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Investigation into the effects of static and electric fields on bone healing process: An experimental tibial fracture model study in Wistar-Albino male rats

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ABSTRACT

OBJECTIVE: In this experimental study, we aimed to investigate whether 0 Hz-Static and 50 Hz-Electric fields have an effect on bone healing.

METHODS: In this study, 45 male Wistar-Albino rats were equally and randomly separated into three groups as follows: a 0 Hz-Static electric field (SEF), a 50-Hz low-frequency electric field (LFEF) and a control group. A manual fracture was performed in the left tibia diaphysis of all rats, and fractures were fixed using circular plaster over the knee. The LFEF group was exposed to 50 Hz electric field for 30 minutes a day, five days a week, for a total of eight weeks. The SEF group was exposed to 0 Hz electric field within the same time interval. The control group was held in identical environmental conditions, without exposure to electric field. Periodic radiographs were taken from all the animals. At the end of this study, rats were sacrificed and mechanical/histopathologic examinations were performed.

RESULTS: Radiologic, mechanical and histologic scores of the LFEF group were lower than those of the SEF and control groups; however, no significant difference was found in group comparisons in terms of average histologic and radiologic scores ($p>0.05$).

CONCLUSION: Results extracted from the current study suggest that 0-hz static and 50-hz electric field exposures affect bone healing tissue of tibial fracture models in rats, although it is not significant.

Keywords: Bone fracture healing; experimental study; low frequency electric field; static electric field.

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Pathophysiology and healing stages of bone fracture are well defined; however, some points remain unclear. There are many local and systemic, positive and negative factors that affect fracture recovery [1, 2]. We encounter many studies in the literature performed using various biologic systems and addressing the effects of several energy types on bone fracture healing [2, 3]. It has been reported that low frequency static, electric and electromagnetic fields had certain biological effects on bone cells [4–7].

Electric fields originate from many natural/artificial sources, and play an important role in our lives. The principal sources of 50-Hz low frequency electric fields (LFEF) are energy distribution cables, high voltage transmission lines and electrical home appliances [8–10]. On the other hand, static electric fields (SEF) are naturally

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presents in the atmosphere and may also be produced through friction. We are namely exposed to static electric fields in our daily lives because of the rail systems and with unidirectional current and monitors or TVs containing cathode ray tubes [8–11]. It has been mentioned in some experimental and epidemiologic studies that interactions of low frequency-electric and electromagnetic fields with biologic tissues have certain negative consequences [9, 12]. Bone is a potential absorber for environmental hazardous materials and fracture healing can be affected from these electric and static field sources [6, 13]. In this experimental study, we aimed to investigate whether 0 Hz-Static and 50 Hz-Electric fields have an effect on the bone fracture healing process.

MATERIALS AND METHODS

Electric and Static Fields Setup and Exposure

Limit values and electric field strength levels, which have been identified in the guideline released by The International Committee on Non-Ionizing Radiation Protection (ICNIRP), were used as a main reference for the experimental setup and the application method [8]. The paper by Lorrain and Corson [14] was cited for the theoretical analysis and the LFEF measurements between the parallel and capacitor plates. The study conducted by Polk [15] was referred to for the linear dielectric constant calculations. Whole measurements, calculations, analyzes and design of the experimental setup were performed in the Suleyman Demirel University Electronics and Communication Engineering Research Laboratory. The electric field setup was performed by parallel plate capacitors based on basic electromagnetics, also well known as a "parallel plate setup". A 6–10 kV/m ELF band setup with 50 Hz frequency was used in Group-1, similar to the setting reported by Aslan et al. [10] and the experimental setup is presented in Figure 1. In Group-2, DC was obtained using a 5 kV DC supply as described in the study by Okudan et al. [5]. Both Group-1 and Group-2 were exposed to 0 Hz (static) and 50 Hz electric fields for 30 minutes a day, five days a week, for eight weeks. Rats in the control group were also kept under the same conditions as the other groups, although they were not exposed to an electric field. All experiments were conducted in a certified clean room, shielded electromagnetically, at Suleyman Demirel University. Electric and static fields were set up under different laboratory conditions and they were isolated against any environmental effects to avoid unsolicited interaction by panel at a 80 dB isolation level

Highlight key points

- Bone tissue can absorb the environmental electric and static field sources.
- Low-frequency 50 Hz EF have no effect on fracture healing in rat models.
- The results about 0 Hz (Static) field are similar to 50 Hz EF.

FIGURE 1. General room setting for the experiment design. Separated parts and animals housed in cages are seen.

was placed. In addition, the cages were placed in the part of the laboratory with the lowest environmental EF level to minimize the undesirable environmental EF effects.

Animal Model

Before this study, the protocol was reviewed and approved by the Ethics Committee of the Suleyman Demirel University (SDU/11-08-05), where the experiments were performed. In this study, 45 adult Wistar-Albino male rats, aged five months (range: 4 to 6) and weighing an average of 255 g (±20 g), were included. Male rats were preferred for this study because they have no short periodic or cyclic hormonal changes, which occur in females, and they are commonly used in animal models of experimental orthopedics [1]. Rats were randomly separated into three groups and each one of them was given a number: Group-1 (50 Hz LFEF exposure), Group-2 (0 Hz. SEF exposure) and Group-3 (controls). Rats were kept under standardized laboratory conditions and their adaptation was ensured

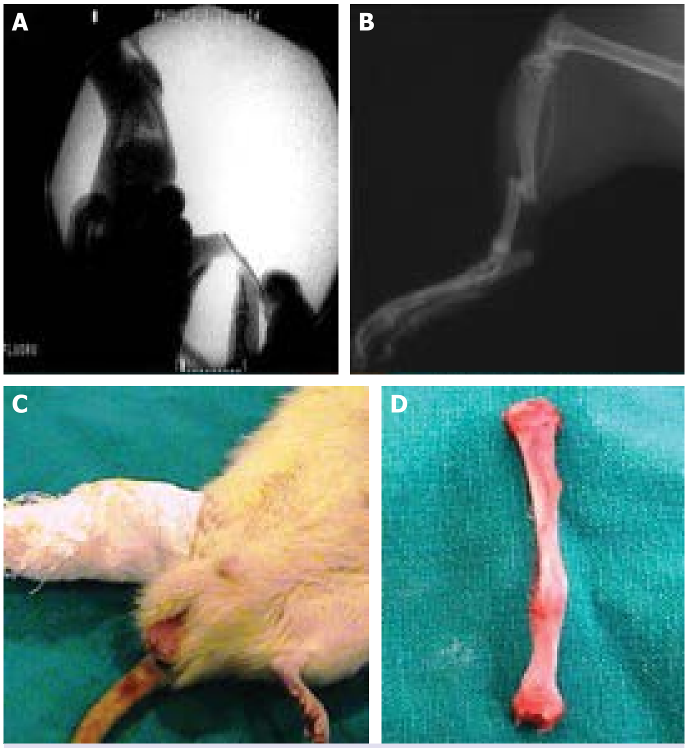

Figure 2. **(A)** Creation of fractures under fluoroscopy control. **(B)** Radiological image of the fracture of the rat tibia. **(C)** Extremities with fracture were fixated with circular plaster. **(D)** Removal of rat tibia as a block at the end of the study.

Figure 3. Periodic radiographs from the LFEF group; final score: 7.

before the experiment. They were kept under ideal humidity and circadian rhythm conditions (temperature: 22±2°C, 12 h light-dark cycle, humidity: 30–70%) and activity and/or loading-stress was not restricted during the study period. They had access to standard rat food and tap water was provided ad libitum.

Rat Tibial Bone Fracture and Fixation Model

Following the sevoflurane (Sevorane®) application to rats under fluoroscopy and/or X-ray equipment, bone fractures were made in tibial diaphysis according to the three-point principle (Fig. 2A) [16]. Fractures were evaluated by modifying the method which Leisner et al. [17] used. One fracture line at the junction point of distal 2/3 and proximal 1/3 parts of the bone was regarded as good (Fig. 2B), while incomplete, segmental fractures and fractures extending to the joint were regarded as bad. Extremities with fracture were fixated with circular plaster over the knee (Fig. 2C). Following periodic radiographs, at the end of this study, the right tibia of all rats was removed as a block, together with healing tissue (Fig. 2D).

Radiologic Evaluation

Rats were anaesthetized using Sevoflurane (Sevorane®) and placed under an X-ray device in the prone position. Anterior and posterior extremities were fixated (tube distance was determined as 50 cm and energy exposure was adjusted to 44 kV-1.25 mA/sec), and conventional direct X-ray imaging of the fractured lower extremity was performed in anterior projection (Fig. 2B, C). Direct X-ray imaging of all rats was performed at 2nd, 4th and 8th weeks to perform follow-up and scoring of bone healing tissue formation (Fig. 3–5). At the end of this study, evaluations according to the modified radiographic scoring system [3] were performed by an independent radiologist and two orthopedists, who did not know which image belongs to which group. Imaging with the same score given by at least two of the authors was included in the evaluation.

Manual Mechanical Evaluation

The mechanical evaluation was performed with the method used by Aslan et al. [3]. One orthopedic surgeon who participated in this study while blinded to the groups macroscopically evaluated the union tissues in tibial fracture sites in two planes. When the evaluations

FIGURE 4. Periodic radiographs from the SEF group; final score: 10.

FIGURE 5. Periodic radiographs from the control group; final score: 10.

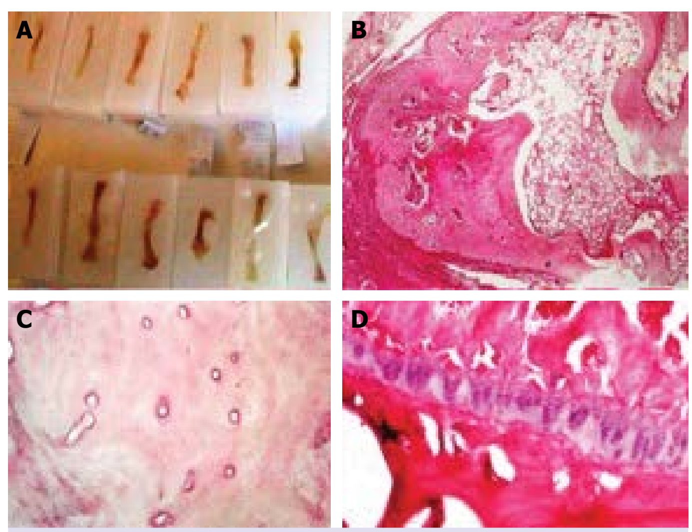

FIGURE 6. **(A)** Image of paraffin-embedded rat tibia bone blocks. **(B)** Proliferation zone and bone marrow cells (Group LFEF; Score: 8; x40 HE). **(C)** Cortical bone-like mature bone trabeculae (Group SEF; Score: 10; x40 HE). **(D)** Spongy bone tissue with fibroblastic proliferation (Group Control; Score: 10; xHE).

from the external specialists were not consistent, another researcher involved in this study provided the final decision on the evaluation of the results. The same scores were taken statistically.

Histological Evaluation

Tibia and fracture healing tissues of all rats were removed and isolated from the surrounding soft tissues without damaging the fracture zones (Fig. 2D). Manual mechanical examinations and evaluations were made and routine histological follow-up procedures were performed after the decalcification process, using nitric acid (10%). Specimens were then embedded in paraffin blocks (Fig. 6A); 6-mm-thick sections were longitudinally cut with a microtome by centering the fracture line, stained with hematoxylin-eosin and all three groups of preparations were evaluated under a light microscope (Fig. 6 B–D). Tissue slides were then evaluated by two independent histologists and a pathologist according to the scoring system recommended by Huo et al. [18]. The protocol described by Deibert [19] was used as a histological examination method. Cells were counted using a squared glass slide. Cartilage, bone and fibrous tissue cell percentages were calculated in three different squares and mean values were recorded.

Table 1. Comparison of histological, radiological and mechanical score means

	Group LFEF Mean±SD	Group SEF Mean±SD	Group C Mean±SD	p*
Radiological score	6.93±1.77	7.31±1.49	7.33±1.29	0.828
Histological score	7.00±3.06	7.67±2.57	7.07±3.71	0.668
Mechanical score	1.67±1.15	1.75±0.87	1.75±0.45	0.961

LFEF: Low frequency electric fields; SEF: Static electric fields; C: Control; SD: Standard deviation; *: Kruskal-Wallis Test.

Statistical Analysis

The SPSS v11.00 (SPSS Inc, Chicago, IL, USA) statistics package program was used. Definitive analyses were presented as mean±standard deviation. Compatibility to normal range in continuous variables was checked using the Kolmogorov-Smirnov test and the Kruskal Wallis test was used for comparison of the groups. P-values less than 0.05 were accepted as statistically significant.

RESULTS

Radiological Evaluation Results

Radiographic evaluations were performed on all the rats in the control group, on 14 rats in Group 1 and 13 rats in Group 2, since some rats died during the study process. Especially mean radiologic scores of the LFEF group were worse than the control group. However, when groups were compared, no significant statistical difference in radiologic scores was found (p=0.828; Table 1).

Histological Evaluation Results

Microscopic tissue slide evaluations were performed for 14 rats in Group 1/Group 2 and all rats in the control group, as a result of excluded or deceased rats and poorly-made sequences. As it is seen in the example case in Figure 6 B–D, mean histological scores of the EFLF group were worse than those of the control group; however, there was no significant statistical difference (p=0.668; Table 1).

Mechanical Evaluation Results

Although 15 rats were mechanically examined, 12 mechanical scores were statistically evaluated, and no significant statistical difference was found between the groups (p=0.961; Table 1).

DISCUSSION

Bone fracture healing usually results in bone tissue that is indistinguishable from the original healthy bone. However, some substances are able to inhibit this healing process [20, 21]. In some previous studies, it has been reported that low-frequency, low-energy and pulsed electric or electromagnetic fields may stimulate calcium uptake, bone formation and fracture healing [4, 22–24]. Impulses originated from direct current, static, electric, magnetic or electromagnetic fields have a certain positive effect on fracture healing [4, 25–28]. There are many studies conducted to investigate the possible effects of various EF and EMF applications on fracture recovery [24, 29]. Finally, both positive [24, 29–31] and negative [17, 32] effects of electric, magnetic and electromagnetic fields on the recovery of bone tissue and fracture have been reported.

In a study conducted by Bilgin et al. [30] in which the effects of the pulsed electromagnetic field and local vibration on bone fracture have been compared, it has been reported that the pulsed electromagnetic field application at 50 Hertz frequency for four hours daily for 21 days in total gave rise to an increment in callus formation in fracture sites of the rat tibial bones, when compared with the controls. They have also mentioned that the mean serum osteocalcin levels had significantly elevated in the experiment groups. Aydin and Bezer [32] have reported that a static magnetic field, applied along with an intramedullary implant, ameliorated bone healing during the initial two weeks following an experimental femoral osteotomy, and that said the static magnetic field had no major effect on bone mineral density. In another study conducted by van der Jagt et al. [33], it has been reported that a pulsed electromagnetic field did not have any effect on cortical or cancellous bone.

Results established from the current study suggest that a 50 Hz EF has no significant effect on bone recovery (Table 1). Our results are in accordance with the studies reporting low-frequency electric, magnetic or electromagnetic fields have no effect on fracture recovery and bone tissue. However, these studies were performed using electric, magnetic or electromagnetic fields with low frequency and intensity, but also with pulsation. In most of these studies, the hypothesis was that the electrical potential has a signal role in the regulation of cellular procedures associated with the bone recovery and reconstruction [4]. The electric field with low frequency (50 Hz), which we used in our study was not a pulsed field.

Zhang et al. [34] mentioned that a four-week moderate magnetic field exposure did not affect bone biomechanical properties or bone microarchitecture in male C57BL/6 mice that were 5–6 weeks old. Ince et al. [35] have exposed rats to an electric field and found that the 50 Hertz electromagnetic field can have a prominent effect on the element composition of rat teeth.

In all of the bone recovery and bone tissue studies mentioned above, it is unknown whether the observed effect following the exposure to external fields resulted from fields on the surface of the body or fields and currents induced within the body. It is necessary to consider both the superficial and internal fields in the interaction between the EF and the tissue until the identification of the exact mechanism is achieved [8]. On the other hand, EMF frequency, magnitude, duration, exposure and other factors may variate the effect occurring on biological tissues [10]. Thus, differences between results reported in the literature and our study should also be evaluated in this manner. Finally, we should mention that we used rats as our animal model in this study, as they have long constituted a popular model in experimental orthopedic studies and, additionally, as they constitute an appropriate option as a model for human bone recovery, bone mass as well as bone mineral density research [3, 5, 16].

Limitations

During the evaluation and preparation process of the histopathological slides, examinations of only twelve samples were performed, the result of rat deaths and other issues; this scantiness in the sample is a limitation in our study. Performing manual mechanical evaluations instead of objective biomechanical evaluations is also considered as a limitation.

Conclusion

Results extracted from our study suggest that low-frequency 50 Hz and 0 Hz (Static) EF have no significant effect on fracture recovery in rat models. However, this result does not mean that EF exposure in daily life is completely safe.

Ethics Committee Approval: The Suleyman Demirel University Clinical Research Ethics Committee granted approval for this study (date: 08.11.2005, number: 08).

Conflict of Interest: No conflict of interest was declared by the authors.

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Authorship Contributions: Concept – AA, VK; Design – AA, SC; Supervision – AA, VK; Fundings – AA, VK; Materials – AA, AK; Data collection and/or processing – AA, AK, SC; Analysis and/or interpretation – AA, AK; Literature review – AA, SC, AK; Writing – AA, AK, VK; Critical review – AK, SC.

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