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Research Article

EFFECT OF MODERATE STATIC MAGNETIC FIELD ON HUMAN BONE MARROW MESENCHYMAL STEM CELLS: A PRELIMINARY STUDY FOR REGENERATIVE MEDICINE

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Abstract: Static Magnetic Field (SMF) is one of the biophysiological stimulants which modulates physiological processes in different cell lines. Mesenchymal stem cells (MSCs) are important biological tools for regenerative medicine. Although it is known that SMFs cause a change in cellular membrane polarization, oxidative product concentrations, gene expression patterns and cell propagation rates, depending on exposure time and intensity, their effects on MSCs have not been properly explained yet. In this study, MSCs derived from human bone marrow were treated with moderate 328 mT SMF by using cylindric Neodymium Iron Boron ($Nd_2Fe_{14}B$) magnets to investigate its influence on orientation, proliferation rates and morphologies. Results showed that the treated cells gained more homogenous orientation than the non-treated cells, however SMF influence did not significantly change proliferation rates.

The cells were grown under both chemically osteogenic induction and SMF to observe the osteogenic differentiation and biomineralization. Alkaline phosphatase (ALP) activity decreased significantly in the cells treated with SMF compared to the control groups. Alizarin Red S staining showed that mineralization also decreased in the cells. The results showed that an easily produced moderate SMF can be a useful physical stimulant to control the fate of MSC both *in vitro* and *in vivo*.

Özet: Statik Manyetik Alan (SMA), farklı hücre hatlarında fizyolojik süreçleri düzenleyen biyofizyolojik uyarıcılardan biridir. Mezenkimal kök hücreler (MKH'ler) rejeneratif tıp için önemli biyolojik araçlardır. SMA'ların yoğunluğuna ve süresine göre hücre membran polarizasyonunu, oksidatif ürün konsantrasyonlarını, gen ekspresyon modellerini ve hücre çoğalma oranlarını değiştirdiği bilinmesine rağmen, MKH'ler üzerindeki SMA etkileri henüz tam olarak açıklanmamıştır. Bu çalışmada, insan kemik iliği kaynaklı MKH'ler, silindirik Neodimyum Demir Bor ($Nd_2Fe_{14}B$) mıknatıslar kullanılarak orta derecede 328 mT SMA etkisinde bırakıldı ve hücrelerin oryantasyonu, çoğalma oranı ve osteojenik farklılaşma potansiyelleri incelendi. Sonuçlar, tedavi edilen hücrelerin, tedavi edilmeyen hücrelerden daha homojen bir yönelim kazandığını, ancak SMF etkisinin çoğalma oranlarını önemli ölçüde değiştirmediğini gösterdi.

MKH'ler, osteojenik farklılaşmayı ve biyomineralizasyonu gözlemlemek için hem kimyasal olarak osteojenik indüksiyon hem de SMA altında büyütüldüğünde, Alkalin Fosfataz (ALP) aktivitesi kontrol gruplarına kıyasla önemli ölçüde azaldı. Alizarin Red S boyaması, uyarılan hücrelerde mineralleşmenin de azaldığını gösterdi. Sunulan sonuçlar, kolayca üretilen orta düzeyde bir SMA'nın *in vitro* veya *in vivo* olarak MKH kaderini kontrol etmek için yararlı bir fiziksel uyarıcı olabileceğinin altını çizmektedir.

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Introduction

In medicine, morphological and functional repair techniques, as well as regeneration of damaged or aged cells, tissues, or organs, are rapidly growing. These approaches need available cell sources that provide appropriate new tissue components or paracrine effects. Due to this reason, stem cells have been gaining much attention for the last three decades (Rajabzadeh *et al.* 2019, Suman *et al.* 2019).

Stem cells are commonly characterized by their unspecialized nature and their high ability for self-renewal and differentiation into functional cell types of the organism. After the embryonic development stage, multipotent stem cells reside in numerous tissues of the body. Tissues conserve their continuity and regulate their micro-environment by the functions of these stem cells (Rajabzadeh *et al.* 2019, Suman *et al.* 2019).

A new type of multipotent stem cells derived from bone marrow was discovered by the pioneering studies of Friedenstein *et al.* in the 1960s. These cells adhere to plastic surfaces and have a fibroblastic appearance. Although Friedenstein called them osteoprogenitors, subsequent studies showed that they can differentiate into osteoblasts, chondrocytes and adipocytes, and regulate the hematopoietic microenvironment in the bone marrow. This special stem cell type is currently known as mesenchymal stem/stromal cells (MSCs) (Keating 2017, Trohatou & Roubelakis 2017).

Although MSCs were first isolated from bone marrow, they can also be isolated by less invasive techniques from adipose tissue, placenta, Wharton jelly, umbilical cord blood, and amniotic fluid. In addition to their high self-renewal and differentiation potential *in vitro* and *in vivo*, they have paracrine effects that promote immunomodulation in addition to anti-apoptotic and anti-oxidative effects (Brown *et al.* 2019). These properties of MSCs make them a good tool for regenerative medicine.

During embryonic development and through the entire life of an organism, cells are constantly exposed to a variety of mechanical stimulations, e.g., muscle force, gravity, blood flow and other physical forces or processes. The interactions between cells and mechanical or physical factors are critical to the health and function of various tissues and organs of the body and are believed that they have important roles in diseases, e.g., atherosclerosis, osteoarthritis and osteoporosis (Guilak *et al.* 2009).

A static magnetic field (SMF) is described as a constant, non-changing vector field of an electrical current or a permanent magnet. The SMF is also a force that interacts with biological systems (Lohmann & Lohmann 2019). Magnetic Resonance Imagining (MRI) presents one of the interaction examples between SMF and tissues, cells or biomolecules (Marycz *et al.* 2018). Magnetism and its effects on healing also have a place in both traditional and modern medicine (Markov 2007, 2015).

We aimed to seek interaction between bone marrow-derived mesenchymal stem/stromal cells (BM-MSC) and moderate SMF in this preliminary study.

Materials and Methods

Bone Marrow Mesenchymal Stem/Stromal Cells Cell Culture

Cryopreserved human BM-MSCs were purchased from Stem Cell and Gene Therapy Research and Application Center, Kocaeli University-Turkey. NutriStem Cell XF Basal Medium (Cat.# 05-200-1A, Biological Industries-USA) supplemented with NutriStem XF Supplement XF (Cat.# 05-201-1U, Biological Industries-USA) for BM-MSC propagation or Stempro Basal Medium (Cat.# A10069-01, Thermo Fisher-USA) supplemented with Stempro Osteogenesis Supplement (Cat.#A10066-01, Thermo Fisher-USA) for osteogenesis induction of BM-MSCs were used in cell cultures. The cells were incubated at 37 °C with 5 % CO_2 and 100 % humidity (Biosan ES20 incubator-Latvia) in all experiments.

Static Magnetic Field Source and Magnetic Flux Density (B)

Two cylindrical Neodymium-Iron-Boron ($Nd_2Fe_{14}B$) magnets with 22 mm diameters (Mıknatıs Teknik-Turkey) were used as SMF sources. The magnets were on top of each other underneath the 12-well plate (TPP-USA) (Figs 1a, b). Each of the magnets has enough surface area to cover one of the assay wells of 12-well plate (Fig. 1c). The diameter of each well in the 12-well plate was 21 mm. The magnetic field flux density or magnetic induction (B) produced by two magnets on top of each other was measured as 328 mT by AC/DC Magnetic Meter PCE-MFM 3000. B is the number of lines of force passing through a unit area of material (Stefanita CG 2012; Wills & Finch 2015). All control groups were grown in Earth's magnetic field.

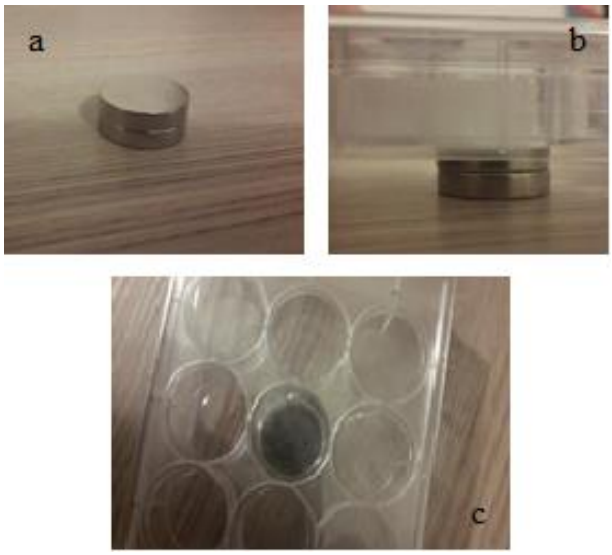

Fig. 1. Two cylindrical Neodymum-Iron-Boron ($Nd_2Fe_{14}B$) magnets provided the 328 mT SMF. **a)** All magnents were placed top on top, **b)** Assay wells of 12-well plates were put on surface of the magnets, **c)** Each of the magnets had enough surface area to cover interior surface of a well.

Determination of Cell Orientation under 328 mT SMF Influence

To observe and measure whether human BM-MSCs gain an orientation under 328 mT SMF, the cells in 12-well plates were grown in NutriStem Cell XF Basal Medium supplemented with NutriStem XF Supplement

XF with or without the SMF influence for 6 days. Two separate 12-well plates were used to minimize the magnetic exposure of the control group. On day 0, 10780 cells/well were plated into the three wells of each 12-well plates. One of the plates was used as the control and was not exposed to SMF. The second plate was placed on top of the $Nd_2Fe_{14}B$ magnets. On the 6th day, the cells were photographed under an inverted microscope with a camera attachment (Zeiss Axiovert A01-Germany). The camera was operated in 20x magnification.

All images were pre-processed with the open-source software ImageJ [http://imagej.nih.gov/ij/index.html] and a fully automated analysis of cell orientation was obtained utilizing the OrientationJ plugin.

Determination of Cell Proliferation and Growth under 328 mT SMF Influence

Two separate 12-well plates were used to minimize the magnetic exposure of the control group. On day 0, 10780 cells/well were plated into the three wells of each 12-well plates. One of the plates was used as the control without exposure to magnetic influence. The second plate was placed on top of the $Nd_2Fe_{14}B$ magnets. The cultures were fed with The NutriStem Cell XF Basal Medium supplemented with NutriStem XF Supplement XF. On the 6th day, the cell growth and propagation were measured after incubation for 3 hours with MTT (3-(4,5-dimethylthiazol-2-yl)- 2,5-diphenyltetrazolium bromide) salt (Cat# M2003, Sigma-USA). After the incubation, purple/blue formazan crystals were solubilized by the addition of 300 µL DMSO (dimethyl sulfoxide) (Cat# D2650, Sigma-USA). 100 µL solution was taken and measured at 570 nm and 650 nm in a spectrophotometric plate reader (BIOTEK-USA).

Determination of Biomineral Accumulation and Osteogenic Differentiation under 328 mT SMF Influence

To determine and measure biomineral accumulation and osteogenic differentiation, two control groups and two assay groups in separate 12 well plates were prepared. The cells were plated intro the three wells of each plates.. On day 0, 70000-80000 human BM-MSCs/ well were incubated in NutriStem Cell XF Basal Medium supplemented with NutriStem XF Supplement XF. On the 1st day, all medium was changed according to the experiment setting.

The 1st control group was the BM-MSCs which were incubated in NutriStem Cell XF Basal Medium supplemented with NutriStem XF Supplement XF. The 2nd control group was the BM-MSCs which were incubated in Stempro Basal Medium supplemented with Stempro Osteogenesis Supplement. The 1st assay group was the BM-MSCs that were incubated in NutriStem Cell XF Basal Medium supplemented with NutriStem XF Supplement XF under 328 mT SMF influence. The 2nd assay group was the BM-MSCs that were incubated in Stempro Basal Medium supplemented with Stempro Osteogenesis Supplement under 328 mT SMF influence. The experiment duration was 14 days in total.

Biomineralization was determined with Alizarin Red S (pH: 4.1-4.3) staining. On the 14th day, all medium was poured out. The cells were fixed by 500 µL 4 % buffered formalin solution for 30 minutes. After washing two times with dH_2O, 1 mL Alizarin Red S staining solution was added to every culture well and incubated for 20 minutes. After incubation, the control and assay wells were washed with dH_2O four times. Stained control and assay wells were photographed.

Osteogenesis was determined with Alkaline Phosphatase (ALP) activity. On the 14th day, all medium was poured out. The cells were lysed by two freeze and thaw cycles in phosphate buffer containing 1 % (v/v) Triton x-100. 1 mg/mL ALP substrate (p-nitrophenol phosphate, p-npp) (20-106, Sigma-USA) solution was prepared in a solution containing 1 M diethanolamine, 0.5 M $MgCl_2$ (pH 9.8). 75 µL cell lysate and 25 µL ALP substrate solutions were mixed and incubated for 20 seconds. ALP activity was measured at 405 nm by a spectrophotometric plate reader.

Statistical Analysis

An unpaired student t-test was used to determine the statistical significance of the differences between control and assay groups with $P<0.05$ accepted as significant.

Results

328 mT SMF Effects on Human BM-MSCs Orientation

The human BM-MSCs groups grown in wells without 328 mT SMF influence (Fig. 2a) and the human BM-MSCs groups grown in wells with 328 mT SMF influence (Fig. 2c) were photographed by 20x magnification after 6 hours of SMF exposure. OrientationJ, a plugin of ImageJ was used to infer the preferred orientation of structures in these images. OrientationJ computed histograms for the control groups (Fig. 2b) and the assay groups (Fig. 2d). The preferred orientation in Fig. 2d was seen as a homogenous histogram with a peak. However, the control group revealed a heterogenous histogram with multiple peaks (Fig 2b).

OrientationJ was also used to produce an orientation map. The images shown in Fig. 2a and Fig. 2c were colored according to their local directionality. The multi-directionality in Fig. 2a was seen as a multi-chromic plane. However, increased mono-directionality was seen as a mono-chromic plane (Fig. 2c).

328 mT SMF Effects on Human BM-MSCs Proliferation and Growth

Cell viability and growth in both the control and assay groups were determined by the MTT analysis after 8 hours of SMF exposure. Cell count in the control and assay groups reached to mean 199300 cells /well and 253744 cells/well, respectively (Table 1). The difference between the groups was not statistically significant (two-tailed $P = 0.2055$).

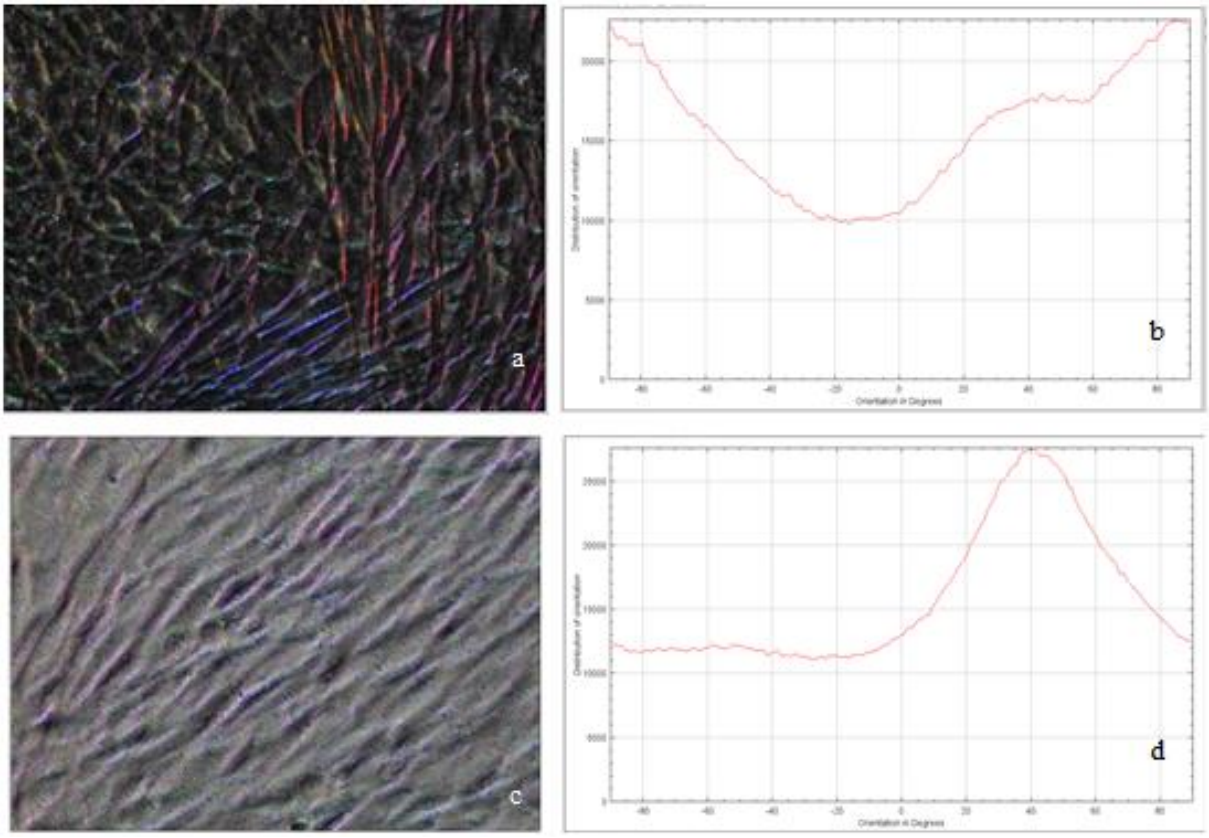

Fig. 2. 328 mT SMF effect on the human BM-MSC orientation. All microscopic photos were taken by 20x magnification. **a)** The cells grown as control group without artificial SMF influence. Multichromatic cells represent multi-directionality of the cells. **b)** shows the orientation, in degrees, of the cells in a. **c)** The cells were grown as assay group with artificial 328 mT SMF influence. Monochromatic cells irepresent mono-directionality of the cells. **d**) shows the orientation, in degrees, of the cells c.

Table 1. Compression of viable vell counts between the control and assay groups.

Control Groups (Without 328 mT SMF Influence)	Viable Cell Count/well	Assay Groups (With 328 mT SMF Influence)	Viable Cell Count/well
1st control well	134,300	1st assay well	256,633
2nd control well	213,800	2nd assay well	232,300
3rd control well	249,800	3rd assay well	272,300
Sample Number	**3**	**Sample Number**	**3**
Mean	**199,300**	**Mean**	**253,744**
Standard Deviation	**59,099.49**	**Standard Deviation**	**20,155.85**
two tailed P = 0.2055			

328 mT SMF Effects on Biomineral Accumulation and Osteogenic Differentiation of Human BM-MSCs

To observe the effect of SMF on osteogenesis, two different cell culture mediums i) Stempro Basal Medium supplemented with Stempro Osteogenesis Supplement, an osteogenesis-stimulating medium, ii) NutriStem Cell XF Basal Medium for proliferation were compared. The osteogenesis process of BM-MSCs under the 328 mT SMF was evaluated by measuring ALP activity and biomineralization.

ALP activity of the cells were compared on the 14th day of incubation in NutriStem Cell XF Basal Medium supplemented with NutriStem XF Supplement XF with or without continuous 328 mT SMF treatment. The mean values of ALP activities of the cells treated and non-treated by continuous 328 mT SMF for 14 days were 11.844 IU/L and 13.575 IU/L, respectively (Table 2). Statistical evaluation of the ALP activities between the groups showed that the effect of SMF exposure was not significantly different (two-tailed P= 0.1015).

Osteogenesis was induced using the Stempro Osteogenesis Differentiation Kit and ALP activities of the cells were compared after 14 days of incubation in Stempro Basal Medium supplemented with Stempro Osteogenesis Supplement with or without continuous 328 mT SMF treatment. The mean ALP activities of the cells treated and non-treated by continuous 328 mT SMF for 14 days were 31.367 IU/L and 25.966 IU/L, respectively (Table 3). Results showed that the difference between ALP activities of the two groups was statistically significant (two-tailed P = 0.0336).

Table 2. IU/ALP activities of human BM-MSCs were grown in NutriStem Cell XF Basal Medium supplemented with NutriStem XF Supplement XF without or with 328 mT SMF treatment.

1st control (Without 328 mT SMF Influence)	1st control IU/ALP Activity	1st assay (With 328 mT SMF Influence)	1st assay IU/ALP Activity
Control well	10.549	Assay well	13.216
Control well	11.701	Assay well	13.589
Control well	13.280	Assay well	13.920
Sample Number	**3**	**Sample Number**	**3**
Mean	**11.844**	**Mean**	**13.575**
Standard Deviation	**1.371**	**Standard Deviation**	**0.352**
two-tailed P = 0.1015			

Table 3. IU/ALP activities of human BM-MSCs were grown in Stempro Basal Medium supplemented with Stempro Osteogenesis Supplement without or with 328 mT SMF treatment.

2nd control (Without 328 mT SMF Influence)	2nd control IU/ALP Activity	2nd assay (With 328 mT SMF Influence)	2nd assay IU/ALP Activity
Control well	30.709	Assay well	26.517
Control well	32.768	Assay well	23.051
Control well	30.624	Assay well	28.331
Sample Number	**3**	**Sample Number**	**3**
Mean	**31.367**	**Mean**	**25.966**
Standard Deviation	**1.214**	**Standard Deviation**	**2.682**
two-tailed P = 0.0336			

Biomineralization was compared by Alizarin Red S staining at the end of the 14th day of incubation in NutriStem Cell XF Basal Medium supplemented with NutriStem XF Supplement XF with or without continuous 328 mT SMF treatment. No difference was observed in staining patterns between the control and assay groups (Fig. 3a, b). When biomineralization was compared by Alizarin Red S staining in Stempro Basal Medium supplemented with Stempro Osteogenesis Supplement with or without continuous 328 mT SMF treatment, the results showed that the staining in the groups treated continuous 328 mT SMF for 14 days was less than the groups grown without 328 mT SMF influence (Figs 4a, b).

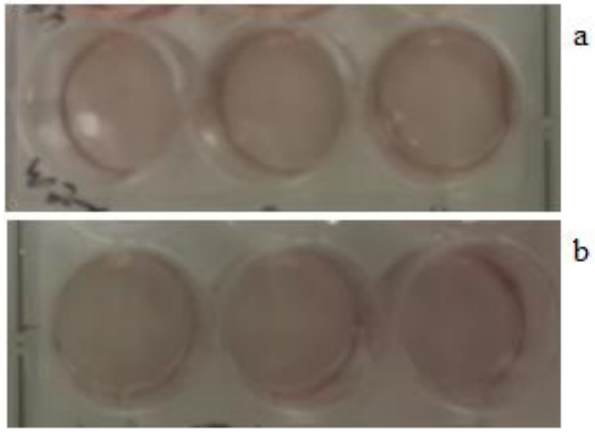

Fig. 3. After Alizarin Red S staining, comparison of the 1st control and 1st assay. **a)** 1st control, human BM-MSCs were grown in NutriStem Cell XF Basal Medium supplemented with NutriStem XF Supplement XF without 328 mT SMF Treatment, **b)** 1st assay, human BM-MSCs were grown in NutriStem Cell XF Basal Medium supplemented with NutriStem XF Supplement XF with 328 mT SMF treatment.

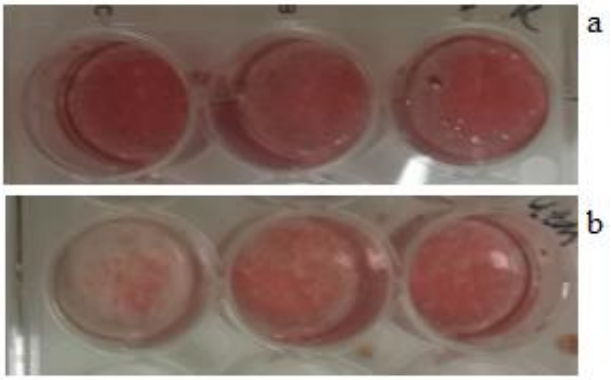

Fig. 4. After Alizarin Red S staining, comparison of the 2nd control and 2nd assay. **a)** 2nd control, human BM-MSCs were grown in Stempro Basal Medium supplemented with Stempro Osteogenesis Supplement without 328 mT SMF treatment, **b)** 2nd assay, human BM-MSCs were grown in Stempro Basal Medium supplemented with Stempro Osteogenesis Supplement with 328 mT SMF treatment.

Discussion

A natural magnetic field (geomagnetic field) or an artificial magnetic field is a physical parameter of the environment just as temperature, humidity, or altitude. Magnetic fields impact biological or organic systems as well as inorganic systems or matters. These influences are determined by magnetic susceptibility of the objects, magnetic field intensity or magnetic field flux density, and gradient. However, the kind of a magnetic field source (an electrical flux or a permanent magnet) has no special effect on the results. Salmons and turtles determine their natal homing behavior by the geomagnetic changes or intensities (Lohmann & Lohmann 2019). Cells can give responses to any magnetic field with their various structures or biomolecules (membranes, mitochondria, nucleic acids, and proteins) (Zhang *et al.* 2017a). In this

preliminary study, $Nd_2Fe_{14}B$ magnets easily produced a SMF. In the SMF, the magnetic field intensity or magnetic field flux density does not change according to time. The geomagnetic field and magnetic field used in Magnetic Resonance Imagining (MRI) are the SMF. It is a more suitable magnetic field with less changeable parameters to observe its influence or effects on biological systems. The SMFs are classified according to their magnetic flux density (B) as weak (<1 mT), moderate (1 mT-1 T), strong (1 T-5 T), and ultrastrong (>5 T) (Zhang *et al.* 2017b). Although there is substantial evidence on the biological effects of moderate SMF, the results of the effects are controversial, and the mechanism of the effects are still not clear. In this study, moderate (328 mT) SMF effects on cells were evaluated.

Mesenchymal stem/stromal cells (MSCs) with differentiation ability to multiple into mesodermal cell types (osteoblast, chondrocyte, and adipocyte) and modulative secretome are the main tools for regenerative medicine and cell therapies (Fitzsimmons *et al.* 2018). Although the MSCs were isolated and propagated from various tissues or tissue areas including bone marrow, adipose tissue, dental pulp, placenta, Wharton Jelly, umbilical cord blood, and other perivascular areas with similar phenotypic characteristics and differentiation abilities, bone marrow-derived MSCs (BM-MSCs) are the most extensively studied one, especially for bone regeneration and osteogenesis.

The MSCs cultured *in vitro* can be chemically induced to differentiate to the bone and other mesodermal cell types. Common biochemical agents and growth factors for osteogenesis are dexamethasone, indomethacin, and Bone Morphogenic Proteins (BMPs). Tissue regeneration methods utilize these factors to produce tissue constructs *in vitro* that are ready for implantation *in vivo* and to reduce healing time. MSCs are also highly mechanosensitive *in vitro* and *in vivo*. A mechanical stimulation as tensile strain induces MSCs for osteogenesis and tendogenesis but inhibits adipogenesis. Other mechanical stimulations as hydrostatic pressure and compressive loading induce MSCs for chondrogenesis. Therefore, mechanical stimulations are also another effector for tissue regeneration methods to determine or modulate MSCs fate (Delaine-Smith & Reilly 2012). The SMF is one of the mechanical stimulants for cellular structures and electrochemical flux on cellular membranes. However, its effects on especially stem cell physiology are poorly discussed. If there are any SMF effects on stem cell fate, its degree, condition and mechanism should be determined by detailed studies (Marycz *et al.* 2018).

Murayama *et al.* (1965) were the first to report cell orientation under SMF influence. Deoxygenized sickled erythrocytes in a suspension gained a pendicular orientation under 0,35 T SMF influence. Kotani *et al.* (2002) observed MC3T3-E1 cells orientation toward magnetic field flux direction after constant 60 hours of 8 T SMF influence. Ogiue-Ikeda *et al.* (2004) showed that A7r5 cells (smooth muscle cell, spindle shape) were orientated after a 60 hour magnetic field (8T) exposure only when the cells were seeded with high cell density ($1x10^5$ cells/cm^2). On the other hand, when the cells were in confluent condition at the start point of the magnetic field exposure, the cells were not oriented. Sadri *et al.* (2018) showed that Wharton Jelly derived mesenchymal stem cells gain parallel orientation in 8 hours and 18 mT SMF influence. However, orientation in SMF influence depends on cell shape. For example, Human kidney HFK293 cells in 8 T SMF influence and Human glioblastoma cells 10 T SMF influence, which are both polygonal shaped cells preferred orientation was not observed. Also, cellular orientation in the SMF influence depends on magnetic flux density or magnetic intensity. This orientation tendency is produced by the SMF influence on non-global diamagnetic anisotropic particles or molecules. This creates a torque on these structures. This effect is especially seen in membrane proteins, microtubes and actin filaments (Zhang, *et al.* 2017b). In our study, we observed that human BM-MSCs in high density but still proliferating cultures gain orientation in moderate 328 mT SMF influence (Figs 2a-d). Although the floating cells as erythrocytes gain an orientation under a static magnetic field in few seconds, the time for adherent cells such as osteoblasts gaining an orientation is in 10 times longer. The starting cell density and assay duration give a chance for orientation under the static magnetic field exposure.

There are contradictory results about the SMF effect on cell proliferation and growth. Kim *et al.* (2015) observed an increased BM-MSC proliferation during 3 mT, 15 mT, and 50 mT SMF treatment for 1, 3, 5, 7, and 9 days exposures, respectively. Maredziak *et al.* (2017) also determined an increasing proliferation in adipose tissue-derived MSCs in 0,5 T SMF treatment for 7 days. However, Silva *et al.* (2018) observed decreased viability of mouse BM-MSC with or without magnetized nanoparticles in 0.3-0.45 T SMF treatment for 48 hours. Cunha *et al.* (2012) also observed decreased proliferation and growth rate of human osteoblast in 320 mT SMF treatment for 1, 3, and 7 days. Yamamato *et al.* (2003) observed an unchanged proliferation and growth rates of rat osteoblasts in 280 mT or 340 mT SMF treatment for 2, 4, 6, 8, and 10 days. We also observed an unchanged proliferation and growth rate of human BM-MSC in 328 mT SMF for 6 days (Table 1 and Fig. 3). Yamamato *et al.* (2003) explained the unchanged proliferation rates as an increased S phase but non-triggered G2/M transition.

A metalloenzyme Alkaline Phosphatase (ALP) is expressed in high concentrations in bone tissues and hydrolases phosphomonoesters. During biomineralization, ALP local concentration increases and triggers the process. ALP activity decreases and the bones become soft with insufficient biomineralization in a heredity hypo-phosphatase disorder (Golub & Boesze-Battaglia 2007). In this study, the ALP activity was compared between groups treated with or without

continuous 328 mT SMF for 14 days. It was observed that the ALP activity decreased in the groups treated with 328 mT SMF. Decreased biomineralization or staining with Alizarin Red S was also seen in the groups treated with continuous 328 mT SMF for 14 days. Wang *et al*. (2016) observed a decreased osteogenic differentiation in adipose tissue-derived MSCs treated with continuous 0.5 T for 7 days. Also, Yang *et al.* (2018) observed a decreased ALP activity and biomineralization in MC3T3-E1 cells treated continuous 200 mT SMF for 8 days. However, there are also contradictory results in this regard. Increased ALP activity and biomineralization in MC3T3-E1 cells treated with 16 T SMF were observed (Yang *et al.* 2018).

In conclusion, a static magnetic field is an easily obtainable and controllable physical stimulant for organisms and cells. Therefore, it can be an effective medical tool. MSCs have a main role in tissue regeneration and cell therapies by differentiation and paracrine effects. Their affectivity and distribution can be controlled by the SMF influence.

Ethics Committee Approval: Since the article does not contain any studies with human or animal subject, its approval to the ethics committee was not required.

Author Contributions: Design: E.O., Execution: K.M., Data analysis/interpretation: K.M., E.O., Manuscript writing: K.M., E.O.

Conflict of Interest: The authors have no conflicts of interest to declare.

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