Journal of Taibah University Medical Sciences (2025) 20(1), 1–12

Taibah University

Journal of Taibah University Medical Sciences

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Original Article

Pulsed electromagnetic field prevents tooth relapse after orthodontic tooth movement in rat models

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Received 9 May 2024; revised 10 November 2024; accepted 10 December 2024; Available online 26 December 2024

الملخص

أهداف البحث: يظل الانتكاس بعد العلاج التقويمي مشكلة جوهرية. من المعروف أن المجالات الكهرومغناطيسية النبضية تسرع تكوين الخلايا البانية للعظم وتنشط تكوين الخلايا الناقضة للعظم. مع ذلك، لم يتم دراسة تأثيرها على حركة الأسنان خلال مرحلة التثبيت من العلاج التقويمي. هدفت هذه الدراسة إلى بحث دور التحفيز بالمجال الكهرومغناطيسي النبضي في منع انتكاس الأسنان بعد حركة الأسنان التقويمية في نماذج الجرذان.

طرق البحث: تم تقسيم 36 من ذكور جرذان ويستار إلى مجموعات: المجموعة الضابطة، ومجموعة المجال الكهرومغناطيسي النبضي 7 أيام، ومجموعة المجال الكهرومغناطيسي النبضي 14 يوما. تم تحريك الضاحك الأول العلوي للأمام بقوة 50 غرام باستخدام نابض لولبي مغلق من النيكل تيتانيوم لمدة 21 يوما. تم تطبيق التحفيز بالمجال الكهرومغناطيسي النبضي بتردد 15 هرتز وشدة 2.0 ملي تسلا في مرحلة التثبيت لمدة ساعتين يوميا لمدة 7 و14 يوما. بعد ذلك، تم تقييم مسافة انتكاس الأسنان في الأيام 1 و3 و7 و14، وكذلك عدد الخلايا البانية للعظم والخلايا الناقضة للعظم والخلايا الليفية باستخدام صبغة الهيماتوكسيلين والإيوسين، وتعبير عامل نمو الأرومة الليفية-2 والكولاجين-1 باستخدام التحليل الكيميائي النسيجي المناعي. تم تحليل البيانات باستخدام تحليل التباين أحادي الاتجاه واختبار المقارنات البعدية.

النتائج: انخفضت مسافة انتكاس الأسنان بشكل ملحوظ في مجموعتي المجال الكهرومغناطيسي النبضي 7 و14 يوما مقارنة بالمجموعة الضابطة. لوحظت زيادة كبيرة في الخلايا البانية للعظم والخلايا الليفية وعامل نمو الأرومة الليفية-2 والكولاجين-1 في كلتا مجموعتي المجال الكهرومغناطيسي النبضي، بينما انخفضت الخلايا الناقضة للعظم.

الاستنتاجات: يمكن أن يعزى انخفاض انتكاس الأسنان إلى التحفيز بالمجال الكهرومغناطيسي النبضي لمدة 7 و14 يوما من خلال تسريع تكوين العظم السنخي وإعادة تشكيل الرباط السني حول السن.

الكلمات المفتاحية: حركة الأسنان التقويمية؛ المجال الكهرومغناطيسي النبضي؛ الانتكاس

Abstract

Objective: Relapse after orthodontic treatment remains a crucial problem. Pulsed electromagnetic fields (PEMFs) accelerate osteoblastogenesis and inhibit osteoclastogenesis. However, their effect on tooth movement during the retention phase of orthodontic treatment has not been studied. This study investigated the role of PEMF stimulation in preventing tooth relapse after orthodontic tooth movement (OTM) in rat models.

Methods: Thirty-six male Wistar rats were divided into control, PEMF 7, and PEMF 14 groups. The maxillary first molar was moved mesially with a 50 g force of a Nickel Titanium closed coil spring for 21 days. Therefore, PEMF stimulations, including a frequency of 15 Hz and intensity of 2.0 mT, were applied to a retention phase for 2 h daily for 7 and 14 days. The tooth relapse distance was evaluated on days 1, 3, 7, and 14; the number of

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Peer review under responsibility of Taibah University.

Production and hosting by Elsevier

 https://doi.org/10.1016/j.jtumed.2024.12.009

osteoblasts, osteoclasts, and fibroblasts was assessed by hematoxylin and eosin staining; and the expression of fibroblast growth factor 2 (FGF-2) and type I collagen (Col-I) was evaluated by immunohistochemistry. The data were analyzed using one-way analysis of variance and post hoc test with $p < 0.05$ considered statistically significant.

Results: Tooth relapse distance was significantly decreased in the PEMF 7 and PEMF 14 groups compared to the control group. A significant increase was detected in osteoblasts, fibroblasts, FGF-2, and Col-I in both PEMF groups, while osteoclasts decreased ($p < 0.05$).

Conclusion: The reduction of tooth relapse could be attributed to PEMF stimulation for 7 and 14 days by accelerating alveolar bone formation and periodontal ligament remodeling.

Keywords: Orthodontic tooth movement; Pulsed electromagnetic field; Relapse

Introduction

The main problem after orthodontic treatment is relapse, which is a change in tooth position. The etiology of relapse is multifactorial, although the most proposed is the ongoing process of periodontal tissue remodeling and osteogenesis around the moved teeth.[1–5] Previous research on 67 patients who had worn retainers for an average of 8.5 years showed that five patients still experienced relapse after the retainer was removed.[6] The results of research on experimental animals with tooth movement for 12 days and retainer use for 2 and 4 weeks showed relapse of 85% and 24%, respectively.[2]

Alveolar bone deposition and periodontal ligament remodeling play an important role in preventing relapse after orthodontic treatment. The cells involved in osteogenesis are osteoblasts, osteoclasts, and osteocytes,[5] while the cells involved in periodontal ligament remodeling are fibroblasts. Type I collagen (Col-I) and fibroblast growth factor 2 (FGF-2) are the main mediators in the process of periodontal ligament and alveolar bone remodeling. Col-I is responsible for the formation of Col fibers and is also an important mediator of bone formation. FGF-2 plays an important role in the formation of new blood vessels by stimulating fibroblast proliferation and Col production during the process of alveolar bone and periodontal ligament remodeling.[7]

Several efforts are made to prevent relapse. Pulsed electromagnetic fields (PEMFs) are a non-invasive adjunctive therapy for osteoporosis in postmenopausal women,[8] mandibular fractures,[9] and improving osseointegration of implants in the alveolar bone.[10–12] Although the biomolecular effects of PEMF on alveolar bone cells have not been widely investigated, previous studies have reported that PEMF stimulation can influence bone regeneration by accelerating osteoblast cell proliferation[13] and inhibiting osteoclastogenesis.[14,15] In addition, PEMF stimulation can increase fibroblast proliferation activity in fibroblast cell culture.[16] However, it remains unknown whether PEMF stimulation can prevent relapse after orthodontic treatment by increasing osteogenesis and remodeling periodontal ligament.

Therefore, this study investigated the role of PEMF in preventing tooth relapse after orthodontic treatment in rat models through assessing the relapse distance, histological analysis of alveolar bone and periodontal ligament cells, as well as immunohistochemical analysis.

Materials and Methods

Experimental animal models

This study received ethical approval letter from the Ethics Committee, Faculty of Medicine, Universitas Brawijaya (Jawa Timur, Indonesia). A total of 36 male, 3-month-old Wistar rats (*Rattus norvegicus*) were included in the analysis. Rats were acclimatized for 7 days in polycarbonate cages at a room temperature of 20–23 °C, with 12/12 h light–dark cycles. To ensure optimal conditions, rats received adequate nutritional and fluid intake, along with soft foods to prevent difficulty eating during orthodontic tooth movement (OTM).

Experimental animals were divided into a control group (21 days of orthodontic appliance and 7 days of retention phase without PEMF stimulation), PEMF 7 group (21 days of orthodontic appliance and 7 days of retention phase with PEMF stimulation), and PEMF 14 group (21 days of orthodontic appliance and 14 days of retention phase with PEMF stimulation). The anesthetic agent was a mixture of ketamine hydrochloride (Troy Laboratories Pty Ltd., Glendenning NSW, Australia) and xylazine (Interchemie werken "De Adelaar" BV, Venray, Netherlands) in a 1:1 ratio. Intramuscular administration of anesthesia at a dose of 1 mL/kg BW aimed at reducing the discomfort of the rats while installing and removing orthodontic equipment. Meanwhile, euthanasia was carried out with anesthesia that exceeded the dose. The general research scheme is presented in Figure 1.

OTM in rat models

A nickel titanium (Ni–Ti) closed coil spring (American Orthodontics, Sheboygan, WI, USA) of 0.01 inches in diameter and 5 mm in length was inserted between the first molar and the maxillary incisor for mesial movement. The end of the Ni–Ti closed coil spring was tied with a ligature wire and wrapped around the first molar. Subsequently, the other end was wrapped around the incisor and covered with glass ionomer luting cement (GC Glass Ionomer Luting & Lining Cement, Tokyo, Japan), as shown in Figure 2A. The magnitude of the activation force of the orthodontic appliance was a 50 g force. According to previous studies, a 50 g force of orthodontic appliances is ideal to move the rat's maxillary first molars mesially.[17–19] The coil springs

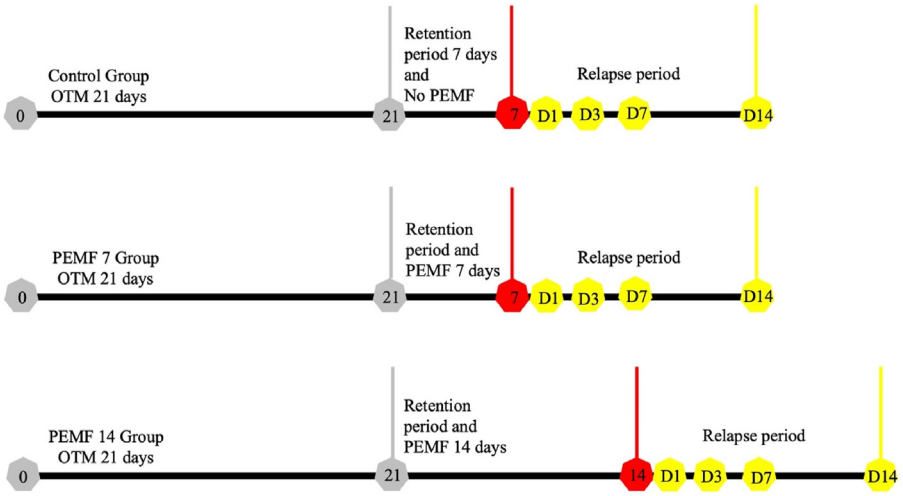

Figure 1: Research scheme. The maxillary first molar was moved bodily mesially with a 50 g force of Ni–Ti closed coil spring for 21 days. During the retention periods of 7 and 14 days, the treatment groups were exposed to PEMF. After passing the retention period, the Ni–Ti closed coil spring was removed and entered the relapse period. D1, day 1 relapse; D3, day 3 relapse; D7, day 7 relapse; D14, day 14 relapse; Ni–Ti, nickel-titanium; OTM, orthodontic tooth movement; PEMF, pulsed electromagnetic field.

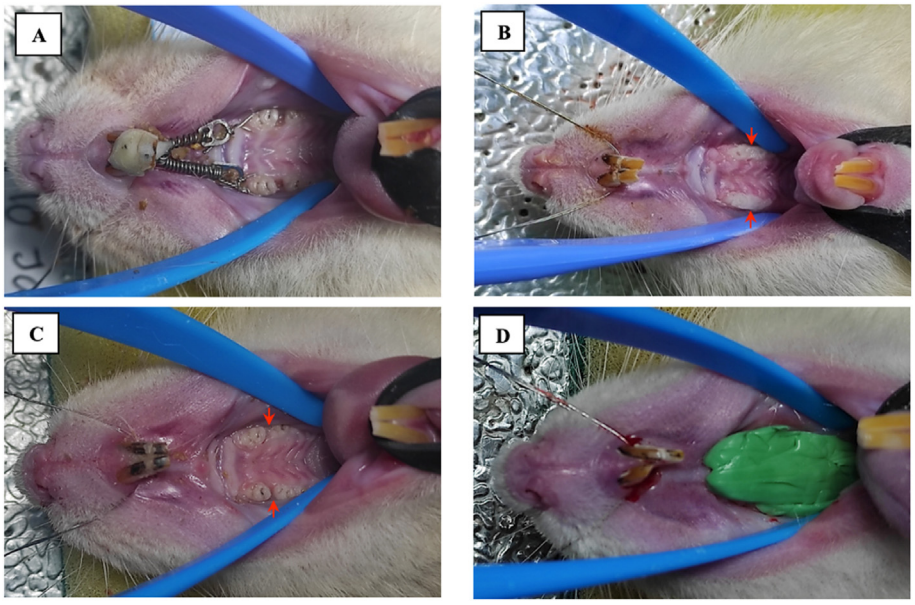

Figure 2: The sequence of research. (A) Tooth movement after using an Ni–Ti close-coil spring for 21 days; (B) retention phase, where the glass ionomer luting cement closed the interdental space between the first and second molars (red arrow); (C) relapse phase with the glass ionomer cement that closed the interdental space between the first and second molars is removed (red arrow); (D) and the maxillary teeth impression with silicone impression materials. Ni–Ti, nickel-titanium.

were measured using a stress and tension gauge (Ormco Co., Glendora, CA, USA) to determine the force magnitude. OTM lasted 21 days. The maxillary molars were impressed using silicone impression materials (Sangchi, Shanghai, China) to measure the distance of the first maxillary molar after 21 days of orthodontic treatment.

Retention phase and PEMF stimulation

The retention phase lasted 7 and 14 days, starting after the completion of active OTM.[2] The retention procedure used the glass ionomer cement type II (GC Glass Ionomer Light-Cured Universal Restorative, Tokyo, Japan) to maintain the interdental space between the maxillary first

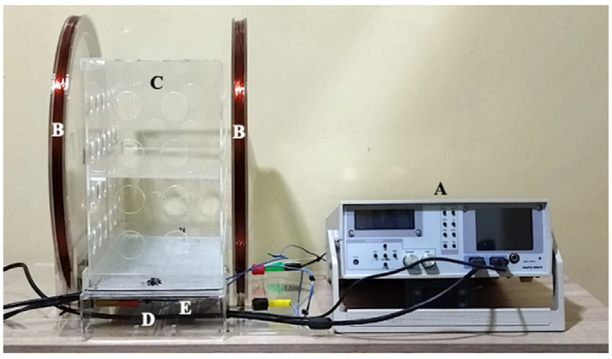

Figure 3: Pulsed electromagnetic field stimulation schematic. (A) Pulse generator, (B) Helmholtz coil, (C) animal cage, (D) electromagnetic wave sensor, and (E) temperature sensor. The waveform was square with 5 ms of burst width, 0.2 ms of pulse width, 0.02 ms of pulse wait, 60 ms of wait burst, 0.3 µs of pulse rise, and 2.0 µs of pulse fall.

and second molars. Subsequently, the animals were anesthetized, and orthodontic devices were removed. Debris attached to the gingiva and teeth between the first and second molars were cleaned and dried. The gingiva in the interdental space was coated with a Teflon tape seal to prevent the glass ionomer from adhering. Glass ionomer was placed in the interdental space, leveled parallel to the occlusal surface, and exposed to a light cure unit for 5 s, as shown in Figure 2B.

The PEMF stimulator was created and modified based on previous studies.[20–23] Specifically, PEMF consists of a pulse generator and two Helmholtz coils of 30 cm in diameter separated at a distance of 15 cm placed coaxially (Figure 3). To produce a suitable Helmholtz coil, 200 turns of copper wire are required. The Integrity Design Research 324 Gauss meter (Integrity Design and Research Corp., Essex Junction, VT, USA) was used to assess the accuracy of the magnetic field intensity created by the Helmholtz coil with a maximum magnetic field intensity

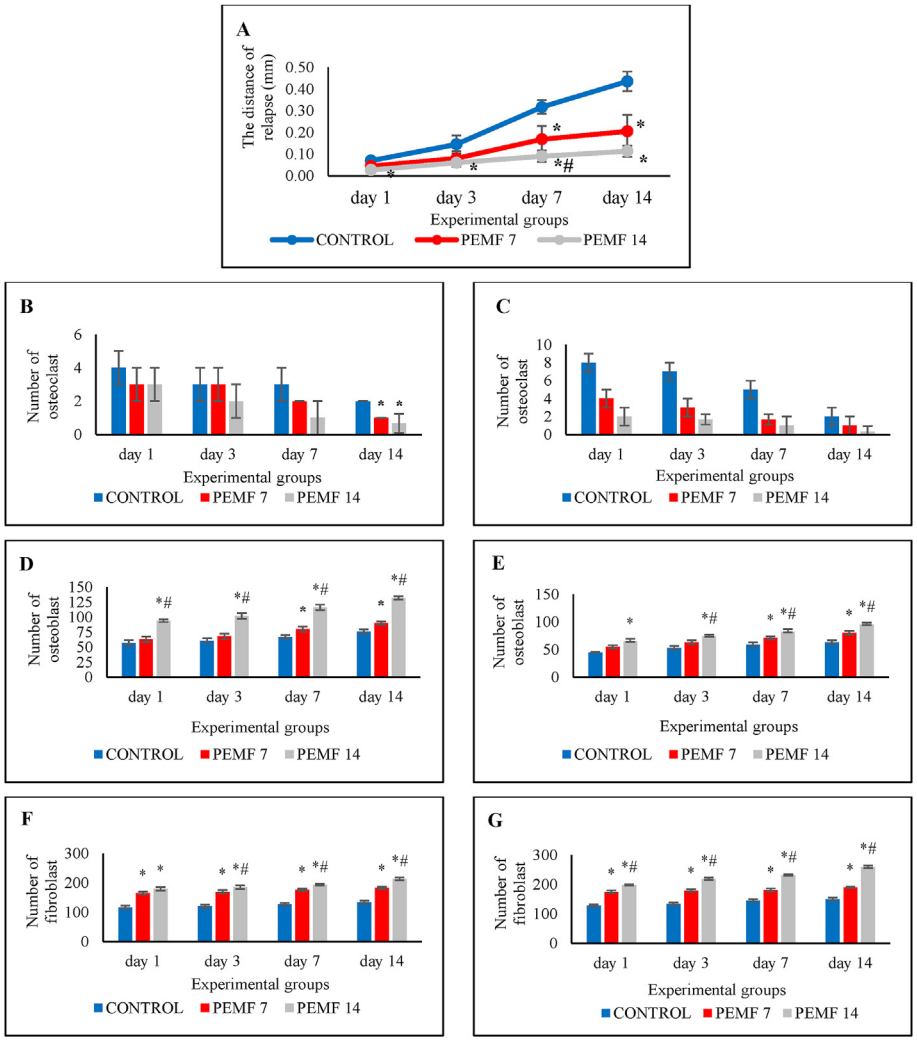

Figure 4: Evaluation of PEMF stimulation on relapse distance and histological analysis. The data are presented as the mean and standard deviation. (A) The relapse distance, histogram of osteoclast numbers on the (B) tension and (C) pressure side, osteoblast number on the (D) tension and (E) pressure side, and fibroblast number on the (F) tension and (G) pressure side. *$p < 0.05$, significant compared with the control group; #$p < 0.05$, significant compared with the PEMF 7 group. PEMF, pulsed electromagnetic field.

Table 1: Evaluation of PEMF stimulation on relapse distance and the number of osteoclasts.

	Relapse distance (mm) (mean ± SD)			Number of osteoclasts on the tension side (mean ± SD)			Number of osteoclasts on the pressure side (mean ± SD)		
	Control	PEMF 7	PEMF 14	Control	PEMF 7	PEMF 14	Control	PEMF 7	PEMF 14
D 1	0.07 ± 0.01	0.05 ± 0.02	0.03 ± 0.01[a]	4 ± 1.0	3 ± 1.0	3 ± 1.0	8 ± 1.0	4 ± 1.0	2 ± 1.0
D 3	0.15 ± 0.04	0.08 ± 0.03	0.06 ± 0.02[a]	3 ± 1.0	3 ± 1.0	2 ± 1.0	7 ± 1.0	3 ± 1.0	1.7 ± 0.6
D 7	0.32 ± 0.03	0.17 ± 0.06[a]	0.09 ± 0.03[a,b]	3 ± 1.0	2 ± 0.0	1 ± 1.0	5 ± 1.0	1.7 ± 0.6	1 ± 1.0
D 14	0.44 ± 0.05	0.21 ± 0.08[a]	0.11 ± 0.03[a]	2 ± 0.0	1 ± 0.0[a]	0.7 ± 1.0[a]	2 ± 1.0	1 ± 1.0	0.3 ± 0.6

Control, control group; D1, day 1 relapse; D3, day 3 relapse; D7, day 7 relapse; D14, day 14 relapse; PEMF, pulsed electromagnetic field; PEMF 7, PEMF 7 group; PEMF 14, PEMF 14 group; SD, standard deviation.

[a] $p < 0.05$, significant compared with the control group.

[b] $p < 0.05$, significant compared with the PEMF 7 group.

Table 2: Evaluation of PEMF stimulation on the number of osteoblasts and fibroblasts.

	Number of osteoblasts on the tension side (mean ± SD)			Number of osteoblasts on the pressure side (mean ± SD)			Number of fibroblasts on the tension side (mean ± SD)			Number of fibroblasts on the pressure side (mean ± SD)		
	Control	PEMF 7	PEMF 14	Control	PEMF 7	PEMF 14	Control	PEMF 7	PEMF 14	Control	PEMF 7	PEMF 14
D 1	57 ± 4.6	63 ± 4.6	94 ± 2.3[a,b]	45 ± 1.0	55 ± 3.0	65 ± 4.4[a]	116 ± 5.29	165 ± 4.58[a]	179 ± 5.57[a]	128 ± 4.00	175 ± 4.58[a]	198 ± 2.00[a,b]
D 3	61 ± 4.0	68 ± 4.6	102 ± 4.5[a,b]	53 ± 3.6	63 ± 3.6	74 ± 2.6[a,b]	120 ± 5.57	169 ± 5[a]	185 ± 6.24[a,b]	134 ± 4.58	179 ± 4.58[a]	219 ± 4.36[a,b]
D 7	67 ± 2.6	80 ± 4.6[a]	116 ± 4.6[a,b]	59 ± 4.0	71 ± 2.6[a]	82 ± 4.6[a,b]	127 ± 4.58	176 ± 57[a]	193 ± 3.00[a,b]	145 ± 5.00	181 ± 5.57[a]	232 ± 2.65[a,b]
D 14	76 ± 3.6	90 ± 2.5[a]	132 ± 2.6[a,b]	63 ± 3.6	80 ± 3.6[a]	95 ± 3.6[a,b]	134 ± 5.57	182 ± 4.36[a]	213 ± 5.29[a,b]	150 ± 4.58	190 ± 2.00[a]	260 ± 4.58[a,b]

Control, control group; D1, day 1 relapse; D3, day 3 relapse; D7, day 7 relapse; D14, day 14 relapse; PEMF, pulsed electromagnetic field; PEMF 7, PEMF 7 group; PEMF 14, PEMF 14 group; SD, standard deviation.

[a] $p < 0.05$; significant compared with control group.

[b] $p < 0.05$; significant compared with PEMF 7 group.

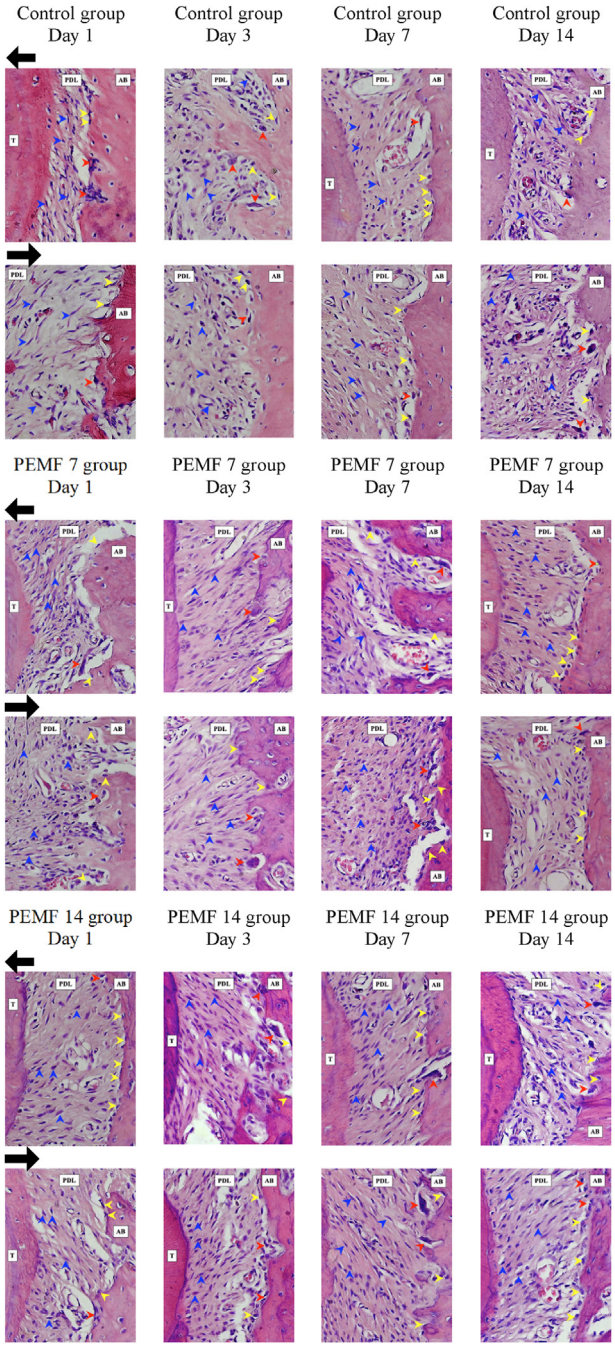

Figure 5: Histological image of osteoblasts (yellow arrow), osteoclasts (red arrow), and fibroblasts (blue arrow) in the control, PEMF 7, and PEMF 14 groups. The direction of tooth movement (black arrow). AB, alveolar bone; PDL, periodontal ligament; PEMF, pulsed electromagnetic field; T, tooth. Scale bar: 50 μm.

of 2.0 mT and repetition frequency of 15 Hz.[21,22] The animals in the retention phase were stimulated by PEMF for 2 h daily for 7 and 14 days. At the end of the retention phase, the glass ionomer cements were removed and the experimental animals entered the relapse phase (see Figure 4).

Relapse measurement

In the relapse phase, on days 1, 3, 7, and 14, the distal shift of the first molar was assessed (Figure 2C). Silicone impression materials (Sangchi, Shanghai, China) were used to cast maxillary molars (Figure 2D). The mesial movement of the first molar was calculated directly from mold models using a digital caliper (Mitutoyo, Kanagawa, Japan). The relapse distance was calculated using the following formula:[17]

$$JR = J1 - J2$$

where JR (relapse distance) is the amount of tooth relapse distance of the first molar (mm); J1 is the distance between the mesial edge tip of the second maxillary molar and the distal edge tip of the first maxillary molar after 21 days of orthodontic treatment; and J2 is the distance between the tip of the mesial edge of the second maxillary molar and the tip of the distal edge of the first maxillary molar after 1, 3, 7, and 14 days in the relapse phase.

Histology and immunohistochemical analyses

Sample fixation was carried out for 24 h using a 10% buffered formalin solution at room temperature. Therefore, samples were decalcified with 14% ethylenediaminetetraacetic acid (EDTA) solution (pH 7.4) for approximately 30 days. This solution was changed every 4 days, while bone density was checked in preparation for cutting tissue slides. After decalcification, the samples were dehydrated with 70%, 80%, and 95% ethanol solution, each for 60 min. Then the samples were cleared with xylene solution for 90 min and embedded in liquid paraffin at 60 °C. After the paraffin hardened, the samples were cut using a microtome in a transverse direction at cervicoapical two-thirds with a thickness of 5 μm for hematoxylin and eosin (H&E) staining and 3 μm for immunohistochemical methods.

H&E staining was conducted to determine the number of osteoclasts, osteoblasts, and fibroblasts. These cells were observed on the tension and pressure sides with a light microscope at 400× magnification equipped with the Optilab 3 camera (PT Miconos, DI Yogyakarta, Indonesia). In the relapse phase days 1, 3, 7, and 14, the numbers of osteoclasts, osteoblasts, and fibroblasts were counted manually in three selected fields of view and calculated as the average. Furthermore, an immunohistochemical procedure was performed to analyze the expression of Col-I and FGF-2 on days 1, 3, 7, and 14 of the relapse phase. The slides were deparaffinized with xylene solution and 100%, 90%, 80%, and 70% ethanol solution and incubated in 3% hydrogen peroxide for 20 min at room temperature. Then the slides were incubated overnight at 4 °C with primary antibodies against Col-I (COL1A1 mouse monoclonal antibody, sc-293,182; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) and FGF-2 (sc-74412; Santa Cruz) and at room temperature for 20 min with polymer (*N*-Histofine Simple Stain MAX PO; Nichirei Biosciences Inc., Tokyo, Japan). Subsequently, the samples were incubated with a substrate and Mayer solutions, each for 5 min at room temperature. The expression of Col-I and FGF-2 was observed with a light

Table 3: Evaluation of PEMF stimulation on the expression of Col-I.

	Col-I expression on the tension side (Arb. unit) (mean ± SD)			Col-I expression on the pressure side (Arb. unit) (mean ± SD)		
	Control	PEMF 7	PEMF 14	Control	PEMF 7	PEMF 14
D 1	1,767,119 ± 100,037	2,044,518 ± 428,526	3,144,221 ± 198,194[a,b]	1,771,499 ± 233,143	2,382,961 ± 292,337	3,449,294 ± 344,199[a,b]
D 3	1,993,493 ± 250,190	2,395,580 ± 198,997	3,437,339 ± 375,283[a]	1,990,024 ± 261,822	2,563,960 ± 285,614	3,815,561 ± 217,231[a,b]
D 7	2,114,574 ± 356,843	2,652,564 ± 362,902	4,129,845 ± 559,669[a,b]	2,096,774 ± 265,876	2,777,874 ± 281,748	4,072,273 ± 295,289[a,b]
D 14	2,271,241 ± 235,879	2,912,669 ± 480,909	5,133,853 ± 568,891[a,b]	2,156,901 ± 308,524	3,030,282 ± 186,305[a]	4,774,964 ± 311,720[a,b]

Arb. unit, arbitrary unit; Col-I, type I collagen; Control, control group; D1, day 1 relapse; D3, day 3 relapse; D7, day 7 relapse; D14, day 14 relapse; PEMF, pulsed electromagnetic field; PEMF 7, PEMF 7 group; PEMF 14, PEMF 14 group; SD, standard deviation.

[a] $p < 0.05$, significant compared with the control group.

[b] $p < 0.05$, significant compared with the PEMF 7 group.

Table 4: Evaluation of PEMF stimulation on the expression of FGF-2.

	FGF-2 expression on the tension side (mean F0B1SD)			FGF-2 expression on the pressure side (mean ± SD)		
	Control	PEMF 7	PEMF 14	Control	PEMF 7	PEMF 14
D 1	61.67 ± 4.51	68.00 ± 2.00	135.67 ± 3.799[a,b]	21.33 ± 3.51	61.33 ± 3.21[a]	122.33 ± 3.21[a,b]
D 3	65.33 ± 5.51	72.33 ± 2.52	143.00 ± 5.13[a,b]	24.00 ± 3.00	62.00 ± 5.29[a]	133.67 ± 4.04[a,b]
D 7	71.67 ± 4.73	79.67 ± 5.51	186.33 ± 3.51[a,b]	30.00 ± 1.00	68.67 ± 3.51[a]	148.67 ± 4.16[a,b]
D 14	72.67 ± 4.16	88.33 ± 4.51[a]	197.67 ± 2.08[a,b]	33.33 ± 1.15	76.33 ± 4.73[a]	165.00 ± 5.00[a,b]

Control, control group; D1, day 1 relapse; D3, day 3 relapse; D7, day 7 relapse; D14, day 14 relapse; FGF-2, fibroblast growth factor 2; PEMF, pulsed electromagnetic field; PEMF 7, PEMF 7 group; PEMF 14, PEMF 14 group; SD, standard deviation.

[a] $p < 0.05$, significant compared with the control group.

[b] $p < 0.05$, significant compared with the PEMF 7 group.

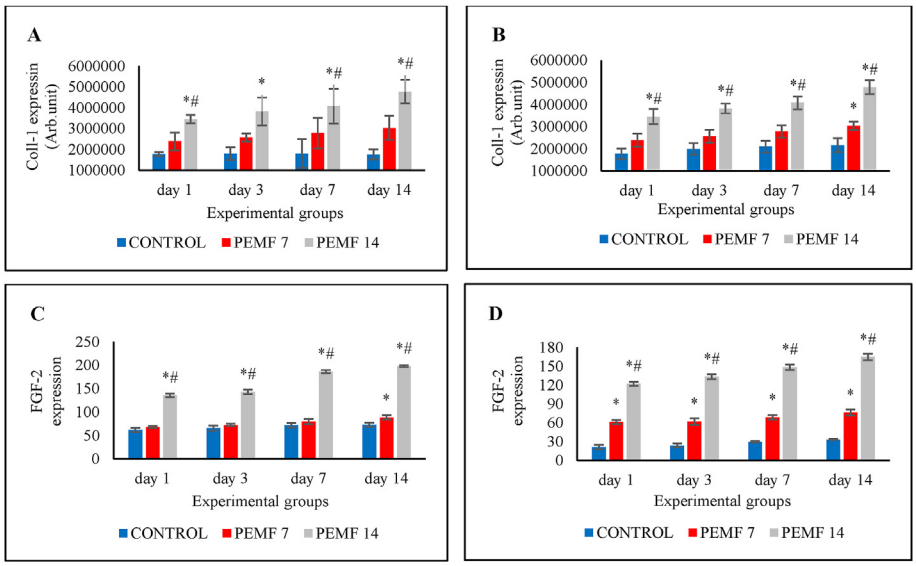

Figure 6: Results of immunohistochemical analysis. The data are presented as the mean and standard deviation. The histogram of Col-I expression on the (A) tension and (B) pressure side and FGF-2 expression on the (C) tension and (D) pressure side. *p < 0.05, significant compared with the control group; #p < 0.05, significant compared with the PEMF 7 group. Arb. Unit, arbitrary unit; FGF-2, fibroblast growth factor-2; PEMF, pulsed electromagnetic field.

microscope at 400× magnification equipped with the Optilab 3 camera for three selected pictures, and the average was taken on the pressure and tension sides. Positive Col-I expression were counted by the intensity of the brown extracellular matrix in the periodontal ligament using ImageJ software version 4.0 (National Institutes of Health, Bethesda, MD, USA). Positive FGF-2 expression was observed manually by counting the number of periodontal ligament cells, which were stained brown.

Statistical analyses

The average relapse distance; the number of osteoclasts, osteoblasts, and fibroblasts; and the expression of Col-I and FGF-2 are presented as the mean and standard deviation. The data were analyzed using SPSS Statistics version 26.0 (SPSS Inc., Chicago, IL, USA). The Kolmogorov–Smirnov test was used to analyze the normality, and one-way analysis of variance was used to analyze the variance, followed by a post hoc test using Tukey's Honestly Significant Difference test. The data on relapse distance was non-homogeneous, so a post hoc test was performed using the Games–Howell test. The number of osteoclasts was not normally distributed, so the Mann–Whitney U test was performed. $P < 0.05$ was considered statistically significant.

Results

Relapse distance

Table 1 shows a decrease in tooth relapse distance in the PEMF 7 and PEMF 14 groups compared to the control group. Statistically, a significant difference in tooth relapse distance was detected between the control and PEMF 7 groups on days 7 and 14, and control and PEMF 14 groups on days 3, 7, and 14 in the relapse phase ($p < 0.05$), as shown in Figure 4A.

Number of osteoclasts, osteoblasts, and fibroblasts

The number of osteoclasts in the PEMF 7 and PEMF 14 groups was decreased compared to the control group on the tension and pressure sides, as shown in Table 1. Statistically, the results showed significant differences between PEMF 7 and control groups, as well as PEMF 14 and control groups on day 14 on the tension side ($p < 0.05$) (Figure 4B, C). Meanwhile, the number of osteoblasts in the PEMF 7 and PEMF 14 groups was increased compared to the control groups on both sides, as shown in Table 2. Thus, there were significant differences between the PEMF 7 and control groups on days 7 and 14 as well as the PEMF 14 and control groups on days 1, 3, 7, and 14 on the tension and pressure sides ($p < 0.05$). Moreover, the number of osteoblasts in the PEMF 14 group showed significant differences compared to the PEMF 7 group on days 3, 7, and 14 on the tension and pressure sides ($p < 0.05$; Figure 4D, E).

The number of fibroblasts in the periodontal ligament was increased in the PEMF 7 and PEMF 14 groups compared to the control group on the tension and pressure sides, as shown in Table 2. Statistical results showed a significant difference between the PEMF 7 and control groups, PEMF 14 and control groups, as well as the PEMF 7 and PEMF 14 groups on both sides on days 3, 7, and 14 ($p < 0.05$; Figure 4F, G). Histological images of osteoclasts, osteoblasts, and fibroblasts on the tension and pressure sides are presented in Figure 5.

Expression of Col-I and FGF-2

The expression of Col-I was elevated in the PEMF 7 and PEMF 14 groups compared to the control group on the

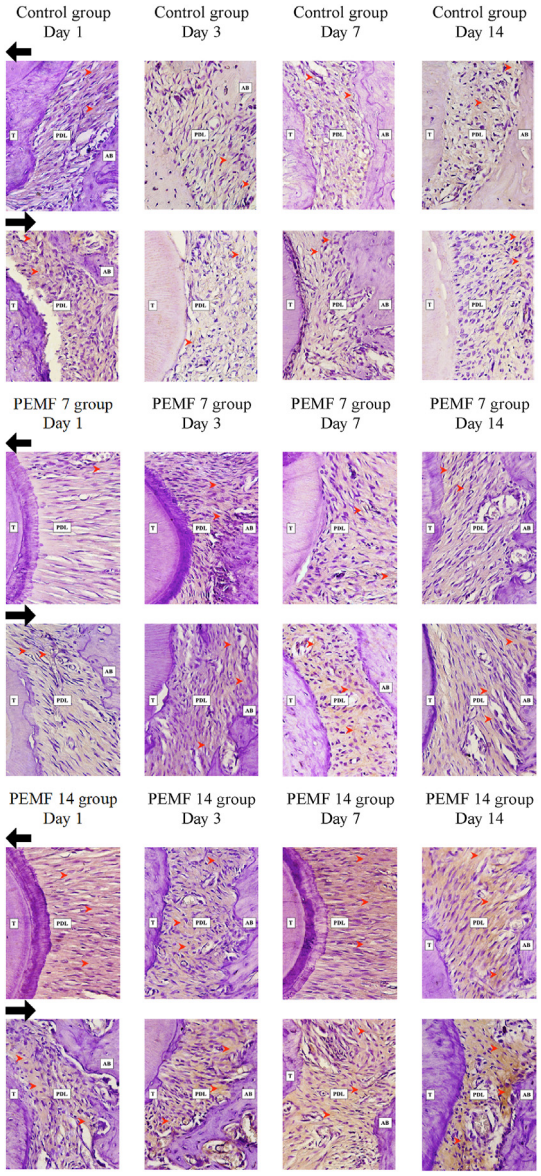

Figure 7: Histological observation collagen-I expression (red arrow) in the control, PEMF 7 and 14 groups. The direction of tooth movement (black arrow). AB, alveolar bone; PDL, periodontal ligament; PEMF, pulsed electromagnetic field; T, tooth. Scale bar: 50 μm.

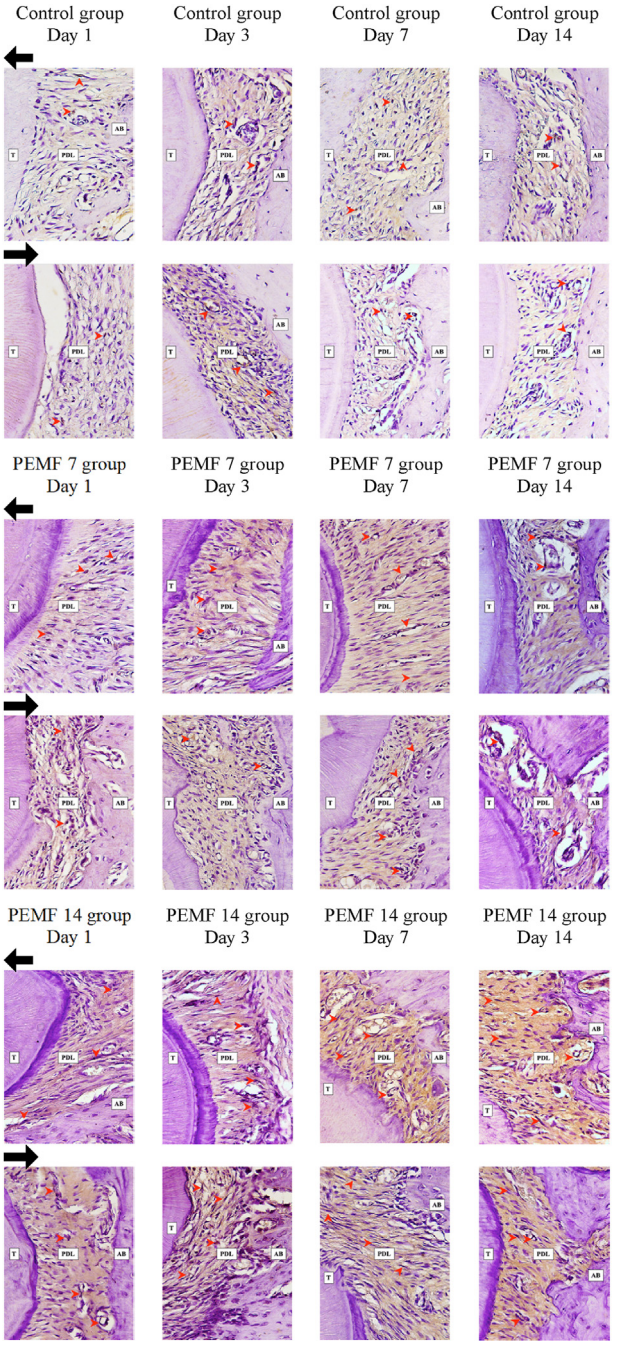

Figure 8: Histological observation of FGF-2 expression (red arrow) in the control, PEMF 7 and 14 groups. The direction of tooth movement (black arrow). AB, alveolar bone; FGF-2, fibroblast growth factor-2; PDL, periodontal ligament; PEMF, pulsed electromagnetic field; T, tooth. Scale bar: 50 μm.

tension and pressure sides, as shown in Table 3. Based on the results on the tension side, there were significant differences between the PEMF 14 and control groups on days 1, 3, 7, and 14, as well as between the PEMF 7 and 14 groups on days 1, 7, and 14 ($p < 0.05$). However, on the pressure side, the expression of Col-I showed a significant difference between the PEMF 14 and control groups on days 1, 3, 7, and 14, as well as between the PEMF 14 and 7 groups on days 1, 3, 7, and 14 ($p < 0.05$) (Fig. 6A, B). The expression of Col-I in the tension and pressure sides is presented in Figure 7.

The expression of FGF-2 in PEMF 7 and 14 groups was increased compared to the control group on the tension and pressure sides, as shown in Table 4. Statistically, there was a significant difference in expression between the PEMF 14 and control groups, as well as the PEMF 7 and PEMF 14 groups on the tension and pressure sides on days 1, 3, 7, and 14. The expression of FGF-2 also showed a significant difference between the PEMF 7 and control groups on days 1, 3, 7, and 14 ($p < 0.05$) on the pressure side (Figure 6C, D). The expression of FGF-2 on the tension and pressure sides is presented in Figure 8.

Discussion

Preventing relapse is crucial after completing orthodontic treatment to maintain the corrected position of the tooth. In this study, OTM in rats, which continued with the retention and relapse phases, was used to explore the effects of PEMF stimulation on relapse. The results of this study showed that PEMF stimulation for 2 h/day during the retention phase for 7 and 14 days reduced the relapse distance compared to control groups. Moreover, this stimulation also decreased the osteoclast number, as well as increased the number of osteoblasts and fibroblasts and the expression of Col-I and FGF-2 on the tension and pressure sides.

Several studies have reported that PEMF stimulation can reduce the number and viability of osteoclasts.[24,25] An in vitro study evaluated the effects of PEMF on osteoclast cells in women aged 18–68 years and showed that the number of these cells decreased at all ages. Another in vitro study showed that PEMF stimulation decreased osteoclast activity.[26] Jiang et al. (2016) also reported that PEMF stimulation resulted in increased OPG expression and decreased receptor activator of nuclear factor kappa B ligand expression, reducing osteoclast differentiation.[20]

The findings of this study are in agreement with previous research. Zhai et al. (2016) showed that PEMF with a frequency of 15.83 Hz and intensity of 2 mT for 2 h/day optimally increased the osteoblast proliferation of MC3T3-E1 cells in vitro, as confirmed by staining with alkaline phosphatase and alizarin red.[22] Exposure of MC3T3-E1 cells to PEMF at a frequency of 15 Hz and intensity of 5 mT has been proven effective in promoting the growth and differentiation as well as maturation of osteoblasts.[27] Meanwhile, exposure to PEMF at 50 Hz, 4.0 mT, and 40 min daily increased osteoblasts through the Wnt signaling pathway in vitro and in vivo. The Wnt signaling pathway acts as a crucial regulator of osteoblast formation by activating transcription factors, The role of the Wnt signaling pathway is to regulate osteoblast formation by activating transcription factors.[20,28] Based on the dual actions of osteoclasts and osteoblasts, PEMF has shown significant potential in alveolar bone deposition after OTM.

Restoring the structural integrity of the periodontal ligament after orthodontic treatment is a complex process, which requires rapid regeneration and restoration of function to inhibit relapse after the orthodontic device is removed. The periodontal ligament consists of Col fibers, mainly Col-I, located in the periodontal space between the alveolar socket and the root. The primary cells comprising the periodontal ligament include fibroblasts, which play a role in repairing alveolar bone and cementum.[29] This study showed a significant increase in fibroblasts in the PEMF 14 compared to the control and PEMF 7 groups. Costantini et al. (2019) investigated an in vitro wound model and reported that PEMF exposure induced the early phase of fibroblast proliferation and accelerating wound healing.[30]

The current study also reported that PEMF stimulation increased Col-I expression in the PEMF 7 and PEMF 14 groups. This result is in line with a study by Choi et al. (2015), who studied PEMF in the healing process of diabetic wounds and showed increased Col-I in the PEMF group compared to the control group.[31] PEMF stimulation for 4 weeks at a frequency of 3.85 kHz and intensity of 1.19 mT in a rat model of acute bilateral supraspinatus injury showed a significant increase in Col-I at the injury site compared to the control.[32] Furthermore, PEMF stimulation for 5 days at 3 min/day in rat tenocyte cultures showed a significant increase in Col-I expression.[33]

Generally, alveolar bones experience remodeling throughout life similarly to bone. Immediately after OTM, the alveolar bone requires accelerated bone formation to prevent relapse. This study showed that there was an increase in FGF-2 expression on the tension and pressure sides after 7 and 14 days of PEMF stimulation. FGF/FGFR signaling is also essential in the process of osteogenesis, indirectly increasing osteoblast differentiation. One of the family members, FGF-2, is the most widely used FGF ligand in the field of regenerative medicine, including regeneration of periodontal ligament, alveolar bone, cementum, and neovascularization.[34–36]

The results of this study showed that PEMF stimulation for 7 and 14 days in the orthodontic retention phase can prevent tooth relapse by increasing the number of osteoblasts and fibroblasts and the expression of FGF-2 and Col-I, as well as decreasing osteoclasts numbers after OTM in rat models. This study had several limitations. The investigations using molecular and microcomputed tomography analysis of alveolar bone and periodontal ligament, which are needed to determine the molecular and structural mechanisms of tooth relapse after OTM, were not assessed. Further research also needs to focus on local exposure to PEMF in the oral cavity so that it can become the basis for research on the clinical use of PEMF stimulators by dentists.

Conclusions

In conclusion, this study indicated that the reduction in tooth relapse could be attributed to PEMF stimulation for 7 and 14 days in the orthodontic retention phase after OTM in rat models by accelerating alveolar bone formation and periodontal ligament remodeling.

Source of funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Conflict of interest

The authors have no conflicts of interest to declare.

Ethical approval

The research protocol was approved by the Ethics Committee of the Faculty of Medicine, Universitas Brawijaya, Malang, Indonesia (Certificate No. 218/EC/KEPK-S3/09/2022) on September 4, 2022.

Authors contributions

HM: Conceptualization, Data curation, writing—original draft. YY: Investigation, Formal analysis, Validation, writing—review & editing. NP: Conceptualization, Methodology, Project administration, writing—review & editing. HS: Supervision, Visualization, writing—review & editing. All authors have critically reviewed and approved the final draft and are responsible for the content and similarity index of the manuscript.

Acknowledgment

The author thanks the Ministry of Education, Culture, Research and Technology, Indonesia for offering postgraduate scholarship programs through the Beasiswa Pendidikan Pascasarjana Dalam Negeri (BPPDN).

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How to cite this article: Maulana H, Yueniwati Y, Permatasari N, Suyono H. Pulsed electromagnetic field prevents tooth relapse after orthodontic tooth movement in rat models. J Taibah Univ Med Sc 2025;20(1):1–12.