Int J Clin Exp Med 2015;8(5):7394-7404
www.ijcem.com /ISSN:1940-5901/IJCEM0007170

Original Article

50 Hz electromagnetic field exposure promotes proliferation and cytokine production of bone marrow mesenchymal stem cells

Wenxia Fan[1], Fenghua Qian[1], Qinlong Ma[2], Pei Zhang[1], Tingting Chen[1], Chunhai Chen[2], Yong Zhang[1], Ping Deng[2], Zhou Zhou[2], Zhengping Yu[2]

[1]Department of Hematology, Southwest Hospital, Third Military Medical University, No. 30 Gaotanyan Avenue, Chongqing 400038, China; [2]Department of Occupational Hygiene, College of Military Preventive Medicine, Third Military Medical University, Chongqing 400038, China

Received February 17, 2015; Accepted April 13, 2015; Epub May 15, 2015; Published May 30, 2015

Abstract: Objective: To investigate the effects of extremely low frequency electromagnetic field (ELF-EMF) on the proliferation and cytokine production of mesenchymal stem cells (MSC) and the effects of mesenchymal stem cell conditioned medium (MSC-CM) on the proliferation and migration of macrophagocytes (RAW264.7). Methods: Bone marrow derived-mesenchymal stem cells (rBMSC) were isolated from rats, cultured and randomly divided into two groups: SHAM group (absence of electromagnetic field exposure) and EMF group. Cells in EMF group were exposed to ELF-EMF (50 Hz, 1 mT, 4 h/d) under sXc-ELF. Mouse mesenchymal stem cells (mMSC) were exposed to EMF for 3 days. Results: The cell viability, DNA synthesis and proportion of cells in S phase in EMF group increased markedly when compared with SHAM group ($P<0.05$). When compared with SHAM group, the mRNA expressions of M-CSF and SCF increased markedly at 2 days after EMF exposure ($P<0.05$), the mRNA expressions of SCF, M-CSF, TPO, LIF, IL-11 and IL-7 increased dramatically, but the mRNA expressions of IL-6, SDF-1, IFN-γ and TNF-α remained unchanged ($P>0.05$) in mMSCs at 3 days after EMF exposure. In EMF group, the viability of RAW264.7 after MSC-CM treatment increased markedly as compared to SHAM group ($P<0.05$), and the ability to migrate of RAW264.7 after MSC-CM treatment in EMF group also increased significantly when compared with SHAM group ($P<0.05$). Conclusion: EMF is able to promote the proliferation of rBMSCs, up-regulate the expressions of hematopoietic growth factors in rBMSC and mMSC and increase the mMSC induced proliferation and migration of RAW264.7.

Keywords: Electromagnetic field, mesenchymal stem cells, cell proliferation, cytokines

Introduction

Bone marrow-derived mesenchymal stem cell (BMSCs) is a group of pluripotent stem cells different from hematopoietic stem cells. BMSCs have been widely used in the bone healing and repair of myocardium, nerves and spinal cord after injury, and play important roles in the regulation and construction of hematopoiesis. BMSCs have been important seed cells in the tissue engineering and cell therapy. In severe GVHD patients, transplantation with both mesenchymal stem cells (MSCs) and hematopoietic stem cells (HSCs) achieved a better survival as compared to transplantation with MSCs alone [1]. This may be related to a lot of cytokines secreted by MSCs (such as macrophage colony-stimulating factor [M-CSF], stem cell factor [SCF], leukemia inhibitory factor [LIF], thrombopoietin [TPO], stromal-derived factor-1 [SDF-1, CXCL12], interleukin-6 (IL-6), IL-7, IL-8, IL-11, IL-12, IL-14 and IL-15) because these cytokines may promote the homing, localization, proliferation and differentiation of HSCs and are important for the formation and repair of hematopoietic microenvironment [1, 2]. With the wide application of MSCs in clinical practice, the optimal conditions for in vitro culture and massive expansion of MSCs have been challenges in tissue engineering and cell therapy [3, 4].

In recent years, the clinical application of extremely low-frequency electromagnetic field (ELF-EMF) as a non-invasive physiotherapy, has been paid attention to. Several studies have shown that ELF-EMF may promote the proliferation of MSCs, and induce the differentiation of MSCs into osteoblasts, in which the synthesis

of bone morphogenetic protein, alkaline phosphatase and collagen, playing important roles in the therapy of bone non-healing and osteoporosis [5]. In addition, ELF-EMF may up-regulate the expression of some cytokines [6-9]. However, whether ELF-EMF may affect the secretion of cytokines by MSCs is still unclear. In this study, MSCs were cultured in vitro and the effect of ELF-EMF on the secretion of cytokines by MSCs was investigated. In addition, the conditioned medium (CM) was collected from MSCs after ELF-EMF and then the influence of CM on the proliferation and migration of RAW264.7 were investigated. Our findings may provide evidence for the clinical application of ELF-EMF treated MSCs.

Materials and methods

Materials

Animals and cell lines: SD rats aged 4-6 weeks (specific pathogen free) and weighing 80±20 g were purchased from the Experimental Animal Center of the Third Military Medical University. The animal procedures were in accordance to the guideline for the use and care of animals. mMSCs were kindly provided by Chen CH in the Department of Labor and Hygiene of the Third Military Medical University. RAW264.7 were kindly provided by Cao ZW in the Department of Labor and Hygiene of the Third Military Medical University.

Main reagents: DMEM, fetal bovine serum (FBS), 0.25% trypsin (Gibco, USA), penicillin, streptomycin (Beyoutime Biotech Co., Ltd), FITC-CD44, FITC-CD29, FITC-CD45, PE-CD31, isotype negative control antibody (BD, USA), CCK-8 kit (Dojindo, Japan), EdU (Guangzhou Ruibo), RNA extraction reagent and reagents used for Real-time PCR (TRIzol, reverse transcription kit, SYBR Green fluorescence quantification kit) (TaKaRa, Japan) were used in the present study.

Preparation of electromagnetic field (EMF): Electromagnetic device was purchased from IT'IS Company (Zurich, Switzerland). The rationale of the operation of this device was described by the Schuderer company [10]. This device is composed of two independent electromagnetic field (EMF) generators and connected to a computer via a specific sensor. It can produce an EMF of 3-1250 Hz and 0.04-3.5 mT. In the present study, the sinusoidal EMF of 50 Hz and 1 mT was used.

Methods

Separation and identification of rBMSCs: The primary rBMSCs were separated according to the previously described [5]. When the cell confluence reached 80-90%, cells were digested with 0.25% trypsin and passaged at a ratio of 1:2. Cells were observed under an inverted phase contrast microscope during the culture. rBMSCs of the 3rd generation were independently incubated with FITC-CD29, FITC-CD44, FITC-CD45 and PE-CD31, and isotype control was also included. Cells were then subjected to flow cytometry.

Preparation of MSC-CM: mMSCs were seeded into 35-mm dishes at a density of 4×10^5/dish and exposed to EMF. Three days later, the supernatant was harvested and centrifuged at 500 g for 10 min to remove debris, and then mixed with normal medium at a ratio of 1:1.

CCK-8 assay of cell proliferation

Detection of rBMSC proliferation by CCK-8 assay: rBMSCs of the 3rd generation were seeded into 96-well plates at a density of 2×10^3/well (100 μL/well). After exposure to EMF, 10 μl of CCK-8 solution was added to each well, followed by incubation at 37°C in an environment with 5% CO_2 for 1.5 h. The absorbance was measured at 450 nm.

Detection of RAW264.7 proliferation by CCK-8 assay: RAW264.7 were seeded into 96-well plate at a density of 5×10^3/well, and medium was refreshed 12 h later: (1) normal control group (CONTROL); (2) SHAM group: supernatant of mMSCs was mixed with normal medium at a ratio of 1:1; (3) EMF group: cells were exposed to EMF and the supernatant of mMSCs was with normal medium at a ratio of 1:1. Cells in different groups were maintained at 37°C in an environment with 5% CO_2 for 24 h, 48 h or 72 h, and then 10 μl of CCK-8 solution was added to each well, followed by incubation for 2 h. The absorbance was measured at 450 nm.

Detection of DNA synthesis by EdU integration assay: Cells were seeded into 24-well plates at

Table 1. Primers used for real-time PCR

Genes	Forward (5'→3')	Reverse (5'→3')	Length
Rat M-CSF	TAGCGAGCAAGGAAGCGAAC	GAGCCCATCCATGTCGAAGA	117 bp
SCF	TGTTCTTGCTACCCGTGACC	TGCTCCGTGCAACTTCTTCT	110 bp
GAPDH	TGCCACTCAGAAGACTGTGGATG	GCCTGCTTCACCACCTTCTTGAT	249 bp
Mouse M-CSF	AAGGAGGTGTCAGAACACTG	TAGCATTGGGGGTGTTGTCTTT	223 bp
SCF	CTCCTATTTCAATCCTCTTGTC	CCAAGTTTCGCTATGATGGAGTAA	267 bp
TPO	TCTGTCCAGCCCCGTAGCTC	CTTGCTCTGTTCCGTCTGGG	184 bp
LIF	GCCACCTGTGCCATACGCACCC	TCCACGTTGTTGGGAAACGGCTC	141 bp
IL-11	TCCAGTAGACCTGGGTGAGG	GTTCCCTGCTCTTCAGGGTC	106 bp
IL-7	GCCTGTCACATCATCTGAGTGCC	TGTATCATCACATACATGTTTTCT	163 bp
IL-6	TCCAGTTGCCTTCTTGGGAC	AGTCTCCTCTCCGGACTTGT	74 bp
SDF-1	CCATGGACGCCAAGGTCGTC	GGGCTGTTGTGCTTACTTGTTT	284 bp
TNF-α	TGACAAGCCTGTAGCCCACG	TCTTTGAGATCCATGCCGTTG	108 bp
IFN-γ	AGCAAGGCGAAAAAGGATGC	TCATTGAATGCTTGGCGCTG	83 bp
β-actin	CATCCGTAAAGACCTCTATGCCAAC	ATGGAGCCACCGATCCACA	171 bp

a density of 1×10^4/well, and exposed to EMF. Three days later, cells were subjected to EdU staining, and then observed under a fluorescence microscope. The EdU positive cells were counted.

Detection of cell cycle by flow cytometry: Cells were seeded into T25 flasks at a density of 1×10^6/mL and exposed to EMF. Three days later, single cell suspension was prepared and cells were washed in PBS once. Then, cells were fixed in 1 ml of 70% pre-cold ethanol for 24 h at 4°C and cells were harvested by centrifugation. Cells were re-suspended in 200 μL of PBS and incubated with PI in dark for 30 min at 4°C, followed by flow cytometry.

Detection of mRNA expressions of growth factors of MSCs by Real-time PCR: rBMSCs were seeded into 35-mm dishes at a density of 4×10^5/dish and then harvested at 0, 1, 2, and 3 d after EMF exposure. mMSCs were seeded into 35-dishes at a density of 4×10^5/dish and then harvested 3 days later. Total RNA was extracted with Trizol, and 1 μg of total RNA was used for reverse transcription into cDNA according to manufacturer's instructions with a 20-μL mixture. Conditions for reverse transcription were as follows: 42°C for 2 min, 37°C for 15 min and 85°C for 5 s. Then, 1 μL of cDNA was used as templates for Real-time PCR, and the mixture used for Real-time PCR was 25 μL in volume. The conditions for Real-time PCR were as follows: pre-denaturation at 95°C for 30 s, 40 cycles of denaturation at 95°C for 15 s, annealing at 59°C for 20 s and extension at 72°C for 10 s, and a final extension at 72°C for 90 s. Fluorescence was measured once every increment of 0.5°C during 65°C-95°C. The Ct value was used to calculate the relative expression of target gene as follow: $\Delta\Delta Ct_{target\ gene} = (Ct_{target\ gene}\text{-}Ct_{reference\ gene})_{EMF} - (Ct_{target\ gene}\text{-}Ct_{reference\ gene})_{SHAM}$, and GAPDH and β-actin were used as internal references. The expression of target gene in EMF group was $2^{-\Delta\Delta Ct}$ folds that in SHAM group. Primers for rat M-CSF, SCF, GAPDH, mouse SCF, M-CSF, TPO, LIF, IL-11, IL-7, IL-6, SDF-1, IFN-γ, TNF-α and β-actin are shown in **Table 1**.

Detection of RAW264.7 migration after MSC-CM treatment by wound healing assay: RAW264.7 were seeded into 12-well plates at a density of 5×10^5/well and incubated for 24 h. A wound was made with a 10-μl pipette tip, and the shedding cells were removed by washing in PBS. The remaining cells were maintained in 1.0 ml of fresh medium. In SHAM group and EMF group, MSC-CM was added. Cells were observed under an inverted phase contrast microscope, and photographed at 24 h, 48 h and 72 h. The width of wounds was measured.

Statistical analysis

Data are expressed as mean ± standard deviation. Statistical analysis was performed with SPSS version 13.0, and the Student's t-test was employed for comparisons. A value of $P< 0.05$ was considered statistically significant.

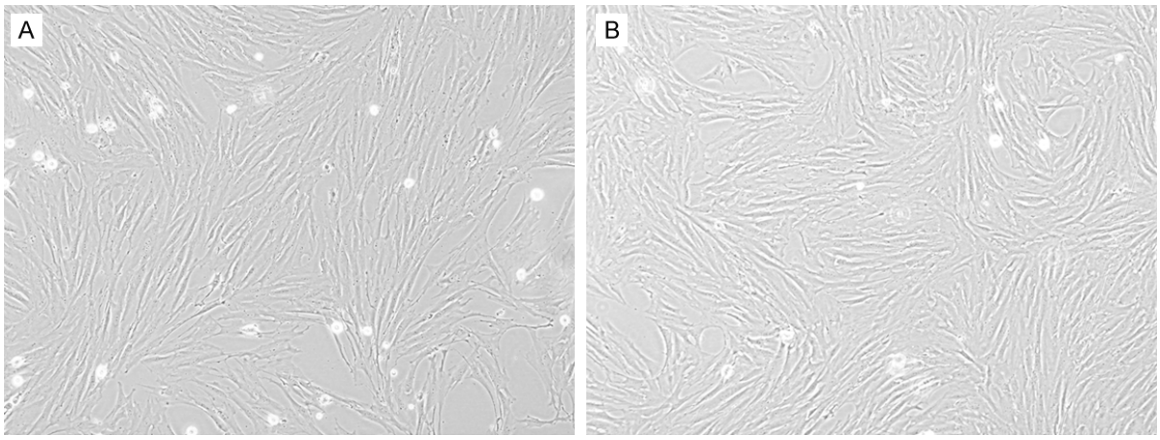

Figure 1. rBMSCs observed under an inverted phase contrast microscope (×100). A: After culture for 10 days, rBMSCs were fish-like and spindle-shaped; B: rBMSCs of the 3th generation were fibroblast-like.

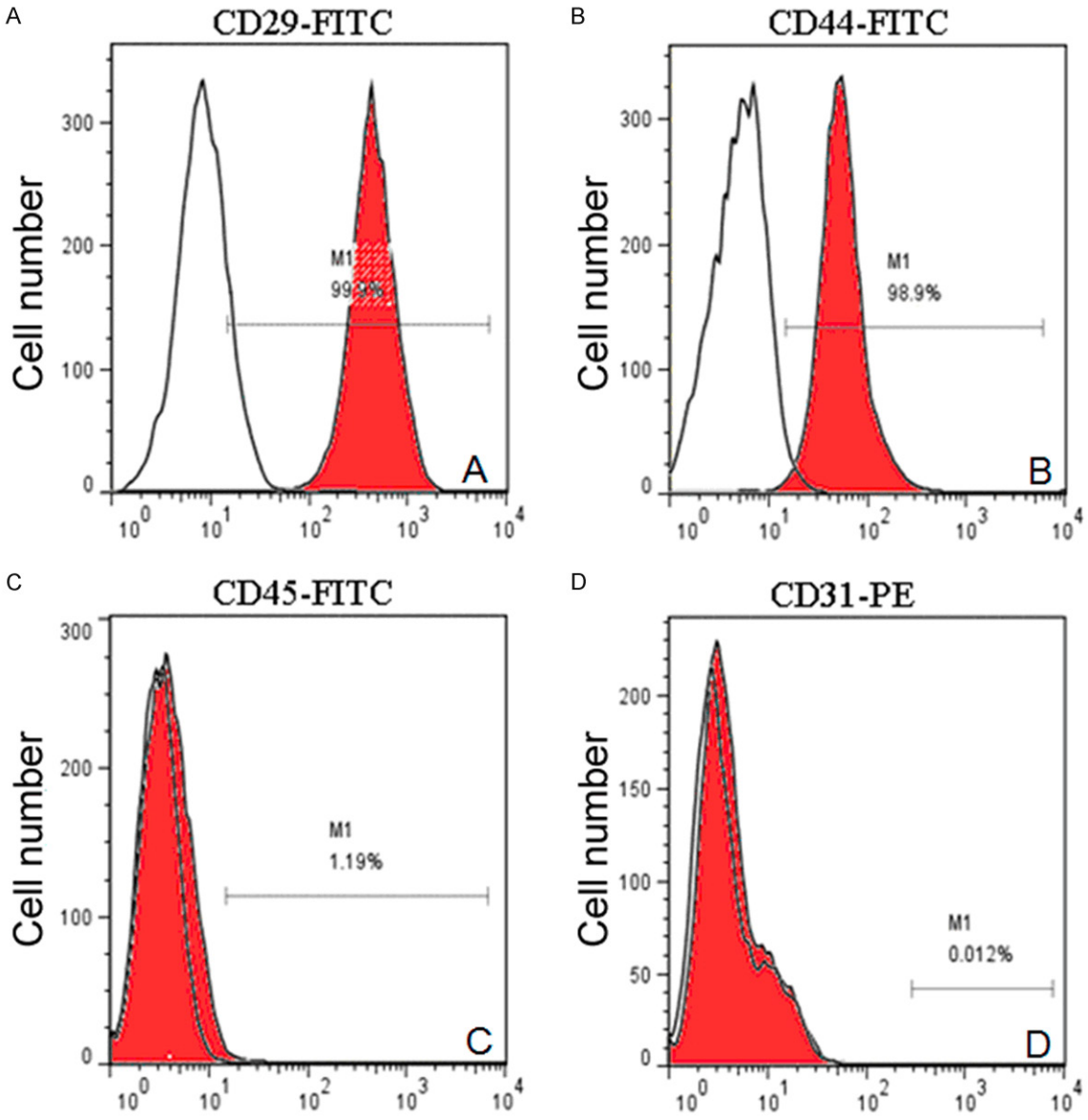

Figure 2. Detection of markers of BMSCs by flow cytometry. A: High CD29 expression; B: High CD44 expression; C: No CD45 expression; D: No CD31 expression.

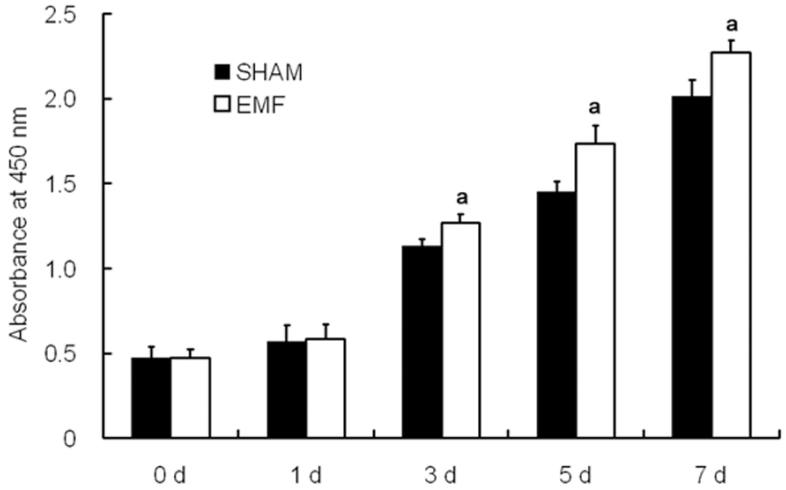

Figure 3. Detection of rBMSCs viability after ELF-EMF exposure by CCK-8 assay. A: *P*<0.05 vs. SHAM group.

Results

Morphology of rBMSCs

rBMSCs were adherent to the wall of dishes. Two days later, the medium was refreshed to remove suspended cells, and remaining cells were observed under an inverted phase contrast microscope. Cells had diverse shapes: Triangular, polygonal or spindle-shaped. Cells were further cultured, and they became long spindle-shaped. After culture for 10-12 days, cells showed fish or swirl-like growth (**Figure 1A**). rBMSCs of the 3rd generation were evenly distributed on the bottom and became fibroblast-like (**Figure 1B**). After passaging, the growth latency became shorter and their proliferation increased.

Identification of rBMSC

rBMSCs of the 3rd generation were subjected to flow cytometry and results showed the proportions of cells positive for CD29, CD44, CD45 and CD31 were 99.9%, 98.9%, 1.19% and 0.012%, respectively (**Figure 2**). The proportions of cells positive for CD29 and CD44 were higher than 95%, suggesting that these cells were homogeneous and had the surface markers of MSCs.

ELF-EMF promotes the proliferation of rBMSCs

ELF-EMF increases the viability of rBMSCs: After exposure to ELF-EMF, CCK-8 assay was performed to detect cell viability. As shown in **Figure 3**, the rBMSC viability in EMF group was more potent than that in SHAM group at 3 d after exposure to EMF (increment of 12.26% at 3 d, 19.78% at 5 d and 12.65% at 7 d) (P<0.05).

ELF-EMF increases the DNA synthesis in rBMSCs: As shown in **Figure 4**, the proportion of EdU positive cells at 3 d after EMF exposure (39.78±6.73%) was significantly higher than that in SHAM group (26.14±5.08%; *P*<0.05), suggesting that ELF-EMF may increase the DNA synthesis in the rBMSCs.

Cell cycle after exposure to ELF-EMF: Flow cytometry showed the cell cycle of rBMSCs changed at 3 d after ELF-EMF exposure. The proportion of cells in S phase after ELF-EMF exposure (28.43±1.34%) was significantly higher than that in SHAM group (21.89±0.99%) (**Table 2**).

mRNA expression of cytokines after ELF-EMF

After ELF-EMF exposure, the mRNA expressions of M-CSF and SCF were measured by rBMSC (**Figure 5A**). At 2 d after exposure, the mRNA expressions of M-CSF and SCF began to increase and were 1.48 and 1.61 times those in SHAM group, respectively (P<0.05). The mRNA expressions of M-CSF and SCF further increased with the prolongation of exposure. At 3 d after exposure, the mRNA expressions of M-CSF and SCF were 1.52 and 1.78 times those in SHAM group, respectively (P<0.01).

At 3 d after ELF-EMF exposure, Real-time PCR was done to detect the mRNA expressions of M-CSF, SCF, TPO, LIF, IL-11, IL-7, IL-6, SDF-1,

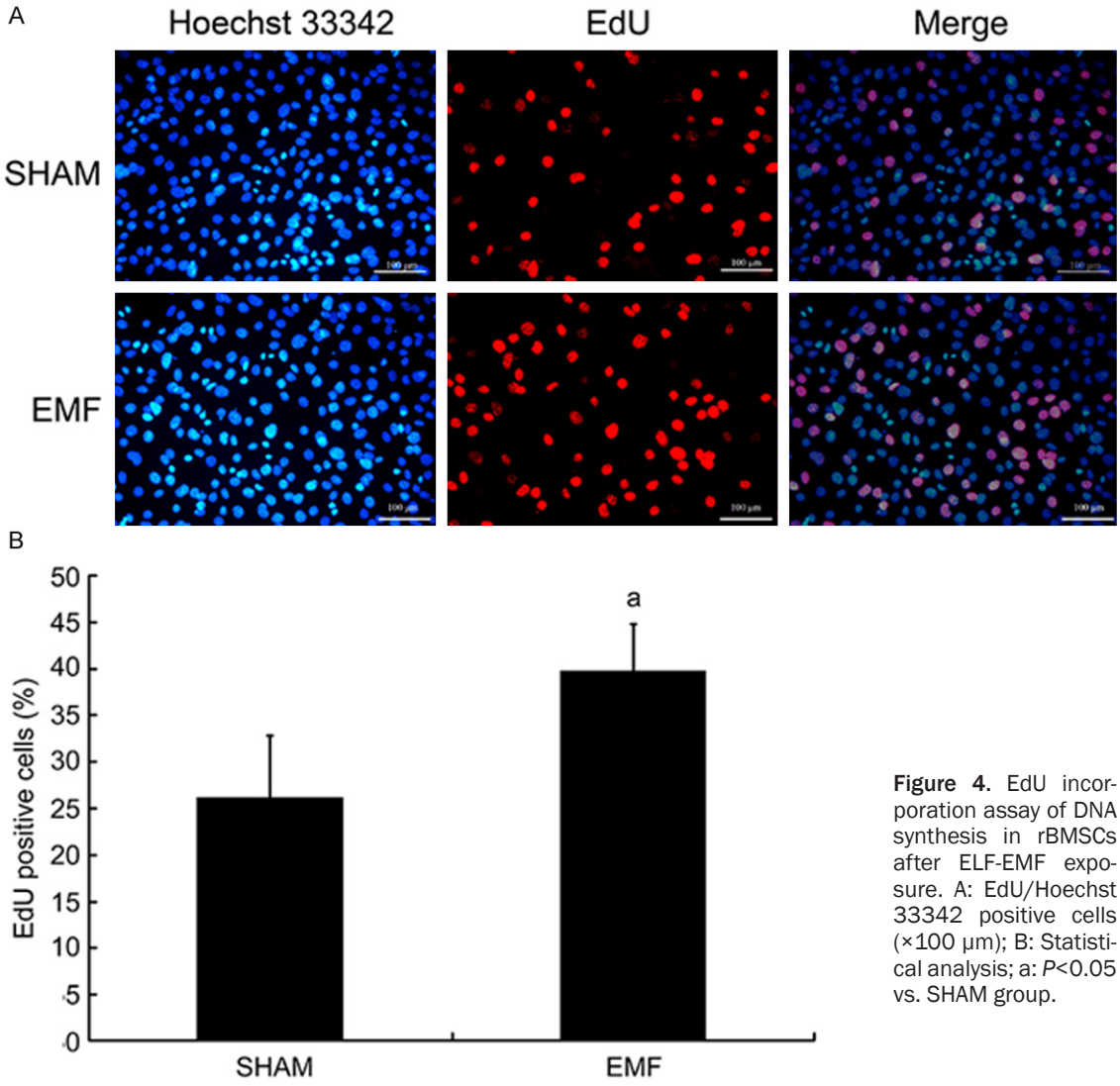

Figure 4. EdU incorporation assay of DNA synthesis in rBMSCs after ELF-EMF exposure. A: EdU/Hoechst 33342 positive cells (×100 μm); B: Statistical analysis; a: *P*<0.05 vs. SHAM group.

Table 2. Influence of ELF-EMF on the cell cycle of rBMSCs (n = 3, $\bar{x} \pm s$, %)

Group	G_0/G_1	S	G_2/M
EMF	70.66±1.76	21.89±0.99	7.45±1.06
SHAM	65.89±1.77[a]	28.43±1.34[b]	5.68±1.22

Footnotes: a: *P*<0.05, b: *P*<0.01 vs. SHAM group.

TNF-α and IFN-γ (**Figure 5B**). Results showed the mRNA expressions of M-CSF, SCF, TPO, LIF, IL-11 and IL-7 after ELF-EMF exposure were significantly higher than those in SHAM group (P<0.05).

CM from ELF-EMF treated mMSCs increases the proliferation of RAW264.7

CCK-8 assay was performed to measure the influence of CM from ELF-EMF treated mMSCs on the proliferation of RAW264.7. As shown in **Figure 6**, the absorbance increased over time (0 h, 24 h, 48 h and 72 h). When compared with normal RAW264.7, the absorbance in SHAM group and EMF group increased markedly after incubation with MSC-CM (P<0.05). Moreover, after culture for 72 h, the absorbance in EMF was significantly higher than that in SHAM group (P<0.05).

CM from ELF-EMF treated mMSCs increases the migration of RAW264.7

mMSCs were exposed to EMF and the supernatant (CM) was collected. The influence of this CM on the migration of RAW264.7 was investigated. As shown in **Figure 7**, the wound width reduced over time (0 h, 24 h, 48 h and 72 h). When compared with normal RAW264.7, the

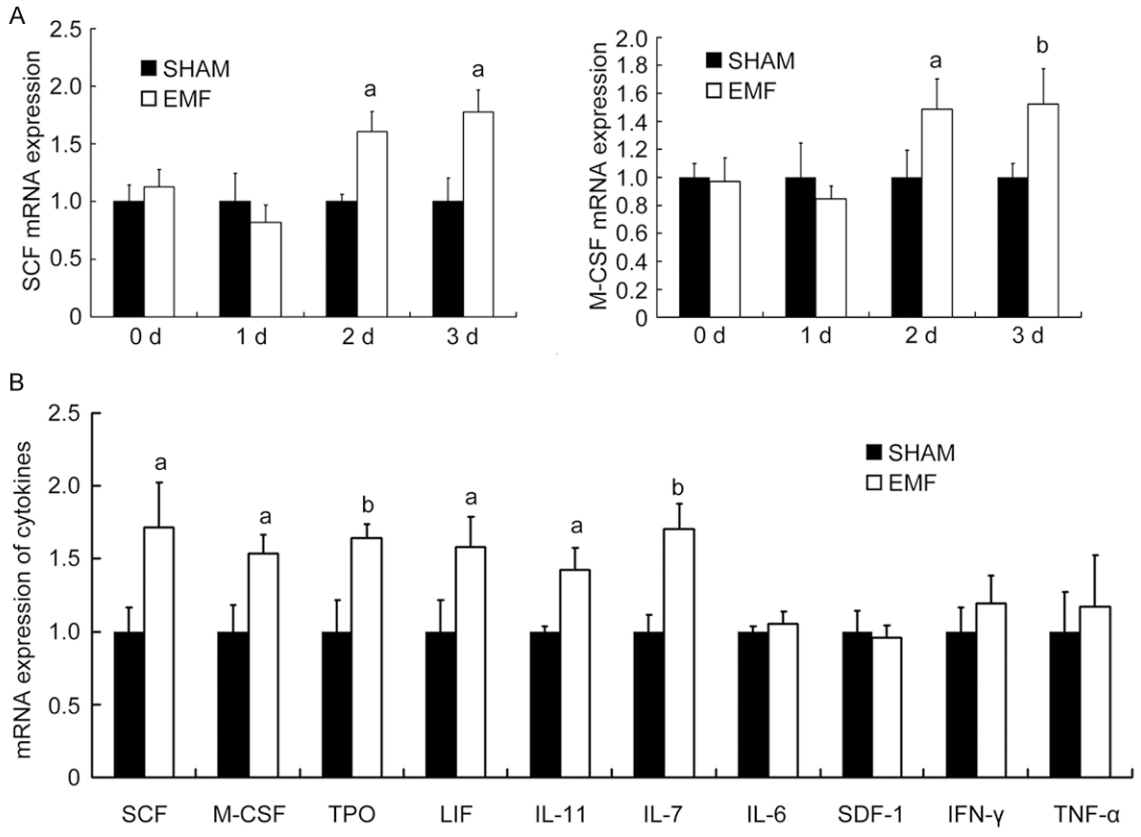

Figure 5. mRNA expressions of cytokines secreted by MSCs (Real-time PCR). A: mRNA expressions of M-CSF and SCF in rBMSCs; B: mRNA expressions of SCF, M-CSF, TPO, LIF, IL-11, IL-7, IL-6, SDF-1, IFN-γ and TNF-α in mMSCs; a: $P<0.05$, b: $P<0.01$ vs SHAM group.

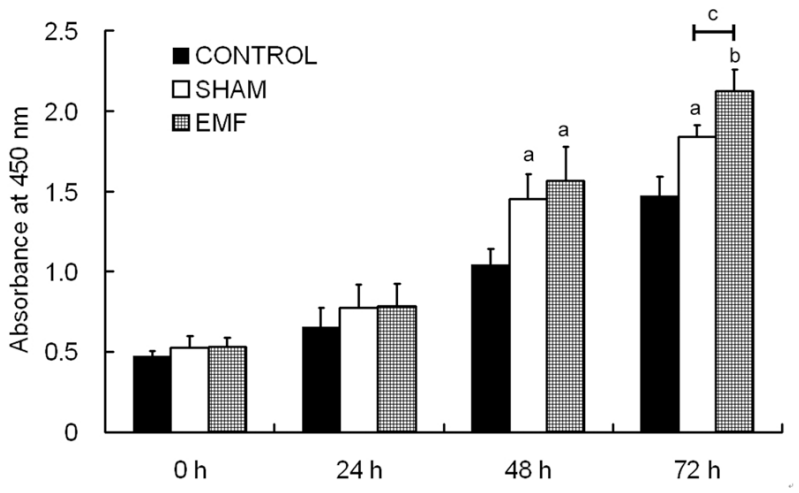

Figure 6. Proliferation of RAW264.7 (CCK-8 assay). A: $P<0.05$ vs. CONTROL group, B: $P<0.01$ vs. CONTROL group; C: $P<0.05$ vs. SHAM group.

wound width in EMF group reduced markedly at 24 h after incubation with MSC-CM (P<0.05). Moreover, after culture for 72 h, the wound width in EMF was significantly reduced as compared to SHAM group (P<0.05).

Discussion

Mesenchymal stem cells (MSCs) are a group of stem cells with the multi-lineage differentiation and are derived from the mesoderm. MSCs have a high plasticity and exogenous genes are easy to be introduced to these cells to achieve stable expression of target gene. These MSCs have been ideal seed cells in tissue engineering and cell therapy. MSCs are easy to be separated from the bone marrow and thus bone marrow has been a major source of MSCs. However,

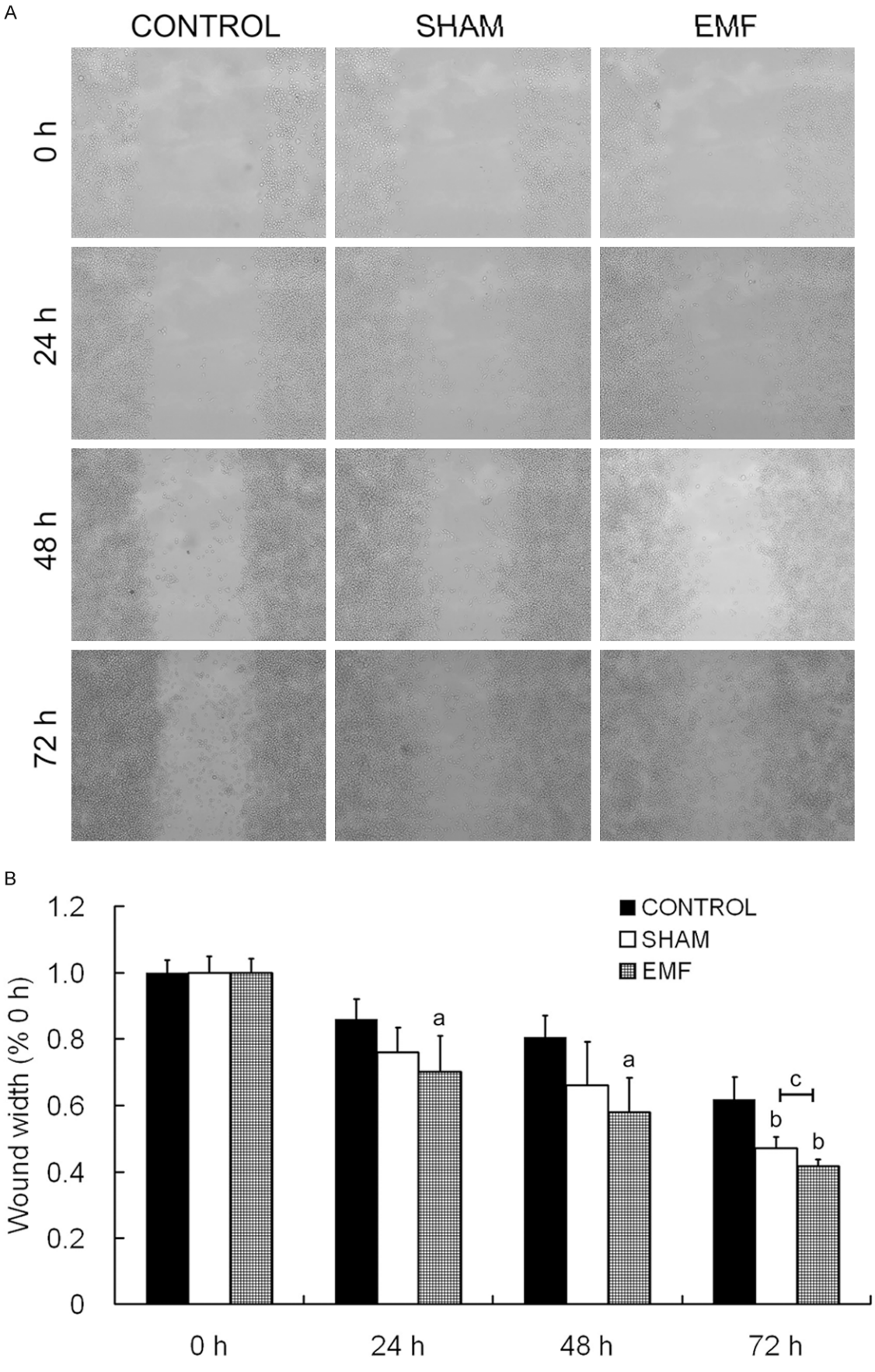
A
CONTROL
SHAM
EMF
0 h
24 h
48 h
72 h
B
CONTROL
SHAM
EMF
Wound width (% 0 h)
1.2
1.0
0.8
0.6
0.4
0.2
0
a
a
b
b
c
0 h
24 h
48 h
72 h

Figure 7. Migration of RAW264.7 (wound healing assay). A: Wound width in different groups (×100); B: Statistical analysis; a: $P<0.05$ vs. CONTROL group, b: $P<0.01$ vs. CONTROL group; c: $P<0.05$ vs. SHAM group.

the cell compositions are complex in the bone marrow, and rBMSCs have a low proportion in the bone marrow and account for only 0.001%-0.01% of nucleated cells. Thus, the separation, purification and in vitro expansion of MSCs of bone marrow are of great importance. In the present study, classic whole bone marrow adherent culture was employed to separate and purify rBMSCs, and flow cytometry showed rBMSCs had high expressions of surface markers of MSCs (CD29 and CD44), but low or no expressions of surface markers of hematopoietic cells and endothelial cells (CD45 and CD31). This suggests that rBMSCs with a high purity were separated. In addition, with the increase in passaging, the proliferation of MSCs reduced and their ability of multi-lineage differentiation was also compromised [11]. In addition, the expressions of bone morphogenetic protein and alkaline phosphatase reduced and the secretion of hematopoietic growth factors was also compromised in MSCs [12]. Thus, to develop strategies to maintain the stemness has been a focus in studies. The maintenance of stemness of MSCs is dependent on their self-renewal.

Our results showed ELF-EMF increased the viability of rBMSCs, promoted the DNA synthesis, and increased the proportion of cells in S phase, which were consistent with previously reported [5]. The proliferation of MSCs is also related to the expressions of relevant cytokines. Callaghan et al [6] and Goto et al [7] found EMF could up-regulate the mRNA expression of fibroblast growth facter-2 (FGF-2). Of note, FGF-2 may inhibit cellular senescence and promote cell proliferation via PI3K/AKT-MDM2 signaling pathway, which plays an important role in the maintenance of self-renewal and stemness of MSCs [13].

ELF-EMF may also increase the expressions of some cytokines including factors related to osteogenesis (BMP, ALP, RUNX2 and DLX5) [5, 8], which play important role in the therapy of bone non-healing and osteoporosis. Li et al [9] found ELF-EMF could up-regulate the mRNA expressions of ACTN2, α-actin and TNNT2 and promote the differentiation of rBMSCs into cardiomyocyte-like cells. Bai et al [5] also proposed that EMF could act synergistically with neural induction factor to promote the differentiation of MSCs into neurons which expressed specific markers of neurons. Our results also revealed that ELF-EMF up-regulated the expressions of some hematopoietic growth factors (M-CSF, SCF, TPO, LIF, IL-11 and IL-7), but the mRNA expressions of SDF-1, IL-6, TNF-α and IFN-γ remained unchanged. This may be related to the pattern of EMF and the time points. Studies have shown that the biological effects of EMF are related to the time of exposure, waveform, frequency, amplitude, cell type and cell status [14]. Both SCF and c-kit (SCF receptor) are involved in the migration, proliferation and differentiation of hematopoietic cells. The proliferation and survival of early hematopoietic cells are controlled by c-kit signaling pathway [15]. Exogenous SCF and G-CSF may increase the proliferation of MSCs [16]. In the present study, results showed ELF-EMF increased the proliferation of MSCs, which may be partially related to the increase in the expression of proliferation-related genes. Colony-stimulating factors (CSFs) are a group of glycoproteins controlling the proliferation and differentiation of monocyte-macrophages, neutrophils and hematopoietic cells. M-CSF (CSF-1) is involved in the regulation of proliferation and differentiation of the mononuclear phagocytic system [17]. The binding of CSF-1 to its receptor (CSF1R) plays crucial roles in the proliferation, differentiation and migration of cells [18]. Our results also showed MSCs could promote the proliferation and migration of macrophages, and the abilities of MSCs after EMF exposure to promote the proliferation and migration of macrophages were more potent than that in SHAM group, which might be ascribed to the up-regulated M-CSF mRNA expression after EMF exposure. The proliferation and differentiation of MSCs require the continuous expressions of some cytokines such as SCF and M-CSF, and EMF-EMF could increase the expressions of some cytokines. Although we did not confirm the causative relationship between MSCs proliferation and cytokines secreted by these cells, our results suggested that the increase in these cytokines could further facilitate the proliferation of MSCs.

Taken together, ELF-EMF may promote the proliferation of MSCs and up-regulate the mRNA

expressions of M-CSF, SCF, TPO, LIF, IL-11 and IL-7. In addition, CM from mMSCs is able to increase the proliferation and migration of RAW264.7. Our findings provide a new strategy for the in vitro expansion of MSCs and reliable experimental evidence for the clinical use of MSCs.

Acknowledgements

The study was supported by the National Basic Research Program of China (National 973 Program) (Grant No.: 2011CB503700).

Disclosure of Conflict of Interest

None.

Address correspondence to: Yong Zhang, Department of Hematology, Southwest Hospital, Third Military Medical University, No. 30 Gaotanyan Avenue, Chongqing 400038, China. E-mail: zhangyongxnyy@163.com

References

[1] Ringden O, Uzunel M, Rasmusson I, Remberger M, Sundberg B, Lonnies H, Marschall HU, Dlugosz A, Szakos A, Hassan Z, Omazic B, Aschan J, Barkholt L and Le Blanc K. Mesenchymal stem cells for treatment of therapy-resistant graft-versus-host disease. Transplantation 2006; 81: 1390-1397.

[2] Pontikoglou C, Deschaseaux F, Sensebe L and Papadaki HA. Bone marrow mesenchymal stem cells: biological properties and their role in hematopoiesis and hematopoietic stem cell transplantation. Stem Cell Rev 2011; 7: 569-589.

[3] Horwitz EM, Maziarz RT and Kebriaei P. MSCs in hematopoietic cell transplantation. Biol Blood Marrow Transplant 2011; 17: S21-29.

[4] Hao L, Sun H, Wang J, Wang T, Wang M and Zou Z. Mesenchymal stromal cells for cell therapy: besides supporting hematopoiesis. Int J Hematol 2012; 95: 34-46.

[5] Bai WF, Xu WC, Feng Y, Huang H, Li XP, Deng CY and Zhang MS. Fifty-Hertz electromagnetic fields facilitate the induction of rat bone mesenchymal stromal cells to differentiate into functional neurons. Cytotherapy 2013; 15: 961-970.

[6] Callaghan MJ, Chang EI, Seiser N, Aarabi S, Ghali S, Kinnucan ER, Simon BJ and Gurtner GC. Pulsed electromagnetic fields accelerate normal and diabetic wound healing by increasing endogenous FGF-2 release. Plast Reconstr Surg 2008; 121: 130-141.

[7] Goto T, Fujioka M, Ishida M, Kuribayashi M, Ueshima K and Kubo T. Noninvasive up-regulation of angiopoietin-2 and fibroblast growth factor-2 in bone marrow by pulsed electromagnetic field therapy. J Orthop Sci 2010; 15: 661-665.

[8] Yang Y, Tao C, Zhao D, Li F, Zhao W and Wu H. EMF acts on rat bone marrow mesenchymal stem cells to promote differentiation to osteoblasts and to inhibit differentiation to adipocytes. Bioelectromagnetics 2010; 31: 277-285.

[9] Li K, Jiang J, Yao XL, Qiu FY, Yan ZQ, Wu WC, Liu XJ and Li L. [Expression of ACTN2, alpha-actin and TNNT2 in rat bone marrow-derived mesenchymal stem cells induced by low frequency pulsed electromagnetic fields]. Sichuan Da Xue Xue Bao Yi Xue Ban 2012; 43: 670-674, 710.

[10] Schuderer J, Oesch W, Felber N, Spat D and Kuster N. In vitro exposure apparatus for ELF magnetic fields. Bioelectromagnetics 2004; 25: 582-591.

[11] Zaim M, Karaman S, Cetin G and Isik S. Donor age and long-term culture affect differentiation and proliferation of human bone marrow mesenchymal stem cells. Ann Hematol 2012; 91: 1175-1186.

[12] Vacanti V, Kong E, Suzuki G, Sato K, Canty JM and Lee T. Phenotypic changes of adult porcine mesenchymal stem cells induced by prolonged passaging in culture. J Cell Physiol 2005; 205: 194-201.

[13] Coutu DL, Francois M and Galipeau J. Inhibition of cellular senescence by developmentally regulated FGF receptors in mesenchymal stem cells. Blood 2011; 117: 6801-6812.

[14] Song MY, Yu JZ, Zhao DM, Wei S, Liu Y, Hu YM, Zhao WC, Yang Y and Wu H. The time-dependent manner of sinusoidal electromagnetic fields on rat bone marrow mesenchymal stem cells proliferation, differentiation, and mineralization. Cell Biochem Biophys 2014; 69: 47-54.

[15] Lennartsson J and Ronnstrand L. Stem cell factor receptor/c-Kit: from basic science to clinical implications. Physiol Rev 2012; 92: 1619-1649.

[16] Orlic D, Kajstura J, Chimenti S, Limana F, Jakoniuk I, Quaini F, Nadal-Ginard B, Bodine DM, Leri A and Anversa P. Mobilized bone marrow cells repair the infarcted heart, improving function and survival. Proc Natl Acad Sci U S A 2001; 98: 10344-10349.

[17] Moffat L, Rothwell L, Garcia-Morales C, Sauter KA, Kapetanovic R, Gow DJ and Hume DA. Development and characterisation of monoclonal antibodies reactive with porcine CSF1R (CD115). Dev Comp Immunol 2014; 47: 123-128.

[18] Pierce JH, Di Marco E, Cox GW, Lombardi D, Ruggiero M, Varesio L, Wang LM, Choudhury GG, Sakaguchi AY, Di Fiore PP, et al. Macrophage-colony-stimulating factor (CSF-1) induces proliferation, chemotaxis, and reversible monocytic differentiation in myeloid progenitor cells transfected with the human c-fms/CSF-1 receptor cDNA. Proc Natl Acad Sci U S A 1990; 87: 5613-5617.