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Effects of vibrating stimulus and pulsed electromagnetic fields on tooth movement

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A thesis submitted in fulfilment of the requirements for the degree of

MASTER OF DENTAL SCIENCE
(Orthodontics)

Discipline of Orthodontics
Faculty of Dentistry
University of Sydney

February, 2005
Dedication

I dedicate this thesis

To my beautiful son, Christopher John, whose every smile, every response and every move makes a cloudy day looks brighter. Thank you for your unconditional love, something so precious that I will cherish it forever. Happy first birthday!

To my husband, Paul Bowker, for the love, support, encouragement and guidance you have provided me. Thank you.
Declaration

CANDIDATE CERTIFICATE

This is to certify that the aforementioned candidate has carried out the work described in this thesis under the auspices of the Discipline of Orthodontics at The University of Sydney. This work has not been submitted to any other university or institution for a higher degree.

Angelina Wen Tzu Zea
February 2005
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Abbreviations

Ca$^{2+}$: Calcium 2+ ion

CAMP: Cyclic Adenosine Monophosphate

cm: Centimetre

DC Current: Direct current

g: Earth's gravitational field

H&E: Haematoxylin and eosin

Hz: Hertz

min: Minute

mm: Millimetre

mT: MilliTesla

N: Newton

Nd-Fe-B: Neodymium-Iron-Boron

PEMF: Pulsed electromagnetic fields

T: Tesla
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1. Literature review

1.1 Introduction

A review of the literature concerning the topic "Effects of vibrating stimulus and pulsed electromagnetic fields on tooth movement" revealed little published material dealing directly with the subject of the proposed experiments; however, there has been significant amount published dealing with the effect of mechanical vibrations, electric currents, and magnetic fields, both static and pulsed on bone. These agents are classified as examples of Energy Medicine.

The aim of the proposed experiments was to use several specific factors i.e. magnets bonded to teeth, pulsating electro-magnetic fields and orthodontic force in a variety of statistically determined combinations so as to assess the relative effects of these different factors on tooth movement using a rat model. Prior to undertaking these experiments, a search was conducted to assess the relevant literature. The review of the literature search is presented here.
1.2 Background Literature

1.2.1 Mechanism of Orthodontic Tooth Movement

It is believed that orthodontic tooth movement is accompanied by site-specific bone remodelling in the absence of inflammation. Alveolar bone remodelling is essential for tooth movement and is characterised by tandem periods of osteoclastic recruitment, bone resorption, reversal and bone formation.\textsuperscript{1} This process involves the periodontal membrane and is dependent on the magnitude and consistency of the force being applied. In the area of periodontal ligament compression, osteoclasts proliferate and an initial resorption of superficial bone occurs.\textsuperscript{2-5} In the region of periodontal ligament tension, the periodontal fibres unwind, fibroblasts appear, and osteoblasts form a non-mineralised collagenous matrix called osteoid. The osteoid is later mineralised, trapping some osteocytes in lacunae within the bone. However, the precise mechanisms of control of bone remodelling remain unclear.

1.2.1.1 Orthodontic tooth movement in a rat model

According to Vignery and Baron\textsuperscript{6}, the alveolar bone around the molar has a balanced bone resorption and formation activity and a very high rate of bone turnover. Rat molars drift distally under physiological conditions; however, this normal pattern of bone resorption on the distal alveolar surface is rapidly reversed with the application of a mesially directed orthodontic force on the rat molar\textsuperscript{7}. Bone formation occurs in areas of tension due to the inhibition of bone resorption and the stimulation of bone formation,
with marked changes occurring late in the orthodontic tooth movement. In areas of pressure, alveolar bone loss is due to the stimulation of resorption and the inhibition of formation and this occurs early in the orthodontic tooth movement. This suggests that the normal degree of bone formation and resorption is disturbed by orthodontic tooth movement\(^6\). The amount of bone resorption and formation during orthodontic tooth movement is characterised by a period of little change of 1-3 days, followed by a period that is mainly resorptive (5-7 days) and a period that is primarily formative (after 5-7 days)\(^7\).

Lu et al\(^8\) applied orthodontic forces of 50g to mandibular rat molars and found a higher distribution of osteoclastic cells on the alveolar bone than on tooth root surfaces, indicating that the alveolar bone is more readily removed than the root surface.

In a study performed by Ashizawa and Sahar\(^9\), three different magnitudes of mechanical stress (27, 60, 136g) were used to move rat molars mesially with fixed coil springs. They found little relationship between the magnitude of the initial mechanical force and the amount of new bone formation in the alveolar wall on the tension side during the initial stage of tooth movement. The heavy force (136g) produced a wide necrotic area of periodontal ligament, and delayed bone remodelling on the pressure side.

### 1.2.2 Energy medicine

Interest in energy medicine has existed for centuries in some parts of the world. Investigations reported in the literature have shown that applied electrical fields can
alter the normal electrical states of bone and cartilage, induce increased rates of cellular division and metabolism, and thus promote increased healing of bony and cartilaginous defects.\textsuperscript{11-12} The technology of using electric and electromagnetic fields to heal fracture non-unions emerged as a result of studies demonstrating the piezoelectric properties of dry bone by Yasuda, 1953\textsuperscript{13} and Fukada and Yasuda, 1957\textsuperscript{14}. Bassett and Becker in 1962\textsuperscript{15} described asymmetric voltage waveforms from mechanically deformed live bone.\textsuperscript{16-17} These changes were presumed to occur in bone during physical activity as a result of mechanical forces, and it was postulated that these forces were linked to modifications in bone structure. Their experiments indicated that the amplitude of the electrical potentials created in the stressed bone was dependent on both the rate and magnitude of the deformation. Polarity was dependent on the direction of bending; areas under pressure are in a electropositive state which is usually associated with osteoclastic activity and areas under tension are in a electronegative state which is associated with osteoblastic activity. They deduced, as did Fukada and Yasuda, that there is a relationship between piezoelectricity and callus formation. They postulated that the fundamental mechanism underlying all callus formation, regardless of the initial cause, is electric stimulation. They also proposed that these stress-induced bioelectric potentials controlled both bone cell activity and the orientation of their macromolecular byproducts. Bassett in 1989 proposed that tissue integrity and function could be restored by applying electrical and/or mechanical energy to the area of injury.\textsuperscript{17-19} Electrical currents were applied to non-healing fractures in animal studies\textsuperscript{20-22} and in clinical trials\textsuperscript{23-24} and successfully helped the healing process. It appears that electrical energy, whether applied as direct current or pulsed electromagnetic fields (PEMF)\textsuperscript{21, 25-35}, has the ability to affect both the depository and resorptive activities of bone and cartilage cells\textsuperscript{20}. However, the mechanism of action remains unclear. Despite the differences
between the methods for stimulating bone healing, all signals result in a change of intracellular ions, leading to an altered cell metabolism\textsuperscript{21,35-39}.

Energy medicine is used in the treatment of numerous health problems, such as pain, inflammatory diseases and epilepsy. Overall success ranges up to 50\% for chronic pain problems and up to 80\% for acute pain\textsuperscript{40}. A substantial number of clinical studies have been done suggest that electric and electromagnetic fields may accelerate bone formation and healing\textsuperscript{41}, especially in osteotomies and spinal fusions. Many of these studies have used randomised, placebo controlled designs. However, the long term success rate in this area of medicine is still unknown.

1.2.2.1 \textit{Vibration Effects}

Early studies concluded that high frequency (30Hz), low magnitude vibrations induce increased anabolic activity in bone\textsuperscript{42-43}. Mechanical perturbations, act as extracellular signals to a variety of cells, including bone cells\textsuperscript{11}. Animal and clinical studies have shown that mechanical stimulation, in particular low intensity pulsed ultrasound, improves the rate of bone healing via up-regulation of cartilage formation and maturation of endochondral bone formation\textsuperscript{44-47}.

1.2.2.1.1 Animal Experiments

1.2.2.1.1.1 \textit{Low intensity ultrasound}

Cook et al\textsuperscript{46} evaluated the effect of ultrasound therapy on the repair of full-thickness osteochondral defects. Bilateral, 3.2mm diameter by 5.0mm deep osteochondral defects
were created in the patellar groove of 106 adult male New Zealand rabbits. The defects were treated with daily low-intensity pulsed ultrasound therapy on the right knee and the left knee was not exposed to ultrasound as controls. In Part I, the effect of ultrasound therapy was evaluated 4, 8, 12, 24, and 52 weeks after surgery. In Part II, the effect of the length of treatment (5, 10, or 40 minutes of daily ultrasound therapy) compared with standard 20 minutes therapy was evaluated. Ultrasound treatment was shown to improve the morphological features and histological characteristics of the repair cartilage compared with non-treated controls. Moreover, doubling the treatment time to 40 minutes daily significantly increased the histologic quality of the repair cartilage.

Azuma et al\textsuperscript{48} used sixty-nine Long-Evans male rats that have bilateral closed femoral fractures to examine the rate of bone healing with low intensity pulsed ultrasound. The right femur was exposed to low intensity pulsed ultrasound and the left femur was used as control. They concluded that low intensity pulsed ultrasound accelerates rat femoral fracture healing by acting on the various cellular reactions involved in each phase of the healing process such as inflammatory reaction, angiogenesis, chondrogenesis, intramembranous ossification, endochondral ossification, and bone remodelling.

In one study performed by Tanzer et al\textsuperscript{49}, twenty-two pairs of fully porous transcortical implants were inserted bilaterally into the femora of 12 dogs. One femur of each animal was exposed daily to ultrasound stimulation for 2, 3, or 4 weeks and the contralateral side was used a control. The ultrasound stimulated implants demonstrated an 18% increase in bone growth compared with their contralateral control (p=0.02). Noninvasive low intensity ultrasound had its greatest effect in the first 2-3 weeks of
stimulation. They concluded that low intensity ultrasound had a positive effect on bone ingrowth.

In another canine study conducted by Tanzer et al\textsuperscript{50}, the possibility that low intensity ultrasound could enhance bone growth into porous intramedullary implants was examined. Fully porous intermedullary rod implants were used bilaterally in the ulnae of six dogs. Each dog received 20 minutes of daily low intensity ultrasound on one ulna and the contralateral side was used as a control. After 6 weeks of treatment, serial transverse sections showed an average of 119% more bone growth into ultrasound treated implants as compared with the contralateral controls (p<0.001). There was a significantly greater amount of bone ingrowth on the ultrasound-stimulated side. It was concluded that ultrasound therapy could augment biological fixation.

In the experiment performed by Takikawa et al\textsuperscript{51}, a consistently reproducible non-union was produced in rat tibias by muscle interposition with osteotomy. One limb was exposed to low-intensity pulsed ultrasound for 20 minutes daily and the other one was not. Rats were sacrificed after 2 and 6 weeks of treatment. Seven out of nine nonunion fractures showed healing on radiographic assessment. All control tibias remained in a state of non-union during the same period. It was concluded that low-intensity pulsed ultrasound promotes healing in the non-union fracture model.

Duarte\textsuperscript{52} investigated bone growth under the influence of low-intensity pulsed ultrasound using 45 rabbits. They were divided into bilateral osteotomy of fibula (n=23) and bilateral drilled holes on the cortex of femur (n=22) and were exposed to ultrasound 15 minutes per day. Radiological and histological evaluations of the progress of the
callus were conducted. It was concluded that pulsed ultrasound of low intensity with the
temperature variation at the osteotomy site was of the order of 0.01 degrees Celsius, reinforced the assumption that the stimulation mechanism due to the appearance of electric potentials is of non-thermal origin such as that caused by piezoelectricity.

Zorlu et al\textsuperscript{53} performed a study to compare the effects of direct current with ultrasound on fracture healing. Thirty-two rats were used with half exposed to ultrasound and the other half exposed to electrostimulation. Fibular osteotomy was performed on the rats. The right legs were used as the experimental sample and the left legs used as controls. Rats were sacrificed on the 7\textsuperscript{th} and 14\textsuperscript{th} days to investigate the macroscopic, radiologic and histopathologic parameters of fracture healing. There was a difference (p<0.05) between electrostimulation and the electrostimulation control groups on the 7\textsuperscript{th} day. There was a difference (p<0.05) between the ultrasound and ultrasound control groups on the 14\textsuperscript{th} day. It was found that in both the ultrasound and the electrostimulation groups, the fracture healing was accelerated by the stimuli as compared with the control groups. There was no statistical difference between ultrasound and electrostimulation effects.

1.2.2.1.1.2 Mechanical vibration

Flieger et al\textsuperscript{54} conducted a study to evaluate the influence of nonphysiological mechanical stimulation, in the form of low intensity vibration of frequency 50Hertz (Hz), acceleration at 2g (g is Earth's gravitational field) for 30 minutes per day for 5 days per week, on the prevention of bone loss in an animal model of postmenopausal osteoporosis. In the ovariectomised groups of rats, a statistically significant (p<0.05)
decrease in bone density (femur and tibia) was recorded at 5 weeks postovariectomy and the effect was maintained for the 12 week duration of the study; whereas vibration did not effect the bone density of sham operated rats.

Fifty-three female New Zealand rabbits were divided into four separate studies to assess the effects of 20 and 60 minutes of vibration per day in both implant and osteotomy models as compared with the respective non-vibrated controls by Usui et al.\textsuperscript{55}. For the implant group, coral hydroxyapatite goniopora rods were implanted bilaterally into tibiae and for the osteotomy model, bilateral fibulae were osteotomised. A resonant frequency of 25 Hz mechanical vibration was used. After the periods of 2, 3, 4, and 6 weeks of vibration, the rabbits were sacrificed and examined. For the implant model, there was no significant difference between control, 20 or 60 minutes of vibration per day with respect to the rate or amount of new bone ingrowth. For the fracture model, 60 minutes of vibration per day produced a significantly larger callus as compared with the non-vibrated controls. The results suggested that mechanical vibration had a beneficial effect on callus volume, possibly due to the stimulation of secondary bone healing processes, but does not appear to promote bone ingrowth into porous hydroxyapatite implants.

Using high frequency, low magnitude stimulus, Goodship and Kenwright\textsuperscript{56} examined the influence of controlled micromotion on fracture healing in two groups of sheep in which tibial diaphyseal fractures had been created. The experimental group was subjected to controlled axial mechanical vibrations for seventeen minutes a day (500 cycles at 0.5 Hertz). Enhanced fracture healing in the experimental group compared
with the control group was demonstrated by radiographic, histological, and biochemical analysis.

Goodship et al.\textsuperscript{57-58} found that short term infragentary cyclic micromovement applied at a high strain rate induced a greater amount of periosteal callus than the same stimulus applied at a low strain rate. In the first part of the study, they used three groups of skeletally mature female sheep, osteotomies were performed in the tibia, and a displacement rate of 2 millimetres (mm), 40mm and 400mm per second were applied using a microporcessor controlled servohydraulic actuator. An initial displacement of 1mm was used, applied with a force of 200N for 500 cycles at 0.5Hz for five consecutive days per week for 12 weeks. The stimulus was applied from 1 week postoperatively in all three groups. In the second part of the study, an additional group of six skeletally mature females were used. The osteotomies in this group were subjected to the same stimulus (400mm/second of applied cyclic micromovement) as the preceding groups, but the stimulus was initiated at 6 weeks postoperatively when periosteal bridging had started. The high strain rate applied later in the healing period significantly inhibited the process of healing. This beneficial effect applied early in the healing period may be related to the viscoelastic nature of the differentiating connective tissues in the early endochondral callus.

In a randomised, prospective in vivo animal study performed by Augat et al.\textsuperscript{59}, which examined the osteogenic effects of high-frequency mechanical stimuli, it was found that induced cyclic tensile strains did not produce a relevant enhancement of bone healing under external fixation. Twenty-four skeletally mature Merino sheep were randomly assigned to six groups of four animals, which received cyclic interfragmentary
movements of 0.2mm and 0.8mm and stimulation frequencies of 1, 5, and 10 Hz respectively. Twelve animals did not receive any externally applied stimulation and served as controls. Bone density and callus cross-sectional area were measured with quantitative computed tomography. The stimulation had no significant influence on the mechanical properties of the healing bone and varying the frequency had no influence on the healing process.

Wolf et al.\textsuperscript{60} conducted an animal study of twelve sheep, each had a transverse osteotomy with a 3mm gap in the right metatarsus, externally stabilized by a rigid circular fixator. External ground-based vibration was performed in six sheep and the interfragmentary movement produced was approximately 0.02mm at 20 Hz frequency. Healing was assessed postmortem by densitometric and mechanical examinations. No significant differences were found between the two groups, although callous formation was slightly enhanced (11%) in the stimulated group compared with the control group. These results did not confirm the findings of Goodship et al.\textsuperscript{61}, who reported significant enhancement of the bone healing process by high frequency, low magnitude mechanical stimulation. Wolf et al. explained the design differences of the two studies. In the experiment of Goodship et al., the ovine tibia was stimulated with axial movement of approximately 0.02mm and a frequency of 30Hz, with stimulation started one week after surgery and 30000 loading cycles applied daily. In contrast, Wolf et al. applied 0.02mm movement with 20Hz, and the number of loading cycles was 6000 daily starting two weeks after surgery. It was assumed that the number of loading cycles (6000) was too low in combination with the small movement magnitude of 0.01mm to 0.02mm. Moreover, stimulation started one week after surgery in the Goodship et al. study; whereas Wolf et al. started the stimulation 2 weeks after surgery.
Rubin et al\textsuperscript{44, 62} conducted a study to determine the effects of extremely small (0.3g, where \( g \) is the Earth's gravitational field), high frequency (30Hz) mechanical stimulation on long bones. The hindlimbs of 6-8 years old adult female sheep were exposed to ground based vertical oscillation for 20 minutes per day for 5 days per week. When the experimental group was not treated by stimulation, they joined the controls to roam freely over a pasture area. After one year of the mechanical stimulation regime, the density of trabecular bone in the proximal femur was 34.2 \% greater in the experimental sheep than in controls, quantified by computer tomography. (\( P<0.01 \)) The strong “anabolic” response was substantiated by undecalcified bone histology of the same region, which showed a 32 \% increase in trabecular bone volume, a 45\% increase in trabecular mesh number and a 36\% reduction in mesh spacing, indicating an increase in the mean width of each trabecular element and the addition of new trabeculae. The mechanical stimulation increased the rate of bone formation by 2.1 fold (\( P<0.2 \)) and the mineralising surface by 2.4 fold (\( P<0.1 \)). Bone trabeculae were also shown to have closer spacing, which is consistent with stronger bone. Histomorphometric studies of bone turnover suggest that this effect, though not statistically significant, was more than two fold, and may be due to the increased bone formation and mineralisation. There were no changes in cortical bone. In terms of clinical relevance, it was mentioned that the bone-generating capacity of these small mechanical signals suggested that biomechanical intervention might help to strengthen bone in osteoporosis sufferers without the side effects associated with pharmacological treatment.

In a subsequent randomised, animal study using adult female rats, Rubin et al\textsuperscript{45} examined the potential of low-magnitude, high frequency mechanical signals to restore anabolic bone cell activity inhibited by disuse. Adult female rats (6-8 months old) were
used and assigned to six groups; baseline controls (n=15), long term normal weight bearing controls (n=30), normal weight bearing animals subjected to 10 minutes per day 90Hz stimulation at 0.25g (n=21), animals subjected to 24 hours per day disuse via hind limb suspension (n=11), animals subjected to disuse interrupted by 10 minutes per day of normal weight bearing (n=7) and disuse interrupted by 10 minutes per day of 90 Hz stimulation at 0.25g (n=19). All protocols ran for 28 days. Ten minutes per day of mechanical stimulation significantly increased bone formation rates versus long-term control animals by 97% (P<0.001) and the mineralising surface by 76% (P<0.001) but not the mineral apposition rate (2%). Because mechanical stimulation increased bone formation primarily by increasing the percentage of mineralising surfaces, this indicates that the low-level, high frequency mechanical signals recruited additional osteoblasts rather than increasing the activity levels of existing osteoblasts.

In an experiment conducted by Chen et al63, 76 rabbits with radial fractures were used. Mechanical vibration at five different frequencies were applied to different groups of animals. The effects were evaluated by means of radiographical and pathological examinations. The results showed that mechanical vibration promoted fracture healing in rabbits regardless of the frequency with both bone strength and speed of fracture healing better than those of the controls. Bone strength was elevated by approximately 20%-30% with the most effective frequencies as 25Hz and 50 Hz.
1.2.2.1.2 In Vitro Studies

1.2.2.1.2.1 Low intensity ultrasound

Low intensity ultrasound (30mw/cm²) can speed the healing of fresh fractures. In vitro studies showed that ultrasound stimulation also accelerates trabecular bone regeneration. Sun et al. used bilateral femora from 36 mature male Wistar rats and created a bone defect at the centre of each distal metaphysis. The femora were maintained for either 7 or 14 days in in vitro tissue culture and received 15 minutes of ultrasound stimulation or a sham exposure. Healing of the bone defect was evaluated by histomorphological examination and by analysis for the synthesis and secretion of prostaglandin E2. The results showed that ultrasound stimulation accelerated both defect healing and trabecular bone regeneration. They found that with ultrasound stimulation of Wistar rats’ femur in vitro, prostaglandin E2 secretion decreased significantly. These changes in prostaglandins synthesis and concentration were found to correspond to changes in the amount of trabecular regeneration and acceleration of bone healing.

In another study, Sun et al. used an in vitro bone cell culture model of rat alveolar mononuclear cell-calvaria osteoblasts to study the effect of low-intensity pulsed ultrasound. The bone cells were cultured for 3 days before ultrasound treatment to facilitate their attachment and differentiation. Ultrasound treatment or sham exposure was applied to samples for 20 minutes per day. At the end of treatment, the osteoblast cell counts were significantly increased, whereas the osteoclast cell counts were significantly decreased. The result of this study suggested that low-intensity ultrasound treatment may have a stimulatory effect on bone healing.
Nolte et al\textsuperscript{42} examined the \textit{in vitro} the influence of low-intensity ultrasound on endochondral ossification of 17-day old fetal mouse metatarsal rudiments. Forty-six triplets of paired metatarsal rudiments were resected "en block" and cultured for 7 days with and without low-intensity ultrasound stimulation. At days 1, 3, 5, and 7, the total length of the metatarsal rudiments, as well as the length of the calcified diaphysis were measured. Histology of the tissue was undertaken to examine its vitality. The increase in length of the calcified diaphysis during 7 days of culture was significantly higher in the ultrasound-treated samples as compared with the untreated controls (p=0.006). They stated that the low intensity ultrasound treatment stimulated endochondral ossification of fetal mouse metatarsal rudiments \textit{in vitro}. This might be due to the stimulation of activity and/or differentiation of osteoblasts and hypertrophic chondrocytes.

1.2.2.1.3 Clinical Studies

According to the registry of orthopaedic prescription of ultrasound use, low intensity ultrasound can enhance the prospects for healing in the presence of diseases that compromise bone healing or in patients required to take medications that will negatively influence healing. The results show high success bone healing rates when diabetes (92\%), osteoporosis (95\%), alcholohism and drug use (92\%), nonsteroidal antiinflammatory drugs (89\%), or calcium channels blockers (90\%)\textsuperscript{38} was affecting or taken by patients.
1.2.2.1.3.1 Low intensity ultrasound

Mayr et al\(^6\) stated that low intensity pulsed ultrasound was shown to accelerate fresh fracture healing both clinically and experimentally. They reported the use of low intensity pulsed ultrasound in the therapy of 951 delayed unions and 366 nonunions on the prescription registry. The overall success rate for delayed unions was 91% and for nonunions was 86%. With patients taking medication such as calcium channel blockers, non-steroidal anti-inflammatory drugs and steroids, the fracture healing rate of nonunions declined, as well as renal and vascular insufficiency. Patients who were smokers during ultrasound therapy had lower healing rates than those who never smoked.

1.2.2.1.3.2 Mechanical vibration

1.2.2.1.3.2.1 Fracture healing

Under the conditions of rigid stabilization, direct osteonal remodeling of the fracture line can occur with little or no external callus, a process known as direct bone repair. The second pattern of repair involves bridging the fragments with an external callus and formation of bone in the fracture site by endochondral healing. This type of repair is known as indirect bone healing and occurs under less rigid interfragmentary stabilization. Recently, flexible fixation has become more common in operative treatment of long bone fracture\(^6\). Some studies have reported successful fracture healing under flexible fixation that supports natural repair process by callus formation (indirect bone healing). It has been shown that the extent and quality of the callus is
heavily dependent on the mechanical conditions at the fracture site. Interfragmentary movements with a significant magnitude of movement will occur under flexible fixation without weightbearing. For patients who are immobilized, lack of an adequate mechanical stimulus was thought to be the reason for delayed healing. Wolf et al.\textsuperscript{67} stated that when cyclical interfragmentary movements of a magnitude to 1 mm and a frequency of approximately 1 Hz were applied to osteotomies in the animals that were stabilized by relatively rigid external fixators, and this accelerated bone healing. However, the positive effect was not shown when the same mechanical signal was applied to osteotomies that were stabilized by flexible external fixators.

Kenwright and Goodship\textsuperscript{66} conducted a prospective, randomised study of 102 tibial fractures stabilised by an external frame modified to apply controlled axial micromovement with displacement of 1.0 mm at 0.5 Hz for 30 minutes per day, starting one week after application of the frame. Axial mechanical vibrations significantly reduced the healing time and was associated with a lower rate of secondary surgery. They concluded that the application of appropriate applied strain to clinical tibial fractures at a time shortly after injury, when most patients would be very inactive, appears to enhance the healing process when using external skeletal fixation. Experimentally, fractures fixed with different degrees of rigidity lead to different callus responses within three weeks of fracture.

Kenwright et al.\textsuperscript{68} in a clinical, prospective, randomised, controlled trial, compared the effects on tibial diaphyseal fracture-healing in thirty-nine tibiae treated with controlled axial micromotion and external fixation with the control group of 41 tibiae treated with external fixation. Fracture healing was assessed clinically, radiographically, and with
biomechanical measurement of the stiffness of the frame. The mean healing time in the experimental group was twenty-three weeks compared to twenty-nine weeks (p<0.05). The difference in healing time was independently related to the method of treatment and no significant differences were found in the rates of complications between the two groups.

1.2.2.1.3.2.2 Bone density

In a clinical study done by Ward et al\textsuperscript{69}, twenty pre-or postpubertal disabled, ambulant children were randomized to standing on an active vibrating platform (0.3g, 90Hz) or a placebo group for 10 minutes per day, 5 days per week for 6 months. One difficulty encountered was poor compliance (44% of expected time on machine). After 6 months, the mean change in proximal tibial volumetric trabecular bone mineral density in children who stood on active devices was 6.27 mg/ml; whereas the children who stood on placebo devices had proximal tibial volumetric trabecular bone mineral density decreased by -9.45 mg/ml. The results of this pilot study showed that low-magnitude, high frequency mechanical stimuli are anabolic for trabecular bone in children, possibly by providing a surrogate for suppressed muscular activity in the disabled.

1.2.2.1.3.2.3 Osteoporosis

In another clinical study, a 1 year prospective, randomized, double-blind and placebo-controlled trail of 70 postmenopausal women were randomly divided to experimental and control groups\textsuperscript{70}. The experimental subjects were exposed to short-duration of two, 10-minute treatments of low magnitude, and 30Hz vertical vibration. The control group
stood on placebo devices for the same duration. The results showed that mechanical vibration could be beneficial in the intervention management of osteoporosis.

1.2.2.2 Electric current effects

1.2.2.2.1 Animal experiments

1.2.2.2.1.1 Direct current

An electric current was applied with an electrode, the cathode surgically implanted into the nonunion site and the anode left at a distant site, usually on the skin\textsuperscript{71}. Direct current (DC current) was applied to create the necessary electrical potential around the bony wound. Osteogenesis or chondrogenesis was frequently observed at the cathode. Due to the invasiveness of this direct current technique, two alternative external methods were considered to achieve a non-invasive means of altering the electrical environment of a bony fracture to increase the rate of repair. They were capacitive coupling of electrostatic and electrodynamic fields and pulsed electromagnetic fields (PEMF). In 1968, Bassett and Herman\textsuperscript{72} reported that capacitive coupled fields could increase DNA and collagen synthesis in fibroblasts grown in culture and increase the repair rate of fibular osteotomies in rabbits.

Davidovitch et al\textsuperscript{73-74} have investigated the effects of DC current at the anode on bone resorption and tooth movement in cats. Using immunohistochemical as well as physical measurements, they showed an increase in bone remodelling, a slight enhancement of tooth movement and increased periodontal nucleotide levels over the two weeks experimental period.
Jacobs and Norton\textsuperscript{75} and Kopczk\textsuperscript{76} attempted to replace lost alveolar bone in periodontically diseased beagle dogs using two different direct current devices. Some increase was seen in endosteal bone but the studies were inconclusive.

1.2.2.2.1.2 \textit{Direct current versus PEMF}

Yonemori \textit{et al}\textsuperscript{22} conducted a study on 105 rabbits separated into five groups: direct current stimulation by Kirshner wire insertion group; pulsed electromagnetic fields (PEMF) stimulation group; PEMF with Kirshner wire insertion group; Kirshner wire insertion group; and an intramedullary drilling control group. In the direct current stimulation group and the PEMF with Kirshner wire insertion group, alkaline phosphatase activity in the bone marrow and argyrophilic nuclear organizer region staining increased at 7 days after surgery. Fourteen days after surgery, alkaline phosphatase activity and proliferative activity of osteoblasts were significantly higher in these two groups than in the other groups. Intramedullary new bone formation was most active in the direct current stimulation group. Electromagnetic stimulation of the inserted Kirshner wire also promoted bone formation significantly. The Kirshner wire insertion alone group and the intramedullary drilling group showed bone formation but it was less significant. Electromagnetic stimulation without the insertion of the Kirshner wire showed little bone formation. It was concluded that the degree of osteogenesis induced by electrical stimulation is influenced by the tissue environment. Osteogenesis is promoted when electrical stimulation is provided in the environment of inflammation and reactive cells.
1.2.2.2.2 Clinical Studies

1.2.2.2.2.1 Bone nonunions

In a study performed by Zamora-Navas et al\textsuperscript{78}, twenty-two established nonunions were treated with a capacitively-coupling electrical signal. A gap of 0.5 centimetre (cm) or more between the fragments was present in all nonunions. After an average of 26 weeks of treatment with capacitive coupling, radiographic assessment showed solid bone union in 72.7% of the cases. The results were better when the fracture site was metaphyseal. When the site was diaphyseal, bone healing was mainly achieved by bone trabeculae invading the gap. When the site was metaphyseal, healing occurred by the formation of a peripheral callus. The results were not affected by the presence of infection.

Twenty-three patients at the Royal London Hospital with long bone nonunion were entered into a prospective, double-blind trial in which electrical capacitive coupling was used for treatment\textsuperscript{23}. Twenty-one patients were actively treated and eleven were managed with a placebo regime. The nonunion group healed in six out of ten patients in the experimental group, but no fractures healed in the control group. The results showed that the rate of healing was significantly higher in the active treatment group than the control group.

In a study undertaken by Brighton et al\textsuperscript{79}, 178 nonunions in 175 patients treated with constant direct current at the University of Pennsylvania in 1970, 149 (83.7%) achieved solid bone union. Patients with a history of osteomyelitis had a healing rate of 74.4%. The presence of inserted metallic fixation devices did not affect the healing rate. Review of the nonunions treated unsuccessfully with constant direct current suggested that
inadequate electricity, the presence of synovial pseudarthrosis or infection, and dislodgment of the electrodes were the causes of failure with the procedure.

1.2.2.2.2 *Fresh mandibular fracture*

In oral surgery, Masuriek *et al*\(^{80}\) used a direct-current device to enhance healing of fresh mandibular fractures anterior to the mental foramen. Electrical stimulation enhanced primary healing as measured by mobility, but no difference was found between control and stimulated groups at the end of the usual intermaxillary fixation time.

1.2.2.2.3 *Osteoarthritis*

According to Hulme *et al*\(^{24}\) Cochrane Database System Review 2002, electrical stimulation therapy has small to moderate effects on outcomes for knee osteoarthritis. Three studies were included in the review. Those results were all statistically significant with clinical benefit ranging from 13%-23% greater with active electrical stimulation treatment than placebo.

1.2.2.3 *Magnetic field effects*

1.2.2.3.1 Animal Studies

1.2.2.3.1.1 *Orthodontic Tooth Movement*

In the experiment performed by Darendeliler *et al*\(^{81}\), 18 young male Hartley guinea pigs were divided into three groups, one with an orthodontic coil spring to move maxillary
central incisors, the second group with a pair of samarium-cobalt magnets to move the central incisors, while the third group, coil springs was used in combination with pulsed electromagnetic field. The results showed that both static magnetic fields produced by samarium-cobalt magnets and the pulsed electromagnetic fields used in combination with coil spring groups had an increased rate of tooth movement over that produced by the coil springs alone. The mechanism producing this increase of tooth movement was reported to involve a reduction of lag phase often seen in orthodontic tooth movement. Both magnetically stimulated groups also showed an increase in the organisation and the amount of new bone deposited in the area of tension between the orthodontically moved maxillary incisors.

1.2.2.3.1.2 Bone mass

The animal model of disuse osteopenia was used by Rubin et al\textsuperscript{20-21} to determine the ability of a complex pulsed electric field to inhibit loss of bone in comparison with the remodelling response generated by low-power, low frequency sinusoidal electrical fields induced exogenously by means of magnetic induction. Exposure to pulsed electrical fields prevented osteopenia and stimulated 10 per cent mean increase in the bone area of thirty turkey ulnae. The osteogenic influence of the sinusoidal electrical fields was dependent on the frequency; the 150, 75, 15 hertz sinusoidal fields respectively, generated a \(-3\) per cent, \(+5\) per cent and \(+20\) per cent mean change in the bone area. The frequency and intensity range of the sinusoidal fields producing the greatest osteogenic response are similar to the levels produced intrinsically by normal functional activity. It was concluded that because fields of these frequencies and
intensities were indigenous to bone tissue, such exogenous treatment could promote bone quantity and quality with minimal risk.

A controlled blinded protocol nonunion of bone after 3-4 weeks of bilateral mid-diaphyseal fibular osteotomy in aged rats was used with PEMF exposure to one limb and sham treatment to the contralateral limb. Bone volumes were assessed using high resolution micro-computed tomography (micro CT) over the course of treatment. It was found that there was a significant reduction in the amount of time-dependent bone volume loss in PEMF-treated non-union sites as compared with sham-treated bones. Osteotomy gap size was significantly smaller in hind limbs exposed to PEMF compared with sham-treatment.

In the study performed by Sandrey et al, daily pre-exposure and postexposure mass measurements of 65 rats were recorded during exposure to a 0.1mT, 60Hz sinusoidal magnetic field, pulsed electromagnetic fields (PEMF) or their control field for 4 hours per day for 21 days. The results showed that the body mass changes of young rats over time when exposed to PEMF decreased more and recovered it more slowly in comparison with controls than did PEMF exposed rats or any 60Hz exposed rats.

1.2.2.3.1.3 Nonunions and delayed unions

Cane et al investigated the influence of pulsed low frequency electromagnetic fields (PEMF) on bone formation in the healing process of transcortical holes at the diaphyseal region of metacarpal bones of six adult horses. A pair of Helmholtz coils was used to provide PEMF to the left metacarpal for 30 days with a pair of inactive
Helmholtz coils as controls. The histomorphometric analysis showed the amount of bone formed during 30 days and mineral apposition rate during 10 days were significantly greater (p<0.01 and p<0.0001, respectively) in the PEMF-treated holes than in the controls. It was concluded that PEMFs at low frequency not only stimulate bone repair but also seem to improve the osteogenic phase of the healing process.

Fredericks et al.\textsuperscript{28} investigated the effect of PEMF exposure on healing tibial osteotomies in New Zealand rabbits. Sixty minutes PEMF treated osteotomies had significantly higher torsional strength than did sham controls at 14 and 21 days postoperatively. Thirty-minute PEMF treated osteotomies were significantly stronger than sham controls after only 21 days of exposure. Normal intact torsional strength was achieved by 14 days in the sixty minute PEMF group, by 21 days in the thirty minute PEMF group and by 28 days in the sham controls. It was concluded that low frequency, low amplitude PEMF significantly accelerated callus formation and osteotomy healing in a dose-dependent manner.

A transverse mid-diaphyseal tibial osteotomy with a 2mm gap was performed unilaterally in 12 adult mixed-breed dogs and stabilized with external fixation in a study done by Inoue et al.\textsuperscript{29}. Animals in the experimental group were treated with PEMF for 1 hour daily starting 4 week after surgery for a total of 8 weeks. Histomorphometric analyses revealed greater new-bone formation (p<0.05) in the osteotomy gap tissue and increased mineral apposition rate (p<0.04) and decreased porosity in the cortex adjacent to the osteotomy line (p<0.02) in the PEMF group.
1.2.2.3.1.4 Healing of recent fractures

PEMF have been shown to be useful in the promotion of healing in nonunions and delayed unions. However, there is conflicting and less information in the literature regarding the benefit of using PEMF to accelerate the healing of fresh fractures.

Grace et al\textsuperscript{83} investigated the effects of PEMF on osteochondral defects placed in the patellofemoral groove of the rat. The results indicated that PEMF enhanced early chondrogenesis, and bone formation was consistently stimulated, plus restoration of normal bone trabeculae advanced. They concluded that PEMF could be useful in advancing repair during the early proliferative stage.

In a study conducted by Leisner et al\textsuperscript{84}, 2-3 mm ulnar fractures were created in rats and randomly assigned into two treatment groups, namely a PEMF group and a sham treatment control group. The radiographic results demonstrated bridging callus formation in both control and treatment groups; however, it was found that the healing process was faster in rats that were not treated by PEMF. Histological evaluation demonstrated that the fibrous content of the callus in rats of the treatment group was significantly higher than that in the rats of the control group. The results of this study do not support the claim that PEMF stimulates osteogenesis and bone healing in fresh bone fractures in rats.
1.2.2.3.1.5 *Cutaneous wound healing*

In an experiment undertaken by Patino *et al*\(^{85}\), twenty-two male Wistar rats were used, and soft tissue circular lesions were made on the back of each animal. The rats were divided into three groups: PEMF and sham treatments for 35 minutes twice a day were used in Group 1 and Group 2 respectively and the third experimental group was treated by topical nitrofurazone solution. The absolute and relative values of the area and perimeter of the wounds showed significantly lower values in the PEMF group at days 7, 14, 21 compared with the control no treatment group. The PEMF group showed significantly lower values at day 21 compared with the group having treatment with topical nitrofurazone solution. It was concluded that PEMF treatment is beneficial to cutaneous wound healing in rats.

1.2.2.3.1.6 *Spinal fusion*

In the study performed by Glazer *et al*\(^{86}\), ten New Zealand white rabbits were randomly assigned to undergo spinal fusion using either autologous bone with electromagnetic fields, or autologous bone without PEMF. The rate of pseudarthrosis, as evaluated radiographically and manually in a blinded fashion, decreased from 40%-20% with PEMF, but this decrease in the non-union rate was not statistically significant given the small sample size. Biomechanical analysis of the fusion mass showed that PEMF resulted in 35% increase in stiffness, which was deemed significant.
1.2.2.3.1.7 Osteoporosis

Thirty-five 3 month old female Sprague-Dawley rats were randomly divided into five groups: intact, ovariectomy, aspirin treated, PEMF stimulation using Helmholtz coils and PEMF stimulation with aspirin. All rats were subjected to bilateral ovariectomy except those in the intact group. Histomorphometric analysis showed that PEMF stimulation augmented and restores proximal tibial metaphyseal trabecular bone mass with increased hard tissue percentage, bone volume percentage and trabecular number, and bone architecture with increased trabecular perimeter, trabecular thickness and decreased trabecular separation. Trabecular bone mass of PEMF rats was restored to the levels closely matched by the intact rats after 30 days of stimulation. PEMF exposure also attenuated the higher serum prostaglandin E2 concentrations of the ovariectomised rats and restored it to levels of intact rats. It was concluded that PEMF may be useful in the prevention of osteoporosis and that prostaglandin E2 might relate to these effects.

Rubin et al. conducted a study on prevention of osteoporosis by pulsed electromagnetic fields induced at a physiological frequency and intensity to disuse turkey ulnae with a loss of bone of 13% compared with the contralateral side functional ulnae. Using a treatment regime of one hour per day of exposure to pulsed electromagnetic fields, an osteogenic dose-response to induced electrical power was observed, with a maximum osteogenic effect between 0.01 to 0.04 tesla 2 per second. Pulse power levels of more or less than these levels were less effective. The results suggested that short daily periods of exposure to appropriate electromagnetic fields could be beneficial in preventing osteoporosis.
1.2.2.3.2 Clinical Studies

Inductively coupled pulsed electromagnetic fields (PEMF) produce electric currents in tissues that lie within the field without the need for surgical placement of electrodes. This non-invasive method was approved by FDA in the United States as a safe and effective treatment for treating nonunions, congenital pseudarthrosis and failed fusions in 1979\textsuperscript{89}. In 1986, it was reported that over one third of all bone nonunions were being treated by PEMF rather than by conventional surgery.

According to a Quittan et al\textsuperscript{25} literature review on magnetic field therapy, electromagnetic fields are applied in numerous studies to promote bone healing, to alleviate pain, to enhance healing of ulcers and to reduce spasticity. Most of the studies confirmed that electromagnetic fields promote bone healing and pain alleviation. Satake\textsuperscript{90} stated that low energy PEMF at frequencies of 60-90 Hz significantly increase healing of chronic fracture non-unions in humans. However, the results of PEMF in treatment of other disorders are contradictory.

1.2.2.3.2.1 Bone density

In a study performed by Tabrah et al\textsuperscript{91}, the nondominant forearms of 20 osteoporosis-prone female subjects were exposed to 72Hz PEMF, 10 hour daily for a period of weeks. Bone density of treated radii measured by single-photon densitimetry increased significantly in the immediate area of the field during the exposure period and decreased during the following 36 weeks. The data suggested that PEMF may have a clinical application in the prevention and treatment of osteoporosis.
1.2.2.3.2.2 Bone healing

Aaron et al\textsuperscript{92} stated that uncontrolled longitudinal cohort studies of delayed and non-unions reported average union rates of approximately 75% to 85% in fractures previously refractory to healing. In osteotomy trials, greater bone density, trabecular maturation, and radiographic healing were observed in actively treated subjects compared with placebo-treated patients.

PEMF is useful in treating cases of fracture non-union. Meskens et al\textsuperscript{90} reported on 45 patients with tibial shaft fractures, all treated conservatively and with union delayed for more than 16, but less than 32 weeks, which were entered in a multi-centre clinical trial. Treatment was by plaster immobilisation in all, with active electromagnetic stimulation treatment in 20 patients and sham treatment for 12 patients. Radiographs were assessed blindly and independently by a radiologist and an orthopaedic surgeon. The results showed that PEMF significantly influenced healing in tibial fractures with delayed union.

Bassetti\textsuperscript{32} stated that PEMF is a highly effective (more than 90% success in adult patients) non-invasive treatment option in the management of ununited fractures, failed arthodeses and congenital pseudarthroses. When union fails to occur with PEMF alone at approximately four months, fresh bone grafts, used in conjunction with PEMF, insures a maximum failure rate of 1 to 1.5%. Unions occur because the weak electric currents induced in tissues by the time-varying effect calcification of the fibrocartilage in the fracture gap, thereby setting the stage for the final phases of fracture healing by endochondral ossification.
A prospective series of 32 consecutive patients with 33 long bone fractures suffering with delayed or non-union were treated by PEMF or PEMF with surgery. Nineteen fractures treated with surgery and PEMF united within nine months. Fourteen fractures were treated with PEMF alone. Twelve (86%) united within ten months and two failed to unite. Colson et al\textsuperscript{33} concluded that the stimulating waveform is less critical than was claimed by Bassett et al\textsuperscript{32} and that a simpler management regime for PEMF treatment can be just as effective.

Heckman et al\textsuperscript{34} stated PEMF treatment was effective in promoting healing of nonunion in 64.4\% of 149 patients. The experimenters stated that the success of treatment depended on several variables. The location of nonunion was important, as the healing rate was found to be higher in the tibia than in the femur or humerus. Young patients healed more rapidly than older patients. It was stated that bone grafts combined with electrostimulation was more effective in some situations. Electrostimulation was more effective when used within two years of the original fracture than if used in a prolonged nonunion. Moreover, infection, either quiescent or actively draining, did not appear to affect the overall results. It was important that the patients adhered to treatment protocols and an adequate immobilization of the fracture and absolute nonweight-bearing during treatment was necessary.

Ito et al\textsuperscript{35} obtained similar results in their study with thirty ununited tibial fractures. Union was achieved in 25 cases (83.3\%). Patient age and gender, the presence of surgical hardware, the length of disability, and the number of surgical procedures did not affect the outcome.
Rubin et al\(^2\) used an *in vivo* model of osteopenia to demonstrate that bone resorption which normally parallels disuse can be prevented or even reversed by the exogenous induction of electric fields. The manner of response (i.e. formation, turnover, resorption) is sensitive to subtle changes in electric field parameters. Reducing the frequency to 15 Hz made the field extremely osteogenic. The frequencies and field intensities most effective in the exogenous stimulation of bone formation is similar to those produced by normal functional activity. This lends strong support to hypothesis that endogenous electric fields serve as a critical regulatory factor in both bone modelling and remodelling processes.

In a study performed by Bassett et al\(^3\), one hundred and twenty-five patients with one hundred and twenty-seven ununited fractures of the tibial diaphysis were treated exclusively with PEMF. The overall success rate in healing of fracture using this out-patient method was 87%. The success rate was not materially affected by age or gender of the patient, the period of prior disability, the number of previous failed operations or the presence of infection or metal fixation\(^4\).

1.2.2.3.2.3 *Unsuccessful treatment by PEMF*

According to Madronero et al\(^5\), PEMF is effective in promoting healing of delayed union and non-union of bone in about 70% of cases, but with no clear explanation of the failures in the literature. Their experimental results on radius non-union and delayed unions suggested that PEMF failure could be associated with metallic implant plates. They suggested that the conducting plates created a uniform bone biopotential around the fracture and therefore prevented the negative polarity which stimulated callus
formation. It was suggested that implant plate removal should be considered before PEMF stimulation.

Contrary to the conclusion of Madronero et al\textsuperscript{95} that metallic implants could interfere with PEMF therapeutic effect, Ito and Shirai\textsuperscript{35} found that union was achieved in 25 cases (83.3\%) of ununited tibial fractures and the presence of surgical hardware, patient age and gender, the length of disability, and the number of surgical procedures did not affect the outcome. Treatment failures occurred only among lesions with a poor blood supply in necrotic areas or in cases diagnosed as radiographically unsuitable.

Fifty-three ununited fractures with a median time since injury of 28 months were treated by pulsed electromagnetic stimulation\textsuperscript{31}. Union was achieved in 38 cases (71.7\%). For ununited fractures of the tibia, the success rate was higher at 86.7\%. Previous or active sepsis, the presence of plates and nails, the age of the patient or the time since the injury did not affect the results. However, it was suggested that inadequate immobilisation, a fracture gap of more than five millimetres or the presence of a screw in the fracture gap was responsible for the failure of treatment by PEMF.

1.2.2.3.2.4 Cutaneous Healing

Forty-four patients with skin ulcers of venous origin participated in the study, with half of the group exposed to active low frequency PEMF and the other half exposed to dummy stimulator as controls\textsuperscript{96}. The success rate was higher in the experimental group and no ulcers worsened, whereas there were ulcers which became worse in control
group. It was concluded that PEMF stimulation is a useful adjunctive therapy in the management of cutaneous ulcers of venous origin.

1.2.2.3.2.5 Osteoarthritis

Hulme et al.\textsuperscript{24} found that pulsed electrical stimulation therapy had a small to moderate effect on the outcomes for knee osteoarthritis in their literature review of the topic. The three experiments used were all statistically significant with improved clinical benefit ranging from 13-23% greater with electrical stimulation treatment than with placebo.

In a double blind, randomised, controlled clinical trial, forty-three subjects with chronic pain in one or both knee joints wore pads containing magnets or placebos over their painful knee for 2 weeks\textsuperscript{97}. Multivariate analysis of covariance revealed significantly greater improvements in the group wearing magnets (p=0.002). It was concluded that static magnets over painful knee joints appears to reduce pain and enhance functional movement.

One hundred and seventy-six patients with knee pain due to osteoarthritis were randomly assigned to the placebo group (magnet off) and the active group (magnet on)\textsuperscript{98}. The results showed that reduction in pain treatment session was significantly (P<0.001) greater in the magnet-on group (46%) compared to the magnet-off group (8%). It was concluded that low amplitude, low frequency magnetic fields are safe and effective in treating patient with chronic knee pain due to osteoarthritis.
1.2.2.3.2.6 Spinal Fusion

A case described by Mackenzie and Veninga\textsuperscript{99} of anterior cervical C6-C7 fusion nonunion was treated by PEMF stimulation. The patient wore a PEMF stimulation device for three hours per day for 10 months. After 3 months of treatment, the patient's symptoms were resolved and radiographs obtained at 31 weeks after stimulation showed even bone density around the C7 screws.

In the prospective, randomised, double-blind, placebo-controlled trial, Linovitz et al\textsuperscript{100} evaluated the effect of combined magnetic field in the healing of primary lumbar spine fusion. The magnetic field device used a single posterior coil centered over the fusion site, with only 30-minutes of treatment per day for 9 months. Randomization was stratified by site and number of levels fused. Evaluation was performed 3, 6, 9 months after surgery and 3 months after the end of treatment. It was concluded that the use of combined magnetic field device was statistically beneficial in the overall patient population. More importantly, the data demonstrated that the female study population responded positively to the adjunctive combined magnetic field treatment, whereas the male population in this experiment was not significant.

Aaron et al\textsuperscript{92} stated in spine fusions, the average union rate of 80%-90% was observed in actively treated patients across numerous studies compared with 65% to 75% in placebo-treated patients.

Sixty-one randomly selected patients who underwent lumbar fusion surgery between 1987-1993 were studied retrospectively by Marks\textsuperscript{101}. Forty-two patients received
adjunctive therapy with PEMF stimulation, and nineteen received no electrical stimulation. Average follow-up time was 15.6 months after surgery. Fusion succeeded in 97.6% of the PEMF group and in 52% of the control group ($P<0.001$). It was concluded that the use of PEMF enhanced bony bridging in lumbar spinal fusion.

1.2.2.4 Dental Applications of Energy Medicine

1.2.2.4.1 Animal Studies

1.2.2.4.1.1 Orthodontic Tooth Movement

Darendeliler et al.\textsuperscript{81} found the rate of orthodontic upper incisor movement was significantly increased compared with the controls in the presence of PEMF and static magnetic fields. They demonstrated a reduction in the "lag phase" which is common in orthodontic tooth movement between the third and sixth day. There was also an increase in the organization and amount of new bone in the area of tension between the orthodontically moved maxillary incisors.

The effect of PEMF on orthodontic tooth movement in guinea pigs was observed through transmission electron microscope for 14 days. The authors indicated that PEMF accelerated the rate of orthodontic tooth movement as a result of increased quantity of active cells without changing the ultrastructure of cells.

Chen\textsuperscript{102} observed the effect of PEMF on orthodontic tooth movement of guinea pigs through transmission electron microscope for 14 days. He found that PEMF could accelerate the rate of orthodontic tooth movement and this could be due to an increase
in the numbers of active cells without changing the ultrastructure of cells. No unfavourable effects on periodontal tissues was detected.

Shapiro et al\textsuperscript{103} described a patient who received a pulsating force theoretically designed to act as a piezoelectric stimulus to bone in order to enhance tooth movement. However, it was never determined whether a measurable piezoelectric current was induced in the patient by the device.

1.2.2.4.1.2 Bone Mass

Vingerling et al\textsuperscript{104} undertaken a study in the control of residual ridge reduction by bioelectric means. An inductive-coupled device adjacent to the alveolar ridge of partially edentulous beagle dogs was used in an attempt to decrease ridge resorption secondary to the loss of buccal teeth. The ridge reduction was decreased by this treatment. Though statistically significant, the effect clinically was small.

1.2.2.4.1.3 Condylar growth

Haas\textsuperscript{105} performed a study to evaluate the influence of PEMF on condylar bone and cartilage. Eight cats were bilaterally condylotomized and unilaterally at random received electric stimulation. Bonemarkers were implanted in the zygomatic process and above the condylotomy site before surgery. 4 cats were then exposed to PEMF. After 5 weeks, implant separation indicated increased condylar growth on the treated side of all eight cats. Histologically, the treated condyles had an irregular hypertrophic
zone and vascularisation increased. The treated condyle showed significantly more
bonemarker separation than the control condyle by 1.24mm.

The modulation and control of stimulation of cellular proliferation were attempted to
increase the amount of mandibular condylar growth in Hartley guinea pigs by Gerling et
al\textsuperscript{106}. PEMF with a frequency of 100 Hz was applied for 8 hours per day to mandibular
area of rapidly growing male guinea pigs. 10 guinea pigs were exposed for ten days and
another 10 animals for 30 days, with 5 animals as controls. After 10 days of PEMF,
there was an increase in vascularity, secretion of cartilaginous intercellular matrix and
woven-bone formation in the condyles of the experimental group. After 30 days of
exposure, there were continued but attenuated vascular and calcification responses with
an increase in bone marrow hemopoietic elements. An increase in the number of
osteoclasts was noted after 10 days; however, the effect was transient and the increase
was not present at the end of the 30 day experimental period. The results suggested that
it is possible to affect condylar cartilaginous and bony metabolism through the
application of PEMF.

1.2.2.4.1.4 Osteointegration of hydroxyapatite implants in cancellous bone

Twelve rabbits were exposed to PEMF (75Hz, 1.6mT) after hydroxyapatite implants
were placed in their femoral condyles\textsuperscript{107}. The animals were sacrificed at 3 and 6 weeks
for histomorphometric analysis and microhardness testing around the implants.
Histomorphometric analysis did not highlight any significant changes. In the PEMF
stimulated animals, the microhardness measured in trabecular bone at distance 200 and
500 micros from the implants were significantly higher than the controls. It was
concluded by that PEMF had a positive therapeutic effect in accelerating hydroxyapatite osteointegration in trabecular bone.

1.2.2.4.1.5 Bone healing around titanium dental implants

Rough-surfaced dental implants were inserted into the femur of Japanese white rabbits bilaterally\textsuperscript{108}. PEMF was applied for 4 hours or 8 hours per day, at magnetic intensity of 0.2mT, 0.3mT or 0.8mT. The animals were sacrificed 1, 2 or 4 weeks after implantation. The bone contact ratios of the PEMF-treated femurs were significantly larger than those of the control groups. Both the bone contact ratio and bone area ratio of the 0.2mT and 0.3mT-treated femurs were significantly larger than those of 0.8mT treated femurs (p<0.001). No significant difference in bone contact ratio or bone area ratio was observed whether PEMF was applied to 4 hours or 8 hours per day. Although a significantly greater amount of bone had formed around the implant of the 2-week treated femurs than the 1-week treated femurs, no significant difference was observed between the 2-week and 4-week treated femurs. It was suggested that PEMF stimulation may be useful in promoting bone formation around rough-surface dental implants; however, the appropriate magnetic intensity, duration per day and length of treatment are important.

In a study done by Buzza \textit{et al}\textsuperscript{109} forty-eight dental implants fixtures with titanium surface were implanted in tibiae metaphysis of 12 New Zealand white rabbits divided into experimental PEMF and controls groups. The animals were killed at 21 day and 42 day after implantation. The mechanical tests were performed in all animals and bone biopsies were prepared for decalcified sections analysis. Mechanical tests did not show

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significant differences between the groups (P<0.05); however, statistically significant differences were observed over time (P< 0.0001). The results suggested that PEMF stimulation does not improve the bone healing process around commercially pure dental implants.

1.2.2.4.1.6 Distraction osteogenesis

In a rabbit tibial distraction model stabilized with external fixator after mid shaft tibial osteotomies, 0.25mm of distraction was performed twice daily for 21 days, with the experimental group exposed 1 hour per day to low amplitude, low-frequency PEMF. Tibiae were tested for torsional strength after 9, 16, 23 days post-distraction. PEMF-treated tibiae were significantly stronger than shams at all three time points. It was concluded that short daily PEMF exposures accelerated consolidation of regenerated bone.

1.2.2.4.2 Clinical Studies

1.2.2.4.2.1 Temporomandibular disorder

A multicenter clinical trial of patients with TMD with pain in one or both joints, was comparing active treatment of 36 patients using PEMF to placebo treatment of 42 patients. For both the PEMF and placebo treatment, significant improvements were seen in the subjective data (P<0.001). It was concluded that PEMF had no specific effects in patients with temporomandibular disorders, despite the therapeutic benefit demonstrated in the treatment of numerous forms of osteoarthritis.
1.2.3 Mechanism of action

1.2.3.1 Mechanism of action of ultrasound

The physical process through which low-intensity pulsed ultrasound interacts with living tissue remains unknown. Many in vitro and in vivo studies were conducted to study the effect of osteogenesis under exposure of ultrasound. Ryaby et al\textsuperscript{112} reported that low intensity ultrasound increased calcium incorporation in differentiating cartilage and bone cell cultures. This increased second messenger activity was paralleled by the modulation of adenylate cyclase activity and TGF(beta) synthesis in osteoblastic cells.

Recent studies by Wu et al\textsuperscript{113} showed that exposure of chondrocyte cultures to low intensity ultrasound stimulates an upregulation of aggrecan gene expression. During endochondral ossification, this large chondroitin sulfate molecule aggregates with hyaluronan, decorin, and biglycan, creating key proteoglycan scaffolding elements for Type II collagen which is a key constituent in fracture callus. The results showed that ultrasound signals had an effect on the expression of specific genes involved in the healing process.

Yang et al\textsuperscript{114} used the rat bilateral femur fracture model to validate the hypothesis that low intensity ultrasound will elevate aggrecan gene expression under the hostile conditions of wound healing. The result also demonstrated an increase in the torsional strength of the ultrasound treated calluses in comparison with their contralateral untreated control. They also suggested that ultrasound exposure stimulated chondrogenesis and cartilage hypertrophy, which would result in an earlier onset of endochondral formation, leading to an increase in stiffness and strength of the fracture.
Rawool et al.\textsuperscript{115} showed that low intensity ultrasound treatment over a 10-day period stimulates an increased degree of vascularity in an osteotomised dog ulnae model of fracture healing. Although it was hypothesized that the ultrasound would serve to increase blood flow during treatment, greater blood flow was seen for an extended period after removal of the stimulus. This increase in blood flow monitored by high resolution power Doppler was paralleled by greater callus formation and an improved blood flow distribution around the fracture, thus enabling the delivery of key components such as growth factors and cytokines, which are essential to the normal healing process. This data implied that greater blood flow serves as a principal factor facilitating the acceleration of fracture healing by ultrasound.

1.2.3.2 Mechanism of Action of PEMF

At the cellular level, PEMF is believed to cause a change in membrane permeability, allowing for an increased flow of calcium, sodium and potassium ions across the cell membrane. Rodan et al.\textsuperscript{116} observed that cyclic nucleotides and calcium vehicles carry extracellular messages across the cell membrane. Interactions of the membrane with the bone matrix or with diffusible ions, such as Ca\textsuperscript{2+} and K\textsuperscript{+}, may convey an electric signal for DNA synthesis and cell division. He also suggested a subsequent rise in cAMP to be a signal for cytodifferentiation. Therefore, mechanical stimuli that are transduced unto electric signals may be a message, at the cellular level, for cytodifferentiation through cAMP\textsuperscript{74}.

In combination with the activation of cell membrane bound enzymes, these ions are thought by Davidovitch et al.\textsuperscript{117} to induce changes in the levels and activity of
intracellular cyclic nucleotides such as cAMP and cGMP. However, the precise mechanism of control remains unclear.

1.2.3.2.1 Osteoblast-like cells

According to Satake\textsuperscript{90}, PEMF promoted the growth of osteoblast-like cells. There was an increase in the basal level of [Ca\textsuperscript{2+}]i due to a decrease in intracellular Ca\textsuperscript{2+} transient modulated by the decrease of response towards epidermal growth factor (EGF) and serum. These effects of PEMF were mimicked by 12-O-tetradecanoyl phorbol 13-acetate (TPA), a potent activator of protein kinase C. Pretreatment of TPA enhanced the cell growth and suppressed the intracellular Ca\textsuperscript{2+} transient induced by EGF receptors of these cells. It was stated that both PEMF and TPA decreased the level of EGF binding to these cells down to about 65%. It was concluded that PEMF acted at cell membrane and modulated the receptors which was essential for cell growth and DNA synthesis.

In a study done by Lohmann \textit{et al}\textsuperscript{118}, confluent cultures of MG63 human osteoblast-like cells were placed between Helmholtz coils and exposed to pulsed electromagnetic signal with bursts of 20 pulses at 15 Hz for 8 hours per day for 1, 2, or 4 days. The PEMF signal caused a reduction in cell proliferation on the basis of cell number and \textsuperscript{3}H thymidine incorporation. Cellular alkaline phosphatase-specific activity increased in the cultures exposed to PEMF with maximum effects at day 1. Enzyme activity in the cell-layer lysates, including alkaline phosphatase- enriched extracellular matrix vesicles, continued to increase with the time of exposure to the signal. After 1 and 2 days of exposure to PEMF, collagen synthesis and osteocalcin production were greater than in the control cultures. Prostaglandin E2 in the treated cultures was significantly reduced at
1 and 2 days, whereas transforming growth factor beta 1 was increased. At day 4, both local factors were similar to the level of controls.

It was concluded that PEMF enhanced cell differentiation on osteoblasts, as evidenced by decreased proliferation and increased alkaline phosphatase specific activity, osteocalcin synthesis and collagen production.

A PEMF with a frequency of 15 Hz was applied to neonatal mouse calvarial bone cell cultures for 14 days in a study done by Chang et al\textsuperscript{119}. It was shown that PEMF significantly increased the osteoblasts' proliferation by 34.0, 11.5, and 13.3% over the control after 3, 5, and 7 days' culture. Alkaline phosphatase activity of the bone cells decreased significantly after PEMF stimulation. In conclusion, the treatment by PEMF of osteoblasts may accelerate cellular proliferation, but did not affect the cellular differentiation. This was opposite to the findings by Lohmann et al\textsuperscript{118} as stated above.

The effects of PEMF (15Hz) stimulation on bone tissue-like formation and on osteoblasts in different stages of maturation were assessed to determine whether the PEMF stimulatory effect on bone tissue-like formation was associated with increase in the number of cells and/or with the enhancement of the cellular differentiation\textsuperscript{120}. The cellular proliferation (DNA content), differentiation (alkaline phosphatase activity), and bone tissue-like formation (area of mineralised matrix) were determined at different time points. PEMF treatment of osteoblasts in the active proliferation stage accelerated cellular proliferation, enhanced cellular differentiation, increased bone tissue-like formation. PEMF treatment of osteoblasts in the differentiation stage enhanced cellular differentiation and increased bone tissue-like formation. PEMF treatment of osteoblasts
in the mineralization stage decreased bone tissue-like formation. It was concluded that PEMF had a stimulatory effect on the osteoblasts in the early stages of culture, which increased bone tissue-like formation. This stimulatory effect was most likely to be associated with enhancement of cellular differentiation, and not the increase in the number of cells.

1.2.3.2.2 Osteoclast-like cells

The effects of extremely low frequency PEMF on osteoclastogenesis, were examined using cultures of murine bone marrow cells $^{121}$. Primary bone marrow cells were cultured from mature Wistar rats and exposed to PEMF stimulation daily with different intensities of induced electric field (4.8, 8.7, and 12.2 V/cm rms) and stimulation times (0.5, 2, and 8h/day). The result suggested that PEMF could both enhance (approximately 50%) and suppress (approximately 27%) the formation of osteoclast-like cells in bone marrow culture, depending on the induced electric field intensity.

In another study, adult female Wistar rats were subjected to bilateral or sham ovariectomy, and primary bone marrow cells were harvested at Day 4 (Subgroup 1) and Day 7 (Subgroup 2) after surgery$^{122}$. Primary bone marrow cells were subjected to PEMF (300us, 7.5Hz) for 1 hour per day for 9 days. Other samples from intact animals, sham ovariectomy and ovariectomized animals were exposed to no PEMF. The PEMF caused significant reductions in osteoclast formation Subgroups 1 and 2. It was suggested that osteoclastogenesis could be inhibited by PEMF stimulation.
1.2.3.2.3 Human articular chondrocyte

Human nasal and articular chondrocytes were exposed to low energy, low frequency pulsed electromagnetic fields\textsuperscript{123}. The cells were exposed to PEMF for time periods ranging from 6 to 30 hours, in medium supplemented with 10% or 0.5% fetal calf serum and in serum-free medium. The ratios between the $^3$H thymidine incorporation in the presence of PEMF and control cultures show an increase of the cell proliferation in culture exposed to PEMF when serum is present in culture medium, whereas no effect was observed in serum-free conditions. The data showed that PEMF induced an increase in the proliferation of both cell types and the concentration of fetal calf serum in the culture medium greatly influences the proliferation response of human chondrocytes to PEMF exposure.

In one study, human articular chondrocyte cultures in medium containing 10% FBS, were exposed to PEMF (75Hz, 2.3mT), which is the method of treatment of some orthopedic pathologies\textsuperscript{124}. Cell proliferation of low density chondrocyte cultures was found after induction by PEMF for 6 days. However, in high density cultures, PEMF induced increase in cell proliferation was observed in the first three days of exposure. It was concluded that growth factors and environmental constrictions affect the cellular response to PEMF.

1.2.3.2.4 Periodontal ligament fibroblasts

There is a widely held hypothesis on orthodontic tooth movement that mechanical stress generates an electrical signal which sets in motion a series of reactions; similar to that
demonstrated when bone is exposed to mechanical forces, electrical currents are produced resulting in bone growth and remodelling. This hypothesis implies a transduction mechanism which translates the electrical signal into a biochemical message, recognizable by the cells. Satake et al.\textsuperscript{125} described the effect of PEMF on the periodontal ligament fibroblasts at a cellular level. Calcium concentration in human periodontal ligament fibroblast cells was increased in the presence of PEMF. A decreased in response towards epidermal growth factor and serum was found with the presence of PEMF. With similar results to the study mentioned above on osteoblastic-like cells, it was concluded that fibroblasts of periodontal ligament have osteoblastic features.

Panagopoulos et al.\textsuperscript{126} suggested that the basic mechanism is the forced-vibration of all the free ions on the surface of a cell's plasma membrane, caused by an external oscillating field. This coherent vibration of electric charge is able to irregularly gate electrosensitive channels on the plasma membrane and thus cause disruption of the cell's electrochemical balance and function.

It was thought that the magnetic field initiates increased localised calcium deposition, which neutralizes the tissues net negative charge, allows for subsequent vascularization, and initiation of osteogenesis\textsuperscript{17,127}.

The clinical benefits of electric and electromagnetic fields have been claimed for 20 centuries but still the mechanism of action remains unclear. Balcavage et al.\textsuperscript{36} described a simple mechanism, based on the Hall Effect, that static and low frequency (50-60Hz) PEMF can modify cation flow across biological membranes, changing the steady state
concentration of cellular cations and alter cell metabolism dependent on cation concentrations.

Stark and Sinclair\textsuperscript{37} stated that the increase in cellular metabolism and proliferation appears to be linked to changes in calcium and sodium ion transport rates at the cell membrane. These ions may then exert their effects on the cell via the levels of enzymes that are responsible for regulating intracellular metabolism.

Nelson \textit{et al}\textsuperscript{38} described that PEMF and capacitive coupling induce fields through soft tissue, resulting in low magnitude voltage and currents at the fracture site. PEMF was shown to be effective in managing extremity non-union. Capacitive coupling appears to be effective both in extremity nonunions and lumbar fusions. Low intensity ultrasound has been used to speed normal fracture healing and manage delayed unions and has been approved in the United States for management of nonunions. Despite the different mechanisms for stimulating bone healing, all signals result in increased intracellular calcium, thereby leading to bone formation.

Otter \textit{et al}\textsuperscript{39} stated that the body of evidence showed that steady direct current time varying electric fields are generated in living bone by metabolic action and mechanical deformation. Externally supplied direct current used to treat nonunions, appeared to trigger mitosis and recruitment of osteogenic cells, possibly via electrochemical reactions at the electrode tissue interface. Time varying electromagnetic fields also have been used to treat nonunions, spinal fusion, osteonecrosis and osteoarthritis. Research evidence showed that the mechanism(s) of action of these time varying fields has concentrated on small, extremely low frequency sinusoidal electric fields. The
osteogenic capacity of these fields does not appear to involve changes in the transmembrane electric potential. It appears to require coupling with the cell interior via transmembrane receptors and mechanical coupling to the membrane itself.
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3. Manuscript

Effects of vibrating stimulus and pulsed electromagnetic fields on tooth movement
Effects of vibrating stimulus and pulsed electromagnetic fields on tooth movement

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Abstract

A number of published papers have claimed that applied mechanical, electrical and magnetic energies affect bone metabolism. Vibrating stimulus was already used in the prevention of osteoporosis and bone healing. It has been hypothesized that vibrating stimulus would affect the rate of tooth movement if combined with conventional force system. The first aim was to develop an in vivo model to apply vibrating mechanical stimulation on a rat molar. The second aim was to determine whether a Nd-Fe-B magnet bonded onto a tooth and vibrated using PEMF affects tooth movement in a Wistar rat. The third aim was to evaluate whether a Nd-Fe-B magnet vibrated by PEMF will affect conventional orthodontic tooth movement produced by a closed coil spring.

Forty four 7-week old Wistar rats weighing 210-250g were randomly divided into four groups. Permanently demagnetised sham magnets, Nd-Fe-B magnets and 25 g Sentalloy closed coil springs were attached to the mesial aspect of the first molars in relevant groups. Half of the animals were exposed to PEMF in a Helmholtz configuration (experimental group) and half of them were not. After 14 days, the animals were sacrificed and the change of distance of the interdental space between the most mesial cervical point of the first molars and the palatal midpoint of the upper incisors was measured with digital callipers. Statistical analysis of the results of these experiments yielded the following conclusions.

1. A new model to demonstrate the application of vibrating stimulus on rat molars was successfully developed by using Neodymium-Iron-Boron magnets in the presence of pulsed electromagnetic fields.

2. The interdental space between incisor and first molar showed an increase in the presence of vibrating mechanical stimulus with Nd-Fe-B magnets and PEMF (Group
1P). This increase seemed to be less than the control (Group 1 NP) and is statistically significant.

3. Continuous mechanical stress applied by 25g Sentalloy coil springs resulted in a decrease in the interdental space.

4. Mechanical stimulus combined with a vibrating mechanical stimulus to the first molar (Group 4P) seems to result in a decrease of tooth movement as compared with the control.

*Keywords:* Vibrating mechanical stimulus, static magnetic fields, pulsed electromagnetic fields, orthodontic tooth movement.
Introduction

Interest in energy medicine has existed for centuries in some parts of the world. Investigations reported in the literature have shown that applied electrical fields can alter the normal electrical states of bone and cartilage, induce increased rates of cellular division and metabolism, and thus promote increased healing of bony and cartilaginous defects.\textsuperscript{1-2} The technology using electric and electromagnetic fields to heal fracture non-unions emerged as a result of studies demonstrating the piezoelectric properties of dry bone by Fukada and Yasuda (1957)\textsuperscript{3}. Bassett and Becker (1962) described asymmetric voltage waveforms from mechanically deformed live bone.\textsuperscript{4-5} These changes were presumed to occur in bone during physical activity as a result of mechanical forces, and it was postulated that these forces were linked to modifications in bone structure. Bassett (1989) proposed that tissue integrity and function could be restored by applying electrical and/or mechanical energy to the area of injury.\textsuperscript{6-7} Electrical currents were applied to non-healing fractures in animal studies\textsuperscript{8-10} and in clinical trials\textsuperscript{11-12} and successfully helped the healing process. It appears that electrical energy, whether applied as a direct current or a pulsed electromagnetic field\textsuperscript{9, 13-23}, has the ability to affect both the depository and resorptive activities of bone and cartilage cells.\textsuperscript{8} However, the mechanism of action remains unclear. Despite the difference between the forms of stimulation of bone healing, all signals result in a change of intracellular ions, leading to an altered cell metabolism\textsuperscript{9, 24-29}.

It is believed that orthodontic tooth movement is accompanied by site-specific bone remodelling in the absence of inflammation. Alveolar bone remodelling is essential for tooth movement and is characterised by tandem periods of osteoclastic recruitment,
bone resorption, reversal and bone formation. This process involves the periodontal membrane and is dependent on the magnitude and consistency of the force being applied. In the area of periodontal ligament compression, osteoclasts proliferate and initial resorption of superficial bone occurs. In the region of periodontal ligament tension, the periodontal fibres unwind, fibroblasts appear, and osteoblasts form a non-mineralised collagenous matrix called osteoid. The osteoid is later mineralised, trapping some osteocytes in lacunae within the bone. However, the precise mechanisms of control of bone remodelling remain unclear.

Previous animal and clinical studies have shown that mechanical stimulation, in particular low intensity pulsed ultrasound, improves the rate of bone healing via up-regulation of cartilage formation and maturation of endochondral bone formation. In addition, the results of earlier studies show that high frequency (30Hz), low magnitude vibrations induce increased anabolic activity in bone. Consequently, it is worth investigating the proposition that these stimuli increase the overall metabolic activity of bone including a proportional increase in catabolic activity. Since it has been hypothesized that there is an increase in metabolic activity of bone, there should be an increase in the rate of orthodontic movement of the teeth.

Our first aim was to develop an in vivo model to apply vibrating mechanical stimulation generated by Nd-Fe-B magnets in the presence of pulsed electromagnetic fields (PEMF) on a rat molar. The second aim was to determine whether a Nd-Fe-B magnet bonded onto a tooth and vibrated using PEMF affects tooth movement in a Wistar rat. The third
aim was to evaluate whether a Nd-Fe-B magnet vibrated by PEMF will affect conventional orthodontic tooth movement produced by closed coil spring.

Materials and Methods

Experimental design

Forty four 7-week old Wistar strain rats weighing 210-250g were obtained from the Westmead Animal Holding Facility. The animal groups were housed in circular lexan cages with an internal diameter of 40cm with plastic water distributors and ceramic feeding bowls for at least 48 hours before use in experiments. (Ethics approval: Westmead Hospital Animal Ethics Committee protocol no. 141.04-04) The animals were randomly divided into four groups as shown in Table 1.

Table 1. Distribution and description of sample groups

<table>
<thead>
<tr>
<th></th>
<th>PEMF (P)</th>
<th>No PEMF (NP)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Right</td>
</tr>
<tr>
<td>Group 1</td>
<td>Sham magnet (n=10)</td>
<td>Magnet (n=10)</td>
</tr>
<tr>
<td>Group 2</td>
<td>Magnet (n=4)</td>
<td>Coil+magnet (n=4)</td>
</tr>
<tr>
<td>Group 3</td>
<td>Sham magnet (n=4)</td>
<td>Coil+sham magnet (n=4)</td>
</tr>
<tr>
<td>Group 4</td>
<td>Coil+sham magnet (n=4)</td>
<td>Coil+magnet (n=4)</td>
</tr>
</tbody>
</table>
Group 1: (n=20) permanently demagnetised sham magnets were bonded onto the mesial aspect of the left first molars and Neodymium-Iron-Boron (Nd-Fe-B) magnets were bonded on the contralateral side (Fig. 1). Half of the animals (n=10) were exposed to pulsed electromagnetic fields (PEMF) in a Helmholtz configuration (Fig. 2)41 (Group 1 P) as the experimental group and half of them were not (Group 1 NP). This group was used to evaluate the effect of the vibrating stimulus produced by the interaction of the Nd-Fe-B magnets with the PEMF on molar tooth movement, if any.

Group 2: (n=8) Nd-Fe-B magnets were bonded on the mesial aspect of the left first molars and 25 g Sentalloy closed coil springs (GAC Cat No:10-000-26) plus Nd-Fe-B magnets were placed on the contralateral side (Fig.3). Half of the animals (n=4) were exposed to PEMF (Group 2P) and the other half of the animals were not (Group 2 NP). This group was used to evaluate the effect of vibrating stimulus produced by Nd-Fe-B magnets under the influence of PEMF alone in comparison to the effect of closed coil springs combined with vibrating mechanical stress on molar tooth movement.

Group 3: (n=8) permanently demagnetised sham magnets were bonded onto left first molars and 25g Sentalloy closed coil springs plus permanently demagnetised sham magnets were placed on the contralateral side. Half of the group (n=4) was exposed to PEMF (Group 3P) and the other half (n=4) was not (Group 3NP). This group was used to evaluate the effect of PEMF alone and combined with closed coil springs.

Group 4: (n=8) 25g Sentalloy closed coil springs with permanently demagnetised sham magnets were placed on the left molars and 25g Sentalloy closed coil springs with Nd-Fe-B magnets were placed on the contralateral side. Half of the group (n=4) was
exposed to PEMF (Group 4P) and the other half (n=4) of the group was not (Group 4NP). This group was used to show the difference in molar tooth movement, if any, between the effect of the Nd-Fe-B magnets vibrated under the influence of PEMF together with the effect of closed coil springs, and the effect of closed coil springs with permanently demagnetised sham magnet.

The rats were administered a gaseous anaesthetic agent (halothane). This sedated the rats to an appropriate state whereby they could be weighed. Subsequently, an appropriate amount of anaesthetic agent was injected into the peritoneum. The anaesthetic agents used were Xylazine (10mg/kg) and Ketamine (90mg/kg). This induced sufficient duration of anaesthesia to complete the bonding of Nd-Fe-B magnets, sham magnets and placement of coil springs as appropriate onto the rat molars.

Before bonding of the magnets and placement of coil springs, polyvinyl siloxane hydrophilic impressions of the rat dentition were taken as records (3M Imprint II Garant Quick Step Regular Body Cat No. 9579). Neodymium-Iron-Boron magnets of 1mm length x 1mm width x 0.5mm thickness were bonded onto the mesial surface of experimental rat upper first molars (Group 1) with 3M Transbond light cure composite resin (Ref No. 712036) and 3M Transbond Moisture Insensitive Primer (Ref. No. 712-025). Permanently demagnetised sham magnets of the same dimension were bonded onto maxillary molars on the contralateral side as a control.

Sentalloy closed coil springs of twenty-five grams were used to move the mandibular molars forward, decreasing the length of the interdental space between the first molar and the incisor. The orthodontic tooth movement model was adapted from Kobayashi et
al" and Rygh\textsuperscript{43-46} and modified by Low et al\textsuperscript{47} (Fig. 3). In appropriate groups, Nd-Fe-B magnets and permanently demagnetised sham magnets of 1mm length x 1mm width x 0.5mm thickness were bonded onto 25g SENTALLOY closed coil springs before ligating onto the first mandibular molars. After ligation with 3.0 silk suture, composite resin was used to bond magnets and sham magnets onto the mesial surface of first molars to ensure contact with tooth structure. The springs were then fixed anteriorly using ligature wire loops placed around the incisal thirds of both lower incisors. Composite resin was placed over ligature wire ends to prevent mucosal trauma. Following recovery, the rats were housed in circular lexan cages.

\textit{Apparatus setup}

The electromagnetic field generating coils of internal diameter of 40cm which were placed above and below the cage in the PEMF group were arranged parallel to each other with a separation of 20cm (Fig.2). This design, known as a Helmholtz configuration, produces an even magnetic field in the space between the coils. The principle of apparatus setup is based on Darendeliler et al\textsuperscript{41}. The field coil is driven by a linear amplifier with a variable power output of up to 800 Watts RMS (Panasonic Pty. Ltd.). Field parameters including frequency (duty-cycle) and growth/decay character can be precisely controlled via a computer interface. This waveform generator comprises a MacIntosh computer, hosting a software signal generator (Mac the Scope, Channel D Corporation, Trenton NJ) with the output being passed to the amplifier described above via the standard analogue sound output channel. Field strength is controlled via the amplifier power output. Throughout this experiment, the settings used on the Mac the Scope software were:- frequency 30 Hz, sinusoidal waveform of single mode. The magnetic field intensity is controlled via the amplifier power output to
provide 2 Gauss as measured using Holaday ELF Magnetic Field Meter (Holaday Industries Inc., USA).

The rats were sacrificed on the 14th day by carbon dioxide asphyxiation. Polyvinyl siloxane impressions of rat dentition were taken as post-treatment records. Casts of before and after treatment was prepared in plaster from the impressions. Measurements were taken with digital millimetre callipers (Orthopli Electronic Digital Callipers Model#50001, USA) to determine the distance between the palatal midpoint of the upper incisors to the most mesial cervical point of the first molar (interdental space). Each measurement was recorded three times on different days to reduce error. The data was analysed using the Univariate Analysis of Variance and Estimated Marginal Means analyses of the Statistical Package for Social Sciences program (SPSS for Windows, version 12 SPSS Inc., Chicago III).

Results

Univariate analysis of variance for tooth movement including a Coil spring by Magnet interaction (Table 2) shows that the difference in the amount of tooth movement. There has been a decrease in interdental space when closed coil springs were used with Nd-Fe-B magnets (Group 4) compared with permanently demagnetised sham magnets. This is statistically significant (p=0.014) which indicates that closed coil springs do not have the same effect on molar tooth movement in the presence and absence of magnets. The amount of tooth movement under the effect of PEMF alone, and under the effect of Nd-Fe-B alone are both not statistically significant (PEMF p=0.577, Magnet p=0.097). The change in length of interdental space between different animals with Rat as a variable is
statistically significant (p=0.002). The mesial molar tooth movement resulting in a decrease of interdental space caused by closed coil springs is highly statistically significant (p<0.001). The estimated marginal means shows that when coil spring is absent, there is an indication that PEMF may have an effect (0.089 vs 0.212).

Due to the indication that PEMF could have an effect on molar tooth movement from the results stated above, the univariate analysis of variance of data for tooth movement was prepared (Table 3). It shows that molar tooth movement resulting in a change of interdental space of all variables are statistically significant. [Coil spring (p<0.001), Magnet (p=0.019), Magnet and Coil springs’ interaction (p=0.001) and Rat is (p=0.004)].

Univariate analysis of variance of data for tooth movement when PEMF is absent (Table 3) shows that molar tooth movement resulting in a change of interdental space with Coil spring (p<0.001) and Rat (p<0.05) as variables are statistically significant; but with Magnet alone (p=0.41) and Coil springs and Magnet interaction (p=0.884) as variables, they are not statistically significant.

**Discussion**

Vibrating mechanical stimulation, in particular low intensity pulsed ultrasound has been shown to improve the rate of bone healing. If vibrating mechanical stimulation affects bone modelling and remodelling in fracture healing, such stimulus may affect tooth movement which involves bone remodelling. In this study, a new method has been developed an *in vivo* model to apply vibrating mechanical stimulation generated by Nd-
Fe-B magnets in the presence of PEMF on a rat molar. The study was designed in such a way that the Nd-Fe-B magnet bonded onto the molar tooth of the rat would interact with the PEMF, resulting in mesiodistal vibratory stimulus to that molar. The Nd-Fe-B magnet orientation was selected to vibrate mesiodistally most of the time when the animals' heads were horizontally oriented and when they were moving about in the cages. The direction of PEMF will affect the direction of pulsating movement of the magnet. However, the direction of vibration in relation to the PEMF may have been changed, depending on the orientation of the head. It was shown that Nd-Fe-B magnets bonded onto rat molars vibrating in the presence of PEMF (Group 1P) resulted in an increase in interdental space less than that seen in non-vibrating (sham magnet) group (Group 1NP) (p=0.019) and this was statistically significant. This difference could be due to growth of the animal or mesial movement of the first molar.

Magnets and sham magnets were bonded on the maxillary first molars of six rats of Group 1P and Group 1 NP. Subsequently, it was found that the coil springs were more securely attached to the mandibular teeth than maxillary teeth due to the concavity of the maxillary teeth. Therefore, magnets and coil springs were attached to the mandibular teeth of the remaining sample groups. It is possible that the amount of increase in interdental space due to growth varies between the maxilla and mandible and between individual animal. It was found that the change of interdental space between each animal was large and statistically significant (p<0.002). The silk suture ligation around the molars for attachment of coil spring may induce gingival inflammation and should be confirmed in future experiments by histological studies.
Measurements should be taken from animals exposed to no treatment for the estimation of increase in interdental space due to growth. The weakness of this study is that each sample group was relatively small. Larger sample sizes should be used to confirm the results obtained from this pilot study. During the experimental design stage, attempts were made to obtain a suitable apparatus to detect the pulsation of a magnet bonded tooth reacting to a PEMF in a live animal but these attempts were unsuccessful. If future tests are to be done on a larger scale, such apparatus would be of benefit. Different pulsating frequencies and stimulation times may produce different results\textsuperscript{50-52}.

Bassett and Becker in 1962 described asymmetric voltage waveforms from mechanically deformed live bone\textsuperscript{9-10}. These changes were presumed to occur in bone during physical activity as a result of mechanical forces. Polarity was dependent on the direction of bending; areas under pressure are in a electropositive state which is usually associated with osteoclastic activity and areas under tension are in a electronegative state which is associated with osteoblastic activity. It is possible that under vibrating stimulus, the areas under pressure will shortly become the areas under tension and vice versa. An area of alveolar bone will be in an alternating electropositive and electronegative states; therefore, associated with both increased osteoblastic activity and osteoclastic activity.

There is also a possibility that the static magnetic field exerted by the Nd-Fe-B magnet alone on the first molar tooth could have an effect on its position when a similar pattern of a lesser increase in the interdental space is shown that as in the absence of PEMF, although this is not statistically significant. The effect of magnetic field on bone healing is well documented in the literature \textsuperscript{18,41,49}; however, the mechanism of action is not
clear. Bassett\textsuperscript{10} stated that magnetic field could initiate increased localised calcium deposition, which neutralizes the tissues net negative charge, allows for subsequent vascularization and initiation of osteogenesis. It seems that this static magnetic effect caused by the Nd-Fe-B magnet alone could be accentuated in the presence of PEMF which causes vibration.

It appears that although the effect on tooth movement of PEMF alone is not statistically significant overall, there is an indication that it affects the linear measurements of the interdental space of the experimental samples compared with the controls. It is well documented in the literature that PEMF at low frequency\textsuperscript{18} significantly accelerate bone fracture healing in clinical trials\textsuperscript{23-24, 26-27} and osteotomy healing in animal studies\textsuperscript{19-22}, especially with nonunions and delayed fracture unions. It was also suggested by Rubin \textit{et al}\textsuperscript{14} that bone resorption which accompanies disuse can be prevented or even reversed by the exogenous induction of electric fields. However, the precise mechanism of action remains unclear. Satake \textit{et al}\textsuperscript{33} found that calcium concentration in human periodontal ligament fibroblast cells was increased in the presence of PEMF. At the cellular level, Rodan \textit{et al}\textsuperscript{34} observed that cyclic nucleotides and calcium vehicles carry extracellular messages across the cell membrane. Interactions with diffusible ions, such as Ca\textsuperscript{2+} and K\textsuperscript{+}, may convey an electric signal for DNA synthesis and cell division. He also suggested a subsequent rise in cAMP to be a signal for cytodifferentiation. If PEMF has an effect on bone modelling and remodelling processes, it can be reasonably expected that it would also affect the bone remodelling which accompanies orthodontic tooth movement. The overall effect in terms of linear measurement changes of the interdental space under the influence of PEMF alone could be minute compared with that of the coil spring.
Referring to the boxplots (Fig. 4) showing the overall data, the apparent increase in the interdental space of the animals with exposure to PEMF alone or without applied agents, after the 14 days experimental period, though statistically insignificant, could be due to growth of the animals. According to Stromberg and Hebel 48, depending on the age of the animal, the interdental space varies from 12 to 20 mm in the upper jaw and 10 to 12 mm in the lower jaw. The row of three tightly apposed molar teeth of each quadrant increases in both length and width by about 10% in older animals (from 300 days). The animals used in this experiment were 7 weeks old rats which were in the juvenile stage of development. During the 14 days experimental period, it is logical to assume that there would be an increase in the length of interdental space due to growth but limited increase in both length and width of the three apposed molar teeth. This small increase in interdental space from growth of the animals would be difficult to detect with digital callipers but maybe more accurately detected by more sensitive means of measurement.

The third aim of this study was to evaluate whether a Nd-Fe-B magnet vibrated by PEMF will affect conventional orthodontic tooth movement produced by closed coil spring. This aim has been met with the finding that 25g Sentalloy closed coil springs do not have the same effect when magnets are present compared with when magnets are absent with Coil spring by Magnet interaction statistically significant ($p=0.004$) in the overall data (Table 2). In the presence of PEMF (Table 3), this interaction remains highly significant. This demonstrates a possible synergistic effect of the PEMF on the effects of tooth movement caused by the Nd-Fe-B magnet and coil springs. The experimental results confirms that 25g Sentalloy closed coil springs caused mesial orthodontic movement of the first molars resulting in a decrease of interdental space.
(p<0.01), which was found by other researchers who used a similar animal model.\textsuperscript{43-47} In addition, the result demonstrates that the pulsating action of the magnet on the molar tooth and coil springs hinders orthodontic tooth movement (p=0.001). This suggests that the pulsating effect of the magnet causes the molar tooth to remain relatively stationary.

The pulsating movement caused by the interaction of the PEMF and the Nd-Fe-B magnet could have a greater effect than detected, because the amount and/or direction of movement may be masked by that produced by the coil springs. The Nd-Fe-B magnet alone and together with the closed coil springs appears to reduce the change of the length of interdental space. This effect could be explained by theorising that the pulsating force on the molar tooth cause by the Nd-Fe-B magnet under the influence of PEMF could have a mesial, distal, intrusive or extrusive component, depending on the orientation of the rat to the PEMF. These forces on average could have cancelled each other. As a result, the molar tooth remains relatively stationary in position. As a result, if the coil spring is present, the PEMF effect, if any, will be masked by that produced by the coil springs. Comparing the data boxplots (Fig. 4) of the tooth movement produced by coil springs with and without the influence of PEMF, there is an indication that PEMF increases the linear measurement changes produced by the closed coil springs. This indication of PEMF effect correlates with the findings of Darendeliler et al.\textsuperscript{41} They found that both the static magnetic fields produced by samarium-cobalt magnets and the PEMF used in combination with the coil springs were successful in increasing the rate of tooth movement compared with that produced by the coil springs alone. The mechanism producing this effect was suggested by the researchers to involve a reduction in "lag" phase, which is common in orthodontic tooth movement. In this experiment, the "lag" phase of orthodontic tooth movement could have been reduced as
a result of the influence of the PEMF, resulting in an increased rate of tooth movement compared to the controls with no PEMF. In the current experiment when the PEMF was present, the change in interdental space affected by the Nd-Fe-B magnet alone is statistically significant (p=0.019). We postulate that the PEMF, although it did not appear to produce much effect on its own, has a synergistic effect on tooth movement when applied to samples in conjunction with Nd-Fe-B magnets and closed coil springs.

The Nd-Fe-B appears to reduce the change in interdental space. In orthodontics during space closure, the use of a magnet bonded onto a tooth may reinforce the anchorage requirements. Moreover, the synergistic effect of PEMF with the effect of coil spring and magnet on tooth movement could also be utilised in the area of orthodontics. PEMF could be applied especially at the beginning of treatment. This will decrease the “lag” phase of initial orthodontic and shorten the treatment time. However it must be recognised that there are concerns in the community about the effect on general health of PEMF. These concerns may prevent the use of PEMF as an aid to orthodontic treatment. If future studies could confirm these findings that a vibrating mechanical stimulus, so described, could generate tooth movement without an orthodontic spring system, and/or minimize the complication of root resorption, it would be a significant discovery in the field of orthodontics and of potential benefit to individuals undergoing orthodontic treatment in the future.

Measurements were taken using handheld digital callipers. Despite repeating the measurements three times, the values taken were prone to operator error. The three dimensional image of the before and after treatment of the polyvinyl siloxane impressions would be best scanned with Micro CT. Computer analysis could be done to
calculate the change of dimension in all three planes of the first molar, as well as the linear dimensional changes of the diastema. Moreover, the roots of the molars attached to the bone could be scanned as well. Resorption craters and periodontal ligament space could then be examined and tooth ankylosis could be detected.

Conclusion

In conclusion, A new model to demonstrate the application of vibrating stimulus on rat molars was successfully developed by using Neodymium-Iron-Boron magnets in the presence of pulsed electromagnetic fields.

2. The interdental space between incisor and first molar showed an increase in the presence of vibrating mechanical stimulus with Nd-Fe-B magnets and PEMF (Group 1P). This increase seemed to be less than the control (Group 1 NP) and is statistically significant.

3. Continuous mechanical stress applied by 25g Sentalloy coil springs resulted in a decrease in the interdental space.

4. Mechanical stimulus combined with a vibrating mechanical stimulus to the first molar (Group 4P) seems to result in a decrease of tooth movement as compared with the control.

Acknowledgements

We thank The Australian Society of Orthodontics for funding of this project. We also thank Mr. Paul Bowker, Senior Lecturer (clinical) of University of Sydney; Ms Janice Mathews from the Department of The Cellular and Molecular Pathology Research Unit,
University of Sydney; all the staff of the Westmead Animal Holding Facility; and Dr. Allan Jones from the Electron Microscopy Unit, University of Sydney.

References


9. Rubin CT, Donahue HJ, Rubin JE, McLeod KJ. Optimisation of electric field parameters for the control of bone remodelling: exploitation of an indigenous


Table 2 Univariate Analysis of Variance

Tests of Between-Subjects Effects

<table>
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Table 3 Univariate Analysis of Variance (pemf=1, pemf=0)

Tests of Between-Subjects Effects

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Figure 1  Neodymium-Iron-Boron and permanently demagnetised sham magnet bonded on the mesial surface of the maxillary first molar in a Wistar rat

Figure 2  PEMF generating coils surrounding circular lexan cage
Figure 3  Orthodontic tooth movement model based on Low\textsuperscript{45} in 7 week old Wistar rat, an intraoral view.
Figure 4 The Effect of Ne-Fe-B magnets, 25g Sentalloy coil springs and their interaction in the absence and presence of pulsed electromagnetic fields.

PCM

... control
.m magnet
.c. coil
.cm coil plus magnet
.p. PEMF
.p.m PEMF plus magnet (vibration)
.p.c. PEMF plus coil
.pcm PEMF plus coil and magnet (vibration)
# 4. Appendix

## Table 2 Univariate Analysis of Variance

Tests of Between-Subjects Effects

<table>
<thead>
<tr>
<th>Source</th>
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a .870 MS(RAT(PEMF)) + .130 MS(Error)
b MS(Error)

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a For each source, the expected mean square equals the sum of the coefficients in the cells times the variance components, plus a quadratic term involving effects in the Quadratic Term cell.
b Expected Mean Squares are based on the Type III Sums of Squares.
Table 3 Estimated Marginal Means

1. PEMF

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   a Based on modified population marginal mean.

2. COIL * PEMF

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3. MAG * PEMF

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   a Based on modified population marginal mean.
Table 4 Univariate Analysis of Variance

Tests of Between-Subjects Effects

Dependent Variable: MT Movement

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\[a \quad .872 \text{MS(RAT(PEMF))} + .128 \text{MS(Error)}
\[b \quad \text{MS(Error)}

Expected Mean Squares(a,b)

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a For each source, the expected mean square equals the sum of the coefficients in the cells times the variance components, plus a quadratic term involving effects in the Quadratic Term cell.
b Expected Mean Squares are based on the Type III Sums of Squares.
### Table 5 Estimated Marginal Means

#### 1. PEMF

<table>
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a Based on modified population marginal mean.

#### 2. COIL * MAG * PEMF

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a Based on modified population marginal mean.
Table 6 Univariate Analysis of Variance (pemf=1)

Tests of Between-Subjects Effects

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a .872 MS(RAT) + .128 MS(Error)
b MS(Error)
Table 7 Estimated Marginal Means (pemf=1)

1. COIL

<table>
<thead>
<tr>
<th>COIL</th>
<th>Mean</th>
<th>Std. Error</th>
<th>95% Confidence Interval</th>
<th>Lower Bound</th>
<th>Upper Bound</th>
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2. MAG

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3. COIL * MAG

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a .872 MS(RAT) + .128 MS(Error)
b MS(Error)
Table 9 Estimated Marginal Means (pemf=0)

1. COIL

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2. MAG

<table>
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<tr>
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3. COIL * MAG

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