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ORIGINAL ARTICLE

The effect of electromagnetic fields on survival and proliferation rate of dental pulp stem cells

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ABSTRACT

Aims: Extremely low-frequency electromagnetic fields (ELF-EMF) can affect biological systems and alter some cell functions like proliferation rate. Dental pulp tissue is known as a source of multipotent stromal stem cells (MSCs), which can be obtained by a less invasive and more available process compared to bone marrow-derived stem cells (BMSCs). This study aimed to consider the effect of ELF-EMF on proliferation rates of human dental pulp stem cells (hDPSCs).

Material and methods: ELF-EMF was generated by a system including autotransformer, multi-meter, solenoid coils, teslameter and its probe. The effect of ELF-EMF with the intensity of 0.5 and 1 mT and 50 Hz on the proliferation rate of hDPSCs was assessed in 20 and 40 min per day for 7 days. MTT assay and DAPI test were used to determine the growth and proliferation of DPSCs.

Results: Based on MTT, ELF-EMF has maximum effect with the intensity of 1 mT for 20 min/day on the proliferation of hDPSCs. The survival and proliferation rate in all exposure groups were significantly higher than the control group. Based on the data obtained from MTT and DAPI assay, the number of viable cells in the group exposed to 1 mT for 20 min/day was higher than other groups ($p < .05$).

Conclusions: Regarding to the results of this study, 0.5 and 1 mT ELF-EMF can enhance survival and proliferation rates of hDPSCs.

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KEYWORDS
Human dental pulp stem cells; extremely low-frequency electromagnetic fields; proliferation rate

Introduction

The effects of chronic exposure to electromagnetic fields on humans have been considered since long time. These fields can interfere in various biological functions, such as proliferation [1,2], morphology [3], apoptosis [4], gene expression [5] and cellular differentiation [6]. Multiple studies have investigated the biological effects of these fields on alveolar bone, gingiva and dental pulp [7]. Lew reported that, 0.4 Tesla static magnetic fields (SMFs) enhance dental pulp stem cell proliferation by activating the p38 mitogen-activated protein kinase (MAPK) pathway as its putative mechanism [8]. A study by Hsu et al. demonstrated that 0.29 T SMF does not influence the cell cycle and proliferation but enhances the osteogenic differentiation and mineralization of dental pulp cells [9]. A 1 mT SMF regulates proliferation, migration, differentiation and YAP/TAZ activation of human dental pulp stem cells (hDPSCs) [7].

Adult mesenchymal stem cells are considered as multipotent undifferentiated cells, which are able to self-renew and differentiate into several cell lineages, including adipocytes, chondrocytes and osteocytes [10]. Dental stem cells can be isolated from different dental tissues such as human dental pulp tissue (hDPSCs), human exfoliated primary teeth (SHED), the tissue of the apical papilla (SCAP) and periodontal ligament surrounding the roots of teeth (PDLSC) [10]. Due to easy surgical access, high proliferation and multilineage potential, hDPSCs are regarded as a promising model for tissue engineering and regenerative medicine [7] including the use of a controllable and manageable therapy for peripheral nerve injury [10] or their therapeutic efficacy on immune diseases [11].

Prompting normal pulpal physiologic functions including the continuation of root development and normal sensory perception, as well as improving apical and periodontium conditions are considered as the aim of regenerative endodontic procedures (REPS) [12]. These processes are based on stem cell [12]. To achieve this, it is necessary to proliferate and transfer these cells into the root canal space.

According to a study, in regenerative endodontics, the amount of viable and functional mesenchymal stem cells in the root canal space should be more than 700 times greater [13]. Moreover, the goal of all of the stages in regeneration therapy is to increase survival, proliferation and odontoblastic differentiation of these cells. For instance, these

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stages include irrigation of dentine with 17% EDTA [14,15], using scaffolds [16,17], using $Ca(OH)_2$ as an intracanal medication [18], and even using sealing materials such as MTA and Biodentin [19–21]. The chance of existence of enough DPSCc for regeneration treatment is decreased by time in aging patients [22–25]. According to the increase in the number of elderly people and advances in tooth regeneration methods and materials, we focussed on promoting the proliferation of human dental pulp stem cells (hDPSCs) to be applied in REPS using a physical agent (very low-frequency electromagnetic fields). This study aimed to investigate the proliferation and survival of hDPSCs by placing them in magnetic fields at much lower intensities than similar studies (2×). This reduction has the aim of applying these studies to clinical works so that it is possible to use it for clinical dentistry goals by designing a proper device for intraoral applications. Although this claim needs designing more accurate animal and clinical studies and this conclusion cannot be solely made based on this study, it provides preliminary information for forming the aforementioned hypothesis. Results of this study can pave the way for more collaborations between scientific and clinical institutions and industrial organizations.

Materials and methods

Magnetic field exposure system

A continuous sinusoidal 50 Hz magnetic field was generated by solenoid coils. The solenoid was wound with 720 turns of 1 mm enamel copper wire on a cylindrical core of acrylic tube (inner diameter: 20 cm, height: 24 cm). The solenoid was serially connected to an autotransformer with a voltage percent scale. The autotransformer was connected to 220 V power. The sinusoidal shape of signals to the solenoid was evaluated by an oscilloscope connected to the solenoid. The favourite flux density of the magnetic field was obtained by setting the voltage percentage scale of the autotransformer. The current and voltage to the solenoid for each flux density were assessed by a digital multi-meter (digital HiTESTER.3256-50, Japan) connected to solenoid. Calibration of the system and uniformity was done by a tesla-meter (LEYBOLD DIDACTIC GMBH 51662, Germany) with a probe AXIALE B-SONDE (model: 516.61). The uniformity of the EMF, at the centre of the solenoid, was ±1% where the cultures were located [26].

Culture of human dental pulp stem cells (hDPSCs)

hDPSCs, which were isolated exactly as in our previous study [27], were used in this study. Flow cytometric analysis was used as an acceptable method for characterization of these cells [28–30]. These cells expressed strongly mesenchymal stem cell markers such as CD105, CD90, CD166 and CD73, and only less than 4% expressed endothelial-hematopoietic epitopes including, CD11b, CD34, CD133, CD64, CD106, CD31 and CD45 [25]. Osseo/odonto differentiation potential of these cells is evaluated in our different studies [31,32].

DPS-12 were cultured in Dulbecco's Modified Eagle Medium: Nutrient Mixture F-12 (DMEM/F12, INOCLON, Iran) with 20% foetal bovine serum (FBS, GIBCO, Thermo Fisher Scientific, Waltham, USA) and with 1% penicillin/streptomycin, at 37 °C in the atmosphere at 5% CO_2. At the 3rd passage, hDPSCs were trypsinized with 0.05% trypsin and harvested cells were resuspended in the DMEM/F12.

ELF-EMF exposure

DPSCs were grown to the 96-well plate at the density 5×10^3 cell/well and incubated for overnight. Then, the plates were exposed to the ELF-EMF with intensities of 0.5 and 1 mT for 20 and 40 min per day for 7 days. The exposed cells were in four groups with the different EMF doses. Group 1: 1 mT for 40 min/day, group 2: 1 mT for 20 min/day, group 3: 0.5 mT for 40 min/day and group 4: 0.5 mT for 20 min/day. The control groups were also placed in the turned off solenoid coils for 20 and 40 min/day. Conditions were the same for exposure and control groups. In all experiments, the plates were located at the centre of solenoid where the magnetic field was most uniform.

Cell viability assay

hDPSCs were cultured into 24-well plates at a density of 5 × 103 cells/well to evaluate cell viability. Cells were exposed to the ELF-EMF (0.5 and 1 mT) for 20 and 40 min per day for 7 days. After 7 days, cell viability was assessed through the addition of MTT reagent (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium; Sigma-Aldrich, USA) for 4 h. After the removal of supernatants, DMSO was added to the wells for 10 min. Finally, samples were read at 570 nm using a multi-well plate reader, ELx808 (Biotek Instruments, Winooski, VT, USA).

DAPI staining

To assess genotoxicity, hDPSCs were seeded into 24-well plates at a density of 50 × 104 cells/well. Cells were treated as above. On the 7th day, cells were fixed in 4% formaldehyde for 10 min. Thereafter, the cells, which had been added to the wells, were incubated with DAPI (40,6-diamidino-2-phenylindole, Sigma-Aldrich, USA). Finally, at least 10 photos of each sample were captured using Cytation™ 5 imaging reader (BioTek Instruments, Inc., Winooski, VT, USA) that 2000 cells were considered for the each treated condition and the number of micronuclei was determined using ImageJ software (National Institutes of Health, Bethesda, MD).

Statistical analyses

Statistical analyses were carried out using Prism software (version 6.0, GraphPad, San Diego, CA, USA). Data were represented as means ± SD. The statistical significance was determined using the one-way ANOVA followed by a *post*

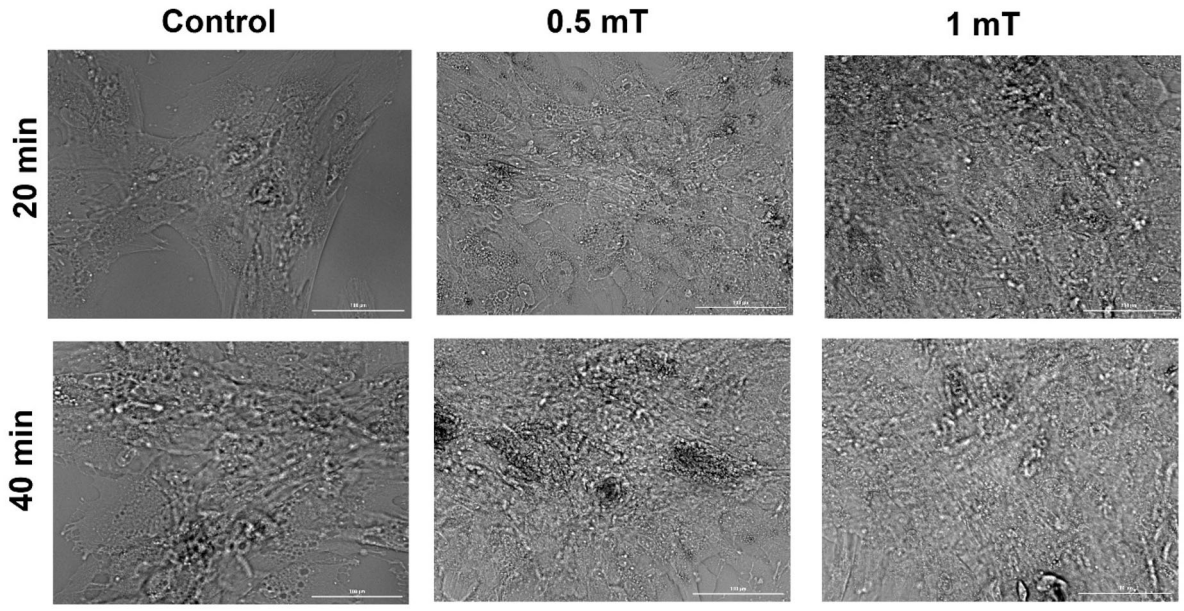

Figure 1. Morphology of exposure and control groups. Cells exposed to ELF-EMF (0.5–1 mT) have been extended on surfaces of the culture vessel, compared to control on the 7th day.

hoc multiple comparisons Tukey test. A p Value of less than .05 was considered statistically significant.

Results

ELF-EMF improves growth of hDPSCs

The DPS-12 have a very homogeneous morphological size in all experimental groups and are expanded with an excellent-expansion attached morphology. In the present experiment, we found that cells exposed to ELF-EMF (0.5–1 mT) have been extended on surfaces of the culture vessel as the fibroblastic cells, compared to control on the 7th day (Figure 1).

ELF-EMF is not cytotoxic and increases cell viability

Figure 2 shows the significant differences in DPSCs viability of groups that were exposed to 1 mT of ELF-EMF for 20 min/day compared to other treated groups and control after 7 days ($p < .05$). Also, all differences between the experimental and control groups were statistically significant ($p < .05$) except the group of 1 mT of ELF-EMF for 40 min/day ($p = .148$). The effects of ELF-EMF were not only toxic but also increased the number of cells in a time- and intensity-dependent pattern.

ELF-EMF induces proliferation

The number of nucleus was evaluated by the fluorescent microscopy using DAPI staining (Figure 3). DAPI staining shows that the nucleus in all groups was spherical or ovoid. The cells treated with ELF-EMF shows a slight rise in cell proliferation with fibroblastic morphology, a high confluence, and no different micronuclei formation after 7 days (Figure 3). The number of micronuclei in the group exposed to 1 mT of ELF-EMF for 20 min/day was higher than that in all the

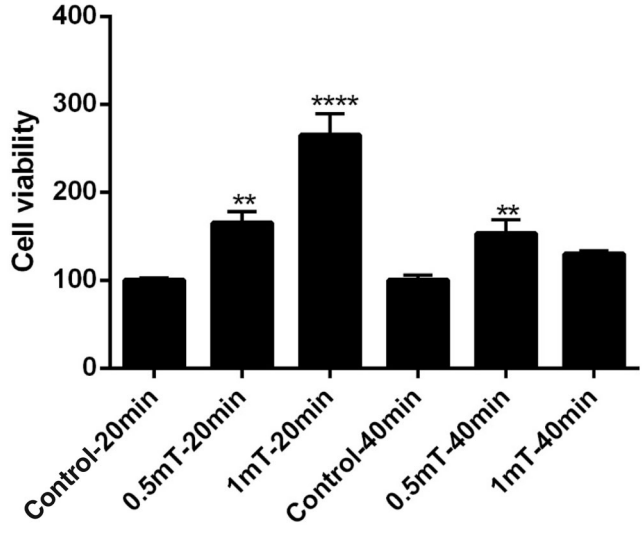

Figure 2. The effect of ELF-EMF on the viability of hDPSCs detected by MTT assay, showing significant differences in DPSCs viability of group exposed to 1 mT of ELF-EMF for 20 min/day compared to other treated groups and control groups after 7 days ($p < .05$).

treatments and the control groups (Figure 4, $p < .05$). Based on the data obtained from genotoxicity assay, there was a significant difference between the 0.5 mT group for 40 min/day with the control groups ($p = .032$) but no significant difference was observed in the groups that received ELF-EMF of 1 mT for 40 min/day with the control groups ($p > .05$).

Discussion

Dental stem cells benefit from the potential to multidifferentiate and are capable of raising at least three distinct cell lineages: osteo/odontogenic, adipogenic and neurogenic, due to a rapidly proliferating cell population compared to other tissue-derived stem cells [33]. The high rates of proliferation

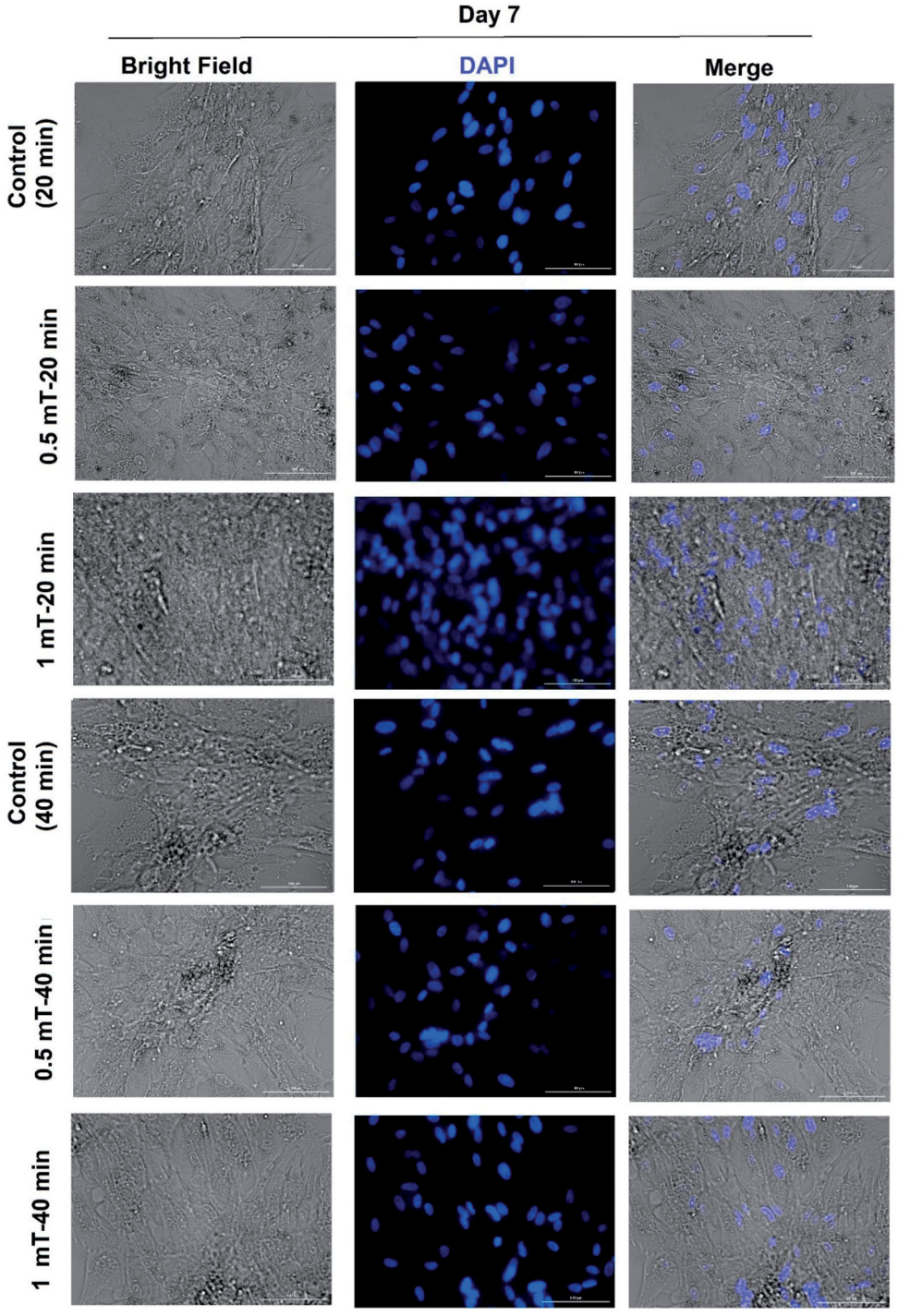

Figure 3. DAPI staining shows that the nucleus in all groups was spherical or ovoid. The cells treated with ELF-EMF shows a slight rise in cell proliferation with fibroblastic morphology, a high confluence, and no different micronuclei formation after 7 days.

as well as ready differentiation of hDPSCs are important factors to be selected as promising candidates in wide therapeutic applications [7]. It has been proved that different agents, such as biochemical and physical factors, can enhance growth and division in DPSCs [34,35]. Physical factors can induce growth factors in cell culture without adding biochemical factors such as magnetic materials [34,35] (e.g. SMF and ELF-EMF waves). So far, the previous studies in dental fields applied SMF, as a positive effect, on proliferation, cell membrane stability and the osteogenic differentiation and mineralization of hDPSCs [36]. SMF intensity of high value was used to study cell cycle and proliferation, which sometimes cannot alter cell growth but accelerate osteo/odontogenic differentiation [9]. The effect of SMF in low intensity on hDPSCs could induce proliferation during long time exposure to the magnetic field [7]. According to review of the literature, there are a few published papers and some accessible data relevant to the effects of ELF-MFs on different cells derived from tissue and cell-line. In addition, it seems that SMF is not a suitable magnetic tool for evaluating cell

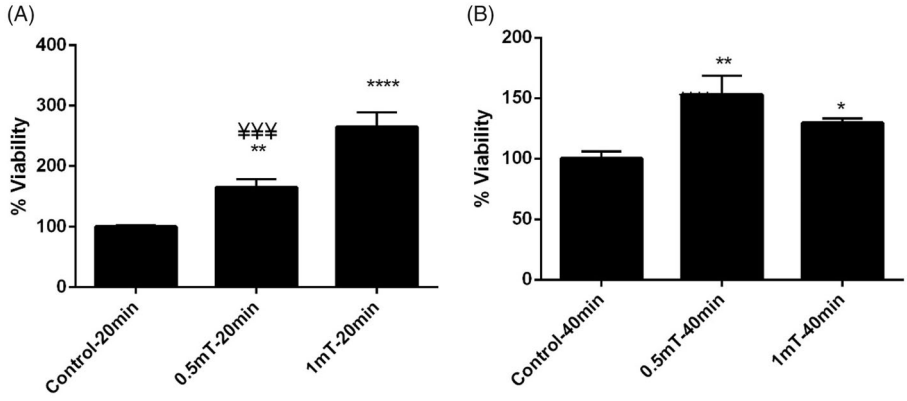

Figure 4. The effect of ELF-EMF on the genotoxicity of hDPSCs detected by DAPI assay. The number of micronuclei in the group exposed to 1 mT of ELF-EMF for 20 min/day was higher than that in all treatments and control groups ($p < .05$). Based on the data obtained from genotoxicity assay, there was a significant difference between the 0.5 mT group for 40 min/day with the control groups ($p = .032$) but no significant difference was observed in the groups received ELF-EMF 0f 1 mT for 40 min/day with control groups ($p > .05$).

proliferation due to the need for high exposure time, which may have an adverse effect on cells [7]. Hence, we studied the effect of ELF-EMS waves on hDPSCs proliferation of first time in low intensity and short time. ELF-EMS waves are a physical factor, its effect on cell proliferation are unclear [36]. In the present study, the effect of these fields with the intensity of 0.5 and 1 mT and power line frequency of 50 Hz was evaluated on survival and proliferation rate of DPSCs for 20 and 40 min for 7 days. Our results of cell viability showed that, the effect of ELF-EMS on cells can be effective to induce proliferation in short time with receiving more intensity. This finding was similar to Shahbazi-Gahrouei et al.'s study in 2013, it was concluded that ELF-EMF increases the survival and proliferation rate of adipose tissue-derived stem cells by 0.5 and 1 mT intensities [26]. In accordance with our study, hDPSCs received 1 mT from SMF source also had a significant increase in cell growth after 24 h [7]. Therefore, the intensity of 1 mT from different sources (e.g. SMF and ELF-EMF) on DPSCs can stimulate cell proliferation in various length of exposure time of magnetic field [7] and different techniques such as culturing DPSCs on titanium surface [37].

In 40 min/day of exposed to ELF-EMS, the cell survival in the group that received 0.5 mT was slightly more than one in the group that was given 1 mT. The cause of this issue may be the situation of solenoid coils that cannot simulate the cell culture incubator. Thus, the survival of cells can be decreased in group exposure to 1 mT for 40 min in comparison with other groups, due to locating a long time out of the incubator. Furthermore, Piacentini et al. reported that exposure to ELF-EMS (1 mT) stimulated growth in neural SCs in the first 6 h and by increasing exposure duration, the difference was decreased in the proliferation rate of neural SCs between the exposure and control groups. These finding proved that the response of biological systems to the ELF-EMS depends on certain density and exposure durations [38]. In contrast, some studies showed that these fields have an inhibitory effect on cell proliferation. For example, based on Yan et al.'s results, the 20 mT ELF-EMS effects on bone marrow-derived inhibited the proliferation rate [39]. Moreover, Zhou et al.'s study in 2011 proved that, SEMFs have a nonlinear effect on osteoblast proliferation and the effects of EMFs on osteoblast proliferation depend on the parameters applied [40]. It seems that, the different effects of ELF-EMS on cell proliferation could be related to the cell types in used studies and the variable parameters of the EMFs applied to the cells. Alterations in cell morphology, size and orientation can indicate cellular responses to ELF-EMS. As shown in this study, the hDPSCs has a very homogeneous morphological size in all experimental groups and proliferated with an excellent-expansion attached morphology. Also, based on the DAPI test, nucleus in all groups was spherical or ovoid and the cells treated with ELF-EMF shows a slight rise in cell proliferation with fibroblastic morphology. The mechanisms of ELF-EMS effects on cell pathway are not well known. It seems that, the SMFs can enhance the proliferation of DPSCs by activating the p38 MAPK pathway [8] or by charging molecules such as salic acid on the outer part of the cell [35]. Another hypothesis is that ELF-EMS is able to stimulate the activity of the calcium channel on the cell membrane. The influx of calcium ions through these channels plays an important role in the expression of specific genes involved in cell differentiation and proliferation [35]. This study has two main limitations; first, the mechanisms involved in increasing the proliferation of dental pulp-derived stem cells from ELF-EMS was not investigated, and second, we could not evaluate the effect of these fields on hDPSCs differentiation *in vivo*.

Conclusion

Generally, our results showed that 50 Hz, 0.5 and 1 mT magnetic fields can promote the survival and proliferation rates of the hDPSCs regarding the duration of exposure; although, the effective mechanisms in this process are still unknown. Further studies were suggested to evaluate the effect of magnetic fields with other intensities and duration on these cells.

Ethical approval

This article does not contain any studies with human participants or animal performed by any of the authors. For this type of study, formal consent is not required (Ethical code: IR.TBZMED.VCR.REC.1398.072).

Disclosure statement

No potential conflict of interest was reported by the author(s).

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