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# Pulsed electromagnetic field (PEMF) treatment for fracture healing

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## ABSTRACT

**Background**

Pulsed electromagnetic field (PEMF) therapy is used as an adjuvant therapy for promoting fracture healing. We performed this study in an animal model to determine the efficacy of PEMF therapy in fracture healing and to identify and quantify growth factor TGF-ß1 and TGF-ß2 by quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis.

**Methods**

The study was conducted in Wistar rats, with six study and six control animals. The right midshaft femur of all 12 animals was osteotomized to create a gap of 5 mm and transfixed with a Kirshner wire. Baseline quantitative RT-PCR analysis was done with the removed bone. The six animals from the study group were given PEMF of 1 hour duration, twice a day for 6 weeks. Radiographic images were obtained in all animals at 1, 3, and 6 weeks. At 6 weeks all the animals were euthanized, and 1.5 cm of bone, including the previous osteotomy site, were analyzed. RT-PCR analysis for TGF-ß1 and TGF-ß2 and histological analysis was done to compare bone healing between the control and study animals. The bone weight also was measured.

**Results**

A significant increase in bone formation was shown by histomorphometry in the study group. Increased expression of TGF-ß1 and TGF-ß2 was present in the study animals. Although bone weight had increased in the study group, it was not statistically significant.

**Conclusions**

PEMF therapy may be useful as an adjuvant treatment for fracture healing. The mechanism of healing seems to be secondary to local expression of growth factor TGF-ß at the fracture site.


**Keywords**

fracture healing, growth factor production, pulsed electromagnetic field therapy, TGF-ß

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## INTRODUCTION

Electric and electromagnetic devices were created to treat fracture nonunions based on observations that electric fields occur in mechanically loaded bones. Beneficial effects of electromagnetic fields include nerve regeneration[1] and wound healing.[2] Preliminary data even suggest possible benefits in neurological disorders.

Electromagnetic fields do modify biological behavior by inducing electrical changes within and around the cell.[3] Animal and cell culture models suggest that endochondral ossification is stimulated by increasing cartilage mass and production of transforming growth factor-ß1 (TGF-ß1).[4] Also, in vitro exposure of osteoblasts to electromagnetic fields stimulates the secretion of numerous growth factors including bone morphogenetic proteins (BMP) 2 and 4,[5] transforming growth factor-ß (TGF-ß), and insulin-like growth factor-II (IGF-II).[6]

Although in vitro culture models have shown an increase in growth factor production, there are few in vivo models to prove this.[7] The purpose of this study was to determine the efficacy of pulsed electromagnetic field (PEMF) in fracture healing and to identify and quantify TGF-ß by quantitative real-time-polymerase chain reaction (RT-PCR) analysis in Wistar rats.

## MATERIALS AND METHODS

A total of 12 male Wistar rats were used. The study was approved by the Institutional Animal Ethics Committee. Six rats formed the study group and six rats the control group. Under anesthesia, the right femur of all 12 animals was exposed from the lateral side. The midshaft of the femur was osteotomized, and 5 mm of bone were removed to create a gap. To keep the bone ends distracted and to prevent abnormal mobility, the femur was fixed with a 1.2-mm Kirschner wire through the defect. The removed bone samples were collected and snap frozen immediately in liquid nitrogen and stored at −70°C. Postoperatively, all rats were given intravenous Cefazolin and intramuscular Ketoprofen for 2 days.

The animals were housed separately, and principles of laboratory animal care followed. Food and water were given ad libitum. Six animals from the study group were given PEMF of 1 h duration twice a day for 6 weeks from day 1. Six animals from the control group were sham treated with only immobilization for 1 hour without PEMF. Radiographs were taken to assess the progression of healing in all animals at 1, 3 and 6 weeks.

At 6 weeks, the animals were euthanized with lethal dose of sodium thiopentone. The midshaft of the femur was exposed, and 1.5 cm of bone including the previous osteotomy were taken for analysis. The weight of bone was measured after carefully removing the soft tissues, using a digital weighing machine (Precisa XB 320 M, Precisa Instruments AG, Dietikon Switzerland). The specimens were cut into half longitudinally, and one half was stored in liquid nitrogen for quantitative RT-PCR analysis, and the other was stored in formalin acetic acid (FAA) for histomorphometric analysis.

The height of maximal callus response was measured in millimeters radiographically by two independent observers, including the author, at 1, 3 and 6 weeks. The digital radiographs were taken in a standard format. They were visualized using the software, GE Path Speed Web Version 8.1 (GE Medical Systems, Milwaukee, WI). Bone histomorphometry was done using Image-Pro® Plus 5.1 software (MediaCybernetics Inc., Bethesda, MD). Quantitative RT-PCR analysis at 0 and 6 weeks for TGF-ß1 and TGF-ß2 also were assessed.

## Apparatus

The pulsed magnetic field enclosure used for this experiment was designed and fabricated according to the parametrical equations of Fanselau and Braunbeck. This apparatus is made up of two larger (inner) coil frames and two smaller (outer) coil frames. This four-coil system is a modified version of the classical Helmholtz coil. The four coils have the same number of turns and are connected in a ''series-aiding'' configuration.

Pulsed electric current from a signal generator energizes the coil system such that the strength, frequency and waveform of the output current can be controlled to any set of desired values, thus offering along the axis of the coil system a pulsating magnetic field of any desired frequency, intensity and wave form. The animals were housed in plastic cages in a completely nonmetallic environment within this highly uniform volume of pulsed magnetic field (Figure 1). The set parameters for the PEMF were frequency 1 Hz, voltage 20 V, sine wave and current +/−30 mA.

## RNA Extraction From Rat Bone

The rat bone samples were collected and snap frozen immediately in liquid nitrogen and stored at −70°C until ready for use. The samples were then ground in RNAase free sterile mortar and pestle using liquid nitrogen to keep the bone frozen (preserving RNA). Once homogenized in the pestle and mortar containing Trizol Reagent® (Sigma, Bangalore, India), the samples were transferred into an RNAase free sterile Eppendorf tube. The total RNA was further isolated from these samples using Trizol Reagent as per the manufacturer's instructions.

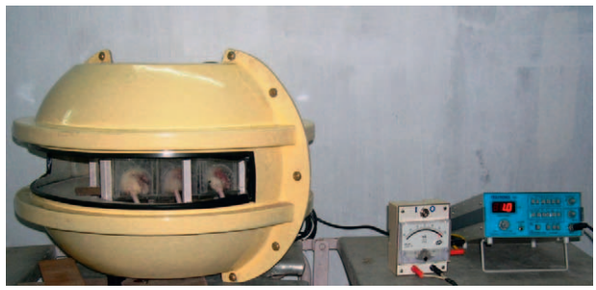

**FIGURE 1.** Photograph of the plastic immobilization cage with animals and the pulsed magnetic field generator.

## cDNA Conversion

Reverse transcription of RNA was performed in a final volume of 20 µl containing 0.5 mM of each nucleotide triphosphate, 40 units of RNAase inhibitor (Ambion, AB Biosystems, Haryana, India) 50–100 ng of Random Hexamers (Amersham Pharmacia, Chennai, India) and 200 units of Moloney Murine Leukemia virus Reverse Transcriptinase (Finnzymes, Chennai, India) to which 15 µl of the extracted RNA or $H_2O$ were added. Samples were incubated at 42°C for 1 hour. Subsequently 1 µl of 1 in 5 diluted cDNA sample was used for amplification.

## Quantitative RT-PCR

Quantitative PCR was performed using the Chromo4 system (MJ Research, Biorad, Hercules, CA). 20 µl of the PCR mix contained 1X buffer containing $MgCl_2$, 0.2 µl of titanium Taq DNA polymerase (Clontech, BD Biosciences, Delhi, India), 200 µM of dNTPs (Finnzymes, Labmate, Chennai, India), 250 nM of reverse primers each of GAPDH, TGF-ß1 and TGF-ß2 given in Table 1 (Sigma Genosys, Bangalore, India), and 1:50 000 diluted SYBR green dye (Molecular Probes, Invitrogen, Gangalore, India). The thermal cycling protocol was as follows: initial denaturation at 95°C for 30 s, followed by 45 cycles of denaturation at 94°C for 30 s, annealing at 61°C for 30 s for GAPDH, TGF-ß1 and TGF-ß2 and extension at 72°C for 30 s, followed by fluorescence measurement. Product specificity was confirmed by the presence of a single peak in the melting curve analysis. The gene expression of TGF-ß 1 and 2 were quantitated by normalizing the CT values of TGF-ß 1 and 2 with that of the housekeeping gene, GAPDH. The results were analyzed using $2^{-\Delta\Delta}$CT method.[8]

**TABLE 1.** Primer sequence

| |
|---|
| GAPDH |
| g1:agatggtgaaggtcggtgtc |
| g2:attgaacttgccgtgggtag |
| TGF beta-1 |
| ß1F:GCGGACTACTACGCCAAAGA |
| ß1R:ACTGCTTCCCGAATGTCTGA |
| TGF beta-2 |
| ß2F:GCAGAGTTCAGGGTCTTTCG |
| ß2R: GCTGGGTTGGAGATGTTAGG |

## Histopathology and Histomorphometric Evaluation

All specimens were decalcified in 10% formic acid, processed in automatic tissue processor Leica ASP300 (Leica Microsystems GmbH, Wetzlar, Germany) and embedded in paraffin. Longitudinal sections of 5 micrometer thickness were cut and stained with hematoxylin and eosin for histological examination. Images were captured using a digital camera Evolution™ MP (MediaCybernetics, Bethesda, MD). Digitized images were analyzed using Image-Pro® Plus 5.1 software (MediaCybernetics, Bethesda, MD). A standard area adjacent to the osteotomy site was mapped out. The areas of trabecular bone and empty spaces were hand drawn by the co-investigators (SA & MM) and the total bone volume was calculated.

## Statistical Methods

SPSS 11.0 (SPSS, Inc., Chicago, IL, USA) for windows was used for statistical analysis with the help of a statistician. Independent samples were compared using the Mann-Whitney test, and paired samples were compared using Wilcoxon's signed ranks test. A *P*-value less than 0.05 was considered statistically significant.

# RESULTS

## Histopathology

In the study rats, an increase in new bone formation was noted at the osteotomy site. Bone deposition was comprised mostly of trabecular bone and soft callus (Figure 2). A moderate amount of osteoblasts and a few osteoclasts were noted. Osteoblastic activity increased (Figure 3). Hemopoietic cells were present in the marrow spaces (Figure 4)

In the control rats, the edge of the osteotomy sites contained both cortical and trabecular bone with hard-tissue callus (Figure 5), and a moderate amount of osteoblastic activity was noted in all. Enchondral ossification also was appreciated (Figure 6).

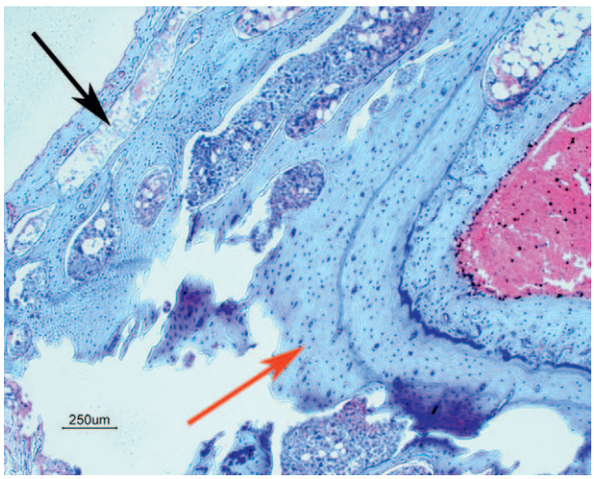


**FIGURE 2.** Study animal with new trabecular (red arrow) and cortical bone (black arrow) at the osteotomy site (hematoxylin and eosin stain).

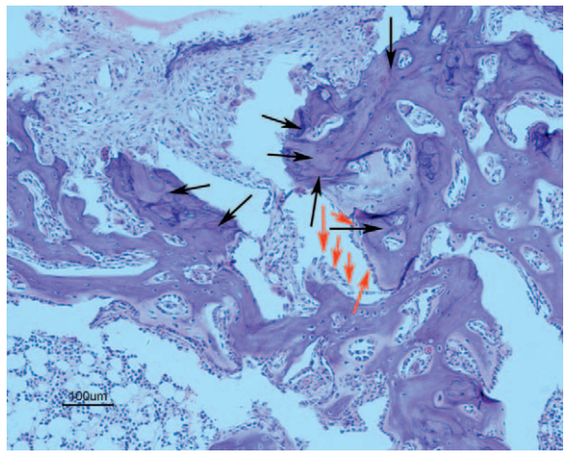


**FIGURE 3.** Study animal with new bone (black arrows) and high osteoblastic (red arrows) activity (hematoxylin and eosin stain).

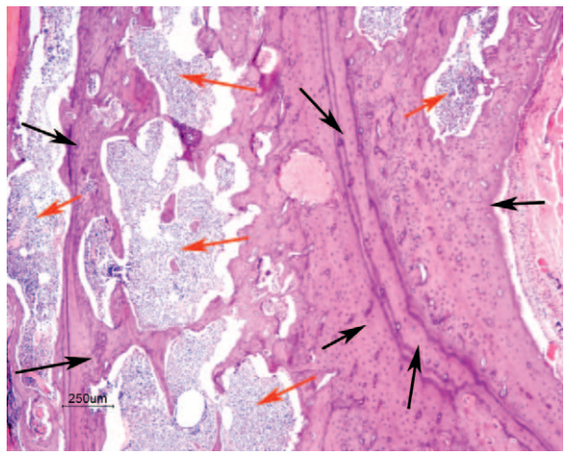


**FIGURE 4.** Study animal with cortical new bone, disoriented arrangement (black arrows) and active bone marrow (red arrows) (hematoxylin and eosin stain).

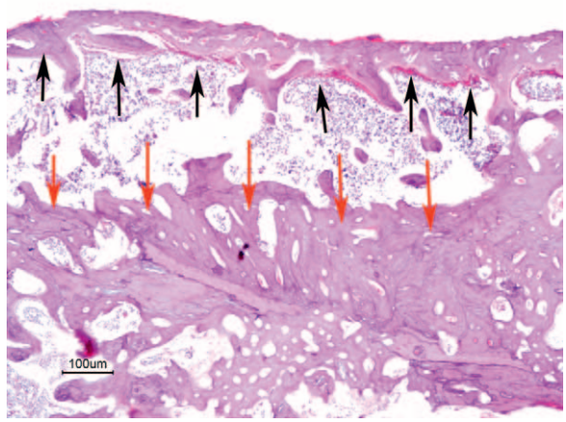


**FIGURE 5.** Control animal with cortical (black arrows) and trabecular bone (red arrows) formation (hematoxylin and eosin stain).

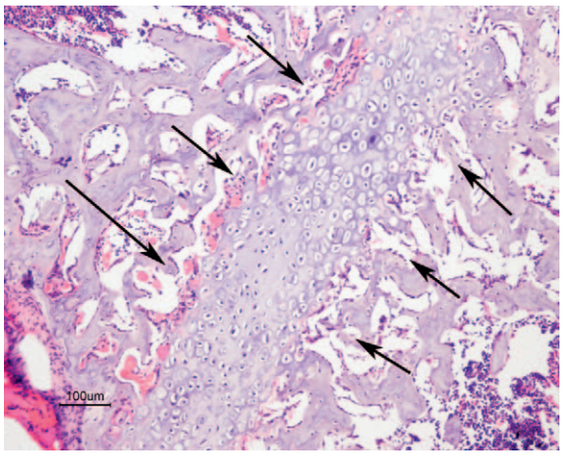


**FIGURE 6.** Control animal with enchondral ossification (black arrows) (hematoxylin and eosin stain).

## Radiographic Callus Measurement

The radiographic callus measurement values doubled in both groups from week 1 to week 6. In control animals it increased from 2.91 mm to 5.10 mm, and in test animals it increased from 2.93 mm to 5.58 mm. The difference in increase in both groups at 6 weeks was not statistically significant ($P = 0.18$).

Radiographic callus measurement at the osteotomy site did not show a statistically significant difference because only the height of the callus at the fracture site was measured. Volume could not be measured (Figure 7). Mineralization of the callus may not have been sufficient to cast a radiographic image, whereas histomorphometry was able to identify and measure the volume of total callus response. Therefore, we used histomorphometry as the method for measuring total bone volume.

## Histomorphometry

The total bone volume calculated by Image-Pro® Plus 5.1 software was significantly increased in the study animals ($P = 0.015$). The mean total volume of bone in the study animals was 1000608 $\mu m^2$ and in control animals, 328091 $\mu m^2$.

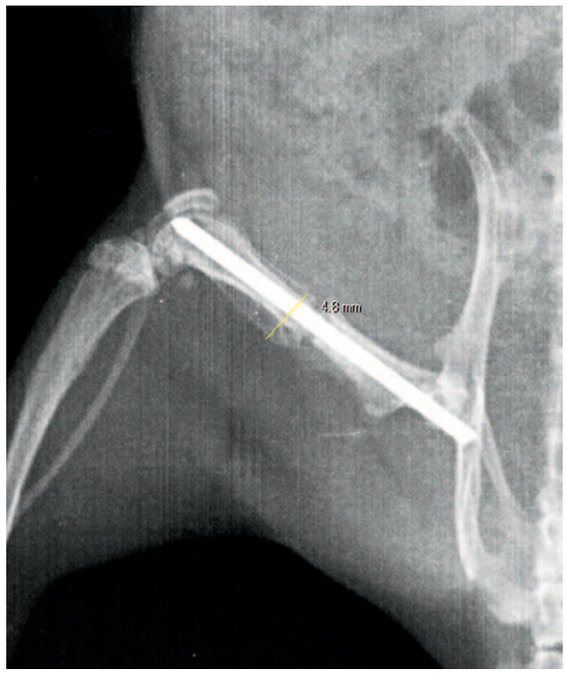


**FIGURE 7.** Radiograph demonstrating callus measurement.

## Bone Weight Measurement

Bone weight measured was 542.5 mg in the study group and 325.5 mg in the control group. This was not statistically significant ($P = 0.65$).

## Expression of TGF-ß: Figures 8 and 9

Expression of TGF-ß1 and 2 was measured at 0 weeks (initial time period) and at 6 weeks (final time period). There was an increased expression of both TGF-ß1 and 2 at 6 weeks in both control and study animals. TGF-ß1 expression was increased $10^5$ times ($P = 0.016$) more in the study animals than in the control animals and TGF-ß2 was increased by 80 times ($P = 0.008$). This indicates that there is a significant increase in TGF-ß production in animals treated with PEMF.

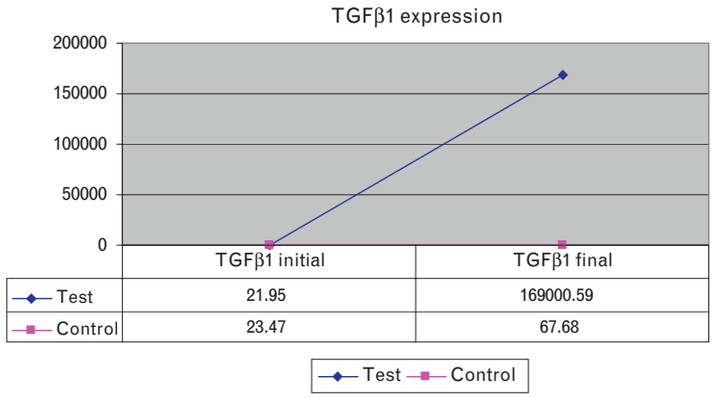


**FIGURE 8.** Graphical representation of TGF-ß1 expression between test and control animals.

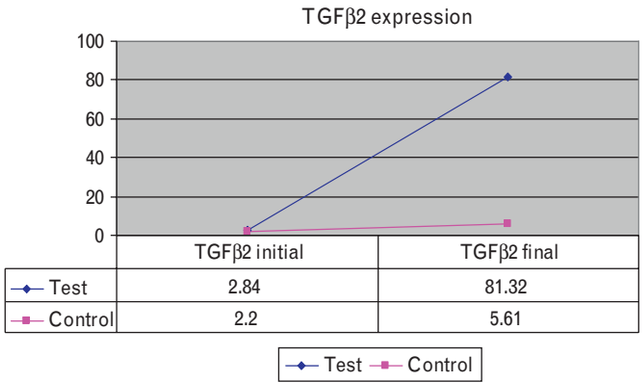


**FIGURE 9.** Graphical representation of TGF-ß2 expression between test and control animals.

## DISCUSSION

The development of alternative techniques for enhancing fracture healing and treating nonunion or bone defects offers promising perspectives for a variety of clinical situations. Although the mainstay of treatment is autologous bone graft, it is associated with morbidity.[9,10] Bone graft substitutes also lack the mechanical and osteoinductive properties of autologous bone grafts, although they play an important role in the replacement of massive bone defects. These techniques are invasive and expensive, and further problems must be overcome before they can be adapted for widespread clinical use. Growth factors used for enhancing bone healing also are expensive and may not be available to all.[11,12]

Physical methods (mechanical loading, ultrasound stimulation)[13,14] have been shown to offer beneficial effects in the healing of fresh fractures. PEMF also is being used in the treatment of hypertrophic nonunion and congenital pseudarthroses.[15] The key to rational use of electromagnetic fields lies in the ability to define the specific treatment parameters (amplitude, frequency, orientation and timing). It is noninvasive and does not require any medications or injections. No side effects have been reported at this extremely low intensity of magnetic field in terms of any radiation hazards.[16]

Electric and electromagnetic devices were created to treat fracture nonunions based on observations that electric fields occur in mechanically loaded bones. Previous studies suggest that the primary effect of PEMF is to promote differentiation but not proliferation.[17]

Dimitriou *et al.*[18] categorized molecular signals involved in bone healing into three groups: (1) The pro-inflammatory cytokines, (2) the TGF-ß super family and other growth factors and (3) the angiogenic factors. During the initial inflammatory phase, platelets release TGF-ß to initiate callus formation. TGF-ß is produced by osteoblasts and chondrocytes and initiates signaling for BMP synthesis by osteoprogenitor cells and may inhibit osteoclastic activity and promote apoptosis of osteoclasts.

In a recent study, patients with low levels of TGF-ß were found to have delayed union compared with patients who healed without any problems.[19] This signifies the role of TGF-ß in fracture healing.

Bostrom *et al.*[20] also stated that TGF-ß and isoforms are important regulators of fracture healing and promote various stages of bone healing.

It is therefore clear that PEMF could stimulate cultured cells to produce TGF-ß[7] and promote fracture healing. This led us to study the in vivo effect of PEMF in an animal model by measuring TGF-ß levels as an indicator of bone healing.

PEMF therapy has been shown to promote callus formation in fractures. It is likely to be useful as an adjuvant therapy in fracture management. The mechanism of healing seems to be secondary to local expression of TGF at the fracture site.

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