the effect on cell differentiation was observed only in NHOC. These observations are contradicted by those from the studies of De Mattei *et al.* in 1999 and Sollazzo *et al.* in 1997. They found different responses of NHOC and human osteosarcoma cell lines to PEMF in terms of cell proliferation, whereas in this study we found a difference in terms of cell differentiation. Further studies need to be carried out to clarify the controversy over these issues in order to understand the real underlying mechanism of PEMF in bone formation.

## 5. Conclusion

In this study, the effect of PEMF (50 Hz, 1.5 mV/cm) on human normal osteoblast cells and human osteoblast-like cells, MG63, was evaluated. The results showed that PEMF effect on osteoblasts might depend on their cell type of origin. For osteoblast-like cell line, MG-63 cells, PEMF increased proliferation rate but not osteocalcin production of the cells. On the other hand, PEMF stimulation effect on human normal osteoblast cells was most likely associated with enhancement of both osteocalcin production and cell proliferation.

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