

Research article

EMF frequency dependent differentiation of rat bone marrow mesenchymal stem cells to astrocyte cells

Nader Asadian^a, Majid Jadidi^{a,*}, Manouchehr Safari^b, Taha Jadidi^b, Mahbobeh Gholami^c^a Department of Medical Physics, Semnan University of Medical Sciences, Semnan, Iran^b Nervous System Stem Cells Research Center, Semnan University of Medical Sciences, Semnan, Iran^c Department of Biochemistry, Semnan University of Medical Sciences, Semnan, Iran

ARTICLE INFO

Keywords:

Electromagnetic field
Mesenchymal stem cell
Cell viability
Proliferation
Differentiation

ABSTRACT

The numerous factors regulate the bone marrow mesenchymal stem cell (BMMSC) self-renewal and differentiation response. We aimed to analyze the influence of electromagnetic field (EMF) as an external inducing factor on rat BMMSC differentiation and proliferation to neuron and astrocyte cells. BMMSCs extracted from the rats femurs and tibias and incubated in a cell-cultured CO₂ incubator. After the third passages, the plates selected randomly and then divided into seven groups (Sham exposed, three groups of square, and three groups of sinusoidal waveform EMF (25, 50, and 75 Hz, 400 μ T, 1 h/day). The BMMSCs exposed to EMF at the middle of a Helmholtz coil for 7 days. The viable cell counting and proliferation performed by the MTT test and BMMSC differentiation into the neuron and the astrocyte cell was studied by immunocytochemistry staining.

The results confirmed BMMSC viability and proliferation rate reduction in sinusoidal 25 Hz, square 50 Hz and sinusoidal 75 Hz EMF groups compare to sham. The maximum BMMSC differentiation to neuron was considered in sinusoidal 50 Hz and 75 Hz EMF groups. The increase of BMMSC differentiation to astrocyte cell was frequency dependent and the most differentiation was shown in square 75 Hz, and sinusoidal 75 Hz EMF groups. In conclusion, the results suggest that both square and sinusoidal EMF could affect BMMSC development and differentiation to neuron and astrocyte cells. Further studies for the consequence of EMF with wider flux density and frequency on BMMSC are recommended.

1. Introduction

In the early 1900s, the first actual stem cell with a capacity of differentiating into frequent cell lineages and self-renewing ability introduced that can make exact copies of it continually and create specialized cells [1]. They can classify by totipotent (differentiate into all possible cell types: zygote), pluripotent (differentiate into almost all cell types: embryonic stem cells), multipotent (differentiate into a closely related family of cells: adult hematopoietic stem cells), and unipotent (only produce cells of their own type: adult muscle stem cells) [1]. Mesenchymal stem cells (MSCs) have been the subject of intense scientific exploration since their preliminary detection by Alexander Friedenstein [2].

The bone marrow mesenchymal stem cell (BMMSC) differentiation into neural cell require the incidence of biochemical factors for instance growth factors, transcription factors, or chemical compounds (Mercaptoethanol, Dimethyl sulfoxide, and Retinoic acid) [3]. Another approach

to arouse stem cells is electromagnetic field (EMF) exposure. EMF as a physical inducing factor could affect the BMMSC proliferation [4–8]. Also, EMF significantly facilitated human and rat BMMSCs to differentiate into neurons [3,6–9], and glial cells [10].

Despite the important character of astrocyte cells in optimize neural information processing, to modulate synaptic transmission and neuronal activity, only a small number of studies examining the effect of EMF on these supportive cells. On the other hand, most of stimulating EMFs are of sinusoidal waveform because alternating current (AC) used at 60 Hz or 50 Hz in the world power lines and few studies examined the effect of EMFs frequency or current waveform on the viability, proliferation, and differentiation of BMMSCs. Therefore, we aimed to investigate the influence of frequency (25, 50 and 75 Hz) and current waveform (sinusoidal and square) of EMF on rat BMMSC differentiation and proliferation into neuron and astrocyte cells.

* Corresponding author.

E-mail address: Jadidim@semums.ac.ir (M. Jadidi).<https://doi.org/10.1016/j.neulet.2020.135587>

Received 14 June 2020; Received in revised form 23 November 2020; Accepted 16 December 2020

Available online 26 December 2020

0304-3940/© 2020 Elsevier B.V. All rights reserved.

2. Methods and materials

2.1. Animals and BMMSC incubation

Four healthy adult male Wistar rats (12–13 weeks of age) with an average body weight of (200 ± 10 g) were housed in polycarbonate cages (2 rats /cage) with free access to tap water and rat chow with a dark/light cycle of 12/12 h (temperature 22 ± 2 °C, relative humidity 50–70 %). The Research Ethics Committee of Semnan University of medical sciences (Iran), approved all procedures (IR.SEMUMS.RES:1396.101), which has harmony with the National Institutes of Health (NIH) guidelines for the care and use of laboratory animals (NIH Publications No. 8023, revised Edition 1978).

Bone marrow extracted from sacrificed animals (ketamine/xylazine, sigma-Aldrich, 30 mg/kg, IP) femurs and tibias by surgery to attain more cells. Bone marrow cells of the 4 experimental animals mixed together and seeded onto PBS (Gibco, 1038500). The cell suspension was filtered through a cell strainer (100 μ m) to remove debris and depleted cells got from filtered suspension incubated in DMEM (1/043 g L-Glutamine, Gibco, 11530556), 20 % FBS (Gibco, 10270106) and penicillin-streptomycin (Bio-Idea) in 25-cm² flasks at 37 °C and CO₂ 5%. Non-adherent cells were discarded after 48 h and adherent cells washed with DMEM. The medium changed every three days and allowed growing until 70–80 % confluence of the flask surface area. For purification of BMMSCs and removing fat, fibroblast and hematopoietic cells, three passages were used. After the third passages, BMMSCs with density of 8,000, allowed adhering overnight in 96 wells plate for MTT assay. At this stage, 50,000 cells in six wells plate incubated for a day to treat with EMF. The plates selected randomly and then divided into seven groups (sham exposed, three groups of square and three groups of sinusoidal waveform EMF at frequencies of 25, 50 and 75 Hz) [6].

2.2. EMF exposure

The electrical currents for EMF exposure provided by a signal generator (GFG-8019 G, Goodwill, Taiwan) and regulated with a 35 W acoustic amplifier (ALFA, Navasaz, Iran). The amplified current connected to the horizontal Helmholtz coil (300 turns, distance of 6 cm, and internal diameter of 16 cm) embedded in an open Plexiglas rectangular frame that placed in a cell culture CO₂ incubator. BMMSCs exposed to EMF (400 μ T, 1 h/day, and 7 days) at the center of Helmholtz coil, apart from the EMF system was turn off in the sham group. The mean flux density of the EMF system measured by a Teslameter (EMF 827, Lutron Electronic Enterprise Co. Taiwan) prior to cell culture exposure [6].

2.3. BMMSC proliferation and differentiation

The proliferation rates and viable cell counting was performed by the MTT dye (Sigma, M5655) colorimetric test. Briefly, after exposure BMMSCs incubated in the presence of MTT (5 mg/mL) 3 h at 37 °C. The absorbance of 570 nm wavelength in each cell culture well was read using an Eliza reader (A.D.TOUCH, apDia, China). To assess the proliferation rates, BMMSCs washed with PBS, trypsinized, staining with Trypan blue and counting done. The mean number of whole cells per volume was considered as a group proliferation rate. BMMSC differentiation into neuron and astrocyte cells was studied by immunocytochemistry (ICC) staining. The fixed cells washed in PBS and incubated at 4 °C overnight with a primary antibody (Anti-NeuN 1:100, ab177487 and Anti-GFAP 1:100, ab7260). Subsequently, they incubated at room temperature for 2 h with the secondary antibody (FITC-conjugate 1:200, Biolegend 406403) to assessing the differentiation of BMMSCs to neuron and astrocyte cells respectively. Cell nuclei counterstained in PBS with 1 μ g/mL DAPI (Sigma, D9542). Morphology of nuclei in all groups examined by fluorescence dye of DAPI and cytoplasm morphology by secondary antibody [6].

2.4. Statistical analysis

The Tukey test used to evaluate normal distribution of data. The statistical differences between groups were examined using one-way ANOVA and Kruskal-Wallis with the SPSS 16.0 software, and statistical differences were considered significant at the $P < 0.05$ level. The data expressed as means $\pm$ SD.

3. Results

To evaluate the effect of EMF on BMMSC proliferation and viability, MTT test were used. However, according to Fig. 1 A, the findings of this study show that EMF exposure reduced the BMMSC viability (with the exception of the 75 Hz square field). The results confirmed cell viability reduction in sinusoidal 25 Hz, square 50 Hz and sinusoidal 75 Hz groups compare to sham. While, in the square 75 Hz group BMMSC viability was increased (118 ± 6.95) in compare to sham ($P < 0.05$). As shown in Fig. 1 B, the sinusoidal 25 Hz, square 50 Hz and sinusoidal 75 Hz groups had a significant proliferation rate decrease in compare to sham (0.36 ± 0.03), and in contrast, the square 75 Hz group had the most increased significant proliferation rate (0.43 ± 0.03) in compare to sham group ($P < 0.05$).

In order to determine whether the BMSC have differentiation potential after EMF treatment, in vitro experiments were conduct for both EMF-treated and non-treated BMSCs. ICC staining and morphology characteristics showed that EMF could induce BMMSC differentiation to neuron cells in all treatment groups. The results confirmed maximum cell differentiation to neuron in sinusoidal 50 Hz (56.67 ± 2.89) and sinusoidal 75 Hz (45.0 ± 5.01) EMF groups compare to sham (1.67 ± 0.58) (Fig. 2). ICC staining and morphology characteristics showed that EMF could induce BMMSC differentiation to astrocyte cells in all experimental groups. The increase of BMMSC differentiation rate to astrocyte cell was frequency dependent and the most differentiation to astrocyte cells occurred in square 75 Hz and sinusoidal 75 Hz EMF groups compare to sham group, 1.33 ± 0.58 (Fig. 3). The differentiation rate in the groups of sinusoidal 25, 50, and 75 Hz, was 30.33 ± 5.51 , 49.33 ± 1.15 , 55.0 ± 5.0 individually; and in the groups of square 25,

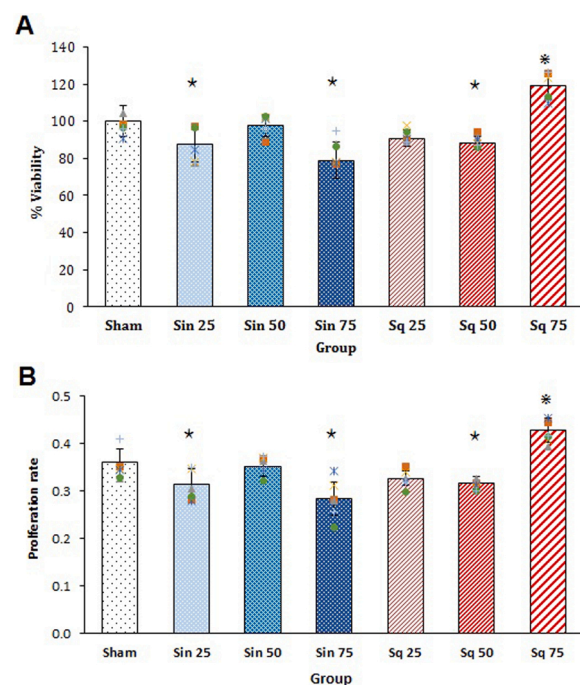


Fig. 1. The BMMSC viability (A) and proliferation rate (B) which evaluated by MTT test. The results represent the mean $\pm$ SD as bar chart and 6 data points as scatter dots. (* reduction, and * increase rate compare to sham, $P < 0.05$).

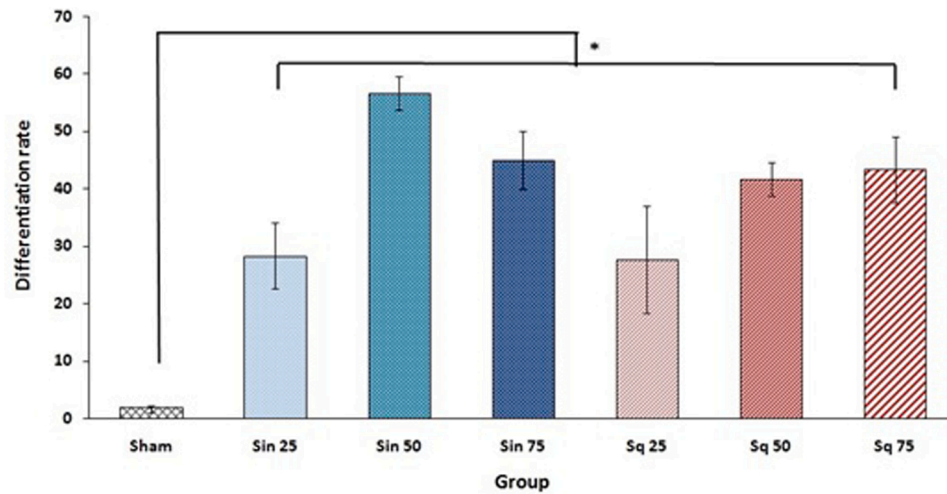
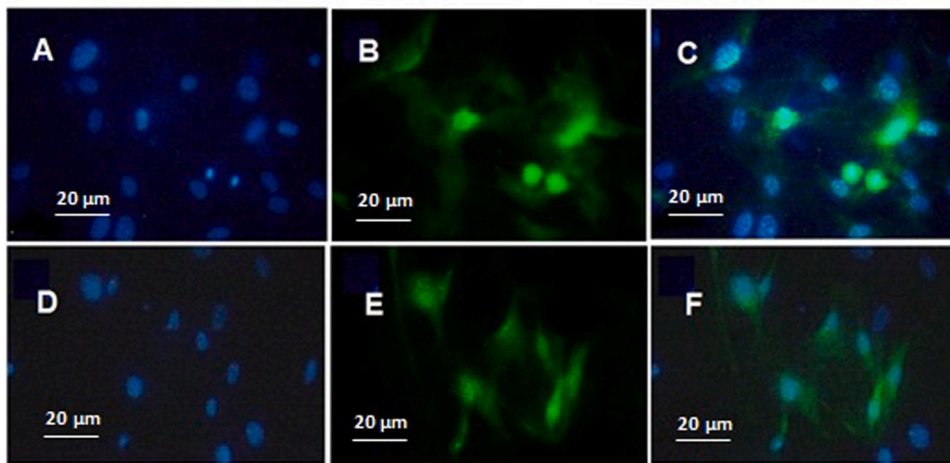


Fig. 2. The BMMSC differentiation to neuron. The most BMMSCs differentiation to neuron showed in sinusoidal 50 Hz EMF group compare to sham. The results represent three wells mean $\pm$ SD (* $P < 0.05$). Fluorescence microscopy images of BMMSC cultured in sham (A-C) and sinusoidal 50 Hz EMF exposed (D-F) groups. Immunocytochemistry staining (Blue indicates nuclei and Green denotes cell cytoplasm), morphology characteristics confirmed BMMSC differentiation to neuron. (x 400).



50, and 75 Hz, was 23.33 ± 1.53 , 36.67 ± 2.89 , 62.67 ± 6.81 respectively. Hence, change of EMF wave-form (square and sinusoidal) had no effect on BMMSC differentiation to astrocyte cell. Groups with 25 Hz EMF treatment had minimum differentiation to neuron and astrocyte cells. Astrocyte/neuron ratio density after BMMSC differentiation represented in Fig. 4. The astrocyte/neuron ratio in the groups of sinusoidal 25 Hz, sinusoidal 75 Hz, and square 75 Hz was 1.07, 1.22, and 1.45 respectively. Then, The neuron/astrocyte ratio in the groups of sinusoidal 25 Hz, sinusoidal 75 Hz, and square 75 Hz was 0.93, 0.82, and 0.69 respectively and lower than that of sham group.

After staining of cell nucleus with DAPI dye, sham-exposed cells had a pale multifaceted cytoplasm, un-recognizable appendages, and central round nucleus. In BMMSCs treated with 50 and 75 Hz sinusoidal EMFs the cell cytoplasm was shown multifaceted, recognizable appendages and round central nucleus. On the other hand, exposed cells to square waveform EMF had an ellipsoid nucleus pushed to side part of multifaceted, recognizable appendages cell cytoplasm (Fig. 5). The cells morphology studied by fluorescent microscope and after imaging with digital camera, diameter of nuclei by Motic image 2.0 plus software calculated and compared.

4. Discussion

We explored the neurogenic potential of BMMSC by using 400 μ T EMF (different frequencies and current waveforms). The findings of this study showed that EMF reduced the BMMSC viability rate. These findings are in agreement with investigation of other researchers and it is imaginable that cell viability decreases because of apoptosis and

necrosis [11–13]. After expose of external EMF, membrane gated electrosensitive channels irregularly and disrupt cell functions [14]. Markhova et al. reported transmembrane K^+ influxes changes during the growth of stem cell culture and suggested that cell K^+ could also be important for successful cell proliferation [15]. In addition, Grassi et al. found that human neuroblastoma cells exposure to 50 Hz EMF increased cell proliferation. He concluded that enhanced expression of voltage gated Ca^{+2} channels in EMF exposed cell membrane and following increase in Ca^{+2} influxes are responsible for the cell proliferation and apoptosis [16]. The findings of present study showed that EMFs declined the BMMSCs proliferation rate in the sinusoidal 25 Hz, square 50 Hz and sinusoidal 75 Hz groups' exception to the 75 Hz square wave. In agreement, researchers like Wu et al. (50 Hz, 2 m T), Cheng et al. (50 Hz, 1.4–2.2 m T), and Safari et al. (50 Hz, 400 μ T) expressed a decrease in cell proliferation [6,9,17]. While, investigators as an example Liu et al. (10 Hz, 1 m T), Sun et al. (15 Hz, 1.8 m T), Zhang et al. (50 Hz, 5 m T), and Shahbazi et al. (50 Hz, 0.5–1 m T) reported a rise in cell proliferation [11,18–20]. In another study, exposure to EMF at 50 Hz increased the proliferative response compared to exposure at 1 and 10 Hz. This inconsistency could also be reason to the two main parameters; a: biological system sensitivity to EMF exposure [21], and b: different specifications of EMF (magnetic flux density, waveform, frequency, and duration of exposure). However, The BMMSCs are the prominent cell line in the mentioned studies and then, biological system sensitivity to EMF exposure may be equal. Beside, dissimilar frequencies were applied in these observations and rise or decline in proliferation rate wasn't a correlation to frequency. Hence, in the present study, we assessed the effect of 400 μ T EMF in one sort of cells (BMMSCs) viability

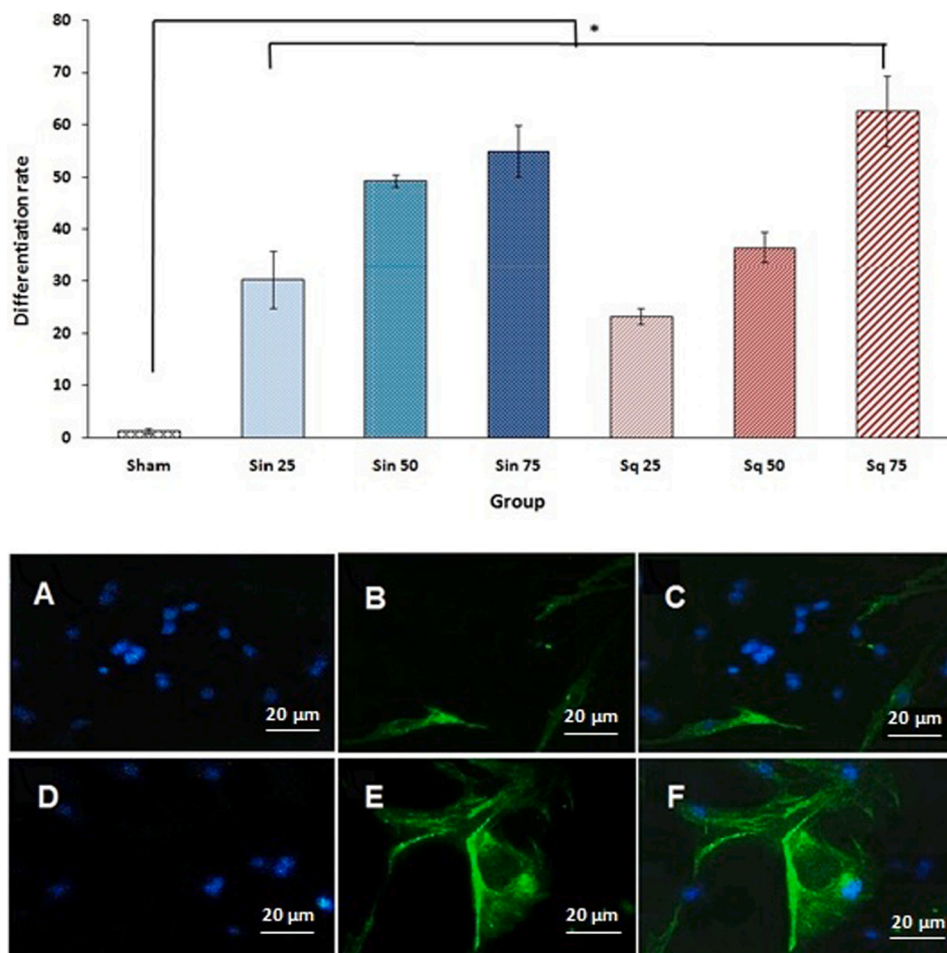


Fig. 3. The BMMSC differentiation to astrocyte cell. The maximum BMMSCs differentiation occurred in square 75 Hz. The results represent three wells mean $\pm$ SD (* $P < 0.05$). Fluorescence microscopy images of BMMSC cultured in sham (A-C) and square 75 Hz EMF exposed (D-F) groups. Immunocytochemistry staining (Blue indicates nuclei and Green denotes cell cytoplasm), morphology characteristics confirmed BMMSC differentiation to astrocyte. (x 400).

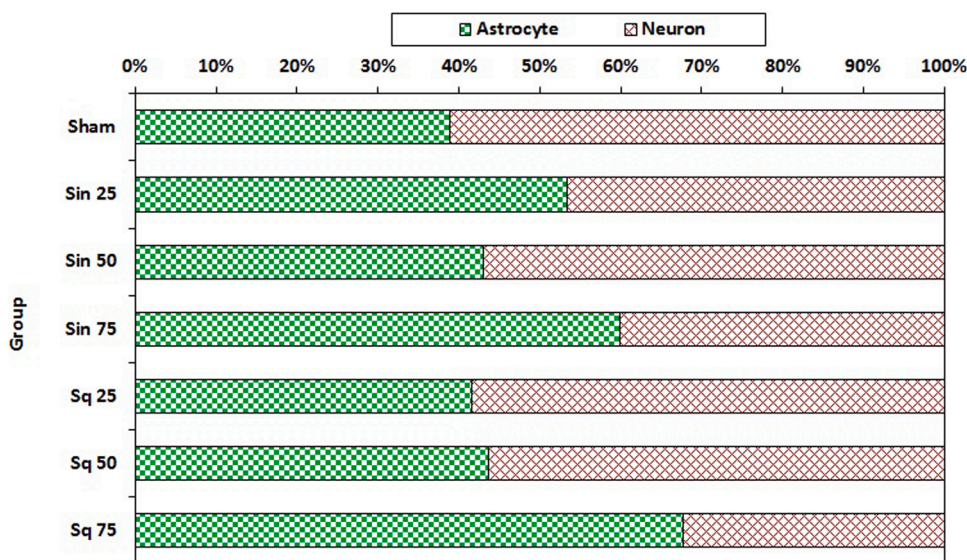


Fig. 4. Astrocyte/neuron ratio after BMMSC differentiation. The astrocyte/neuron ratio in the groups of sinusoidal 25 Hz, sinusoidal 75 Hz, and square 75 Hz was 1.07, 1.22, and 1.45 respectively.

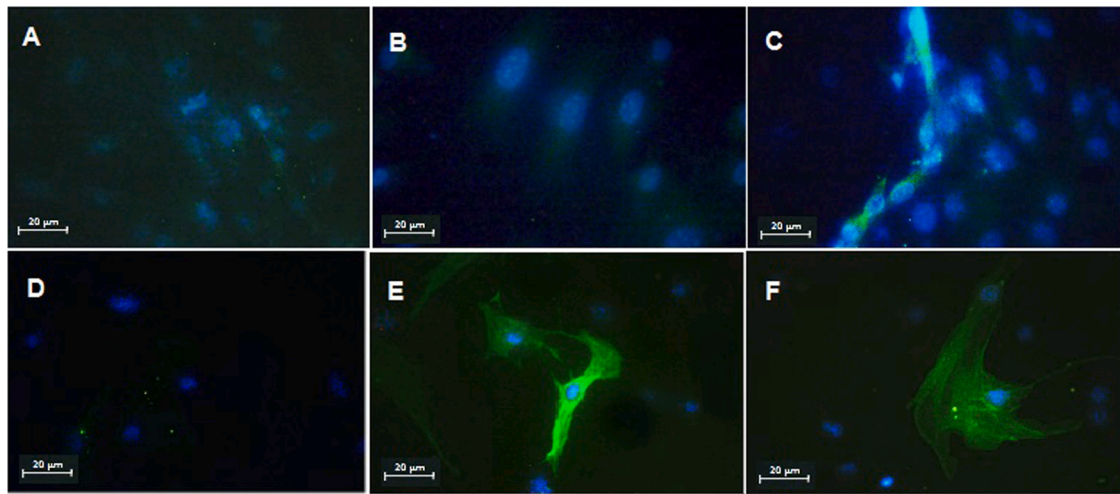


Fig. 5. Morphology of BMMSCs after immunocytochemistry staining of neuron (A-C) and astrocyte (D-F) cells: sham, sinusoidal, and square EMFs exposed respectively, (Blue indicates nuclei and Green denotes cytoplasm). (x 400).

and proliferation rate, and different attainment would be dependent to vary of current waveform or frequency.

The current research also examined the frequency-response relationship between EMF and BMMSCs differentiation. ICC staining confirmed maximum cell differentiation to neuron in square 25 Hz, sinusoidal 50 Hz, and square 50 Hz EMFs groups as the sham exposed, but the merely frequency that causes both increase in BMMSC proliferation and astrocyte differentiation was square 75 Hz. Besides, the astrocyte/neuron ratio or differentiation to astrocyte cells was frequency dependent and increased with the rise of frequency (25–75 Hz). These findings suggested that dissimilar frequencies of EMF could affect BMMSC differentiation differently. In the studies of Safari et al. (50 Hz, 400 μ T), Bai et al. (50 Hz, 5 m T), and Urnukhsaikhhan et al. (60 Hz, 10 m T) enhancement of BMMSC differentiation to neuron observed [6–8], but in the experiment of Barthelemy et al. the greatest increase was detected in differentiation to glial cell after exposure to 900 MHz EMF [10]. Astrocytes and neurons have a mutualize relationship to optimize neural information processing to modulate synaptic transmission and neuronal activity, and pro-inflammatory molecules released by astrocyte cells may affect these functions [10]. Astrogliosis aims at protecting the central nervous system against harmful processes as excitotoxic glutamate neurotransmitter excess, neuron lesions, oxidative stress, and neuroinflammation [10].

Stem cells response to the EMF stimulation is dependent on their situation of differentiation and it may be that EMF moderates action of transcription factors and the level of cell cycle regulatory genes [18]. EMF could accelerate electron transfer and causes threaten the Hydrogen bonds of cellular macromolecules, which will lead to stimulation of transcription and protein expression. Consequently, genotoxic effects facilitated by indirect mechanisms as microthermal processes and generation of reactive oxygen species (ROS). Small amounts of ROS activate signaling cascades involved in growth and differentiation of many cell types. Additionally, EMF induction of stem cell differentiation into specific cell types promotes its application in central nervous system injuries [22]. The temporal mechanisms of the cell physical stimuli (as frequency and duration) to produce a positive effect on differentiation must be appropriate for each cell type and cell cycle. If the temporal component is not acceptable, it could have a negative effect on their differentiation [23]. However, EMF Parameters such as duration of exposure, flux density, waveform, and practice frequency in these procedures are dissimilar, and this would clarify most discrepancies and we cannot clearly conclude how the EMF affects stem cell differentiation. Of course, there are still ambiguities about the function of the EMF on the BMMSC proliferation and differentiation, and wish further investigate to

seek out the precise mechanisms.

5. Conclusion

In conclusion, we presented a low flux density EMF (400 μ T) to assess the potential of BMMSC proliferation and differentiation. The findings showed that EMF inhibited the BMMSCs viability and proliferation rate, while the results suggest that both square and sinusoidal EMFs could affect rats BMMSCs development and differentiation to neuron and astrocyte cells. Further studies for the effects of EMF with wider flux density and frequency on BMMSCs are recommended.

Declaration of Competing Interest

The authors declare no conflict of interest regarding the publication of this paper.

Acknowledgements

This work supported by Semnan University of Medical Sciences, Iran (1276). We would like to thank the Nervous System Stem Cells Research Center of Semnan University of Medical Sciences for cooperation and make available facilities to this work.

References

- [1] K. Kalra, P.C. Tomar, *Stem cell: basics, classification and applications*, *AJPCT* 2 (7) (2014) 919–930.
- [2] L.J. Spees, R.H. Lee, C.A. Gregory, Mechanisms of mesenchymal stem/stromal cell function, *Stem Cell Res. Ther.* 7 (2016) 125, <https://doi.org/10.1186/s13287-016-0363-7>.
- [3] J.E. Park, Y.K. Seo, H.H. Yoon, C.W. Kim, J.K. Park, S. Jeon, Electromagnetic fields induce neural differentiation of human bone marrow derived mesenchymal stem cells via ROS mediated EGFR activation, *Neurochem. Int.* 62 (2013) 418–424, <https://doi.org/10.1016/j.neuint.2013.02.002>.
- [4] C.L. Ross, M. Siriwardane, G. Almeida-Porada, C.D. Porada, P. Brink, G.J. Christ, B. S. Harrison, The effect of low-frequency electromagnetic field on human bone marrow stem/progenitor cell differentiation, *Stem Cell Res.* 15 (1) (2015) 96–108, <https://doi.org/10.1016/j.scr.2015.04.009>.
- [5] Y.K. Choi, D.H. Lee, Y.K. Seo, H. Jung, J.K. Park, H. Cho, Stimulation of neural differentiation in human bone marrow mesenchymal stem cells by extremely low-frequency electromagnetic fields incorporated with MNPs, *Appl. Biochem. Biotechnol.* 174 (2014) 1233–1245.
- [6] M. Safari, M. Jadidi, A. Baghian, H. Hasanazadeh, Proliferation and differentiation of rat bone marrow stem cells by 400 μ T electromagnetic field, *Neurosci. Lett.* 612 (2016) 1–6.
- [7] W.F. Bai, W.C. Xu, Y. Feng, H. Huang, X.P. Li, C.Y. Deng, M.S. Zhang, Fifty-Hertz electromagnetic fields facilitate the induction of rat bone mesenchymal stromal cells to differentiate into functional neurons, *Cytotherapy* 15 (2013) 961–970.

- [8] E. Urnukhsaikhan, H. Cho, T. Mishig-Ochir, Y.K. Seo, J.K. Park, Pulsed electromagnetic fields promote survival and neuronal differentiation of human BM-MSCs, *Life Sci.* 151 (2016) 130–138.
- [9] H. Wu, K. Ren, W. Zhao, B. Ge, S. Peng, Effect of electromagnetic fields on proliferation and differentiation of cultured mouse bone marrow mesenchymal stem cells, *J. Huazhong Univ. Sci. Technol. Med. Sci.* 25 (2) (2005) 185–187.
- [10] A. Barthélémy, A. Mouchard, M. Bouji, K. Blazy, R. Puigsegur, A.S. Villégier, Glial markers and emotional memory in rats following acute cerebral radiofrequency exposures, *Environ. Sci. Pollut. Res.* 23 (2016) 25343–25355, <https://doi.org/10.1007/s11356-016-7758-y>.
- [11] C. Liu, J. Yu, Y. Yang, X. Tang, D. Zhao, W. Zhao, H. Wu, Effect of 1 mT sinusoidal electromagnetic fields on proliferation and osteogenic differentiation of rat bone marrow mesenchymal stromal cells, *Bioelectromagnetics* 34 (2013) 453–464.
- [12] S. Tofani, D. Barone, M. Cintorino, M.M. de Santi, A. Ferrara, R. Orlassino, P. Ossola, F. Peroglio, K. Rolfo, F. Ronchetto, Static and ELF magnetic fields induce tumor growth inhibition and apoptosis, *Bioelectromagnetics* 22 (2001) 419–428.
- [13] J. Kaszuba-Zwoinska, P. Chorobik, K. Juszczak, W. Zaraska, P.J. Thor, Pulsed electromagnetic field affects intrinsic and endoplasmic reticulum apoptosis induction pathway in Monomac6 cell line culture, *J. Physiol. Pharmacol.* 63 (2012) 537–545.
- [14] D.J. Panagopoulos, L.H. Margaritis, The effect of exposure duration on the biological activity of mobile telephony radiation, *Mutat. Res.* 699 (2010) 17–22.
- [15] I.I. Marakhova, A. Domnina, A. Shatrova, A. Borodkina, E. Burova, N. Pugovkina, V. Zemelko, N. Nikolsky, Proliferation-related changes in K⁺ content in human mesenchymal stem cells, *Sci. Rep.* 9 (2019) 1–11, <https://doi.org/10.1038/s41598-018-36922>.
- [16] C. Grassi, M. D'Ascenzo, A. Torsello, G. Martinotti, F. Wolf, A. Cittadini, G. B. Azzena, Effects of 50 Hz electromagnetic fields on voltage-gated Ca²⁺ channels and their role in modulation of neuroendocrine cell proliferation and death, *Cell Calcium* 35 (2004) 307–315.
- [17] G. Cheng, K.Z. Chen, J. Li, Z. Zhou, M. Wei, H. Zhao Bai, Enhancement of osteoblastic differentiation of bone marrow mesenchymal stem cells in rats by sinusoidal electromagnetic fields, *J. Biomed. Eng.* 28 (2011) 683–688.
- [18] L.Y. Sun, D.K. Hsieh, P.C. Lin, H.T. Chiu, T.W. Chiou, Pulsed electromagnetic fields accelerate proliferation and osteogenic gene expression in human bone marrow mesenchymal stem cells during osteogenic differentiation, *Bioelectromagnetics* 31 (2010) 209–219.
- [19] M. Zhang, X. Li, L. Bai, K. Uchida, W. Bai, B. Wu, W. Xu, H. Zhu, H. Huang, Effects of low frequency electromagnetic field on proliferation of human epidermal stem cells: an in vitro study, *Bioelectromagnetics* 34 (2013) 74–80.
- [20] D. Shahbazi-Gahrouei, S. Razavi, M. Salimi, Effect of extremely low-frequency (50 Hz) field on proliferation rate of human adipose-derived mesenchymal stem cells, *J. Radiobiol.* 1 (2014) 31–37.
- [21] J. Czyza, T. Nikolova, J. Schuderer, N. Kuster, A.M. Wobus, Non-thermal effects of power-line magnetic fields (50 Hz) on gene expression levels of pluripotent embryonic stem cells- the role of tumour suppressor p53, *Mutat. Res.* 557 (2004) 63–74.
- [22] A. Maziarz, B. Kocan, M. Bester, S. Budzik, M. Cholewa, T. Ochiya, A. Banas, How electromagnetic fields can influence adult stem cells: positive and negative impacts, *Stem Cell Res. Ther.* 7 (2016) 54, <https://doi.org/10.1186/s13287-016-0312-5>.
- [23] D. Rodriguez-Sanz, M.E. Losa-Iglesias, R.B. de Bengoa-Vallejo, P. Palomo-Lopez, C. Calvo-Lobo, D. Lopez-Lopez, How is cell proprioception related to cell growth and differentiation? Strong scientific evidence for future clinical activities, *Rev. Assoc. Med. Bras.* 64 (2018) 990–996.