Original Article
J Immunol Clin Microbiol. 2020;5(4):139-149

J Immunol Clin Microbiol

Radiological and histomorphometric investigation of the effectiveness of the Pulsed Electromagnetic Field Therapy (PEMFT) in fracture models that were created in rats

Gulsah Yasa Oztürk [1*], Ferda Ozdemir [2], Selçuk Oztürk [3], Cem Copuroğlu[4], Gulay Durmuş Altun[5], Mehmet Kurkcu [6], Nermin Tuncbilek[7], Necdet Sut[8]

1Department of Physical Medicine and Rehabilitation, Adana City Hospital, Adana, Turkey. 2 Department of Physical Medicine and Rehabilitation, Taksim Acıbadem Private Hospital, İstanbul, Turkey. 3 Department of Cardiology, Tarsus State Hospital, Tarsus,Mersin, Turkey. 4 Department Of Orthopaedic Surgery And Traumatology, Trakya University Faculty of Medicine, Edirne, Turkey. 5 Department of Nuclear Medicine, Trakya University Faculty of Medicine, Edirne, Turkey. 6 Department of Oral and Maxillofacial Surgery, Çukurova University, Adana, Turkey. 7 Department of Radiology, Trakya University Faculty of Medicine, Edirne, Turkey. 8 Department of Bioistatistic, Trakya University Faculty of Medicine, Edirne, Turkey.

Abstract

Backround: In this study, we aimed to investigate the effect of the pulsed electromagnetic field therapy on histomorphometric and radiographic evaluation and bone mineral density of fracture model that were created in rats.

Materials and Methods: In this study, 14 female young-adult Wistar albino rats that were weighing from 250 to 300 g were used. A closed fracture was created in the tibia of all rats with the guillotine device. 8 rats in the first group underwent 15 sessions of pulsed electromagnetic field therapy at a dose of 15 millitesla/30 minutes a day, whereas 6 rats in the second group were not given any treatment. At the end of 15 sessions of treatment, digital mammographic examination, bone mineral density measurement and histomorphometric evaluation of the fracture area of the rats in the second group were performed.

Results: Although there is no statistically significant difference between the two groups after 15 sessions of pulsed electromagnetic field therapy at a dose of 15 millitesla/30 minutes a day, the outcomes of fracture healing were better in the group that received pulsed electromagnetic field therapy than the control group according to radiological score, bone mineral density measurement and histomorphometric evaluation.

Conclusion: Pulsed electromagnetic field is thought to contribute to the healing of fractures.

Key words: *Pulsed electromagnetic field, fracture healing, histomorphometry, radiology, bone mineral density*

***Corresponding Author:** Gülşah Yasa Oztürk, Department of Physical Medicine and Rehabilitation, Adana City Hospital, Adana, Turkey. **Phone:** +90 530 296 22 74 **E-mail:** gulsahyo@hotmail.com, **Received:** Apr, 2020. **Accepted:** Dec, 2020.

Introduction

Today, fracture healing has become a major health problem as a result of increased incidence of fractures due to increase in elderly population as well as in traffic and work accidents. Studies on this issue has focused on accelerating the healing of fractures. The main targets of treatment of fractures in orthopedics clinics are to perform necessary surgical or conservative intervention as soon as possible, and to ensure the patient's mobilization by helping fracture healing as quickly as possible by providing pain relief with physiotherapy and rehabilitation methods (1-5). Many treatment methods are applied in order to accelerate the healing of fractures with evaluation of systemic and local factors positively or negatively effecting the fracture healing, and to provide early return to daily life activities. These methods can be classified in to two groups: pharmaceutical therapies and physical therapies.

Pharmaceutical Treatments: The studies showed that TGF-β, PDGF, IGF, BMP and FGF-2, which are growth factors, affect fracture healing positively (6-10). Coral-derived hydroxyapatite and calcium sulfate dihydrate have been reported to accelerate fracture healing in orthopedics literatures. Calcium (Ca) and vitamin D have an important role in fracture healing. Ca deficiency leads to inadequate calcification of organic bone matrix and an efficient fracture healing can not be provided (11-13). A large number of studies have been done on many agents in fracture healing, and these include gene therapies, systemic zinc therapy, and systemic calcitonin, alendronate and estrogen treatments. 2) Physical Agents: Many physical agents that were used to accelerate fracture healing include exercise and mechanical stimulation, hyperbaric oxygen therapy, extracorporeal shock wave therapy, electrical current therapy, and low-intensity ultrasound**,** low-energy laser and pulsed electromagnetic field therapies (14,15). The voltage that were caused by exercise and mechanical stimulation in the skeletal system causes the osteogenic response (16,17). The application of pulsed electromagnetic field, which is a research issue and is increasingly used in recent years, is one of the methods of physical treatment that is non-invasive and based on the interaction of the magnetic fields which is a natural and sensitive treatment modality (18).

Speed of electromagnetic energy is equal to the speed of light, and presented in 'Gauss or Tesla' units. The forces of the device used for the magnetic field therapy (MFT) are between 500 and 3000 Gauss. An increase in frequency increases the thermal effect and suppresses the effect of the magnetic field. Therefore, frequencies below 100 Hz are preferred for the devices (19). The effects of the magnetic field on the organism in the light of the previous studies are as follows:

1. Earth's crust has a natural magnetism, which allows removal of waste substances and toxins, and the reception of nutrients, oxygen and minerals inside the cells.
2. The application of low-frequency pulsed magnetic field therapy increases euchromatin levels and reduces heterochromatin levels in the nucleus.
3. Adenohypophysis is stimulated, and morphological changes occur.
4. The cellular activity in the adrenal glands is increased.
5. Histological changes occur in the testes.
6. Vasodilation occurs due to the affected autonomic nervous system.
7. It has anti-inflammatory, analgesic and anti-edematous effects. It accelerates the healing of fractures and wounds.

8. The resistance against viral diseases is increased with the application of pulsed magnetic field therapy.
9. Respiratory volume is increased, and the course of bronchial asthma is affected positively.
10. Various intensities and frequencies of pulsed magnetic field application causes sedation in rats.

Magnetic field therapy can be used for bursitis, tendinitis, epicondylitis, fasciitis and muscular injuries caused by tissue lesions; acute, subacute and chronic periods of degenerative or inflammatory rheumatic diseases; soft-tissue injuries of athletes such as sprain, contusion and strain; fractures with poor union; loosening of endoprosthesis; aseptic necrosis of the femoral head; acute and chronic muscle spasms; spinal and paraspinal functional disorders, ankylosing spondylitis, osteoporosis, osteoarthrosis, acute or chronic osteomyelitis, spondylosis, spondyloarthritis and ligament tears (20).

Initially, magnetic field therapy has received approval of Food and Drug Administration only for delayed fracture healing. Aaron et al. (21) have reported in their published review that pulsed magnetic field therapy was more successful than placebo treatment in osteotomy animal experiments, and the success rate was higher by 80 to 90% in patients receiving electromagnetic field therapy. As a result of the investigations, MFT was found to be effective, inexpensive and safe method of treatment without serious side effects (22). It is recommended to sure that patients assigned to receive MFT should not have a history of implanted device (such as insulin pump), acute fever, bleeding disorder, hyperthyroidism, malignancy, pregnancy, and seizures (23). Vertigo and syncope may occur as a result of sudden changes in blood pressure in patients with hypotension and hypertension. This should be resolved within 30 minutes, and the patient is expected to ensure compliance after five applications (18).

Materials and Methods

Samples and study setup

Our study was carried out on 14 femle young-adult Wistar albino rats that were weighing from 250 to 300. A total of two group, including 8 rats in pulsed electromagnetic field therapy (PEMFT) group and 6 rats in the control group, were created. The number of rats was determined by pre-study power analysis. Procedures carried out in this study were as follows, respectively: provision of general anesthesia with intramuscular administration of 5 mg/kg Ketamine hydrochloride and 3 mg/kg Xlazine hydrochloride, the process of creating a closed fracture with a guillotine device, radiological demonstration of the fracture (Figure 1), administration of paracetamol (5mg/kg dose) to provide analgesia after fracture, application of pulsed electromagnetic field therapy (PEMFT) in rats after above-knee circular plaster application (Figure 2), and at the end of treatment sessions, imaging of fractures with digital mammography device (Lorad Selenia Full Fild) for monitoring of fracture healing, bone mineral density measurement of the fracture zone (with Holagic QDR 4500 device), sacrificing and histomorphometric examination of extracted tissues.

Figure 1. Pulsed electromagnetic field digital mammography imaging after treatment.

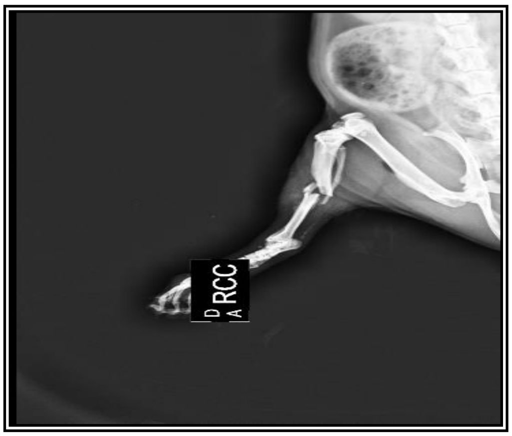

Figure 2. In the control group post-treatment imaging of digital mammography.

In our study, BTL-09 (BTL, Benesov, Czech Republic, AC input 230 v/50-60 Hz, 2x Fuse T6.3A, input power: 600VA) magnetic field device was used for application of PEMFT. In the first group, a total of 15 sessions of PEMFT was applied at a dose of 15mT/30 minutes/once a day at the same time every day for 15 days without an interruption. (Figure 3). The rats were not given sedative agents during this application. An observer was present at the practice room throughout the entire application and ensured the continuous progress of the process.

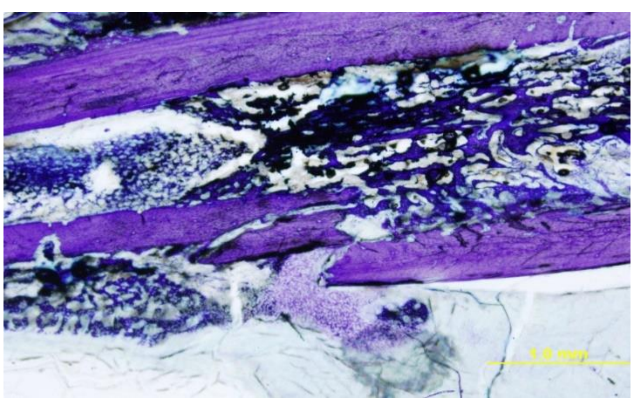

Figure 3. Pulsed electromagnetic field therapy group, an example of the display of the histomorphometric (x4).

Images taken with digital mammography device at the end of the 15th session of pulsed electromagnetic field therapy (Figure 4) were scored numerically by the same radiologist, without knowing the groups, according to the gradual scoring system (14) (Table 1).

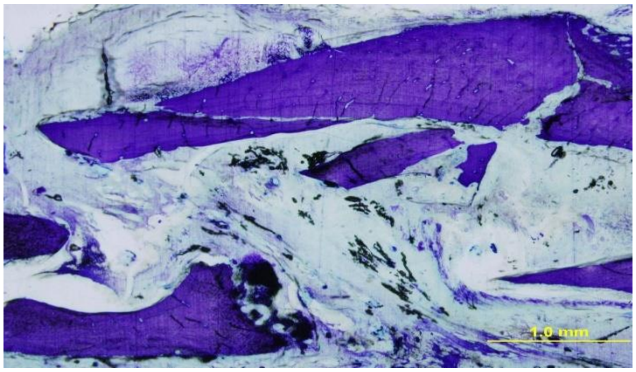

Figure 4. Histomorphometric display of the control group sample (x4).

Table 1. Radiological scoring (79).

0 points	Does not heal
1 points	Callus formation
2 points	Pronounced callus formation, bridging does not
3 points	Bony union

Bone mineral density (BMD) measurements of the fracture zone were performed and were analyzed by the same nuclear medicine specialist, without knowing the groups.

Fractured tibial bones in both groups were fixed with 10% formalin for preparation of cross-section. The fixed specimens were dehydrated in increasing concentrations (70 to 99%) of ethanol alcohol solution in vacuum-packed containers for 12 days. The cross-sections underwent necessary interventions for histomorphometric measurements. Histomorphometric scoring system that was described by Parfitt et al in 1987 and is adopted by American Society of Bone and Mineral Research was used (24). (Table 2).

Table 2. Histomorphometric evaluation parameter

Parameter	Units	Description/Equation
Bone-implant contact (BIC)	%	The percentage of implant length at which there is direct bone-to-implant contact, without intervening tissue, along the total length of the implant
Bone volume (BV)	%	Ratio of mineralized and un-mineralized bone volume to the total tissue volume estimated from the analyzed section
Bone area (BAr)	mm^2	The total bone area of the analyzed section
Bone perimeter (BPm)	mm	The total perimeter of bone in the analyzed section, including void surfaces and excluding artificial edges
Node termination (NTm)	-	End points in a trabecular network or free ends
Trabecular pattern factor (TbPF)	-	(perimeter 1 − perimeter 2)/(area 1 − area 2)

Parathyroid hormone (PTH), osteocalcin, Ca and P (phosphorus) levels in the blood samples taken from the heart were measured immediately after the sacrificing. Some of the measurements could not be performed in clotted and inadequate samples.

Data analysis

Data was performed by using Statistica 7.0 (License No: AXF003C775430FAN2) software package. Kruskal-Wallis test was used for the comparison of BMD measurements, digital mammographic measurements, histomorphometric scores and blood values between the groups (PEMAU, Control) and p value of <0.05 was considered to be statistically significant.

Results

During the study, while reduction in movements and loss of appetite were found in a rat from study group (receiving PEMFT) at 7th day of treatment and within subsequent two days, this rat returned to normal at the end of the second day. All rats in the study tolerated the application well, and there was no significant amount of weight loss in rats until the day of sacrificing.

Above-knee circular plasters that were applied during the study were eroded and opened by rats. Cervical collar was applied to prevent eroding, and this practice was ended because of the shortness of breath and nose-bleeding that occurred while the rats tried to remove cervical collars by pushing up them with their upper extremities. As a solution, circular plasters above the knee have been renewed every 2 days under general anesthesia.

After the plaster applications, follow-up of blood circulation of the exposed parts of the distal extremity were performed every day. The former plasters of the rats with swelling, redness, bruising or edema of their feet have been removed and renewed. Blood circulation of rats was relieved in this way.

As a result of measurements of bone mineral density, there was no statistically significant difference in R1 values showing the mean BMD values of fracture zones (p=0.796), but the mean R1 value of PEMFT group was found to be higher than that of the control group.

Radiographs taken with the digital mammography device were numerically scored with radiological scoring system (14). The evaluation was performed by a radiologist without knowing the groups. While there was no statistically significant difference between the two groups (p = 0.263), the score of PEMFT group was found to be higher than that of the control group. Digital mammographic images of one rat both from PEMFT and control groups are shown in figures 6 and 7.

For the histomorphometric analysis of sections prepared without calcifying, a total of 14 sections (8 from PEMFT group and 6 from the control group) were taken from the tissue samples. Among histomorphometric scores, while bone surface (p = 0.439), the number of nodes (p = 0.796), terminuses (p = 0.156) and trabecules (p = 0.156), and bone circumference (p = 0.439), were higher than that of the control group, histomorphometry examples of PEMFT and the control group are shown in Figures 8 and 9.

As a result of the examination of blood samples, while PTH levels were measured as 0 in all groups, osteocalcin levels were lower than 2, and statistical analysis could not be performed. There was no statistically significant differences in Ca and P levels between the groups, but the mean Ca and P levels of PEMFT group were higher than that of the control group.

Discussion

In our research, the effect of PEMFT at a dose of 15 mT, which has been administered at different dosages in previous studies, on the fracture of the tibia was evaluated. Because thick cross-section is required for histomorphometric analysis, decalcificed histological sections that is required for the histological evaluation could not be prepared, and this examination could not be prepared. For future studies, it has been observed that high number of rats will be needed, if histomorphometric and histologic evaluations will be performed together.

It was seen in recent studies that a variety of methods were used to stabilize the fractures. Erdogan et al (25), have created an incision on the rabbit mandible and fixed the fracture segments rigidly with mini-plates and screws and easily applied low intensity pulsed ultrasound (LIPUS) therapy. In our study, above-knee circular cast method was chosen as internal fixators were not compatible with PEMFT. When above-knee circular plaster casts were eroded and opened by rats, cervical collar was applied to rats to prevent eroding, but this application was ended immediately after observing nasal bleeding in rats. As a solution, above-knee circular plasters have been renewed every two days under general anesthesia. As a result of radiological evaluations performed during the study, it was seen that fracture terminals remained distant from each other, and plaster casting technique increased the risk of nonunion. In such studies, the use of titanium internal fixators, which is a high-cost technique that is difficult to access but compatible with PEMFT, was considered to be more appropriate.

Many physical therapy methods are used today in order to accelerate fracture healing. Recent studies have shown that the electromagnetic field (EMF) application can stimulate osteoblasts. In these studies, it has been shown that this effect takes place through ion transport channels (26, 27). Androjna et al reported that (28), daily PEMF treatments would improve the fracture healing response in skeletally mature OVX (ovariectomized) and by 6 weeks of healing, PEMF treatments resulted in improved hard callus elastic modulus across fibula fractures normalizing the healing process in OVX rats with respect to this mechanical property. The same authors implied that radiographic evidence showed an improved hard callus bridging across fibula fractures in OVX rats treated with PEMF as compared to sham treatments and this can provide a scientific rationale for investigating whether PEMF might improve bone-healing responses in at-risk osteoporotic patients.

Darenderiler et al (29) created an osteotomy at mandible of Guinea pig, and applied PEMFT in the first group for a period of 9 days, the static electromagnetic field treatment in the second group for a period of 9 days, no treatment in the third group and as a result of this study pulsed and static electromagnetic field therapy increased the healing of bone significantly. Cheing et al. (30) PEMF treatment is effective in wound healing and myofibroblast proliferation in diabetic rats. In another study, Maniaras et al. (31), demonstrated that the effects of EMF stimulation depend on the intensity and frequency of the EMF and the time of exposure to it. However, the same authors implied that other factors may affect these processes, such as growth factors, reactive oxygen species, and so forth.

Hilz et. al. (32) by combining electromagnetic field therapy with mechanical stimulation, they have had effecetive results in the healing of chondrocytes and cartilage. Gupta et all (33) applied magnetic field therapy to 45 patients with tibial fracture non-union at a dose of 0.008 weber/m^2/12 hours a day, and ultimately reported that magnetic field therapy accelerates the fracture healing. Ross et. al. (34) Low frequency electro magnetic field therapy affects various biological functions such as gene expression, cell destruction and cell differentiation, but these effects will only induce low frequencies as well as low amplitudes within a certain range. EMF has been reported to be effective in increasing osteogenesis and chondrogenesis without any adverse effects. In our study, the effectiveness of PEMFT in fracture healing was investigated with histomorphometric, radiological, and BMD evaluations. According to the results of histomorphometric measurements, while BS, NNd, NTm, BPm, TbN values were higher in PEMFT group, TbSp measurement that indicates the trabecular seperation and is expected to be inversely proportional to the healing was found to be lower in PEMFT group than other groups. Although there is no statistically significant difference between groups, according to the results of histomorphometry, PEMFT was concluded to accelerate the fracture healing.

In the fracture study of Aro et al (35) conducted in 1989, they have done an evaluation with conventional radiographic evaluation and BMD measurements, they have considered that BMD evaluation, which can provide quantitative data, is a useful index *for* the follow-up of healing, even conventional radiography is important for the follow-up of the fracture healing. In our study, as a result of radiological examination done with digital mammography**,** recovery score of PEMFT group was found to be higher than that of the control group. Although there is no statistically significant difference between groups, PEMFT was considered to accelerate the fracture healing. In the measurements of BMD,

BMD values of PEMFT group was higher than other groups, and PEMFT was found to increase BMD slightly. According to the results of BMD measurements, no statistically significant difference was found between the 2 groups. It was concluded that BMD measurement, which provides objective and quantitative results, can be used in the follow-up of fracture healing.

The effect of external factors on biological systems has been a research issue in recent years. Although there are many studies about the effect of EMF, which is one of these factors, biological systems, no accurate results have been achieved in this regard. EMA is thought to cause electrolyte changes by changing the permeability of the cell membrane (22). In the study of Schober et al (36) on rats, EMA has been found to decrease the level of plasma Ca. Steward et al (37) and Poll et al (38) have shown that the electromagnetic field promotes cell differentiation through the modulation of extracellular calcium entry.

In our study, although Ca levels of PEMFT group was found to be slightly higher that that of the control group, the difference was not statistically significant. It has been concluded that the effect of PEMFT on blood Ca levels can be tolerated by the organism during the acute phase. In this regard, the human studies comparing the doses and durations are required. Burchard et al (39) found an increase in P levels following application of the EMF**.** In the study of Eraslan et al (40), they applied 90 Hz frequency EMF to rats at a dose of militesla and reported an increase in P levels at 15th and 45th days. In our study**,** blood P levels of PEMFT group were found to be higher than that of the PEMFT group, but no statistically significant difference was found between the groups. Short-term administration of PEMFT was thought to affect blood P levels slightly. Chang Tu et al (41) In rats, models treated with tibial brachytherapy had received electromagnetic treatment and showed positive effects on the proliferation of bone marrow mesenchymal stem cells. They have been recommended in the treatment of boiling.

As a result of our study, PEMFT, which was applied for the purpose of fracture healing in the fracture model created in rats ,was observed to be effective in the fracture healing. It was also concluded that PEMFT is effective and easily applicable method and has no negative effect on the tissues. Nevertheless, further more comprehensive studies investigating the usage areas and effectiveness of PEMFT at different doses and durations are needed.

Ethics Committee Approval: Yes
Informed Consent: NA
Peer-review: Externally peer-reviewed.
Conflict of Interest: No conflict of interest was declared by the author.
Financial Disclosure: The author declared that this study has received no financial support.

References

1. Bouxsein ML, Turek TJ, Blake CA. Recombinant human bone morphogenic prtein-2 accelerates healing in a rabbit osteotomy model. J Bone Joint Surg. 2001;83:1219-30.
2. Higgins TF, Dodds SD, Wolfe SW. A biomechanical analysis of fixation of intra-articular distal radial fractures with calcium-phosphate bone cement. J Bone Joint Surg Am. 2002;84:1579-86.
3. Leisner S, Shahar R, Aizenberg I, Lichovsky D, Levin-Harrus T. The effect of short-duration, high-intensity electromagnetic pulses on fresh ulnar fractures in rats. J Vet Med A Physiol Pathol Clin Med. 2002;49:33-7.

4. Doetsch AM, Faber J, Lynnerup N, Watjen I, Bliddal H, Danneskiold-Samsoe B. The effect of calcium and vitamin D3 supplementation on the healing of the proximal humerus fracture: a randomized placebo-controlled study. Calcif Tissue Int. 2004;75:183-8.
5. Larrson S, Kim W, Caja VL, Egger EL. Effect of early axial dynamization on tibial bone healing. Clin Orthop Rel Res. 2001;388:240-51.
6. Nielsen M, Andreassen T, Ledet T, Oxlund H. Local injection of TGF-β increases the strength of tibial fractures in the rat. Acta Orthopaedica. 1994;65(1):37-41.
7. Nash T, Howlett C, Martin C, Steele J, Johnson K, Hicklin D. Effect of platelet-derived growth factor on tibial osteotomies in rabbits. Bone. 2009;15(2):203-8.
8. Wei-Jia C, Seiya J, Ikuo A, Jun A, Goh H, Makoto T et al. Effects of FGF-2 on metaphyseal fracture repair in rabbit tibiae. J Bone Miner Metab. 2004;22(4):303-9.
9. Kloen P, Di Paola M, Borens O, Richmond J, Perino G. BMP signaling components are expressed in human fracture callus. Bone. 2003;33(3):362-71.
10. Mahmoudifar, N. & Doran, P. M. Chondrogenesis and cartilage tissue engineering: the longer road to technology development. Trends Biotechnol. 2012;30:166–76.
11. Yürekli V, Akkuş S, Akhan G, Tamer MN. Osteomalazi and antiepilepticles. SDÜ Medical Fac J. 2005;12(2):34-7.
12. Steward AJ, Kelly DJ, &Wagner DR. The roll of calcium signaling in the chondrogenic response of mesenchymal stem cell to hydrostatic pressure. Eur Cell Mater. 2014; 28, 358-71.
13. Pall ML. Electromagnetic fields act via activation of voltage-gated calcium channels to produce beneficial or adverse effects. J Cell Mol Med. 2013;17, 958–65.
14. TToh, WS Spector M, Lee, E. H. & Cao, T. Biomaterial-mediated delivery of microenvironmental cues for repair and regeneration of articular cartilage. Mol Pharm. 2011;994–1001.
15. Raghothaman D, Leong MF, Lim TC, Toh JK, Wan AC, Yang, Z. Lee EH. Engineering cell matrix interactions in assembled polyelectrolyte fiber hydrogels for mesenchymal stem cell chondrogenesis. Biomaterials 2014;35:2607–16.
16. Kirazlı Y. Medical remedy for osteoporotic hip broken patient. Turkish Phys Rehab Journal. 2009;55(1):46-50.
17. Responte DJ, Lee JK, Hu J. C. & Athanasiou KA. Biomechanics-driven chondrogenesis: from embryo to adult. Faseb J 2012;26:3614–24.
18. Iordonou P, Baltopoulos G, Gionnokopoulou M, Bellou P, Ktenas E. Effect of polarized light in the healing process of pressure ulcers. Int J Nurs Pract 2002;8:49-50.
19. Akgün K. Remedy of Magnetic Area. Physical medical methods in movement system diseases. İstanbul: Nobel Medical Bookstore, 2002;65-71.
20. Aksoy C. Magnetic Area Cure. İstanbul: Nobel Medical Bookstore. 2001;119-27.
21. Aaron RK, Ciombor DM, Simon BJ. Treatment of nonunions with electric and electromagnetic fields. Clin Orthop Relat Res 2004;419:21-9.
22. Kangarlu A. Cognitive, cardiac and physiological safety studies in ultra high field magnetic resonance imaging. Magn Reson Imaging 1999;17:1407-16.
23. American Cancer Society. Magnetic field therapy, bioelectromagnetics, bioenergy therapy, bioresonance guidelines for using compelmantary and alternative methods electromagnetic therapy asp. Cancer 2005.
24. Weintraub MI. Magnetotherapy: A new intervention? Arch Phys Med Rehabil 1998;79:469-70.
25. Erdoğan O, Esen E, Ustun Y, Kurkçu M, Akova T, Gonlusen G et al. Effects of low-intensity pulsed ultrasound on healing of mandibular fractures: an experimental study in rabbits. J Oral Maxillo Fac Surg 2006;64(2):180-8.
26. Bassett CA. Pulsing electromagnetic fields: A new method to modify cell behavior in calcified and noncalcified tissues. Calcif Tissue Int 1982;34:1-8.
27. Daish C, Blanchard R, Fox K. et al. The Application of Pulsed Electromagnetic Fields (PEMFs) for Bone Fracture Repair: Past and Perspective Findings. Ann Biomed Eng 2018; 46, 525–542.
28. Androjna CB, Fort M, Zborowski R and Midura J. Pulsed electromagnetic field treatment enhances healing callus biomechanical properties in an animal model of osteoporotic fracture. Bioelectromagnetics, 2014; 35(6):396–405.

29. Darendeliler MA, Darendeliler A and Sinclair, PM. Sinclair Effects of Static Magnetic and Pulsed Electromagnetic Fields on Bone Healing. Int J Adult Orthodon Orthognath Surg. 1997;12(1):43-53.
30. Cheing GL, Li X, Huang L, Kwan RL, Cheung KK. Pulsed electromagnetic fields (PEMF) promote early wound healing and myofibroblast proliferation in diabetic rats. Bioelectromagnetics. 2014;35(3):161-169.
31. Maziaraz, A., B. Kocan, M. Bester, S. Budzik, M. Cholewa, T. Ochiya, and A. Banas. How electromagnetic fields can influence adult stem cells: positive and negative impacts. Stem Cell Res. Ther. 2016; 7(1):1.
32. Hilz FM, Ahrens P, Grad S, J Stoddart M, Dahmani C, L Wilken F, et al. et *al*. Influence of extremely low frequency, low energy electromagnetic fields and combined mechanical stimulation on chondrocytes in 3-D constructs for cartilage tissue engineering. Bioelectromagnetics 2014; 35:116–28.
33. Gupta AK, Srivastava KP, Avasthi S. Pulsed electromagnetic stimulation in nonunion of tibial diaphyseal fractures. Indian J Orthop 2009;43(2):156-60.
34. Ross CL. Siriwardane M, Almeida-Porada G, D Porada C, Brink P, J Christ G, S Harrison B. The effect of low-frequency electromagnetic field on human bone marrow stem/progenitor cell differentiation. Stem Cell Res 2015;15:96–108.
35. Aro HT, Wippermann BW, Hodgson SF, Wahner HW, Lewallen DG, Chao EY. Prediction of properties of fracture callus by measurement of mineral density using micro-bone densitometry. J Bone Joint Surg Am 1989;71:1020-30.
36. Schober A, Yanic M, Fischer G. Electrolytic Changes in the white mouse under the influence of weak magnetic fields. Zentralbl Bacteriol Microbiol Hyg 1982;176 (4):305-15.
37. Steward, A. J., Kelly, D. J. & Wagner, D. R. The role of calcium signalling in the chondrogenic response of mesenchymal stem cells to hydrostatic pressure. Eur Cell Mater 2014;28,358–71.
38. Pall, ML. Electromagnetic fields act via activation of voltage-gated calcium channels to produce beneficial or adverse effects. J Cell Mol Med 2013;17, 958-65.
39. Burchard JF, Nguyen DH, Block E. Macro- and trace element concentrations in blood plasma and cerebrospinal fluid of dairy cows exposed to electric and magnetic fields. Bioelctromagnetic. 1999;20(6):58-64.
40. Eraslan G, Bülgülü A, Epsüz D, Saltap H. Effects of elektromagnnetic area (90 Hz ve 5 mT) of some electrolit levels (Ca^{++}, P^{+++}, Na^{+}, K^{+}, Cl^{-}) in male rats. Turk J Vet Anim Sci. 2002;1233-1236.
41. Chang Tu, Yifan Xiao, Yongzhuang Ma, Hua Wu, and Mingyu Song. The legacy effects of electromagnetic fields on bone marrow mesenchymal stem cell self-renewal and multiple differentiation potential. . Stem Cell Research & Therapy. 2018;9:215.