

## Effect of extremely low frequency magnetic field in prevention of spinal cord injury-induced osteoporosis

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**Abstract**—The present study was designed to investigate the effect of extremely low frequency (ELF) magnetic field (MF) on spinal cord injury (SCI)-induced osteoporosis in rats. Adult male Wistar rats ( $n = 24$ ) were equally divided into sham, SCI, and SCI+MF groups. Complete transection of spinal cord (thoracic 11 vertebra) was surgically performed under anesthesia, whereas in the sham group only laminectomy was done. Post-SCI day 1, rats were either exposed (2 h/d  $\times$  8 wk) to ELF-MF (17.96 micro-Tesla, 50 Hz; SCI+MF group) or sham exposed (SCI group). Basso, Beattie, and Bresnahan (BBB) score was recorded weekly. All the rats were sacrificed 8 wk post-SCI; tibia and femur bones were isolated for the analysis of bone mineral content (BMC; total calcium [Ca], phosphorus [P], carbon [C]), bone mineral density (BMD), and biochemical status (osteocalcin, collagen I, alkaline phosphatase). The BBB score decreased post-SCI, which partially recovered after ELF-MF. In SCI rats, there was a statistically significant decrease in BMC, Ca, P, C, BMD, and biochemical level in both the bones as compared with the sham group, which was attenuated in SCI+MF rats except the C content. Electron microscopic study revealed the enhancement of microstructural composition and compactness in cortical and trabecular parts of treated bones. The results suggest that the chronic (2 h/d  $\times$  8 wk) ELF-MF exposure (17.96 micro-Tesla, 50 Hz) to SCI rats is effective in attenuating SCI-induced osteoporosis.

**Key words:** alkaline phosphatase, BBB score, bone mineral contents, bone mineral density, collagen I, complete transection, extremely low frequency magnetic field, osteocalcin, osteoporosis, spinal cord injury.

### INTRODUCTION

Spinal cord injury (SCI)-induced osteoporosis is reported as the most rapid and severe form of osteoporosis [1]. Post-SCI, bone loss has been detected only in the distal metaphysis of the femur on day 10, while bone loss has been detected at 3 wk in the metaphysis, epiphysis, and diaphysis of both the femur and tibia of rats [2–3]. A decrease in dry and ash weight are reported as early as 3 wk, with wet weight decreased at 6 wk [3–4]. Deterioration in bone mass and microarchitecture have been detected at 3 wk [3], and increase in water content has been detected at 6 mo [4]. A recent report attributes bone mass deterioration to dysfunction of osteoclast in addition to suppression of osteoblast activity in the development of

**Abbreviations:** ALP = alkaline phosphatase; BBB = Basso, Beattie, and Bresnahan; BMC = bone mineral content; BMD = bone mineral density; C = carbon; Ca = calcium; DXA = dual-energy X-ray absorptiometry; ELF-MF = extremely low frequency magnetic field; MF = magnetic field; OC = osteocalcin; OPG = osteoprotegerin; P = phosphorus; PEMF = pulsed electromagnetic field; RANKL = receptor activator of nuclear factor kappa-B ligand; SCI = spinal cord injury; SEM = scanning electron microscope; T = thoracic.

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post-SCI osteoporosis [2] as reflected by their respective markers. The bone formation markers in the serum include osteocalcin (OC), alkaline phosphatase (ALP), and procollagen, while bone resorption markers include carboxyterminal telopeptide of type I collagen and in urine as hydroxyproline, collagen type I telopeptide, etc. ALP is produced by osteoblasts together with the collagenous bone matrix and extruded into the extracellular matrix, where the enzyme is present prior to mineralization. Ambiguity regarding ALP's activity remains in patients with SCI [5–6], while in rats no change in ALP activity is reported after 8 to 12 d of stromal cell culture from post-SCI (3 wk) tissue [7] and in serum at >6 mo [4]. Serum OC, a small hydroxyapatite-binding protein, increases in urine and serum 3 mo post-SCI in patients [7–8] and 3 wk in rats [3,9], thereby indicating a predominance of osteoblast activity. Serum concentration of carboxy-terminal propeptide of type I collagen and amino-terminal propeptides of type I procollagen is an objective measure of newly formed type I collagen. Substantial increases in the serum type I collagen C-telopeptide at 3 mo and urinary hydroxyproline/creatinine ratios at 1 mo are reported in patients with SCI [8,10], while serum N-terminal telopeptide of type I collagen are increased at 3 wk to 6 mo in rats with SCI [4,11–12]. Intensive exercise or standing regimens may partially prevent bone loss in the femoral shaft but not at the proximal hip, while functional electrical stimulation [7,13–15] and pharmacological agents (etidronate, alendronate) are reported to be ineffective in patients with SCI [16–17], probably because of an exorbitant sublesional bone turnover and osteoclastic activity at all the resorption pits [6].

Pulsed electromagnetic field (PEMF) therapy is reported to promote peripheral nerve regeneration, osteogenesis, and probably recovery from SCI because of increases in nicotinamide adenine dinucleotide (NAD)-specific isocitrate dehydrogenase activity, acetyl cholinesterase at the motor plate, sparing of white matter, number of motor neurons reestablishing connections [18], maturation of bone trabecula, bone volume, bone formation, and decrease in lesion volume [19–21]. PEMF induces the differentiation of cartilage cells and enhances ALP activity in rat osteoblasts [22]. The beneficial effect of PEMF on other models of osteoporosis, namely disuse osteoporosis (15 Hz, 8 ms for 2h/d × 8 wk) [23] and ovariectomy-induced osteoporosis (1–2 mV/cm, 0.3 ms, 7.5 Hz for 8h/d × 30 wk) [24] has been reported in avians and rats, respectively. A recent report suggests intensive

electrical stimulation for 1 h/d for 6 wk can reduce femoral bone mineral density (BMD) loss induced by acute SCI [25] and benefits of chronic extremely low frequency magnetic field (ELF-MF; 50 Hz, 1.5  $\mu$ T, 4 h/d × 6 mo) in ovariectomy-induced osteoporosis in rats [26].

However, to the best of our knowledge, there is no report regarding ELF-MF in SCI-induced osteoporosis. Therefore, we report the efficacy of chronic ELF-MF (50 Hz, 17.96  $\mu$ T, 2 h/d × 8 wk) on bone mineral content (BMC; total calcium [Ca], phosphorus [P], and carbon [C]), BMD, and biochemical status (OC, collagen I, ALP) of SCI-induced osteoporosis in adult male rats.

## METHODS

### Animals

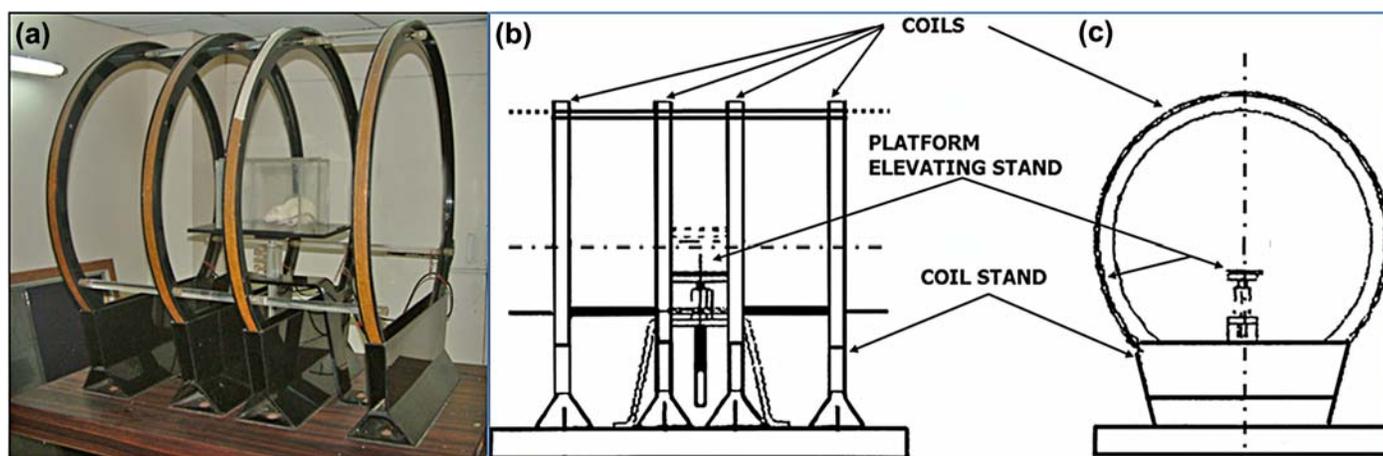
Adult male Wistar rats (body weight 230–250 g) were individually housed in a room at  $24 \pm 2^\circ\text{C}$  on a 14:10 h light:dark cycle and were provided with standard laboratory food pellets and fresh tap water ad libitum daily. Rats ( $n = 24$ ) were equally divided into sham group, SCI group, and magnetic field (MF)-exposed SCI rats (SCI+MF group).

### Extremely Low Frequency Magnetic Field Exposure Chamber

The MF stimulator used has electromagnetic coils mounted on a stand, a movable platform for the rat cage, and a current regulator that maintains constant current through the coils [27]. The coils (2 outer coils, each with 18 turns; 2 inner coils, each with 8 turns) are connected in series and provide a uniform ELF-MF (17.96  $\mu$ T, 50 Hz) of the modified Helmholtz coil in the central area where the eight rats were kept separately in a specially designed polypropylene cage (**Figure 1**).

### Spinal Cord Injury Method

Under deep anesthesia (ketamine + xylazine; 60 + 10 mg/kg body weight, intramuscular), the dorsum of the animals were shaved and a longitudinal midline incision was made through the skin. A selective laminectomy (thoracic [T]10–T12 vertebrae) was done to only expose the spinal cord (sham group). After the laminectomy, complete spinal cord transection (T11 vertebra corresponding to T13 spinal cord) was done with microscissors (SCI group) under magnifying lenses. Both stumps of the spinal cord were gently lifted away to



**Figure 1.** Magnetic field chamber. (a) Original photograph. (b) Diagrammatic side view. (c) Diagrammatic front view. Diameters of coils are 1,000 mm, with 45 mm width. Distance of outer coil from center of structure is 470 mm and of inner coil is 122 mm. Number of turns in outer coil is 18 and turns in inner coil is 8. Current in electromagnetic coil is 1 A at 50 Hz. Magnetic field is 17.96  $\mu\text{T}$  at central exposing area.

create a 1 to 2 mm gap, which was filled with sponge gel. The muscle fascia and skin were sutured, and the rats were returned to their home cages. After surgery, rats received a bolus of Lactate Ringers solution (5 mL, intraperitoneal) to compensate for blood loss, and antibiotic cover (systemic, gentamycin 50 mg/kg body weight, intramuscular; local neosporin ointment) was provided. Manual evacuation of the bladder was regularly done 3 times/day until reflex micturition was restored.

#### Assessment of Locomotor Functions

The quality of locomotion was assessed by Basso, Beattie, and Bresnahan (BBB) locomotor rating score [28].

#### Determination of Volumetric Bone Mineral Content and Bone Mineral Density

The volume of fresh bones was determined by submersion method. After the bone was freeze-dried, dry weight was determined and percent of water was calculated. The bone was ground and kept (50 mg) in muffle furnace (700°C) for 8 h to obtain bone ash, which was weighed (BMC/50 mg), while BMD ( $\text{g}/\text{cm}^3$ ) was calculated from the ratio BMC/bone volume [29].

#### Determination of Element Content (Ca, P, C)

Total Ca content was determined by atomic absorption method as described elsewhere [29]. Briefly, the lyophilized bone powder (50 mg) was digested in aqua

regia, diluted with distilled water, and combined with lanthanum chloride (0.5%). The concentration of Ca was calculated from the standard curve of Ca.

P content was determined using the Vanado-Molybdo-phosphoric acid colorimetric method. Lyophilized bone powder (50 mg) was digested in a mixture of  $\text{H}_2\text{SO}_4$  and  $\text{HNO}_3$ , heated to remove  $\text{HNO}_3$ , neutralized with double-distilled water, and combined with a Vanadate-molybdate reagent.

Total C content in lyophilized bone sample (50 mg) kept in a sterile ceramic boat was determined by a C analyzer (CS 500, ELTRA; Haan, Germany) at 1,200°C.

#### Biochemical Analysis

##### Collagen I

A bone sample (50 mg) was digested with acetic acid (2 mL, 0.5 M, pH 2.0) for 48 h [30]. Soluble collagen was separated by centrifugation; supernatant was transferred to a microcentrifuge tube, and the residue was digested with pepsin (1 mg/10 mg bone powder) in acetic acid for 48 h. The pepsin-soluble collagen was separated by centrifugation, whereas the pepsin-insoluble bone matrix was extracted by guanidine hydrochloride (4.0 M) in Tris-HCl (0.05 M, pH 7.5) for 48 h and separated by centrifugation. The supernatants were pooled for salt precipitation ( $\text{NaCl}$ , 2.6 M, pH 7.4) and dissolved in acetic acid containing sirius red (1%), and stained collagen was

separated by centrifugation (15,000 g, 45 min). The residue was dissolved in acetic acid and the collagen I concentrations were measured in a microplate reader using collagen type I standard (C3867, Sigma-Aldrich; St. Louis, Missouri).

### Osteocalcin

Bone powder was decalcified in ethylenediamine tetra-acetic acid, (10% weight per volume, pH 7.2) and extracted by stirring at 4°C for 48 h. OC in the supernatant was measured by using a rat sandwich enzyme-linked immunosorbent assay kit (BPB Biomedical; Stroughton, Massachusetts).

### Alkaline Phosphatase Activity

The ALP activity in lyophilized bone powder was determined with the plasma ALP kit. Briefly, the bone powder was sonicated in MgCl<sub>2</sub>, 10 mM and Triton X-100, 0.1 percent. ALP activity was determined colorimetrically using p-nitrophenylphosphate substrate. The total protein content was measured using a protein assay kit (Bio-Rad Laboratories; Hercules, California) utilizing bovine serum albumin as the protein standard.

### Scanning Electron Microscopy

The proximal diaphysis sections from the femur and tibia and the acetabulum of the femur were cut for scanning electron microscope (SEM) study in every rat. The bone samples were dried and mounted on circular disc stubs with adhesive. Gold coatings were applied at a thickness of about 20 nm with the help of a sputter coater. SEM images were obtained on low vacuum SEM Leo 435 VP (Cambridge, England) at the National Facilities of Electron Microcopy, All India Institute of Medical Sciences, New Delhi. The cortical area, interpolated polygon area, and perimeter of porosity was obtained utilizing Olympus imaging software (Center Valley, Pennsylvania).

### Study Plan

The rats of the ELF-MF group received MF exposure consecutively for 2 h/d × 8 wk (10:00 am–12:00 pm) from post-SCI day 1, while those of sham and SCI groups were similarly treated but without MF exposure. BBB score was noted weekly pre- and post-SCI for 8 wk. Rats were sacrificed by decapitation under deep ether anesthesia. Tibia and femur bones were freed from soft tissues on both the sides and stored at –20°C until analysis.

### Statistical Analysis

Data are presented as mean ± standard deviation and compared between groups using one-way analysis of variance followed by post hoc analysis by Bonferroni test. *P*-values less than 0.05 were accepted as significant.

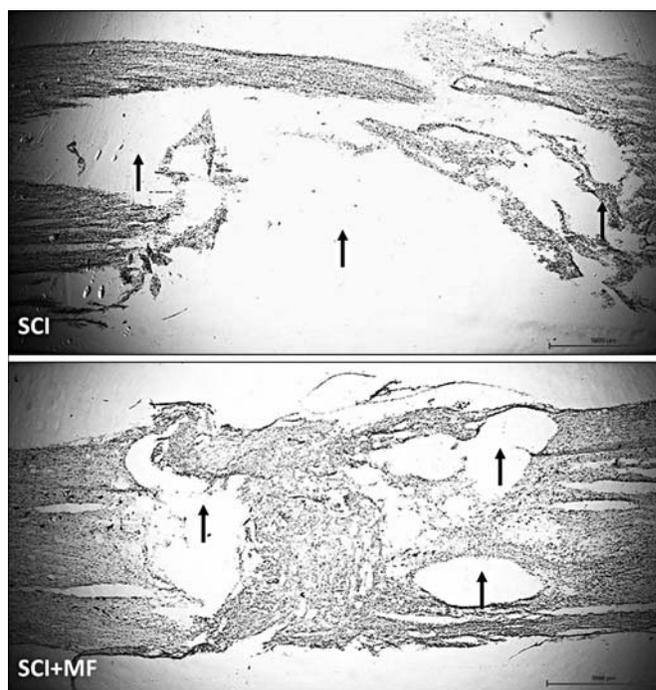
## RESULTS

### Histology

Histology confirmed complete transection of the spinal cord in the SCI group and partial restoration in the SCI+MF group at 8 wk post-SCI (**Figure 2**).

### Basso, Beattie, and Bresnahan Score

BBB score decreased ( $p < 0.001$ ) in the SCI versus sham groups at all time-points, which was restored to the baseline ( $p < 0.001$ ) post-SCI week 2 onwards in the SCI+MF group. However, the BBB score was significantly



**Figure 2.** Represented section of spinal cord post-spinal cord injury (SCI) week 8 in SCI and magnetic field-exposed spinal cord injury (SCI+MF) groups of rats. There are areas of continuity in spinal cord and smaller cavities vis-à-vis degeneration of neurons (arrow mark) in SCI+MF group versus SCI group.

lower in the SCI+MF group versus sham group after SCI and before MF exposure (Figure 3).

### Bone Water Content, Bone Mineral Content, and Bone Mineral Density

Water content of both bones was higher ( $p < 0.001$ ), while BMC and BMD were lower post-SCI versus sham group. As compared with SCI group, the SCI+MF group had less water content and higher BMC and BMD, excluding BMC in the femur. There was no statistically significant difference in the bones of the SCI+MF group versus sham group (Table 1).

### Total Calcium, Phosphorus, and Carbon Content

In both the tibia and femur bones, Ca, P, and C contents significantly decreased post-SCI versus sham group, all of which recovered after MF except for P and C in the femur and tibia, respectively. There was no statistically significant difference in the SCI+MF group versus sham group except for C content in both the bones (Table 2).

### Biochemical Status (Collagen I, Osteocalcin, and Alkaline Phosphatase)

Post-SCI, the levels of collagen I, OC, and ALP decreased ( $p < 0.001$ ) in both the bones versus sham

group, all of which were recovered by MF, except collagen I in the femur. In the SCI group versus the SCI+MF group, the biochemical levels were higher in both the bones, except collagen I in tibia (Table 3).

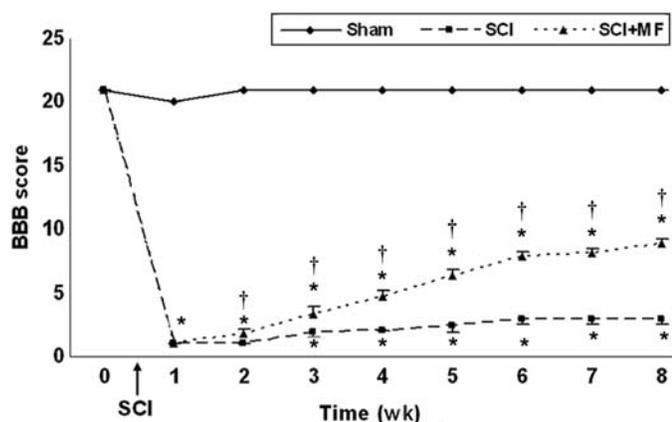
### Scanning Electron Microscope of Bones

SEM images in the transverse section of bone samples show the microstructural changes in bones (Figures 4–6). The femoral neck part and acetabular head showed more compactness and less porosity in SCI+MF versus SCI rat groups (Figure 4). The trabecular bone, the frets (intertrabeculae) of proximal epiphysis in SCI rat, were absorbed and reformed after SCI+MF; however, connectivity was less than control (Figure 5 and Table 4). Cortical thickness was significantly more (Table 5) and bone marrow was attached to the cortex in the SCI+MF versus SCI rat groups (Figure 6). The SEM study indicates lesser mineral deposition and greater porosity in SCI bones, while mineral deposition was recovered in the SCI+MF group.

## DISCUSSION

The present study was proposed to investigate the efficacy of ELF-MF in an SCI model of osteoporosis. SCI produced a significant decrease in BBB score, BMC, BMD, Ca, P, C, and biochemical parameters (collagen I, OC, and ALP) of weight-bearing bones (femur, tibia) as compared with a sham group of rats. Exposure (2 h/d  $\times$  8 wk) to ELF-MF (17.96  $\mu$ T, 50 Hz) restored these parameters, except C content in both the bones and partial recovery in BBB score. Therefore, our results indicate that exposure to ELF-MF has a therapeutic potential in the management of SCI-induced osteoporosis.

Complete SCI at the lower thoracic cord of rats is widely used to study pathophysiology of SCI-induced osteoporosis [3–4,6–7]. It produces the most rapid and severe forms of osteoporosis, resistant even to exercise. Several factors, including unloading, neural lesion, and endocrine disturbances, contribute toward osteoporosis, although the precise mechanism is not known [1]. Mechanical stimulation—which is sensed by mechanosensory cells (osteocytes), transmitted via intracellular and extracellular signals to induce bone formation by osteoblasts—during weight bearing is crucial for bone remodeling. SCI contributes toward osteoporosis directly by denervation of bone or indirectly by disrupting



**Figure 3.** Effect of spinal cord injury (SCI) (interrupted line with filled square) on locomotion as indicated by Basso, Beattie, and Bresnahan (BBB) score (mean  $\pm$  standard deviation) compared with sham operated group (uninterrupted line). Beneficial effect of exposure to magnetic field (MF) is clear (interrupted line with filled triangles). \*Comparison of SCI group and MF-exposed SCI (SCI+MF) group versus sham group. †Comparison between SCI group versus SCI+MF group.  $p < 0.001$ .

**Table 1.**

Effect of spinal cord injury (SCI) and magnetic field (MF) exposure on water content, bone mineral content (BMC), and bone mineral density (BMD) in femur and tibia.

Parameter	Bone	Group			p-Value		
		Sham	SCI	SCI+MF	Sham vs SCI	Sham vs SCI+MF	SCI vs SCI+MF
Water Content (% of wet weight)	Femur	21.90 ± 5.72	55.64 ± 4.6	32.55 ± 5.92	<0.001	0.75	0.03
	Tibia	16.42 ± 3.31	34.94 ± 8.8	23.14 ± 10.96	<0.001	0.2	0.001
BMC (mg)	Femur	407.83 ± 16.4	328.51 ± 51.45	366.38 ± 60.34	0.01	0.27	0.36
	Tibia	276.97 ± 16.9	211.60 ± 30.67	268.59 ± 14.24	<0.001	>0.99	<0.001
BMD (mg/mL)	Femur	1,019.99 ± 112.2	707.11 ± 102.8	971.47 ± 308.9	0.02	>0.99	0.04
	Tibia	780.12 ± 56.85	552.38 ± 80.95	763.06 ± 65.57	<0.001	>0.99	<0.001

Note: p-value of difference between groups of animals (sham vs SCI, sham vs SCI+MF, SCI vs SCI+MF) was calculated using one-way analysis of variance.

**Table 2.**

Effect of spinal cord injury (SCI) and magnetic field (MF) exposure on total calcium (Ca), phosphorus (P), and carbon (C) contents of femur and tibia.

Parameter	Bone	Group			p-Value		
		Sham	SCI	SCI+MF	Sham vs SCI	Sham vs SCI+MF	SCI vs SCI+MF
Ca (mg)	Femur	254.65 ± 14.64	211.42 ± 7.56	244.16 ± 6.78	<0.001	0.16	<0.001
	Tibia	231.58 ± 7.60	193.06 ± 8.20	219.84 ± 15.25	<0.001	0.13	<0.001
P (mg)	Femur	133.25 ± 12.98	117.40 ± 7.48	130.67 ± 10.75	0.02	>0.99	0.06
	Tibia	115.96 ± 8.84	101.59 ± 8.23	113.29 ± 6.13	0.004	>0.99	0.02
C (% of dry weight)	Femur	183.91 ± 6.44	149.65 ± 1.17	174.37 ± 5.34	<0.001	0.02	<0.001
	Tibia	178.99 ± 2.98	147.74 ± 8.69	154.81 ± 4.66	<0.001	<0.001	0.08

Note: p-value of difference between groups of animals (sham vs SCI, sham vs SCI+MF, SCI vs SCI+MF) was calculated using one-way analysis of variance.

**Table 3.**

Effect of spinal cord injury (SCI) and magnetic field (MF) exposure on total collagen, osteocalcin, and alkaline phosphatase (ALP) activity of femur and tibia.

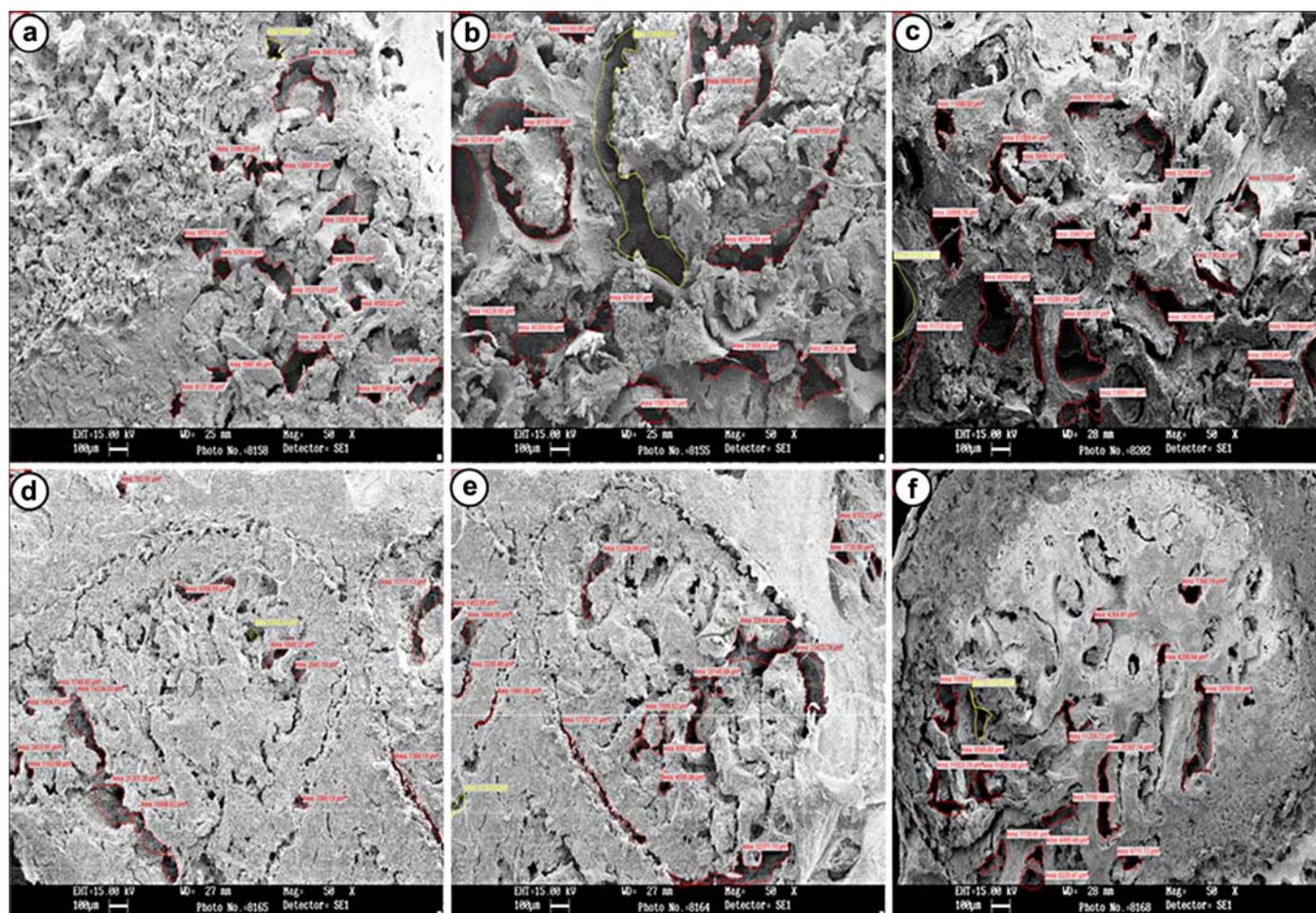
Parameter	Bone	Group			p-Value		
		Sham	SCI	SCI+MF	Sham vs SCI	Sham vs SCI+MF	SCI vs SCI+MF
Collagen I (mg/g)	Femur	296.59 ± 34.80	213.33 ± 22.73	255.17 ± 33.83	<0.001	0.04	0.04
	Tibia	274.83 ± 28.62	221.07 ± 36.99	254.31 ± 34.51	0.01	0.7	0.18
Osteocalcin (µg/g)	Femur	790.14 ± 76.71	459.74 ± 52.99	705.06 ± 84.21	<0.001	0.08	<0.001
	Tibia	850.37 ± 54.49	530.85 ± 51.26	785.28 ± 73.35	<0.001	0.13	<0.001
ALP (IU/g)	Femur	134.68 ± 8.84	93.10 ± 11.57	121.28 ± 11.50	<0.001	0.06	<0.001
	Tibia	124.53 ± 7.32	106.17 ± 3.80	115.67 ± 9.13	<0.001	0.06	0.04

Note: p-value of difference between groups of animals (sham vs SCI, sham vs SCI+MF, SCI vs SCI+MF) was calculated using one-way analysis of variance.

vasoregulation. Hormones also participate in post-SCI osteoporosis, possibly via negative  $Ca^{2+}$  balance caused by increased excretion.

Nonetheless, it is critical to precisely assess and monitor the progression of osteoporosis to limit the incidence of fracture. BMD and BMC are currently the gold standard for objective assessment of osteoporosis and are the best surrogate of fracture risk in vivo of 50 to 80 percent variance in bone strength [31]. The methods used for in situ clinical assessment of osteoporosis are micro-computed

tomography and dual-energy X-ray absorptiometry (DXA), while for in vitro analysis only ash weight is used. DXA-derived BMC values are on average higher than the ash weight and may lead to a dangerous underestimation of fracture risk [32]. Lochmüller et al. have suggested that the ash weight, Ca, and P content provide a better estimate of the femur fracture risk than in situ DXA-derived BMC [32]. Since the aim of this study was to explore the efficacy of ELF-MF in the prevention of SCI-induced osteoporosis, we chose to determine BMC and BMD from the bone ash,



**Figure 4.**

Scanning electron microscope images of femur from sham, spinal cord injury (SCI), and magnetic field (MF)-exposed SCI (SCI+MF) groups. Micrographs from femoral neck part (a–c) and acetabular head (d–f) of rat femur bone in sham, SCI, and SCI+MF bone samples.

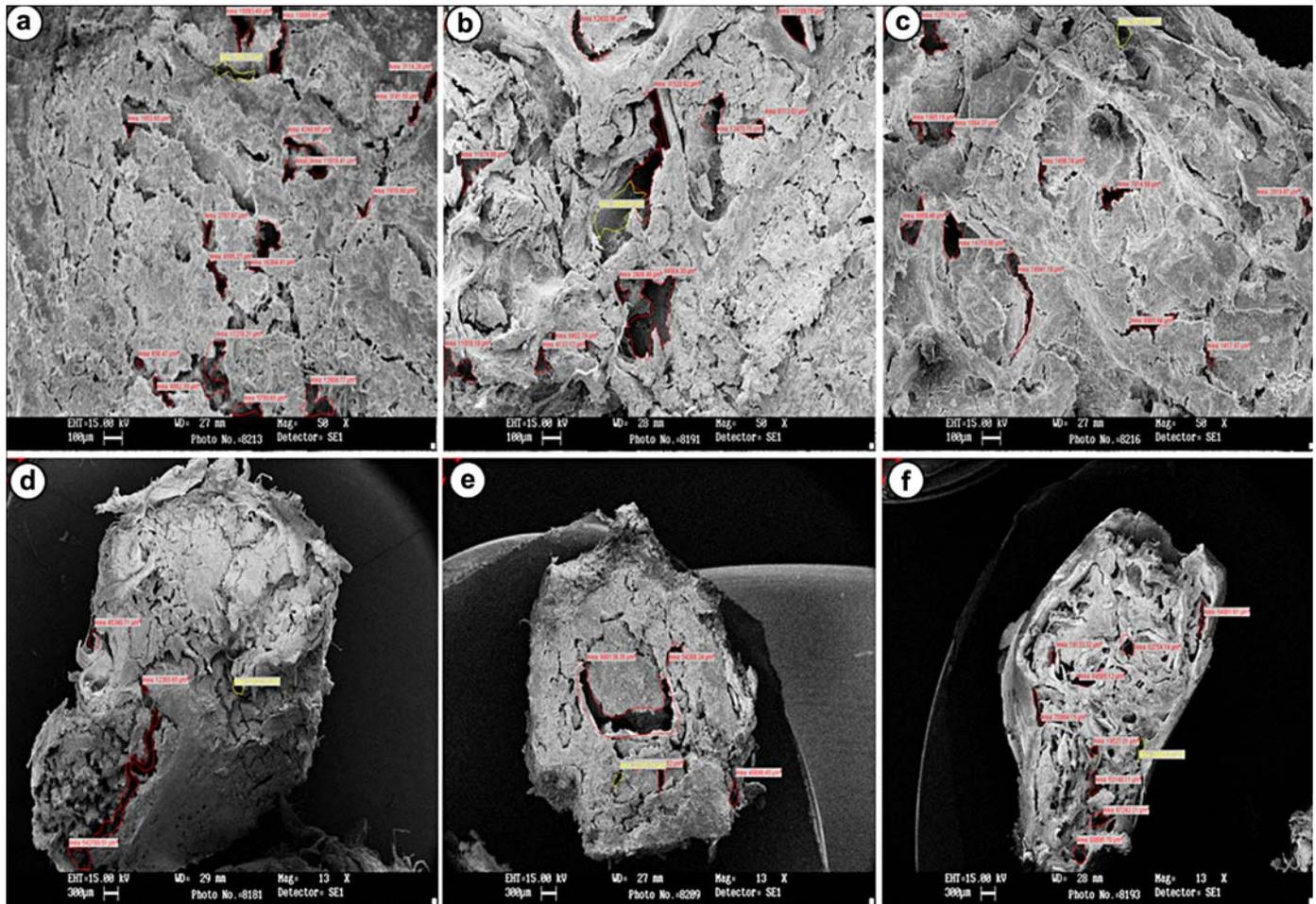
which has the advantage of providing direct sensitive evidence of osteoporosis.

However, osteoporosis is established in patients with SCI on the basis of the biochemical markers reflecting either an increase in osteoclastic or a decrease in osteoblastic activity. Formation, maturation, and destruction products of bone collagen I in serum and urine indicate either osteoblastic or osteoclastic activity. Collagen constitutes about 90 percent of the structural protein of the bone matrix and is secreted by osteoblasts, which are stiffened by integration of the mineral phase [33]. In our study, the water content in the femur and tibia were increased after SCI, which indicates demineralization of these bones. We further support the loss of bone by their microarchitecture.

Post-SCI 3 wk to 6 mo, the serum level of bone collagen I telopeptide was reported to be higher, indicating

predominant osteoclastic activity [3–4]. In consonance with our objective and the limited value of direct translation of circulating/urinary markers into the magnitude of bone gain and resorption, it was pertinent to determine the status of bone activity in the bone itself. In our rats, the concentration of collagen I was significantly reduced in both the sublesional bones, thereby supporting enhanced osteoclastic activity.

A higher serum OC concentration in patients with SCI (immediately, 3–6 mo) and rats (3–4 wk) has been reported [3,7–8,10]. OC serum concentration correlates with static and dynamic parameters of bone formation, but any correlation with matrix synthesis or mineralization is not evidenced. Therefore, we estimated OC concentration directly in the bones to support the possible efficacy of ELF-MF in the management of SCI-induced osteoporosis.



**Figure 5.**

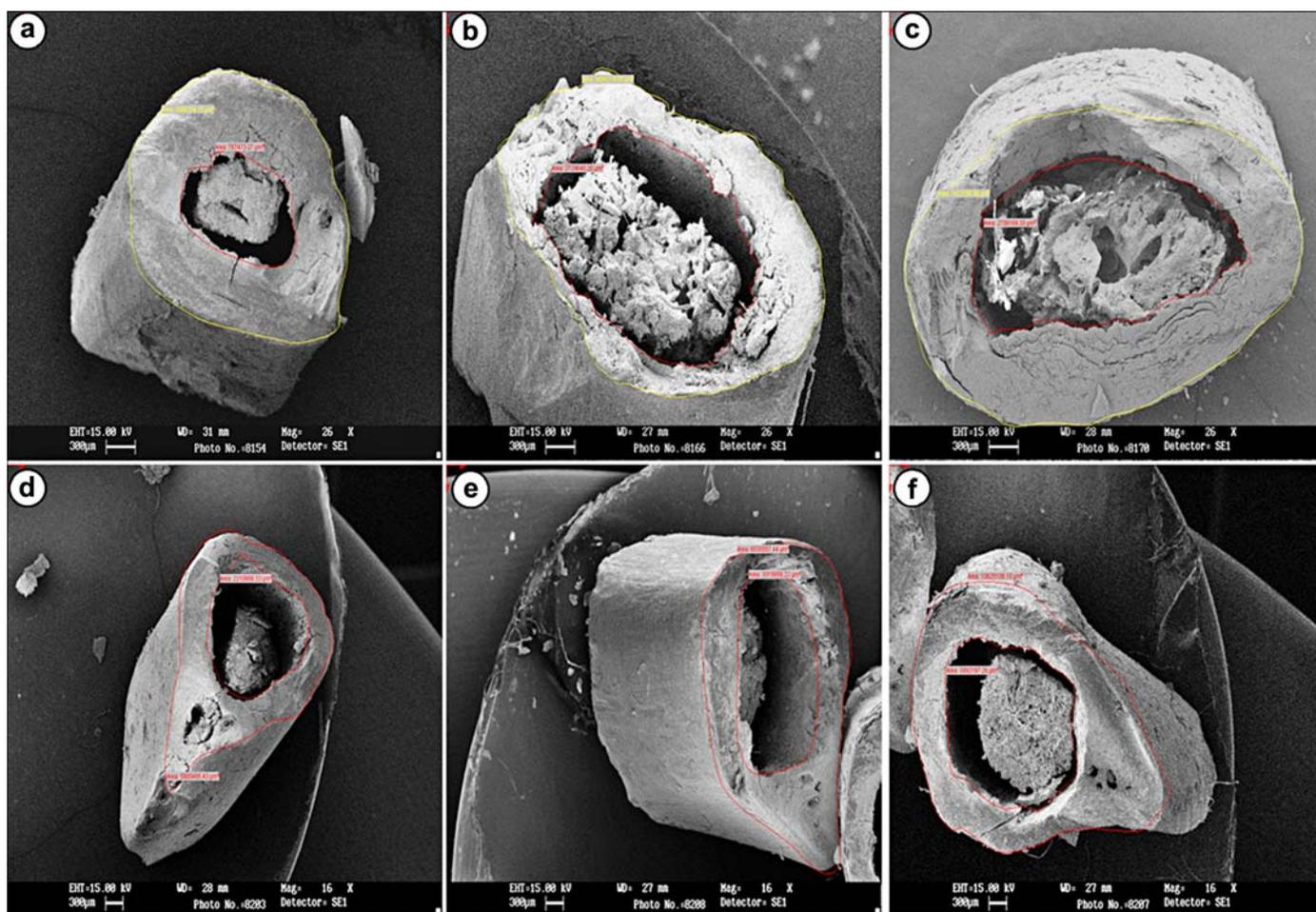
Microarchitecture (microradiograph of tranverse section) from trabecular part of proximal femur (a–c) and tibia (d–f) of sham, spinal cord injury (SCI), and magnetic field (MF)-exposed SCI (SCI+MF) bone samples.

Our study showed that SCI caused a significant decrease in OC concentration in the bones, thereby signifying a probable decrease in bone matrix synthesis in our rats.

Osteoblasts express robust ALP activity, which is anchored to the external surface of the plasma membrane and contributes to bone mineralization [34]. ALP is considered to be an early differentiation marker of osteoblast maturation, while collagen and OC formation represent the end of differentiation. Therefore, ALP in matrix and osteoblast is a good indicator of bone formation and matrix mineralization [35]. On the contrary, no effect on serum bone ALP post-SCI and total ALP activity in culture exist in the literature [6,10]. This discrepancy is explainable since the serum pool consists of several dimeric isoforms, which originate from various tissues besides bones [36].

Our study provides evidence of variation in ALP activity of the bone per se, wherein SCI led to a significant decrease in ALP level in weight-bearing bones, reflecting a low osteoblastic activity.

It is clear from our study that ELF-MF exposure favored osteogenesis, although the specific mechanism is not precisely understood. Nonetheless, the literature suggests a positive effect of PEMF on bone formation in delayed-union bone fractures, failed joint fusions, congenital pseudoarthroses, neurectomy, and ovariectomy-induced osteoporosis [20,37–40]. These processes also lead to an alteration in the expression of genes leading to osteoblast proliferation. Osteoblasts proliferate and differentiate by modification of ion channel activity and enhance the synthesis of matrix formation by PEMF



**Figure 6.**

Cortical part microarchitectural depiction of femur (a–c) and tibia (d–f) of sham, spinal cord injury (SCI), and magnetic field (MF)-exposed SCI (SCI+MF) bone samples.

in other models of osteoporosis [41–42]. ALP expression is considered a good early indicator of osteoblast phenotype. In our SCI+MF rats, local concentration of ALP in bone was higher, indicating predominance of osteoblastic activity. ELF-MF has been suggested to promote the formation of matrix protein, namely collagen, OC, and ALP in bone cell cultures [43–45]. In our model of SCI-induced osteoporosis, ELF-MF also increased the concentration of collagen I, OC, and ALP, indicating the formation of matrix protein in the bones, wherein collagen I specifically provides a site for mineral precipitation and OC and ALP for its mineralization [46]. An increased mineralization in sublesional bones in our ELF-MF exposed rats with SCI is further supported by their increase in BMC and BMD, with a concomitant decrease in water content and microarchitecture. Nonetheless, for

a greater magnitude of effect, exposure sessions may be further increased.

Besides promoting in bone formation, MF stimulation possibly improves bone status by restricting the resorption process. Cytokines required for osteoclast formation—namely, receptor activator of nuclear factor kappa-B ligand (RANKL) and macrophage colony-stimulating factor—bind to the transmembrane receptor of osteoclast to promote its differentiation and induce osteoclast proliferation, respectively. RANKL also binds to osteoprotegerin (OPG), a decoy receptor, which reduces resorption. PEMF is reported to increase OPG concentration, decrease RANKL concentration, and increase osteoclast apoptosis [47–48], and therefore it is probable that ELF-MF not only promotes bone formation but also limits bone resorption.

**Table 4.**

Porosity quantification in different parts of proximal femur and tibia bones of scanning electron microscope micrograph.

Bone Area	Sham Group		SCI Group		SCI+MF Group	
	Interpolated Polygon Area (mm <sup>2</sup> )	Interpolated Polygon Perimeter (mm)	Interpolated Polygon Area (mm <sup>2</sup> )	Interpolated Polygon Perimeter (mm)	Interpolated Polygon Area (mm <sup>2</sup> )	Interpolated Polygon Perimeter (mm)
Trabecular Part of Proximal Femur	155.59 ± 14.54	7.09 ± 0.35	530.47 ± 32.30*†	16.41 ± 0.99*†	306.91 ± 16.51†§	12.03 ± 0.48*†,‡¶
Acetabular Head of Femur	90.58 ± 5.75	5.66 ± 0.19	163.43 ± 4.14*§	10.44 ± 0.41*¶	152.12 ± 4.36‡§	7.83 ± 0.21‡¶
Trabecular Part of Proximal Tibia	118.03 ± 6.15	7.03 ± 0.15	210.24 ± 10.86*¶	8.25 ± 0.16*¶	19.81 ± 1.47†‡	8.71 ± 0.04†‡
Proximal Part of Tibia	6,176.30 ± 23.22	9.47 ± 0.25	1,064.79 ± 147.17*†	11.96 ± 0.12*¶	476.92 ± 101.91*†	9.80 ± 1.60*†

\*Comparison with sham group.

†*p* < 0.05.

‡Comparison with SCI group.

§*p* < 0.001.¶*p* < 0.01.

MF = magnetic field, SCI = spinal cord injury.

**Table 5.**Cortical area quantification (mm<sup>2</sup>) in femur and tibia cortex scanning electron microscope micrograph in different animal groups.

Bone	Sham Group	SCI Group	SCI+MF Group
Cortical Bone in Femur	4,746.25 ± 829.45	972.65 ± 53.99*†	3,819.10 ± 172.38‡§
Cortical Bone in Tibia	6,597.36 ± 403.25	3,760.44 ± 297.38*§	4,858.77 ± 438.55‡§

\*Comparison with sham group.

†*p* < 0.001.

‡Comparison with SCI group.

§*p* < 0.01.

MF = magnetic field, SCI = spinal cord injury.

ELF-MF exposure in our rats could have possibly supported recovery by resumption of weight bearing as revealed by BBB score. Unloading is one of the important causes in the genesis of osteoporosis induced by SCI [18–19,49–52].

## CONCLUSIONS

The present study suggests that SCI-induced osteoporosis in our rats could be limited by chronic (2 h/d × 8 wk) exposure to ELF-MF (17.96 μT, 50 Hz) as revealed by BBB score, BMC, BMD, mineral element contents, and biochemical parameters pertaining to the sublesional bones.

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*Study concept and design:* J. Manjhi, R. Mathur, J. Behari.

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*Analysis and interpretation of data:* J. Manjhi, S. Kumar.

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## REFERENCES

- Jiang SD, Jiang LS, Dai LY. Mechanisms of osteoporosis in spinal cord injury. *Clin Endocrinol (Oxf)*. 2006;65(5): 555–65. [PMID:17054455] <http://dx.doi.org/10.1111/j.1365-2265.2006.02683.x>
- Morse L, Teng YD, Pham L, Newton K, Yu D, Liao WL, Kohler T, Müller R, Graves D, Stashenko P, Battaglini R.

- Spinal cord injury causes rapid osteoclastic resorption and growth plate abnormalities in growing rats (SCI-induced bone loss in growing rats). *Osteoporos Int.* 2008;19(5):645–52. [PMID:17987335]  
<http://dx.doi.org/10.1007/s00198-007-0494-x>
3. Jiang SD, Jiang LS, Dai LY. Spinal cord injury causes more damage to bone mass, bone structure, biomechanical properties and bone metabolism than sciatic neurectomy in young rats. *Osteoporos Int.* 2006;17(10):1552–61. [PMID:16874443]  
<http://dx.doi.org/10.1007/s00198-006-0165-3>
  4. Jiang SD, Jiang LS, Dai LY. Changes in bone mass, bone structure, bone biomechanical properties, and bone metabolism after spinal cord injury: a 6-month longitudinal study in growing rats. *Calcif Tissue Int.* 2007;80(3):167–75. [PMID:17340221]  
<http://dx.doi.org/10.1007/s00223-006-0085-4>
  5. Demirel G, Yilmaz H, Paker N, Onel S. Osteoporosis after spinal cord injury. *Spinal Cord.* 1998;36(12):822–25. [PMID:9881730]  
<http://dx.doi.org/10.1038/sj.sc.3100704>
  6. Jiang SD, Jiang LS, Dai LY. Effects of spinal cord injury on osteoblastogenesis, osteoclastogenesis and gene expression profiling in osteoblasts in young rats. *Osteoporos Int.* 2007;18(3):339–49. [PMID:17036173]  
<http://dx.doi.org/10.1007/s00198-006-0229-4>
  7. Liu D, Zhao CQ, Li H, Jiang SD, Jiang LS, Dai LY. Effects of spinal cord injury and hindlimb immobilization on sublesional and supralesional bones in young growing rats. *Bone.* 2008;43(1):119–25. [PMID:18482879]  
<http://dx.doi.org/10.1016/j.bone.2008.03.015>
  8. Pietschmann P, Pils P, Woloszczuk W, Maerk R, Lessan D, Stipicic J. Increased serum osteocalcin levels in patients with paraplegia. *Paraplegia.* 1992;30(3):204–9. [PMID:1630849]  
<http://dx.doi.org/10.1038/sc.1992.56>
  9. Price PA, Baukol SA. 1,25-Dihydroxyvitamin D3 increases synthesis of the vitamin K-dependent bone protein by osteosarcoma cells. *J Biol Chem.* 1980;255(24):11660–63. [PMID:6969260]
  10. Maïmoun L, Couret I, Micallef JP, Peruchon E, Mariano-Goulart D, Rossi M, Leroux JL, Ohanna F. Use of bone biochemical markers with dual-energy x-ray absorptiometry for early determination of bone loss in persons with spinal cord injury. *Metabolism.* 2002;51(8):958–63. [PMID:12145766]  
<http://dx.doi.org/10.1053/meta.2002.34013>
  11. Roberts D, Lee W, Cuneo RC, Wittmann J, Ward G, Flatman R, McWhinney B, Hickman PE. Longitudinal study of bone turnover after acute spinal cord injury. *J Clin Endocrinol Metab.* 1998;83(2):415–22. [PMID:9467550]  
<http://dx.doi.org/10.1210/jc.83.2.415>
  12. Zehnder Y, Risi S, Michel D, Knecht H, Perrelet R, Kraenzlin M, Zäch GA, Lippuner K. Prevention of bone loss in paraplegics over 2 years with alendronate. *J Bone Miner Res.* 2004;19(7):1067–74. [PMID:15176988]  
<http://dx.doi.org/10.1359/JBMR.040313>
  13. BeDell KK, Scremin AM, Perell KL, Kunkel CF. Effects of functional electrical stimulation-induced lower extremity cycling on bone density of spinal cord-injured patients. *Am J Phys Med Rehabil.* 1996;75(1):29–34. [PMID:8645435]  
<http://dx.doi.org/10.1097/00002060-199601000-00008>
  14. Leeds EM, Klose KJ, Ganz W, Serafini A, Green BA. Bone mineral density after bicycle ergometry training. *Arch Phys Med Rehabil.* 1990;71(3):207–9. [PMID:2317139]
  15. Pacy PJ, Hesp R, Halliday DA, Katz D, Cameron G, Reeve J. Muscle and bone in paraplegic patients, and the effect of functional electrical stimulation. *Clin Sci.* 1988;75(5):481–87. [PMID:3267113]
  16. Pearson EG, Nance PW, Leslie WD, Ludwig S. Cyclical etidronate: its effect on bone density in patients with acute spinal cord injury. *Arch Phys Med Rehabil.* 1997;78(3):269–72. [PMID:9084348]  
[http://dx.doi.org/10.1016/S0003-9993\(97\)90032-0](http://dx.doi.org/10.1016/S0003-9993(97)90032-0)
  17. Sniger W, Garshick E. Alendronate increases bone density in chronic spinal cord injury: a case report. *Arch Phys Med Rehabil.* 2002;83(1):139–40. [PMID:11782844]  
<http://dx.doi.org/10.1053/apmr.2002.26828>
  18. Crowe MJ, Sun ZP, Battocletti JH, Macias MY, Pintar FA, Maiman DJ. Exposure to pulsed magnetic fields enhances motor recovery in cats after spinal cord injury. *Spine.* 2003;28(24):2660–66. [PMID:14673366]  
<http://dx.doi.org/10.1097/01.BRS.0000099385.46102.0D>
  19. Mert T, Gunay I, Gocmen C, Kaya M, Polat S. Regenerative effects of pulsed magnetic field on injured peripheral nerves. *Altern Ther Health Med.* 2006;12(5):42–49. [PMID:17017754]
  20. Aaron RK, Ciombor DM, Jolly G. Stimulation of experimental endochondral ossification by low-energy pulsing electromagnetic fields. *J Bone Miner Res.* 1989;4(2):227–33. [PMID:2728926]  
<http://dx.doi.org/10.1002/jbmr.5650040215>
  21. Tsai MT, Chang WH, Chang K, Hou RJ, Wu TW. Pulsed electromagnetic fields affect osteoblast proliferation and differentiation in bone tissue engineering. *Bioelectromagnetics.* 2007;28(7):519–28. [PMID:17516509]  
<http://dx.doi.org/10.1002/bem.20336>
  22. Lee RC, Gowrishankar TR, Basch RM, Patel PK, Golan DE. Cell shape-dependent rectification of surface receptor transport in a sinusoidal electric field. *Biophys J.* 1993;64(1):44–57. [PMID:8381681]  
[http://dx.doi.org/10.1016/S0006-3495\(93\)81339-0](http://dx.doi.org/10.1016/S0006-3495(93)81339-0)
  23. Shen WW, Zhao JH. Pulsed electromagnetic fields stimulation affects BMD and local factor production of rats with

- disuse osteoporosis. *Bioelectromagnetics*. 2010;31(2):113–19. [PMID:19670410]
24. Chang K, Chang WH. Pulsed electromagnetic fields prevent osteoporosis in an ovariectomized female rat model: a prostaglandin E2-associated process. *Bioelectromagnetics*. 2003;24(3):189–98. [PMID:12669302] <http://dx.doi.org/10.1002/bem.10078>
25. Groah SL, Lichy AM, Libin AV, Ljungberg I. Intensive electrical stimulation attenuates femoral bone loss in acute spinal cord injury. *PM R*. 2010;2(12):1080–87. [PMID:21145519] <http://dx.doi.org/10.1016/j.pmrj.2010.08.003>
26. Akpolat V, Celik MS, Celik Y, Akdeniz N, Ozerdem MS. Treatment of osteoporosis by long-term magnetic field with extremely low frequency in rats. *Gynecol Endocrinol*. 2009;25(8):524–29. [PMID:19903057] <http://dx.doi.org/10.1080/09513590902972075>
27. Kirschvink JL. Uniform magnetic fields and double-wrapped coil systems: improved techniques for the design of bioelectromagnetic experiments. *Bioelectromagnetics*. 1992;13(5):401–11. [PMID:1445421] <http://dx.doi.org/10.1002/bem.2250130507>
28. Basso DM, Beattie MS, Bresnahan JC. Graded histological and locomotor outcomes after spinal cord contusion using the NYU weight-drop device versus transection. *Exp Neurol*. 1996;139(2):244–56. [PMID:8654527] <http://dx.doi.org/10.1006/exnr.1996.0098>
29. Manjhi J, Mathur R, Behari J. Effect of low level capacitive-coupled pulsed electric field stimulation on mineral profile of weight-bearing bones in ovariectomized rats. *J Biomed Mater Res B Appl Biomater*. 2010;92(1):189–95. [PMID:19810112] <http://dx.doi.org/10.1002/jbm.b.31505>
30. Galicka A, Brzóska MM, Sredzińska K, Gindziński A. Effect of cadmium on collagen content and solubility in rat bone. *Acta Biochim Pol*. 2004;51(3):825–29. [PMID:15448742]
31. Majumdar S, Kothari M, Augat P, Newitt DC, Link TM, Lin JC, Lang T, Lu Y, Genant HK. High-resolution magnetic resonance imaging: three-dimensional trabecular bone architecture and biomechanical properties. *Bone*. 1998;22(5):445–54. [PMID:9600777] [http://dx.doi.org/10.1016/S8756-3282\(98\)00030-1](http://dx.doi.org/10.1016/S8756-3282(98)00030-1)
32. Lochmüller EM, Miller P, Bürklein D, Wehr U, Rambeck W, Eckstein F. In situ femoral dual-energy X-ray absorptiometry related to ash weight, bone size and density, and its relationship with mechanical failure loads of the proximal femur. *Osteoporos Int*. 2000;11(4):361–67. [PMID:10928227] <http://dx.doi.org/10.1007/s001980070126>
33. Boivin G, Meunier PJ. The mineralization of bone tissue: a forgotten dimension in osteoporosis research. *Osteoporos Int*. 2003;14(Suppl 3):19–24. [PMID:12730799]
34. Bernard GW. Ultrastructural localization of alkaline phosphatase in initial intramembranous osteogenesis. *Clin Orthop Relat Res*. 1978;135(135):218–25. [PMID:709934]
35. Morris DC, Masuhara K, Takaoka K, Ono K, Anderson HC. Immunolocalization of alkaline phosphatase in osteoblasts and matrix vesicles of human fetal bone. *Bone Miner*. 1992;19(3):287–98. [PMID:1472898] [http://dx.doi.org/10.1016/0169-6009\(92\)90877-G](http://dx.doi.org/10.1016/0169-6009(92)90877-G)
36. Green S, Anstiss CL, Fishman WH. Automated differential isoenzyme analysis. II. The fractionation of serum alkaline phosphatases into “liver”, “intestinal” and “other” components. *Enzymologia*. 1971;41(1):9–26. [PMID:5116110]
37. Bassett CA. Beneficial effects of electromagnetic fields. *J Cell Biochem*. 1993;51(4):387–93. [PMID:8496242]
38. Behari J. *Biophysical bone behaviour: Principles and applications*. Hoboken (NJ): John Wiley & Sons; 2009. 175–239 p.
39. Behari J. Biophysical parameters affecting osteoporosis. *Crit Rev Biomed Eng*. 1994;22(2):69–137. [PMID:7587221]
40. Jayanand BJ, Lochan R. Effects of low level pulsed radio frequency fields on induced osteoporosis in rat bone. *Indian J Exp Biol*. 2003;41(6):581–86. [PMID:15266903]
41. Diniz P, Shomura K, Soejima K, Ito G. Effects of pulsed electromagnetic field (PEMF) stimulation on bone tissue like formation are dependent on the maturation stages of the osteoblasts. *Bioelectromagnetics*. 2002;23(5):398–405. [PMID:12111759] <http://dx.doi.org/10.1002/bem.10032>
42. Diniz P, Soejima K, Ito G. Nitric oxide mediates the effects of pulsed electromagnetic field stimulation on the osteoblast proliferation and differentiation. *Nitric Oxide*. 2002;7(1):18–23. [PMID:12175815] [http://dx.doi.org/10.1016/S1089-8603\(02\)00004-6](http://dx.doi.org/10.1016/S1089-8603(02)00004-6)
43. Lohmann CH, Schwartz Z, Liu Y, Li Z, Simon BJ, Sylvia VL, Dean DD, Bonewald LF, Donahue HJ, Boyan BD. Pulsed electromagnetic fields affect phenotype and connexin 43 protein expression in MLO-Y4 osteocyte-like cells and ROS 17/2.8 osteoblast-like cells. *J Orthop Res*. 2003;21(2):326–34. [PMID:12568966] [http://dx.doi.org/10.1016/S0736-0266\(02\)00137-7](http://dx.doi.org/10.1016/S0736-0266(02)00137-7)
44. Chang K, Chang WH. Pulsed electromagnetic fields prevent osteoporosis in an ovariectomized female rat model: a prostaglandin E2-associated process. *Bioelectromagnetics*. 2003;24(3):189–98. [PMID:12669302] <http://dx.doi.org/10.1002/bem.10078>
45. Chang WH, Jimmy K, Sun JS. Bone defect healing enhanced by pulsed electromagnetic fields stimulation: in vitro bone organ culture model. *J Med Biological Eng*. 2005;25:27–32.

46. Andre-Frei V, Chevally B, Orly I, Boudeulle M, Huc A, Herbage D. Acellular mineral deposition in collagen-based biomaterials incubated in cell culture media. *Calcif Tissue Int.* 2000;66(3):204–11. [PMID:10666496]  
<http://dx.doi.org/10.1007/s002230010041>
47. Chang K, Chang WH, Tsai MT, Shih C. Pulsed electromagnetic fields accelerate apoptotic rate in osteoclasts. *Connect Tissue Res.* 2006;47(4):222–28. [PMID:16987754]  
<http://dx.doi.org/10.1080/03008200600858783>
48. Chang K, Chang WH, Huang S, Huang S, Shih C. Pulsed electromagnetic fields stimulation affects osteoclast formation by modulation of osteoprotegerin, RANK ligand and macrophage colony-stimulating factor. *J Orthop Res.* 2005;23(6):1308–14. [PMID:15913941]
49. Walker JL, Kryscio R, Smith J, Pilla A, Siskin BF. Electromagnetic field treatment of nerve crush injury in a rat model: effect of signal configuration on functional recovery. *Bioelectromagnetics.* 2007;28(4):256–63. [PMID:17265446]  
<http://dx.doi.org/10.1002/bem.20302>
50. Moldovan M, Sørensen J, Krarup C. Comparison of the fastest regenerating motor and sensory myelinated axons in the same peripheral nerve. *Brain.* 2006;129(Pt 9):2471–83. [PMID:16905553]  
<http://dx.doi.org/10.1093/brain/awl184>
51. Bervar M. Effect of weak, interrupted sinusoidal low frequency magnetic field on neural regeneration in rats: functional evaluation. *Bioelectromagnetics.* 2005;26(5):351–56. [PMID:15887258]  
<http://dx.doi.org/10.1002/bem.20108>
52. Güven M, Günay I, Özgünen K, Zorludemir S. Effect of pulsed magnetic field on regenerating rat sciatic nerve: an in-vitro electrophysiologic study. *Int J Neurosci.* 2005;115(6):881–92. [PMID:16019581]  
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