Int J Clin Exp Med 2016;9(7):12521-12531
www.ijcem.com /ISSN:1940-5901/IJCEM0021356

Original Article

Exposure to static magnetic field induces c-Jun expression to promote osteoblast differentiation

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Received December 7, 2015; Accepted June 8, 2016; Epub July 15, 2016; Published July 30, 2016

Abstract: Increasing evidence has shown the effects of pulsed electromagnetic fields (PEMFs) on the biological behaviors of osteoblasts and bone metabolism. This study aimed to investigate the effects of static magnetic fields (SMFs) on osteoblast differentiation and the underlying mechanism. Rat osteoblasts cultured *in vitro* were exposed continuously to a 12.5-, 125-, or 250-mT SMF for different periods. Osteoblast proliferation was evaluated by MTT assay. Alkaline phosphatase (ALP) and osteocalcin (OCN) activities were monitored. c-Jun mRNA and protein expression were measured by quantitative real-time PCR and immunohistochemistry. No significant difference in cell number was found between the exposed and control cells during the culture period. During a 20-day culture, ALP and OCN expression were significantly increased. Furthermore, c-Jun expression was significantly increased in cells exposed to a SMF in a dose-dependent manner. SMF exposure promoted osteoblast differentiation, and this may be related to the induction of c-Jun expression. These results provide insight into a possible mechanism by which SMF exposure affects osteoblast differentiation, which will aid in the selection of a suitable clinical therapeutic SMF flux density for osteogenesis.

Keywords: Magnetic attachments, static magnetic fields, osteoblasts, c-Jun, alkaline phosphatase, osteocalcin

Introduction

Dental magnetic attachments provide a static magnetic force generated by a Neodymium ($Nd_2Fe_{14}B$) permanent magnet and offer many outstanding advantages [1, 2]. For example, they can provide a long-term reliable retention force to significantly improve the retention and stability of removable dentures as well as reduce lateral force to protect abutment teeth and improve masticatory efficiency. Therefore, dental magnetic attachments have been applied in a variety of therapies, especially for the retention of partial and full dentures, implant prostheses or other maxillofacial appliances [3-5].

Increasing evidence has demonstrated the effects of pulsed electromagnetic fields (PEMFs) on the biological behaviors of osteoblasts and bone metabolism [6, 7]. Moreover, PEMFs have been extensively used in the clinical treatment of delayed unions and non-unions [8-10]. Recently, *in vivo* and *in vitro* studies have shown that, like PEMFs, static magnetic fields (SMFs) affect a number of biological processes related to bone growth such as osteoblastic proliferation, differentiation, bone mineral density, and healing of bone fractures [11-13].

The proliferation and differentiation of osteoblasts, which are responsible for the growth, formation, and remodeling of bone, are regulated by a wide variety of systemic and local regulatory factors such as cytokines and hormones [14]. Moreover, osteoblastic proliferation and differentiation represent complicated multistep processes modulated by an integrated gene expression cascade [15, 16]. Proto-oncogene c-Jun is one of the immediate early genes (IEGs), and it forms a complex with c-Fos

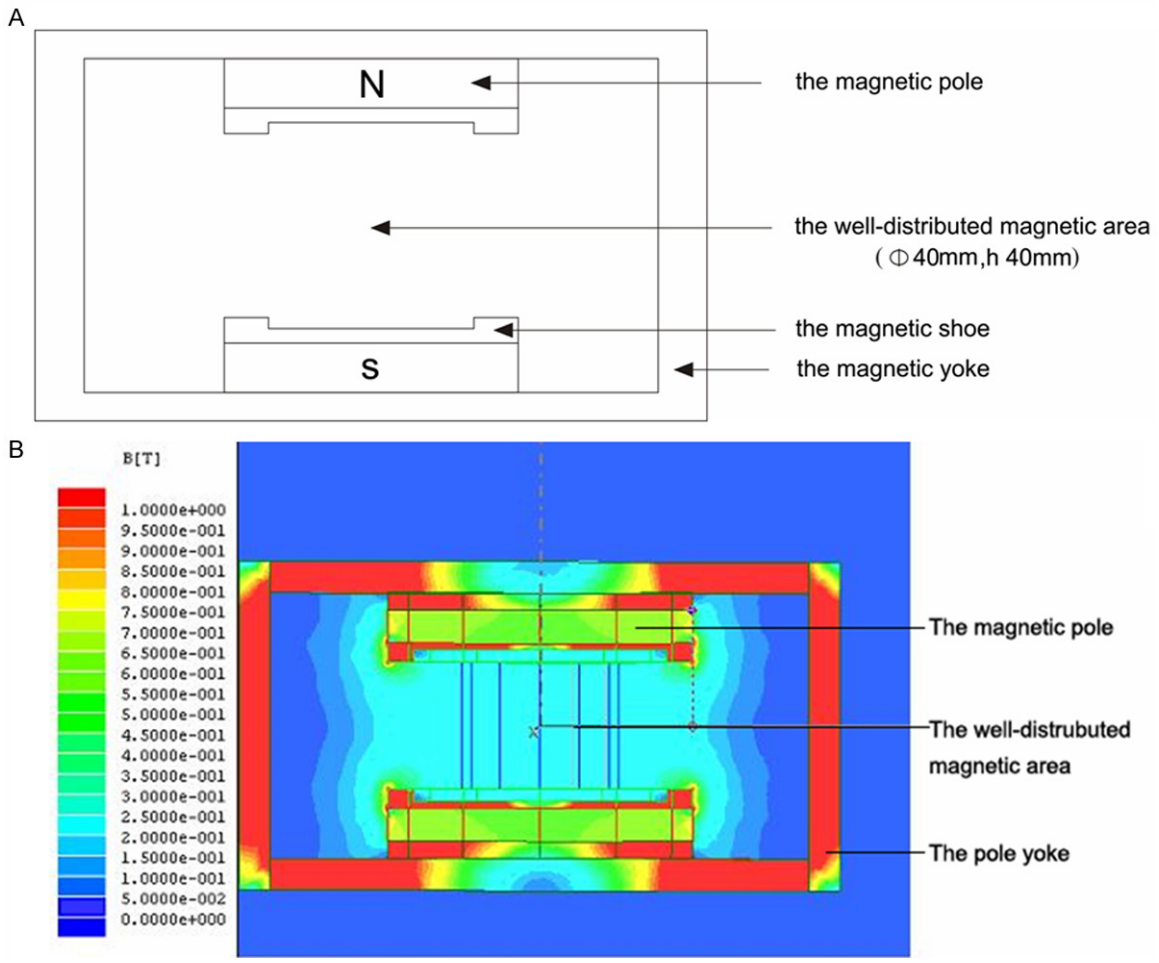

Figure 1. A. The structural diagram of the exposure system for the static magnetic field (SMF). The different parts of the exposure system are indicated with arrows. 3.5-cm cultured dishes were placed directly on the south polar surface of the permanent magnets and in the well-distributed 40-mm diameter magnetic area of the exposure system. B. The magnetic flux density distribution in the cross-sectional figure of the static magnetic field exposure system (e.g., 250 mT). Based on three-dimensional finite element method, the well-distributed magnetic area was 40 mm in diameter and 40 mm in height. The direction of magnetic field was directed perpendicularly to the bottom of the field.

as activator protein-1 (AP-1) to regulate cell proliferation and differentiation [17]. After activation, AP-1 is recruited to the binding sites of its target genes, such as alkaline phosphatase (ALP), osteocalcin (OCN), transforming growth factor-β_1 (TGF-β_1), and insulin-like growth factor-I/II (IGF-I/II), where it activates their expression and ultimately modulates the cellular response. Thus, c-Jun is considered to be a critical regulator of osteoblastic differentiation [18].

In our studies, three magnetic flux densities of magnetic field systems were designed according to the flux densities detected on the surface of Magnedisc 800 magnetic attachments in the clinical application. We have also previously demonstrated that SMF-simulating magnetic attachments have no effects on osteoblast shape, cell cycle distribution, and apoptosis [19]. However, SMF stimulation enhances cell differentiation in the early maturation stage [20], but the underlying mechanism remains unknown. In the present study, our results further confirm that SMF exposure can promote osteoblastic differentiation and reveal that this effect is associated with the induction of c-Jun expression. These results provide insight into the effects of SMFs generated by magnetic attachments on bone formation and will aid the selection of a suitable clinical therapeutic SMF flux density for osteogenesis.

Material and methods

SMF exposure system

For SMF exposure, three independent magnetic field systems were designed with average flux densities of 12.5, 125, and 250 mT, respectively (Southwest Institute of Applied Magnetics, Mianyang, China) (**Figure 1A**, **1B**). Each of them was constructed from magnetic poles and magnetic yokes. The magnetic poles were made of Neodymium ($Nd_2Fe_{14}B$) permanent magnets covered by pole shoes. Permanent magnets were designed to provide three kinds of magnetic flux densities, 12.5, 125, and 250 mT. The pole shoes were used to improve the homogeneity of the magnetic field. Through optimizing the shapes of the poles and pole shoes, a relatively uniform SMF was produced in a large imaging volume between the poles. The well-distributed magnetic area was 40 mm in diameter and 40 mm in height. The magnetic yoke made of pure iron was structured to reduce the loss of the field flux. It also housed and supported magnetic poles. The north magnetic pole was attached on the lower surface of the roof of the magnetic yoke, while the south magnetic pole was connected to the upper surface of the bottom of the magnetic yoke. The direction of magnetic field was directed perpendicularly to the bottom of the field. Based on a previous study [21], the magnetic flux density on the surface of Magnedisc 800 magnetic attachments is 250 mT under the open-field circuit and 12.5 mT under the closed-field circuit. In addition, 125 mT represented the middle flux density when the magnet and sliding keeper were maintained laterally at a certain distance or a sufficient amount of air existed between them. Therefore, there were three independent similar structural magnetic field systems designed according to different magnetic flux densities, 12.5, 125, and 250 mT, respectively.

Cell culture

Osteoblasts were isolated from calvarias of newborn Sprague-Dawley rats by enzymatic digestion. Newborn Sprague-Dawley rats were obtained from the Animal Experimental Center of Sichuan University. All procedures and handling of animals conformed to the guidelines of international conventions on animal experimentation. Animal protocols were approved by the ethics committee of Sichuan University. Cells were plated and cultured in DMEM supplemented with 10% fetal bovine serum (FBS) (Harry Biological Engineering Co. LTD, Chengdu, China) and 1% penicillin-streptomycin (Gibco, Life Technologies, Carlsbad, CA, USA) at 37°C in a humidified atmosphere of 5% CO_2. Cultured osteoblasts were divided into four groups: the control group and three SMF-exposed groups. Three SMF-exposed groups were exposed to flux densities of 12.5, 125 or 250 mT. Briefly, osteoblasts were seeded at a density of 1×10^5 cells/ml in 3.5-cm dishes and cultured in DMEM (Gibco, Carlsbad, CA, USA) supplemented with 10% FBS and 1% penicillin-streptomycin in an unexposed environment in the incubator at 37°C with 5% CO_2 for 24 h. For all SMF-exposed groups, 3.5-cm cultured dishes were placed directly on the south polar surface of the permanent magnets and in the well-distributed 40-mm diameter magnetic area of exposure system. Cultured cells were exposed continuously to a SMF for different periods. The control cells were cultured continually in an identical incubator without SMF exposure at the same time.

Cell proliferation assay

The numbers of viable cells with or without SMF exposure on days 1, 3, 5, 7, and 9 were determined using the methyl-thiazol-diphenyl-tetrazolium (MTT) colorimetric assay. Briefly, for proliferation assay, osteoblasts were seeded at a low density of 1×10^5 cells/ml in 96-well plates. After exposure to the 12.5-, 125-, or 250-mT SMF, the cells were incubated with the tetrazolium salt (MTT) for 4 h according to the supplier's instructions (MTT kit, Sigma-Aldrich, St. Louis, MO, USA). Then the formazan dye was solubilized with 500 µl dimethyl sulfoxide for 10 min, and the absorbance of each well was measured by using a microplate reader (HTS 7000 plus, PE Co., Ltd., Philadelphia, PA, USA) at 490 nm.

ALP activity assay

ALP activity was detected using Sigma-Aldrich kit 85 (AMP buffer, Merit Choice Bioengineering, Hong Kong, China) according to the manufacturer's instructions. The ALP activity of cultured osteoblasts was determined after 1-14 days of exposure to the 12.5-, 125-, or 250-mT SMF. Briefly, osteoblasts were seeded at a density of 1×10^5 cells/ml in 3.5-cm dishes. At each time point, the media were aspirated, cells were washed twice with PBS, and 300 µl 0.05% (v/v) Triton X-100 (Sigma-Aldrich) was added

per dish. After three freeze/thaw cycles, 50-μl aliquots of cell lysate from each dish were transferred to a 96-well plate. Absorbance was measured at 405 nm with a spectrophotometer (HTS 7000 plus, PE Co. Ltd.). ALP enzyme activity was evaluated for 60 min and expressed as V_{max}. Total protein in cell lysate was detected at 595 nm by the Coomassie brilliant blue method. ALP activity values were normalized against protein concentration of total cell lysate.

OCN expression assay

Cultured osteoblasts were exposed continuously to a 12.5-, 125-, or 250-mT SMF for 20 days, and OCN expression was measured with the Iodine [^{125}I] Osteocalcin radioimmunoassay kit (Sigma-Aldrich) according to the manufacturer's instructions. Briefly, the supernatants of each group were collected, lyophilized, and stored at -80°C. Samples were then dissolved in PBS and incubated with 100 μl Iodine [^{125}I] Osteocalcin antibodies (1:250) overnight at 4°C. Subsequently, samples were centrifuged at 1,000× g for 25 min. The radiation dose was detected using a SN-682 γ automatic counter (Shanghai Institute of Nuclear Research on the Environment and Instrument, Shanghai, China).

Quantitative reverse transcription-polymerase chain reaction (qRT-PCR)

Osteoblasts were exposed continuously to a 12.5-, 125-, or 250-mT SMF for 2, 6, 12, 24, or 48 h in the test groups for detecting the mRNA expression of cellular c-Jun, while the control groups not exposed to a SMF were used for comparison. Total RNA was isolated from osteoblasts using the RNA extraction Kit (Promega, Madison, WI, USA). cDNA was synthesized from 1 μg of purified total RNA using oligo dT primers and Improm-II Reverse Transcription System (Bio-Rad, Hercules, CA, USA). For qRT-PCR, a reference pool was generated by combining equivalent amounts of RNA from all the samples. The primer sequences were: 5'-GACTGCAAAGATGGAAACGA-3' and 5'-GGTCATGCTCTGCTTCAGAA-3' for c-Jun; and 5'-TGGGTGTGAACCACGAGAA-3' and 5'-GGCATGGACTGTGGTCATGA-3' for GAPDH. They were designed and analysed by using Primer Premier 5.0 software. The c-Jun level was normalized to that of GAPDH.

Immunocytochemical staining

After exposure to the SMF of 12.5-, 125-, or 250-mT for 2, 6, 12, 24, or 48 h, osteoblasts were washed three times with PBS, fixed with 4% paraformaldehyde in PBS for 1 h and stored at -80°C. c-Jun expression in osteoblasts was detected using the DAB Horseradish Peroxidase Color Development Kit (Sigma-Aldrich) according to the manufacturer's instructions. Briefly, cells were incubated with primary rabbit-anti-mouse c-Jun monoclonal antibody (Sigma-Aldrich) overnight at 4°C, and then incubated with horseradish peroxidase-conjugated secondary antibody (Sigma-Aldrich). Finally, the images were taken using a photomicroscope (Olympus BH 50, Tokyo, Japan). The specimens in each of the four groups were blindly evaluated by two histologists. The number of positive stained cells was calculated by counting the total cells in a 100-μm^2 window through amplification slides at 40× magnification and expressed as the percentage of positively stained cells.

Statistical analysis

Data are expressed as means ± standard deviation (SD), and data were analyzed using the SAS program (SAS version 8.1, Cary, NC, USA). Comparisons between control and SMF-exposed groups were performed by repeated measures analysis of variance tests, and differences with p values less than 0.05 were considered significant.

Results

SMF exposure has no effect on osteoblast proliferation

The number of viable cells in each group with or without SMF exposure showed a continuous increase throughout the experimental period (**Figure 2**). However, no significant difference in cell number was found between exposed and control cells during the culture period of 9 days (P>0.05). These data indicate that SMF exposure has no effect on the proliferation of osteoblasts.

SMF exposure increases ALP activity of osteoblasts

Immunohistochemical assay of ALP activity showed that compared with the control group,

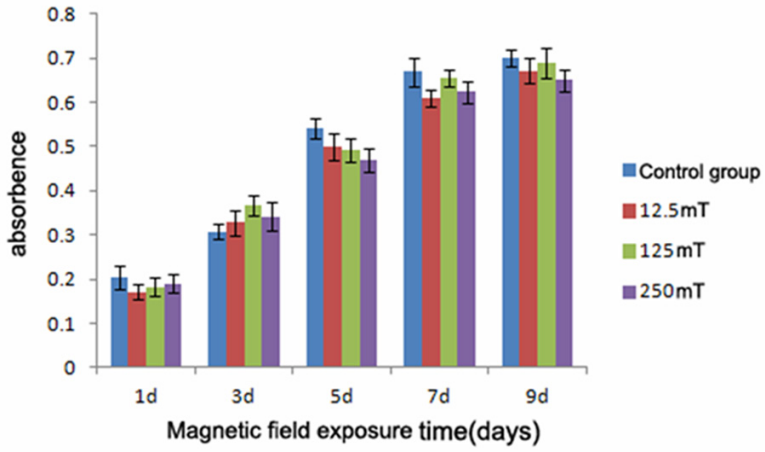

Figure 2. The effects of SMF exposure on osteoblast proliferation. The numbers of viable cells increased continually over the culture period. The cell growth trends of SMF-exposed cells and unexposed cells were similar. No significant differences in viable cell number were found between SMF-exposed and control cells during the culture period ($P>0.05$).

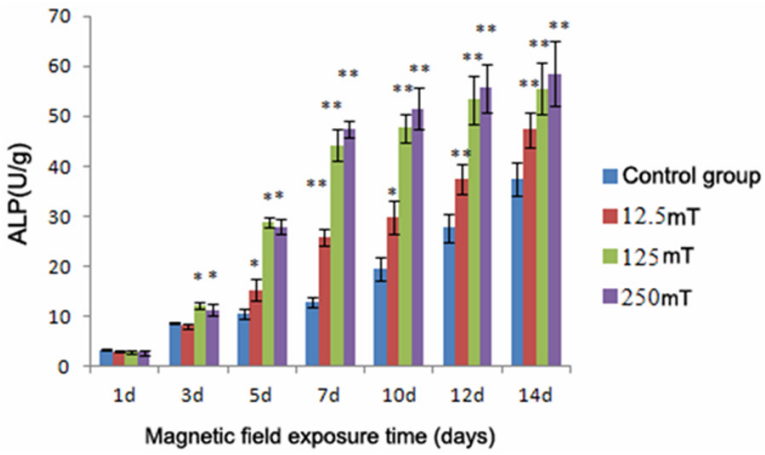

Figure 3. The effects of the various flux densities of the SMF on ALP activity of osteoblasts. After 5 and 7 days of exposure, ALP activity was significantly different in the three groups with different SMF flux densities compared to the control and was gradually up-regulated during subsequent culture periods (10-14 days). This increase was dependent on the flux density of the SMF (*$P<0.05$, **$P<0.01$).

ALP activity in SMF-exposed cells was not significantly different after 1 day of exposure ($P>0.05$), but was slightly increased after 3 days of exposure to the 125-mT and 250-mT SMFs ($P<0.05$). After 5 and 7 days of exposure, ALP activity was significantly different in the three groups with different SMF flux densities compared to the control and gradually up-regulated during the subsequent culture periods (10-14 days), and this increase was dependent on the flux density of the SMF ($P<0.01$, **Figure 3**). These results demonstrate that SMF exposure increases ALP activity of osteoblasts in a time- and dose-dependent manner.

SMF exposure increases OCN expression in osteoblasts

We further investigated late stage differentiation by measuring the osteocalcin expression up to 20 days. Radioimmunoassay showed that compared with the control group, the OCN protein level was not significantly different after 1 or 3 days of exposure to a SMF ($P>0.05$). In contrast to the small differences at 5 and 7 days, the differences at late stage were more profound. The OCN protein level was markedly increased from 12-20 days of exposure to a SMF ($P<0.01$). However, no significant changes were detected in OCN protein expression between the three SMF exposure groups (**Figure 4**). These results demonstrate that SMF exposure increases OCN expression in osteoblasts in a time-independent manner.

SMF exposure increases c-Jun mRNA expression in osteoblasts

Real-time PCR analysis showed that c-Jun mRNA expression in 12.5-mT SMF-exposed cells was significantly increased at 6 and 12 h, compared to that in control cells ($P<0.05$). c-Jun mRNA expression in the 125-mT group was increased to 300% at 2 h and then decreased but remained higher at 6 h than that in the control and 12.5-mT groups ($P<0.05$). However, with longer exposure time, there was no statistical difference in c-Jun mRNA expression between the control and 125-mT groups ($P>0.05$). In addition, when cells were treated with the 250-mT SMF, the c-Jun mRNA level was significantly increased to 310% at 2 h and maintained at higher level at 12 h compared to the control,

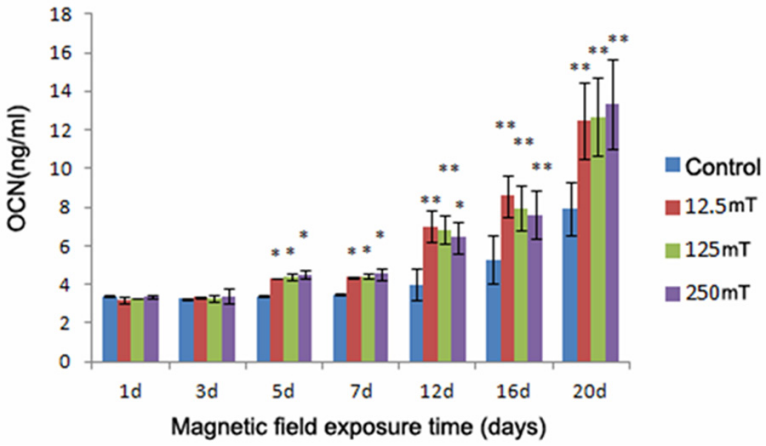

Figure 4. The effects of the various flux densities of the SMF on OCN expression in osteoblasts. In contrast to the small differences at 5 and 7 days, the OCN protein level was markedly increased from 12-20 days of exposure to the SMF ($P<0.01$). However, no significant changes were detected in OCN protein expression between the three SMF exposure groups (*$P<0.05$, **$P<0.01$).

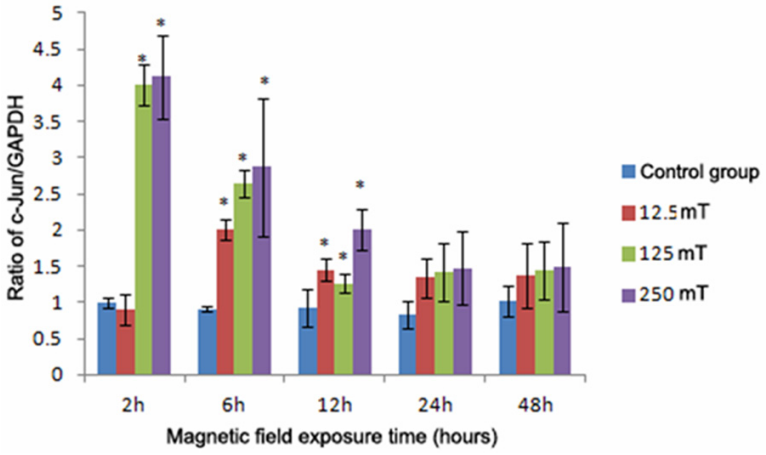

Figure 5. c-Jun mRNA expression in osteoblasts after SMF exposure. c-Jun mRNA levels in the 125-mT and 250-mT groups were significantly increased at 2, 6, and 12 h, compared to those in control cells (*$P<0.05$ and **$P<0.01$).

12.5-mT, and 125-mT groups ($P<0.05$). At 24 and 48 h, no difference in c-Jun mRNA expression was found between the control and SMF-exposed cells (**Figure 5**).

SMF exposure increases c-Jun protein expression in osteoblasts

Immunohistochemical staining showed weak staining of c-Jun in the nuclei of unexposed control cells, but strong staining in the nuclei of SMF-exposed cells (**Figure 6A-D**). The percentages of c-Jun-positive cells were significantly increased after 6 and 12 h of exposure to the 12.5-mT SMF, compared to those among control cells ($P<0.05$). In the 125-mT SMF-exposed group, the percentages of c-Jun-positive cells were significantly higher at 2 and 6 h, and then declined gradually. In the 250-mT SMF-exposed group, the percentages of positively stained cells for c-Jun at 2, 6, and 12 h were significantly higher than those in the control group ($P<0.05$), and then the percentages of these cells stained positively for c-Jun appeared to decrease at 24 and 48 h (**Figure 7**).

Discussion

Osteoblast differentiation is a multistep series of events modulated by an integrated gene expression cascade, which initially supports cell proliferation and then promotes matrix maturation and mineralization of the bone extracellular matrix [22]. ALP activity is a well-known early marker of osteoblast differentiation. In the present study, we found that the ALP activity of SMF-exposed cells was slightly increase from days 3- to 5, and markedly increased from days 7 to 14, whereas the significant increase of unexposed cells was observed after day 10. Thus, the early significant increase in ALP expression of SMF-exposed cells may represent the early onset of osteoblast differentiation. These data suggest that SMF exposure promotes osteoblastic differentiation, consistent with the findings of previous studies [23, 24].

OCN is one of only a few proteins that are expressed only in osteoblasts. OCN is expressed by highly differentiated osteoblasts during the mineralization stage in rat calvaria cells and is a marker of late-stage osteoblast differentiation [17, 25]. We had noted a large differ-

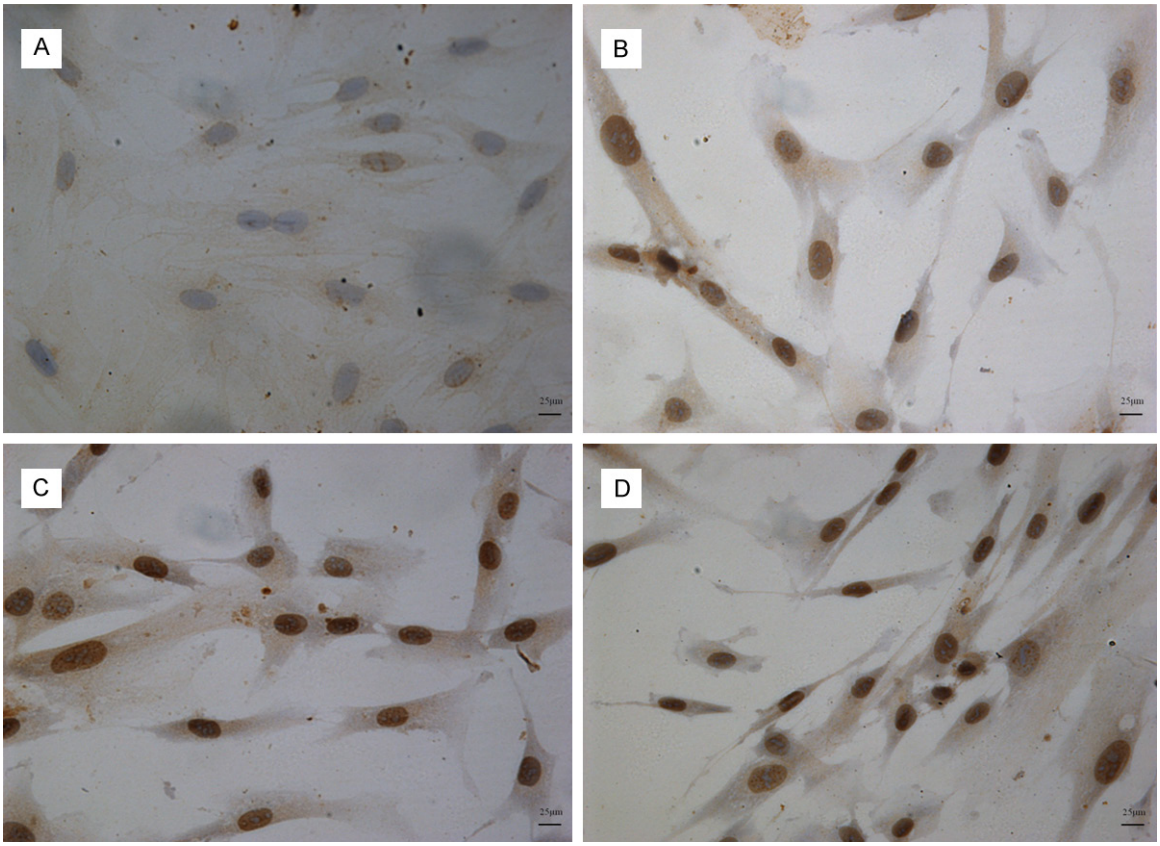

Figure 6. Immunohistochemical staining of c-Jun in osteoblasts after SMF exposure. (A) Weak staining of c-Jun was observed in the nuclei of control cells. Strong staining of c-Jun was observed in cells exposed to: (B) 125 mT at 2 h; (C) 250 mT at 2 h; and (D) 12.5 mT at 6 h. Scale bar = 25 μm.

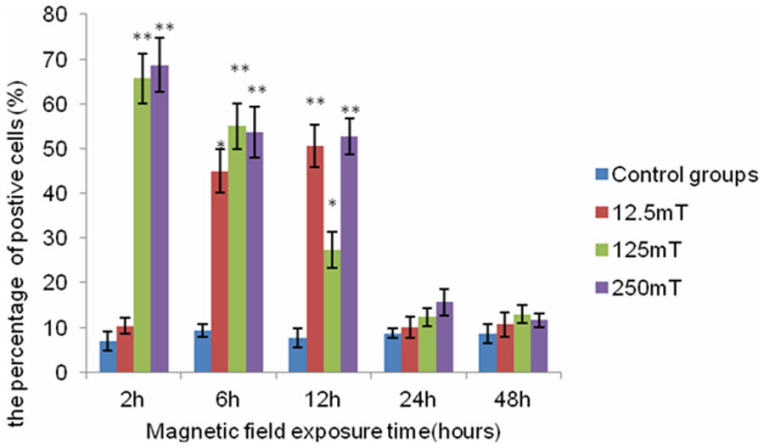

Figure 7. The percentage of positively stained cells among osteoblasts after SMF exposure. The percentage of cells stained positively for c-Jun was significantly increased among cells exposed to a 12.5-mT SMF for 6 and 12 h, and those exposed to 125-mT and 250-mT SMFs for 2, 6, and 12 h, compared to control cells (*P<0.05 and **P<0.01).

ence in OCN expression with SMF exposure. Though the increase in OCN expression was only slight at days 5 and 7, the sharp increase in OCN expression after day 12 may be indicative of its involvement in the onset of increased mineralization, whereas the much more gradual increase observed in the absence of the SMF after day 16 may be responsible for the slower rate of mineralization. These results are in agreement with the report by Yamamoto [14], who found that SMF exposure promoted ALP activity and OCN content in the culture medium.

To further investigate transcriptional factors that regulate the expression of ALP and OCN, we detected the expression of c-Jun by real-time PCR and immunohistochemical stain-

ing. Under normal circumstances, the expression of c-Jun is too low to be detected in most cells. The transcription level of c-Jun has been characterized by rapid activation in response to extracellular stimuli. c-Jun heterodimerizes with c-Fos to form the transcriptionally active complex known as activator protein 1 (AP-1), which binds to AP-1 binding sites of the target genes to activate their expression and ultimately affect cellular function [17, 18].

Recently, both *in vivo* and *in vitro* studies have indicated that c-Jun plays an important role in the induction of osteogenesis. c-Jun is considered to be a critical regulator of the expression of TGF-β, IGF-I, and IGF-II, which all contain AP-1 binding sites. In addition, activated AP-1 protein can bind the DNA sequence 5'-TGA(C/G)TCA-3' in the promoters of osteoblast-specific growth factors such as ALP, OCN, type I collagen, and type II collagen, to activate their expression and promote cell differentiation. Therefore, c-Jun plays an important role in the induction of osteogenesis. However, whether c-Jun is involved in the effects of SMF exposure on osteoblastic differentiation remains unclear.

In the present study, we observed relatively low levels of c-Jun mRNA and low percentages of positively stained cells in control osteoblasts throughout our experiments. However, after exposure to SMFs of different flux densities, the expression of c-Jun mRNA and the percentages of positively stained cells increased dramatically. In cells exposed to a 12.5-mT SMF, c-Jun mRNA and the percentage of positively stained cells were significantly increased at 6 and 12 h compared to those in control cells. Moreover, exposure to both 125-mT and 250-mT SMFs promoted greater expression of c-Jun mRNA and protein than were observed in the control group and cells exposed to a 12.5-mT SMF as early as 2 h from the onset of exposure. In the 250-mT SMF-exposed group, the increased expression of c-Jun mRNA and protein continued until 12 h. The variations in c-Jun expression in response to SMFS of different intensities indicated a dose-dependent relationship. Similarly, Hirose et al. [26] found that c-Jun expression in HL-60 cells is enhanced by exposure to a high 6-T gradient magnetic field from 24-72 h, but not by exposure to high magnetic flux density. The reasons for differential c-Jun expression in response to different SMFs are currently unknown, but these differences may be related to the type and flux density of the magnetic field.

We found that ALP activity in untreated cells did not show a significant increase until 10-14 days. Similarly, OCN production was not significantly improved until 16-20 days. However, SMF stimulation tended to promote an early increase and then later a sharp increase in ALP and OCN production. c-Jun that encodes the major component of AP-1 belongs to the class of immediate early genes that are rapidly and transiently induced by extracellular stimuli. AP-1 acts as a nuclear third messenger, converting cytoplasmatic signals into long-term alterations in gene expression, a mechanism that is essential for gene regulation in response to many extracellular stimuli [27-29]. Because c-Jun is considered to be a critical regulator of osteoblastic differentiation, the sharp increase in c-Jun expression induced by SMFs may be involved in the early increase and later rapid increase in ALP and OCN expression in SMF-exposed cells.

In addition, we found that c-Jun mRNA expression and the percentage of positively stained cells were increased from 2-12 h of exposure to SMFs of different flux densities but not from 24-48 h, suggesting that SMF-induced c-Jun expression is a temporary phenomenon. The expression pattern of c-Jun was consistent with the characteristics of an IEG, which is increased dramatically at the beginning of SMF exposure and then decreases sharply. The exact mechanism underlying the transient expression of c-Jun remains elusive, and it may involve specific receptor desensitization, the shorter half-life of mRNA, an increase in negative transcription factors, or a decrease in positive transcription factors [30, 31].

c-Jun/AP-1-mediated regulation should be considered within the context of a complex dynamic network of signaling pathways, as well as the cell type and its microenvironment [32]. In the present study, there were differences in the time of c-Jun upregulation and the time of ALP and OCN upregulation induced by SMF. We speculate that these are most likely related to the osteoblast matrix microenvironment. During osteoblast proliferation, several genes associated with the formation of the extracellular matrix are actively expressed, such as type I collagen and TGF-β. It is primarily during

the proliferation period that the activity of these growth factors, their regulators, and the associated signal transduction mechanisms influence genes expressed later in osteoblast development, such as ALP [33]. Extracellular matrix biosynthesis during the proliferation period contributes to the onset and progression of osteoblast differentiation. Following type I collagen and TGF-β synthesis, ALP expression is initiated. In the present study, the time lag to ALP expression may therefore be related to type I collagen accumulation and extracellular matrix biosynthesis. Additionally, there are AP-1 bind sites in the type I collagen and TGF-β promoters [34-36]. The up-regulation of c-Jun by the SMF may also result in accelerated type I collagen synthesis and extracellular matrix synthesis. More detailed experiments will be needed to clarify this point.

Numerous studies have shown that the induction of high levels of OCN is dependent upon the formation of a mineralized extracellular matrix. Thus, mineral deposition may be required to signal the expression of a subset of osteoblast phenotype genes such as osteocalcin [33]. ALP and type I collagen may be involved in preparing the extracellular matrix for the ordered deposition of mineral. In the present study, the time of c-Jun activation by SMF was not completely consistent with that of the increased expression of OCN by SMF. It may be related to ALP synthesis, calcium deposition, and formation of collagen matrix. Rapidly induced c-Jun homodimer activates the ALP gene by mediating AP-1-ALP promoter TRE interaction. c-Jun functions as an early response element and may play a key role in subsequent stretch-induced ALP transcriptional control and augmented expression of OCN [35]. This evidence further supports our findings that a SMF may affect the cells as a mechanical force [37].

In our present study, MTT analysis of cells exposed to SMFs for 1-9 days showed no significant difference between the three tested flux densities. Yamamoto and co-workers [14] reported that osteoblast proliferation is not affected when rat calvarias, ROS 17/2.8, and UMR 106 cells are exposed to a SMF (flux density = 160 mT). Furthermore, three studies [24, 38, 39] also suggested that SMF exposure has no effect on the proliferation of osteoblasts and dental pulp cells. Therefore, it is unlikely that SMF exposure affects the proliferation of osteoblasts.

Conclusion

In summary, the present study showed that exposure to a SMF promoted osteoblastic differentiation, and this was associated with increased ALP activity and OCN expression, as well as the up-regulation of c-Jun mRNA expression. These data provide insight into a possible mechanism by which SMF exposure affects osteoblasts, which will be valuable information in the selection of a suitable therapeutic SMF flux density for osteogenesis. Further research is needed to elucidate the signal transduction mechanisms involved in the effects of SMF exposure on bone formation.

Acknowledgements

We thank Mrs. Li Xiao-Yu (West China Stomatological Hospital, Sichuan University) for her technical assistance. This study was supported by funds from the Science and Technology Planning Project of Guangdong Province, China (Grant No. 2012B031800453).

Disclosure of conflict of interest

None.

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References

[1] Boeckler AF, Morton D, Ehring C, Setz JM. Mechanical properties of magnetic attachments for removable prostheses on teeth and implants. J Prosthodont 2008; 17: 608-615.

[2] Takeshita S, Kanazawa M, Minakuchi S. Stress analysis of mandibular two-implant overdenture with different attachment systems. Dent Mater J 2011; 30: 928-934.

[3] Ellis JS, Burawi G, Walls A, Thomason JM. Patient satisfaction with two designs of implant supported removable overdentures; ball attachment and magnets. Clin Oral Implants Res 2009; 20: 1293-1298.

[4] Örtorp A. Three tumor patients with total maxillectomy rehabilitated with implant-supported frameworks and maxillary obturators: a follow-up report. Clin Implant Dent Relat Res 2010; 12: 315-323.
[5] Boeckler AF, Ehring C, Morton D, Geis-Gerstorfer J, Setz JM. Corrosion of dental magnet attachments for removable prostheses on teeth and implants. J Prosthodont 2009; 18: 301-308.
[6] Sollazzo V, Palmieri A, Pezzetti F, Massari L, Carinci F. Effects of pulsed electromagnetic fields on human osteoblastlike cells (MG-63): a pilot study. Clin Orthop Relat Res 2010; 468: 2260-2277.
[7] Lin HY, Lin YJ. In vitro effects of low frequency electromagnetic fields on osteoblast proliferation and maturation in an inflammatory environment. Bioelectromagnetics 2011; 32: 552-560.
[8] Gossling HR, Bernstein RA, Abbott J. Treatment of ununited tibial fractures: a comparison of surgery and pulsed electromagnetic fields (PEMF). Orthopedics 1992; 15: 711-719.
[9] Garland DE, Moses B, Salyer W. Long-term follow-up of fracture nonunions treated with PEMFs. Contemp Orthop 1991; 22: 295-302.
[10] Bassett CA, Mitchell SN, Gaston SR. Treatment of ununited tibial diaphyseal fractures with pulsing electromagnetic fields. J Bone Joint Surg Am 1981; 63: 511-523.
[11] Chiu KH, Ou KL, Lee SY, Lin CT, Chang WJ, Chen CC, Huang HM. Static magnetic fields promote osteoblast-like cells differentiation via increasing the membrane rigidity. J Ann Biomed Eng 2007; 35: 1932-1939.
[12] Huang HM, Lee SY, Yao WC, Lin CT, Yeh CY Static magnetic fields up-regulate osteoblast maturity by affecting local differentiation factors. Clin Orthop Relat R 2006; 447: 201-208.
[13] Leesungbok R, Ahn SJ, Lee SW, Park GH, Kang JS, Choi JJ. The effects of a static magnetic field on bone formation around a SLA treated titanium implant. J Oral Implantol 2013; 39: 248-255.
[14] Yamamoto Y, Ohsaki Y, Goto T, Nakasima A, Lijima T. Effects of static magnetic field on bone formation in rat osteoblast cultures. J Dent Res 2003; 82: 962-966.
[15] Joung YH, Lim EJ, Darvin P, Chung SC, Jang JW, Do Park K, Lee HK, Kim HS, Park T, Yang YM. MSM enhances GH signaling via the Jak2/STAT5b pathway in osteoblast-like cells and osteoblast differentiation through the activation of STAT5b in MSCs. PLoS One 2012; 7: e47477.
[16] Lo KW, Kan HM, Ashe KM, Laurencin CT. The small molecule PKA-specific cyclic AMP analogue as an inducer of osteoblast-like cells differentiation and mineralization. J Tissue Eng Regen Med 2012; 6: 40-48.
[17] Matsuguchi T, Chiba N, Bandow K, Kakimoto K, Masuda A, Ohnishi T. JNK activity is essential for Atf4 expression and late-stage osteoblast differentiation. J Bone Miner Res 2009; 24: 398-410.
[18] Naito J, Kaji H, Sowa H, Hendy GN, Sugimoto T, Chihara K. Menin suppresses osteoblast differentiation by antagonizing the AP-1 factor, JunD. J Biol Chem 2005; 280: 4785-4791.
[19] Zhao Y, Li BY, Chao YL, Du Li. Effect of simulating static magnetic field of magnetic attachment on osteoblastic proliferation, cell cycle distribution and apoptosis ratio. West China Journal of Stomatology 2007; 25: 437-440.
[20] Yao W, Zhao Y, Li BY, Chao YL, Du L. Effect of simulating static magnetic field of magnetic attachment on osteoblastic morphology and surface ultrastructure. Zhonghua Kou Qiang Yi Xue Za Zhi 2008; 43: 48-49.
[21] Yang L, Chao YL, Du L. Study of biologic effects of simulating static magnetic field of magnetic attachment on human periodontal ligament fibroblasts. Hua Xi Kou Qiang Yi Xue Za Zhi 2007; 25: 316-319.
[22] Stein GS, Lian JB, Stein JL, Van Wijnen AJ, Montecino M Transcriptional control of osteoblast growth and differentiation. Physiol Rev 1996; 76: 593-629.
[23] Yang JC, Lee SY, Chen CA, Lin CT, Chen CC, Huang HM. The role of the calmodulin-dependent pathway in static magnetic field-induced mechanotransduction. Bioelectromagnetics 2010; 31: 255-261.
[24] Kotani H, Kawaguchi H, Shimoaka T, Iwasaka M, Ueno S, Ozawa H, Nakamura K, Hoshi K. Strong static magnetic stimulates bone formation to a definite orientation in vitro and in vivo. J Bone Miner Res 2002; 17: 1814-1823.
[25] Nacamuli RP, Fong KD, Warren SM, Fang TD, Song HM, Helms JA, Longaker MT. Markers of Osteoblast differentiation in fusing and nonfusing cranial sutures. Plast Reconstr Surg 2003; 112: 1328-1335.
[26] Hirose H, Nakahara T, Zhang QM, Yonei S, Miyakoshi J. Static magnetic field gradient (41.7 T/m) induces c-Jun expression in HL60 cells. In Vitro Cell Dev Biol Anim 2003; 39: 348-352.
[27] Hartl M, Bader AG, Bister K. Molecular targets of the oncogenic transcription factor jun. Curr Cancer Drug Targets 2003; 3: 41-55.
[28] Karin M, Liu ZG, Zandi E. AP-1 function and regulation. Curr Opin Cell Biol 1997; 9: 240-246.
[29] Shaulian E, Karin M. AP-1 in cell proliferation and survival. Oncogene 2001; 20: 2390-2400.
[30] Sena K, Leven RM, Mazhar K, Sumner DR, Virdi AS. Early gene response to low-intensity pulsed ultrasound in rat osteoblastic cells. Ultrasound Med Biol 2005; 31: 703-708.

[31] Wagner EF. Functions of AP1 (Fos/Jun) in bone development. Ann Rheum Dis 2002; 61: ii40-42.
[32] Hess J, Angel P, Schorpp-Kistner M. AP-1 subunits: quarrel and harmony among siblings. J Cell Sci 2004; 117: 5965-5973.
[33] Stein GS, Lian JB, Owen TA. Bone cell differentiation: a functionally coupled relationship between expressions of cell-growth- and tissue-specific genes. Curr Opin Cell Biol 1990; 2: 1018-1027.
[34] Chen HT, Tsou HK, Chang CH, Tang CH. Hepatocyte growth factor increases osteopontin expression in human osteoblasts through P13K, Akt, c-Src, and AP-1 signaling pathway. PLoS One 2012; 7: e38378.
[35] Peverali FA, Basdra EK, Papavassiliou AG. Stretch-mediated activation of selective MAPK subtypes and potentiation of AP-1 binding in human osteoblastic cells. Mol Med 2001; 7: 68-78.
[36] Stein GS, Lian JB, Owen TA. Relationship of cell growth to the regulation of tissue-specific gene expression during osteoblast differentiation. FASEB J 1990; 4: 3111-3123.
[37] Sakurai T, Terashima S, Miyakoshi J. Enhanced secretion of prostaglandin E2 from osteoblasts by exposure to a strong static magnetic field. Bioelectromagnetics 2008; 29: 277-283.
[38] McDonald F. Effect of static magnetic fields on osteoblasts and fibroblasts in vitro. Bioelectromagnetics 1993; 14: 187-196.
[39] Hsu SH, Chang JC. The static magnetic field accelerates the osteogenic differentiation and mineralization of dental pulp cells. Cytotechnology 2010; 62: 143-155.