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The effect of low static magnetic field on osteogenic and adipogenic differentiation potential of human adipose stromal/stem cells

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ABSTRACT

The aim of this work was to investigate the effects of static magnetic field (SMF) on the osteogenic properties of human adipose derived mesenchymal stem cells (hASCs). In this study in seven days viability assay we examined the impact of SMF on cells proliferation rate, population doubling time, and ability to form single-cell derived colonies. We have also examined cells' morphology, ultrastructure and osteogenic properties on the protein as well as mRNA level. We established a complex approach, which enabled us to obtain information about SMF and hASCs potential in the context of differentiation into osteogenic and adipogenic lineages. We demonstrated that SMF enhances both viability and osteogenic properties of hASCs through higher proliferation factor and shorter population doubling time. We have also observed asymmetrically positioned nuclei and organelles after SMF exposition. With regards to osteogenic properties we observed increased levels of osteogenic markers i.e. osteopontin, osteocalcin and increased ability to form osteonodules with positive reaction to Alizarin Red dye. We have also shown that SMF besides enhancing osteogenic properties of hASCs, simultaneously decreases their ability to differentiate into adipogenic lineage. Our results clearly show a direct influence of SMF on the osteogenic potential of hASCs. These results provide key insights into the role of SMF on their cellular fate and properties.

1. Introduction

Regenerative medicine is currently one of the fastest developing fields of medical science which offers advanced solutions for treating a number of diseases. The potential application of stem cell-based therapies for repair and/or regeneration of various tissues will most probably become an alternative for currently applied medical solutions. The essential components of cell based therapies that stimulate the body's own repair system involve the application of autologous multipotent stem cells (MSCs) [1–3]. The most often applied multipotent stem cells, both as a research tool and in clinical trials, are those isolated from bone marrow multipotent stem cells (BMMSCs) and of adipose origin multipotent stem cells (ASCs). Although both of the above mentioned stem cell populations exhibit many similarities, ASCs are a potentially better candidate for regenerative medicine [2,4–6]. This is because of their high viability and ease of obtaining in large quantities with little donor site related complications or patient discomfort. Cellular therapies based on ASCs have been shown to be both safe and effective in pre- and clinical studies – both in veterinary, as well as human medicine [7–9] These stem cells posses the unique ability to differentiate into chondrocytes, osteoblasts and adipocytes, and recently also their differentiation potential into neuronal precursor cells has been demonstrated [10,11]. Beside self-renewal and multipotency ASCs have been demonstrated to display immunomodulatory effects by

Abbreviations: SMF, static magnetic field; hASCs, human adipose derived mesenchymal stem cells; MSCs, multipotent stem cells; BMMSCs, bone marrow multipotent stem cells; ASCs, adipose origin multipotent stem cells; MVs, microvesicles; BMP-2, bone morphogenetic protein 2; Col-I, collagen type I; OPN, osteopontin; OCL, osteocalcin; ALP, alkaline phosphatase; HBSS, Hanks' balanced salt solution; DMEM, Dulbecco's modified Eagle's medium; FBS, fetal bovine serum; CFU-E, colony forming unit efficiency; SEM, scanning electron microscope; PF, proliferation factor; PDT, population doubling time

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interacting both with natural, as well as adoptive immune responses [12,13]. Additionally, several studies indicate the importance of efficient intercellular signaling, which might be crucial in the context of successful tissue regeneration. Cells communicate with each other via secreted membrane derived microvesicles (MVs) [14–16]. As it has been shown, ASCs release MVs, which contain a wide range of molecules, including cytokines, growth factors, and microRNA (miRNA). They are involved in the improvement of donor cells response, proliferation and differentiation potential [17,18].

In todays' world, musculoskeletal disorders are one of the leading causes of disability [19], especially in the ageing populations. Bone defects can arise from trauma, congenital malformations or diseases such as arthritis or osteoporosis [20,21]. The results obtained by several research groups, including ours, have confirmed the positive therapeutic effect of ASCs in skeletal tissue repair in an animal model [7,8,22]. The positive clinical results of ASCs in the field of musculoskeletal system disorders might result from their ability to differentiate into osteoblasts that produce specific proteins and deposit minerals [23,24]. Thus, searching for factors able to enhance cell viability seems to be essential when application of MSCs in the field of regenerative medicine is considered. In our previous studies we have demonstrated that ASCs proliferation rate and their population doubling time (PDT) are distinctly increased when treated with a static magnetic field (SMF) [25]. It was previously shown that SMF might affect the behavior of cells [26]. It is assumed that SMF might effect biological free radicals [27] as well as influence the concentration of Ca^{2+} [28,29] and filaments. Thus, SMF might play an important role in intracellular signaling [27,28], as well as intercellular communication [29], as well as intercellular communication, and also possibly alter cellular function [30]. Besides improving cell proliferation, SMF has an analgesic and anti-inflammatory effect [31]. Beneficial effects and simplicity of application make SMF a complementary tool, which may support stem cell-based treatment. Effects of SMF as an osteoinductive factor which can support differentiation of stem progenitor cells into mature osteoblasts are still a poorly studied subject. Thus, we were interested if SMF might enhance the osteogenic differentiation potential of human adipose stem cells (hASCs).

The aim of the present study was to determine the activity of hASCs cultured under SMF conditions, including analysis of their proliferation rate, morphology, ultrastructure and osteogenic properties on protein as well as mRNA level. We established a complex approach, which enables obtaining information about SMF and ASCs potential in the context of differentiation into osteogenic and adipogenic lineages.

2. Materials and methods

All procedures were performed in accordance with manufacturers' instructions.

2.1. Static magnetic field exposure

The exposure system used in this study was previously described by Marędziak et al. [25]. A pair of permanent neodymium magnets with a known magnetic polarization vector was used to expose the cells to SMF. The intensity of the obtained SMF was measured using a Hall sensor and reached 0.5 T. Cell culture plates were placed in the gap between the magnets, and the exposure system was placed in a 37 °C CO_2 incubator.

2.2. Isolation of hASCs

Subcutaneous adipose tissue was collected from 10 female healthy donors that underwent total hip arthroplasty. Each donor signed an informed consent form prior to inclusion into the study. This study was approved by the Local Bioethics committee of the Wroclaw Medical School (registry number KB-177/2014), and has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. Adipose tissue samples were placed in sterile Hanks' balanced salt solution (HBSS, Sigma Aldrich Germany) and preserved at 4 °C after surgery, until further mechanical processing. Isolation of adipose-derived mesenchymal stromal cells was performed under aseptic conditions using well established methods [32,33]. In order to isolate the cells, tissue samples were incubated with collagenase type I (1 mg/ml; Sigma Aldrich, Germany). Cells were harvested by centrifugation and plated in a 25 cm^2 flask with Dulbecco's modified Eagle's medium (DMEM)/Ham' s F12 medium, supplemented with 10% fetal bovine serum (FBS) and with 1% penicillin/streptomycin/amphotericin b solution. Cultures were maintained at 37 °C in a humid atmosphere with 5% CO_2. The medium was changed every three days until cells reached approximately 80% confluence. Adherent cells were detached from the flask using TrypLE™ Express (Life Technologies, Poland). Cells were passage three times before the tests.

Table 1
Sequences of qPCR primers used for the amplification of human mRNA.

Gene name	Primer sequentions 5′–3′	Amplicon size (bp)	Accession number
Col-I	F: GTGATGCTGGTCCTGTTGGT R: CACCATCGTGAGCCTTCTCT	123	NM_000088.3
OPN	F: AAACGCCGACCAAGGTACAG R: ATGCCTAGGAGGCAAAAGCAA	213	U20758.1
BMP-2	F: ATGGATTCGTGGTGGAAGTG R: GTGGAGTTCAGATGATCAGC	349	KC294426.1
ALP	F: CGCGCTTGTGCCTGGA R: CCTGCTTTATCCCTGGAGCC	185	XM_006710546

2.3. Cell culture

The following conditions were investigated: (i) MF−: cells maintained without exposition to magnetic field (control conditions). (ii) MF+: ASCs cultured with exposition to magnetic field (both cultured in growth medium). (iii) Osteogenesis MF− cells cultured in osteogenic medium without exposition to magnetic field (control). (iv) Osteogenesis MF+: cells maintained in osteogenic medium, exposed to magnetic field. (v) Adipogenesis MF−: cells cultured in adipogenic medium, not exposed to magnetic field (control). (vi) Adipogenesis MF+: cells maintained in adipogenic medium under magnetic field.

All cells untreated with magnetic field were regarded as control for each condition.

For viability 7-days test cells were cultured in DMEM/Ham' s F12 medium, supplemented with 10% FBS and with 1% penicillin/streptomycin/amphotericin b solution. Cells from third passage were used for experimental purpose. For the test, cells were seeded into 24-well plates suspended at a concentration of 30×10^3 cells per well.

For osteogenic and adipogenic differentiation experiments, hASCs were cultured in osteogenic and adipogenic medium (STEMPRO®, Life Technologies, Poland). The adipo- and osteostimulation of cells was maintained in 24-well plates and the cells were inoculated at concentration of 30×10^3 cells per well. The media were changed every two days. Adipogenic and osteogenic stimulation was conducted for 14 and 21 days respectively.