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Therapeutic potential of electromagnetic fields for tissue engineering and wound healing

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Abstract

Ability of electromagnetic fields (EMF) to stimulate cell proliferation and differentiation has attracted the attention of many laboratories specialized in regenerative medicine over the past number of decades. Recent studies have shed light on bio-effects induced by the EMF and how they might be harnessed to help control tissue regeneration and wound healing. Number of recent reports suggests that EMF has a positive impact at different stages of healing. Processes impacted by EMF include, but are not limited to, cell migration and proliferation, expression of growth factors, nitric oxide signalling, cytokine modulation, and more. These effects have been detected even during application of low frequencies (range: 30–300 kHz) and extremely low frequencies (range: 3–30 Hz). In this regard, special emphasis of this review is the applications of extremely low-frequency EMFs due to their bio-safety and therapeutic efficacy. The article also discusses combinatorial effect of EMF and mesenchymal stem cells for treatment of neurodegenerative diseases and bone tissue engineering. In addition, we discuss future perspectives of application of EMF for tissue engineering and use of metal nanoparticles activated by EMF for drug delivery and wound dressing.

Abbreviations

Anti-GFAP: anti-Glial Fibrillary Acidic Protein

Anti-MAP2: anti-Microtubule-Associated Protein 2

Anti-O4: anti-Oligodendrocyte O4 Marker

CREB: cAMP response element-binding protein

DMSO: dimethyl sulphoxide

ECM: extracellular matrix

EGFR: epidermal growth factor receptor

ELF EMF: extremely low-frequency electromagnetic field

EMF: electromagnetic field

hBM: human bone marrow

MSCs: mesenchymal stem cells

PEMF: pulsed electromagnetic field

ROS: reactive oxygen species

RT-PCR: reverse transcription polymerase chain reaction

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Introduction

The term ‘electromagnetic fields’ (EMFs) indicates a combination of electric and magnetic fields, which are able to give rise to each other under certain conditions. From their time of discovery, EMFs have attracted attention of scientists as a potential therapeutic and diagnostic modality. Particularly, it relates to application of non-ionizing EMFs for induction of various biological effects on cells. It has already been shown that EMF can cause changes in cell proliferation, differentiation, cell cycle, apoptosis, DNA replication and expression, cytokine expression, and more (1–5). A summary of findings on bio-effects induced by EMF is represented in Table 1.

Electric fields as a component of EMF have been employed for manipulation of cells, building artificial

Table 1. Summary of studies of therapeutic potential of electromagnetic fields for tissue engineering and wound healing

Study (author and year)	Processes studied	Biological model	Type of exposure	Frequency	Exposure intensity	Duration	Summary
Fassina *et al.* (2008)	EM stimulation of proliferation and surface modification of osteoblasts	Human osteoblasts SAOS-2	PEMF	75 Hz	2 mT	24 h × 22 days	PEMF caused higher cell proliferation and increased surface coating with type-I collagen, decorin and osteopontin
Ceccarelli *et al.* (2013)	Effects of PEMF on cell viability, cell matrix distribution, and calcified matrix production	Human mesenchymal stem cells (MSCs): bone marrow and adipose-tissue MSCs	PEMF	75 ± 2 Hz	2 ± 0.2 mT	5, 10 and 30 min; 1, 4 and 8 h per day × 21 days	PEMF stimulation of bone extracellular matrix deposition is more efficient in osteoblasts differentiated from bone marrow-MSCs rather than from adipose tissue-MSCs
Kaszuba-Zwoinska *et al.* (2003)	Influence of PEMF on cell viability and apoptosis induction pathways	Monocytic cell line MonoMac6	PEMF	50 Hz	45 ± 5 mT	4 h	PEMF affects induction of apoptosis in MonoMac6 cells stimulated to death with inducing agents
de Girolamo *et al.* (2013)	Bio-effects of PEMF on tendon cells	Human tendon cells	PEMF	75 Hz	1.5 mT	4, 8, or 12 h	PEMF positivly affects on proliferation, tendon-specific marker expression, release of anti-inflammatory cytokines and angiogenic factor
Manjhi *et al.* (2011)	Effects of ELF-EMF on spinal cord injury-induced osteoporosis in rats	Male Wistar rats	ELF-EMF	50 Hz	17.96 μT	2 h × day (8 weeks)	The results demonstrated that ELF-EMF is effective in attenuating spinal cord injury-induced osteoporosis
Tatarov *et al.* (2011)	Effects of EMF on tumour growth and viability in mice injected with breast cancer cells.	Swiss outbred female nude mice with injected metastatic mouse breast tumour cell line EpH4-MEKBcl2	ELF-EMF	1 Hz	100 mT	60, 180, or 360 min × day (4 weeks)	EMF reduced tumour growth and progression. Maximal effects were observed in the group exposed for 360 min daily
Park *et al.* (2013)	Signalling pathways involved in the neural differentiation of bone marrow mesenchymal stem cells induced by EMF	Human bone marrow mesenchymal stem cells	ELF-EMF	50/100 Hz	1 mT	90 min	EMF is capable to induce neural differentiation through activation of EGFR signalling and mild generation of reactive oxygen species

Table 1 (continued)

Study (author and year)	Processes studied	Biological model	Type of exposure	Frequency	Exposure intensity	Duration	Summary
Raus *et al.* (2012)	Behavioural effects of ELF-EMF in gerbils submitted to global cerebral ischaemia	Gerbils (Meriones unguiculatus)	ELF-EMF	50 Hz	0.5 mT	7 days of continuous exposure	ELF-MF decreased motor hyperactivity induced by global cerebral ischaemia, *via* modulation of the processes that underlie this behavioural response
Raylman *et al.* (1996)	Effect of strong magnetic field on viability, growth and DNA fragmentation of malignant cells	Human cell lines: HTB 63 (melanoma), HTB 77 IP3 (ovarian carcinoma), CCL 86 (lymphoma; Raji cells),	EMF	Not provided	7 Tesla	64 h of continuous exposure	Significant decrease of viability in all three cell lines compared to control group. The cell cycle was not altered. Prolonged exposure was capable to inhibit the growth of tumour cell lines.
Jasti *et al.* (2001)	Effects of PEMF on production of cytokines	Female Harlan Sprague Dawley rats	PEMF	Not provided	0.12 mT	4 h	PEMF did not affect the expression of cytokines in spleens of exposed rats
Patino *et al.* (1996)	Bio-effects of PEMF on wound healing	Male Wistar rats	PEMF	50 Hz	20 mT	35 min × 2 times per day × 7, 14 and 21 days	PEMF stimulated wound healing
Callaghan *et al.* (2007)	Bio-effects of PEMF on healing normal and diabetic wounds	Db/db and C57BL6 mice; murine endothelial cells; human umbilical vein endothelial cells	PEMF	15 Hz	0–12 G (0–1200 μT)	8 h × 7, 14 and 24 days	PEMF is capable to accelerate time of wound healing in diabetic and normal mice. It prevents tissue necrosis in response to ischaemic insult
Ahmadian *et al.* (2006)	Effects of ELF/PEMF on the synthesis of epidermal collagen	Male Sprague–Dawley rats	ELF/PEMF	25, 50, 100 Hz	1, 2, 4 mT	2.5 h × 8 days	ELF/PEMF increased the collagen synthesis. It was found out that 25 Hz frequency with intensities of 2 and 4 mT was the most effective
Carlo *et al.* (2012)	Influence of ELF on cell growth and differentiation	Myoblast cells (C2C12)	ELF at calcium-ion cyclotron frequency	13.75 Hz	Static EMF (18 μT); sinusoidal ELF-EMF (2.5 μT)	5 days	ELF-EMF is able to drive C2C12 cell myogenesis both at transcriptional and translational levels through the increase of cell proliferation.
Cho *et al.* (2012)	Induction of differentiation of human bone marrow-derived mesenchymal stem cells into nerve type cells by ELF-EMF	Human bone marrow-derived mesenchymal stem cells (hBM-MSCs)	ELF-EMF	50 Hz	11 mT	5 days of continuous exposure	ELF-EMF exposure significantly increased the neural differentiation of hBM-MSCs, but the differentiation was non-selective.

Table 1 (continued)

Study (author and year)	Processes studied	Biological model	Type of exposure	Frequency	Exposure intensity	Duration	Summary
Luo *et al.* (2012)	Effects of PEMF on the osteogenic differentiation of human mesenchymal Stem Cells	Human bone marrow-derived mesenchymal stem cells	ELF/PEMF	5, 25, 50, 75, 100, and 150 Hz	1.1 mT	30 min per day × 21 days	PEMF induced the expression of alkaline phosphatase and osteocalcin. The maximal effect on cell differentiation was observed for 50 Hz EM frequency
Pirozzoli *et al.* (2003)	Influence of ELF-EMF on apoptosis and proliferation in the human neuroblastoma cell	Human neuroblastoma cell line LAN-5	ELF-EMF	50 Hz	1 mT	7 days of continuous exposure	No effect on apoptosis induction was observed. Antagonistic effect of EMF (in combination with retinoic acid and camptothecin) against the differentiation of cells

bio-scaffolds and drug delivery, over past number of decades (6–8). Recent findings indicate the capability of electric fields to trigger differentiation of stem cells, including neural stem cells (9,10). Magnetic fields, a further component of EMF, has been also the subject of possible therapeutic applications (11–14). A significant body of research conducted has been devoted to studies of bio-effects induced by extremely low-frequency EMFs (ELF EMF) (15–17), which has demonstrated promise in becoming a novel bio-physical tool for stem cell therapy, particularly for treatment of neurodegenerative disorders. Also, ELF-EMF along with pulsed EMF has shown great potential for bone tissue engineering.

This review aims to summarize progress achieved recently with an emphasis on application of EMF for wound healing and tissue engineering.

Bio-effects of extremely low-frequency EMF

ELF-EMF is a form of non-ionizing, low-energy, electromagnetic field capable of inducing physiological effects. Pesce *et al.* identified extremely low-frequency ELF-EMF waves as being sinusoidal in shape (up to 300 Hz) and of low amplitude (0.2–20 mT), and refers to them as EMFs (18).

Several studies have demonstrated that EMF can influence proliferation of cells, which might be effectively employed for cell therapy. De Carlo and co-workers studied results of ELF-EMF on differentiation and proliferation of mouse skeletal muscle cells (C2C12) (19). It was found that exposure of myoblasts (at calcium-ion cyclotron frequency 13.75 Hz) led to reduction in cell growth, while increase in G_0/G_1 phase transition was detected. Results obtained indicate that ELF-EMF is capable of inducing up-regulation of C2C12 differentiation. These authors hypothesized that their findings might have clinical application for treatment of myodegenerative diseases. The results concur with a report of de Girolamo *et al.*, in which they showed that treatment of cultured human tendon cells by pulsed EMF favourably affected cell proliferation along with release of anti-inflammatory cytokines (20).

One form of ELF-EMF is pulsed electromagnetic fields (PEMFs), low-frequency fields with specific wave shapes and amplitude (18). Feasibility of use of PEMFs has been investigated for more than three decades. PEMF frequencies and intensity in ranges less than 100 Hz and 3 mT respectively, have been found to be more effective in accelerating wound repair processes (21–23). PEMFs have been shown to have an effect in reducing healing time, and rate of recurrence of venous leg ulcers, in human clinical studies (24,25). Stiller *et al.* showed that exposure to PEMFs can induce

significant reduction in wound depth and pain intensity, for patients with venous ulcers (25). In addition, patients exposed to PEMFs had significantly higher rates of healing venous leg ulcers and protection from ulcer recurrence, in comparison with the control group, and such patients had their pain reduced or eliminated (24,26).

In further work by Muccioli *et al.* reported successful application of PEMF for reduction of knee pain and necrotic area in a cohort of patients with knee osteonecrosis (27). In a number of *in vivo* studies, it has also been shown that animals treated with PEMFs had significant reduction in wound size compared to the control group (28,29).

In addition, some studies have shown that PEMF treatment stimulated early formation of connective tissue and a vascular network, early collagen synthesis and better maturation, all causing complete re-epithelialization after 12 days exposure (26,30,31). Despite encouraging results mentioned above, in a recent randomized trial study, Gupta *et al.* demonstrated that EMFs did not have any significant effect on tissue repair process (32).

EMF for wound healing

Wound healing is a complex process involving cascades of inflammatory, proliferative and immune reactions. It involves a series of coordinated events, including bleeding, coagulation, the acute inflammatory response, regeneration, migration and proliferation of connective tissue and parenchymal cells, synthesis of extracellular matrix (ECM) proteins and wound remodelling (33).

It has been shown that EMF may have an effect on different components of the healing machinery; particularly, it concerns application of low frequencies and non-thermal effects. It has been hypothesized that EMF may influence nitric oxide signalling, modulation of cytokine profiles, expression of growth factors, cell migration and proliferation, and regulation of mitogen-activated protein kinase/extracellular signal-regulated kinase (18,34–37). Application of ELF-EMF for wound healing may provide anti-inflammatory effects along with enhancement of the re-epithelialization process (38).

Effects of EMF on wound healing through promoting tissue regeneration (39,40) and triggering embryonic stem cell differentiation (41) has led Matos and Cicerone to explore the effect of electric fields on neural stem cell differentiation in mice (9). Initially, neural stem cells were encapsulated into beads of hydrogel followed by application of an electrical field. Beads were placed into electrolytic cells of different frequency (0.1, 0.5, 1 and 10 Hz) filled with differentiation media. For the next stage, beads were stained with calcein-AM and ethidium homodimer for assessment of cell viability and cell differentiation, carried out using fluorescence confocal microscopy and comparing tests to control. Results indicated that, cell viability steadily reduced in fields of frequencies 0.1, 0.5 and 10 Hz, whereas at 1 Hz, number of viable cells increased sharply, indicating that the increase occured due to improved cell viability and accelerated cell proliferation. The authors provided a possible explanation for this change being the result of increase in electrokinetically enhanced mass transport. Regarding effects of electrical fields on cell differentiation, it is important to point out that in neural tissues apart from neurons, there are glial cells which provide neuronal support in terms of nutrition and operation. Normally, neural stem cells differentiate into two types of phenotype (neurons and glial cells); thus, when mimicking this procedure *in vitro*, the main important aspect is to consider the ratio of neurons to glial cells (42). Usually in normal tissue, this parameter is $\geq$1:1 (43).

Results of Matos and Cicerone's experiment demonstrated no clear association of differentiated cell line proliferation rates with magnitude and frequency of applied electric field (9). It was shown that proliferation rate of two cell types either increased or decreased depending on time of incubation and EMF frequency. Nevertheless, these findings provide a basis for further optimization of EMF parameters to control level of proliferation of neural stem cells.

EMF, stem cells and neurodegenerative diseases

Multipotent potential of mesenchymal stem cells (MSCs) has made them attractive for clinical applications, including control of proliferation of MSCs by means of EMF. Wide availability of MSCs of various types of tissues, and their capability for multilineage differentiation, have made them even more appropriate for medical therapies (44). An important aspect worth mentioning, is that MSCs isolated from bone marrow are capable of differentiating into tissues with ectodermal, endodermal and mesenchymal origin (45). Moreover, various manipulations of culture conditions (such as co-culturing MSCs with Schwann cells) have allowed MSCs to pass lineage restrictions and convert to complete neural cell types, including astrocytes, oligodendrocytes, glial cells and neurons.

Recently, there have been attempts to treat neurodegenerative disease *via* differentiation of MSCs by exposing them to ELF-EMF. Indeed, long before using ELF-EMF it had been shown that exposure of MSCs to specific external factors such as growth factors and cytokines (46) or to chemicals such as mercaptoethanol, butylated

hydroxyanisole and dimethyl sulphoxide (DMSO) (47,48) lead to their differentiation into various cell types. However, aforementioned methods cause several problems such as dysfunctional neuron formation, reduction in cell viability and high cost of laboratory work. Thus, attempts to discover more efficient ways of MSC differentiation into neural cells have been of interest. In 2012, Cho and colleagues demonstrated that ELF-EMF triggered conversion of human MSCs into neurons (49). In their experiments, MSCs derived from human bone marrow (hBM-MSCs) were exposed to continuous sinusoidal ELF-EMF. After their treatment with ELF-EMF, differentiation of hBM-MSCs to neuronal stem cells was confirmed by amplification in RT-PCR of RNA, western blotting and immunohistological analysis of 2-fold increased expression of tau and NF-L proteins. Once MSCs had been converted into neuronal stem cells, their differentiation potential into further neural cell types was evaluated by immunohistochemistry using anti-GFAP, anti-O4 and anti-MAP2, markers of astrocytes, oligodendrocytes and neurons respectively. After 12 days exposure to ELF-EMF, the vast majority of cells expressed abovementioned markers, indicating that EMF had the ability to induce differentiation of mesenchymal stem cells into neurons. To determine biological mechanisms behind this initiation of neural differentiation, CREB signalling pathway was investigated. This has been found to play an essential role in the neurotrophic response (49). Activation of the pathway was achieved by phosphorylation of CREB; but in ELF-EMF-treated cells, the CREB pathway was active for 12 h only after treatment, suggesting that it is responsible for expression of protein markers for neural cells. However, a potential mechanism for the whole process was not indicated; thus, further research on this topic is required.

One possible mechanism of ELF-EMF action on hBM-MSCs was proposed by the team lead by Park and his colleagues (50). Researchers aimed to determine the signalling pathway activated by increase of intracellular concentrations of reactive oxygen species (ROS) as a result of EMF exposure.

A number of studies have demonstrated that there is a correlation between increase of ROS and increase in number of proliferating and differentiating cells (51,52). Thus, a similar mechanism might be activated by exposing cells to ELF-EMF, which results in increase in proliferation and differentiation rates. Elevation of intracellular ROS has been indicated with fluorescent product dichlorofluoroscein (DCF) in ELF-EMF-exposed cells (50). Increase in CREB and Akt phosphorylation, downstream molecules of EGFR, was once again demonstrated by western blotting. Moreover, immunohistochemical analysis of EGFR clustering showed that ROS induced EGFR activation. Observed changes indicated that ROS played an essential role in proliferation and differentiation processes. However, little research has been carried out to identify exact effects of ROS on specific pathways.

EMF for bone tissue engineering

One further area in bio-medical application, where mesenchymal stem cells are activated by EMF, is bone tissue engineering. Unlike in neural tissue where continuous EMF as been applied, here a pulsed EMF (PEMF) is widely employed in therapy (53). Initially, PEMF was successfully used in 1977 by the team lead by Basset (54). These researchers demonstrated that low-frequency PEMF application to a group of the patients with congenital and acquired pseudarthrosis, triggered electrically induced changes at the cellular level. This resulted in a high percentage of overall success level. Similar study has been performed by Simmons *et al.* (55), who demonstrated the effectiveness of the method.

It must be noted that bio-effects mediated by PEMF may vary depending on the type of the cell line under investigation. Ceccarelli *et al.* employed bone marrow and adipose-tissue mesenchymal stem cells to compare reactions of cells to stimulation by PEMF (21). Cell viability, matrix distribution and calcified matrix production were analysed after exposure to PEMF (frequency 75 ± 2 Hz; intensity 2 ± 0.2 mT). Acquired results demonstrated an increase in cell proliferation and deposition of extracellular matrix components (along with calcium deposition) in bone marrow-derived mesenchymal stem cells. This indicates that stimulation of bone extracellular matrix deposition by means of PEMF was more effective in osteoblasts differentiated from bone marrow mesenchymal stem cells than from adipose tissue mesenchymal stem cells.

Application of PEMF holds promise of utility for modification of surfaces of biomaterials exploited in orthopaedic practice. First, it relates to optimization properties of materials used for building 3D scaffolds. Fassina and co-workers chose titanium fibre-mesh scaffold as a model for bio-integration testing, due to high biocompatibility of titanium and its extensive use in orthopaedic surgery (23). These workers exposed human osteoblasts (SAOS-2 line) to PEMF to stimulate cell proliferation and enhance surface coating. Data obtained revealed increase in cell proliferation rate and surface coating with type-I collagen, decorin and osteopontin, in the post-treatment period. These findings indicate the feasibility of modification of a scaffold's surfaces, as an alternative approach to biointegration.

Another issue for bone regeneration is a regulation of bone remodelling. Balance between bone resorption and ossification is maintained by natural mechanisms. Application of EMF provides an opportunity to modulate the processes in a safe and non-invasive manner. Kim *et al.* explored effects of ELF-EMF (17.96 μT, 50 Hz) on bone remodelling and prevention of spinal cord injury-induced osteoporosis (56). In their study, ELF-EMF was shown to suppress bone resorption and promote formation of bone tissue.

The formation of new bone tissue is a complex process, where both alkaline phosphatase and osteocalcin play important roles. Alkaline phosphatase is a hydrolyzing enzyme for phosphate and lead to formation of phosphonic acid, an essential metabolite in promotion of bone formation. Thus, its expression marks initiation of bone formation. Moreover, osteocalcin is secreted by osteoblasts during their differentiation; thus, it is a strong marker of osteoblast differentiation. In 2012, Luo and co-workers published experimental results in which they demonstrated effects of PEMF on osteogenic differentiation of human mesenchymal stem cells (53). The team used level of alkaline phosphatase activity and osteocalcin expression as indicators of cell differentiation (53). In their experiments, they isolated mesenchymal stem cells from bone marrow samples collected from healthy volunteers. Range of applied PEMF frequencies varied from 5 to 150 Hz, for 30 min every day for 21 days. Results assessed using inverted and transmission electron microscopies showed that cells after PEMF application increased in size and were more highly differentiated than the control group (53). All experimental groups had time-dependent increasing alkaline phosphatase expression, whereas activity of enzyme in control groups remained low. It was revealed that level of osteocalcin increased steadily and reached its maximum by the third week, when simultaneous formation of nodules occurred. These pieces of evidence demonstrated that PEMF triggered bone differentiation, but differentiation level was dependent on frequency applied.

Overall, conclusions made are as follows: PEMF effect on stem cell differentiation was dependent on its frequency and experimentation provided a fresh perspective for clinical application of this procedure to bone fracture healing. The underlying biological procedure for effect of EMF on stem cell differentiation remains unclear, and it still requires further research.

Future perspectives

Employment of EMF in regenerative medicine opens a new avenue for treatment of various diseases. Due to its non-ionizing and non-invasive nature, the use of EMF has evident advantages compared to current chemical, biological and physical methods of tissue regeneration and wound healing. Electric and magnetic components of EMF could be employed separately or in combination for different therapeutic purposes. Both components have demonstrated a capability for stimulating cell proliferation and differentiation.

The magnetic aspect of EMF deserves special attention. Therapeutic application of this component has been mainly associated with use of magnetic nanoparticles, which have potential to modulate regenerative processes selectively at the target zone.

New generation of magnetic nanoparticles provides a platform for development of therapeutic and diagnostic modalities, based on thermal and non-thermal properties of EMF. For example, nanoparticles can be exploited for stem cell tracking under MRI guidance (non-thermal application). Thermal application traditionally is associated with elevation of local temperature using the response of metal-based nanoparticles to externally applied magnetic field (high-frequency EMF). Controlled moderate hyperthermia can be employed for local stimulation of regenerative processes, including repair of damaged tissues and modulation of immune reactions.

In addition, EMF-induced hyperthermia can provide a basis for developing systems for targeted drug and gene delivery. In this case, magnetic fields could be applied in a non-contact mode to the affected site of the body for further activation of thermo-responsive magnetic nanoparticles loaded with chemical or genetic substances. Therapeutic effects would be monitored in real-time by means of MRI, where nanoparticles act as imaging agents. This strategy opens a new field for developing novel ranges of theranostic agents.

EMF has great potential to be harnessed for wound treatment in combination with metal nanoparticles of noble metals. Gold and silver have been credited with antimicrobial properties for many centuries. Modern technology allows fabrication of stable gold and silver nanoparticles with desired size and shape (57–59). Particle size can provide the possibility of enlarging contacting area, which leads to an increase in anti-bacterial activity and speeding up processes of wound healing (60–62). Noble nanoparticles might be incorporated into wound dressings made of various types of materials such as polymer films, hydrogels, composites and alginates. EMF can be applied externally and non-invasively to wound dressings containing the noble nanoparticles to enhance their antimicrobial action. This approach might serve as a foundation for developing a completely new type of wound dressing.

Despite definite advantages and huge therapeutic capacity of EMF, clinical applications must be carried

out cautiously with respect to possible adverse effects of EMF on DNA. Moreover, development of novel EMF-based techniques requires more study on mutagenic aspects of EMF. It is essential to obtain better understanding of underlying mechanisms of EMF-induced bio-effects. In this regard, more research must be conducted in this direction.

To summarize, taking into account the great clinical potential of EMF, we can expect a rise in new techniques for tissue regeneration and wound healing in close perspective. Such strategy allows combining EMF with various chemical, physical and biological modalities to provide desired synergistic bio-effects and enhanced treatment efficacy.

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