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The Effects of *Hypericum perforatum* L. on the Proliferation, Osteogenic Differentiation, and Inflammatory Response of Mesenchymal Stem Cells from Different Niches

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Keywords
Mesenchymal stem cell · Osteogenic differentiation · Migration · Niche · *Hypericum perforatum* L. · Bone marrow · Dental pulp

Abstract
The aim of this study is to demonstrate and compare the differentiation, proliferation, migration and inflammatory behavior of dental pulp- and bone marrow-derived mesenchymal stem cells (DP-MSCs and BM-MSCs) in response to a *Hypericum perforatum* ethanol extract. Using xCELLigence, a real-time monitoring system, a dose of 10 µg/mL was found to be the most efficient concentration for vitality. The IC50 values and doubling time were calculated. The results showed that *H. perforatum* L. was able to accelerate osteogenic differentiation in DP-MSCs, but calcium granulation was impaired in BM-MSCs. *H. perforatum* L.-induced migration increased when compared to the TNF-α-induced migration in a Transwell migration assay, and the IL-6 cytokine levels between cells also differed. It can be suggested that tissue memory is an important factor in MSCs, and that they differ in their response to external factors. In conclusion, *H. perforatum* L. can be considered an excellent osteoinductive agent for DP-MSCs but should not be used for BM-MSCs. Tissue-specific osteoinductive agents should be discussed in future studies.

Introduction

Mesenchymal stem cells (MSCs) can be considered a promising tool in regenerative medicine due to their high therapeutic potential in the treatment of degenerative and metabolic diseases [Kornicka et al., 2017]. MSCs are capable of self-renewal and differentiation into different lineages, including bone, cartilage, fat, tendon, muscle, and hematopoietic stroma [Deans and Moseley, 2000]. Cellular therapies involving MSCs require their isolation

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Abbreviations used in this paper

BM-MSCs	bone marrow-derived mesenchymal stem cells
DP-MSCs	dental pulp-derived mesenchymal stem cells
DT	doubling time
MSCs	mesenchymal stem cells
OCN	osteocalcin
ON	osteonectin
RTCA	real-time cell analyzer

mainly from bone marrow (BM), adipose tissue, the umbilical cord, or dental pulp (DP), and in vitro expansion for further autologous or allogenic transplantations, and these have the potential to differentiate into a wide variety of cell lineages (i.e., osteoblasts, adipocytes, chondrocytes, tenocytes, neurons and myocytes [Gronthos et al., 2000; Ding et al., 2011; Inoue et al., 2013]. Based on these advantageous properties, techniques to increase MSC proliferation and differentiation are under constant scrutiny. Extensive use of synthetic and semi-synthetic substances, i.e., the recombinant cytokines and growth factors currently used as proliferative and differentiation factors in stem cell therapy, particularly for bone regeneration, may lead to side effects and toxicity while also being exorbitantly expensive. Osteoinductive agents aim to stimulate seeded cell migration, proliferation, and migration, and modulate immune responses, involving the stimulation of MSCs and/or osteoprogenitors to differentiate into osteoblasts. There are many osteogenic proteins that stimulate the proliferation and differentiation of MSCs and/or progenitors in vitro and in vivo [Govender et al., 2002; Giannoudis et al., 2005; Ko et al., 2013]. However, they have a short half-life, and so require either high concentrations or sustained delivery for bone tissue engineering [Itoh et al., 2001]. That said, higher concentrations could lead to increased osteoclastic activity and bone resorption [Kaneko et al., 2000], and so it is apparent that alternative and natural ostoeinductive agents need to be identified.

Recent studies have shown that bioactive compounds, which occur naturally in seaweed, herbs, fruits, and vegetables, have the ability to modulate self-renewal and the differential potential of adult stem cells, targeting a broad range of intracellular signal transduction pathways [Kornicka et al., 2017]. There are a number of ongoing trials, aiming to find a herbal extract that is less toxic and more affordable as a natural therapy [Udalamaththa et al., 2016]. Promoting endogenous stem cell multipotency and differentiation potential may also support regenerative processes after MSC transplantation [Kornicka et al., 2017]. We suggest that natural ostoeinductive agents should be identified from MSCs of different origins if successful outcomes in bone regeneration are to be achieved.

Hypericum perforatum L., also known as St. John's wort [Oztürk et al., 2007], is known to have remarkable wound-healing and anti-inflammatory properties [Knüppel and Linde, 2004; Dole et al., 2015]. It is used to treat anxiety, depression, lacerations, burns, cancer, and bacterial and viral diseases, and is an antioxidant, analgesic, and neuroprotective agent [Rota et al., 2004]. Based on these data, we chose *H. perforatum* L. as a promising natural osteoinductive agent, and compared its effects on proliferation, differentiation, migration, and immune response in MSCs from different niches.

Materials and Methods

Extraction of Plant Samples

H. perforatum L., as naturally growing plants belonging to the Hypericaceae family, were purchased from a local market in Muğla, Turkey, and a voucher specimen (herbarium No: MUH 2796) was deposited in the Herbarium of the Faculty of Science, University of Muğla, Turkey. Air-dried plant samples were extracted with ethanol (Merck, Taufkirchen, Germany) using a Soxhlet apparatus, and the extracts were evaporated and stored in sterile opaque glass bottles under refrigerated conditions until use. The total hypericin amount in the ethanol extract was determined as 0.2 mg (Dr. Yılmaz Klinik, Kayseri).

Isolation and Culture of MSCs

Human DP tissue was obtained from patients (aged 15–20 years) who were undergoing extraction of their third molars for orthodontic reasons at the Department of Oral and Maxillofacial Surgery, Gazi University, Ankara, Turkey. All patients signed an informed consent form. After the tooth surfaces were disinfected (75% ethanol), the tooth was drilled, and the DP extracted gently with forceps. The extracted pulp tissue was rinsed in α-MEM supplemented with 2 nM L-glutamine, 100 U/mL penicillin, 100 μg/mL streptomycin, and 10% FBS (Invitrogen/GIBCO, Grand Island, NY, USA) (hereafter referred to as the MSC culture medium), after which it was minced into fragments 1–2 mm^3 in size. The tissue fragments were cultured on T75 plates (Nunc) in MSC culture medium at 37 °C in a humidified atmosphere containing 5% CO_2.

The human BM-MSCs were a kind gift from the Hacettepe University Center for Pediatric Stem Cell Research and Development. They were suspended in a concentration of 1×10^6 cells/mL in MSC culture medium, and the cultures were monitored regularly with an inverted microscope (Olympus CKX41, Tokyo, Japan). The MSC culture medium was changed every 3 days. After reaching 70–80% confluence, the cells were harvested with 0.05% Trypsin/EDTA (Sigma Aldrich, St. Louis, MO, USA) and subcultured for further experiments. The experiments were done on passage 2–3 cells.

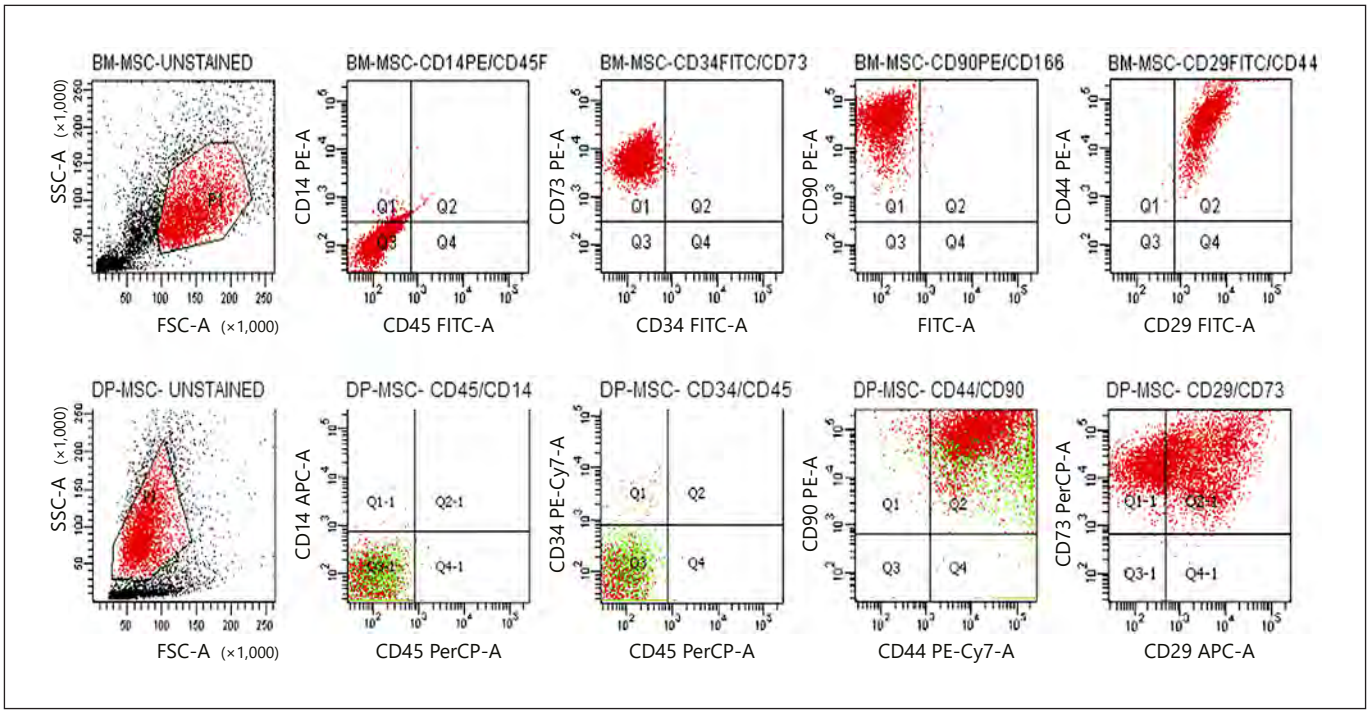

Fig. 1. DP-MSCs and BM-MSCs were identified as MSCs according to their expression of surface receptors. BM-MSCs and DP-MSCs were found positive for MSC markers (CD90, CD44, CD73, and CD29) and negative for hematopoietic stem cell markers (CD14, CD45, and CD34). Also, a subpopulation in DP-MSCs was found positive for MSCs and negative for hematopoietic markers.

Immunophenotypic Analysis

The culture-expanded adherent cells were analyzed on flow cytometry (BD FACSAria, USA), and the antibody panel included CD105-PE (eBioscience, USA); CD44-PE (eBioscience); CD90-PE (BD, USA); CD166-PE (BD); CD146-PE (BD); CD73-PE (BD); and CD29-FITC (eBioscience) as mesenchymal stromal markers, as well as their isotype controls. CD45-FITC (BD); CD14-PE (BD); HLA DR-FITC (Chemicon, USA); and CD34-FITC (BD) were used as hematopoietic markers to exclude any cells of hematopoietic origin. The relative frequencies of the cells that expressed the respective surface markers were analyzed using FACS Diva software v6.0.0 (BD-Biosciences, San José, CA, USA) by acquiring 10,000 events for each sample.

Effect of H. perforatum *L. on MSC Proliferation Using the xCELLigence System*

Initially, the proliferation of DP-MSCs in a 24-well culture microplate seeded at a density of 5,000 cell/cm^2 was examined. DP-MSCs were cultured in different concentrations (1, 3, 5, 10, 25, 50, 75, and 100 μg/mL) of *H. perforatum* L. up to the control group of 90% confluency. The cells were counted using the Trypan blue method (data not shown), and the 3 concentrations (i.e., 5, 10, and 25 μg/mL) that induced the cell number were selected for xCELLigence analysis. The xCELLigence system, made up of an impedance-based real-time cell analyzer (RTCA), an RTCA single plate (E-plate 96), an RTCA computer and a tissue-culture incubator, was used according to the manufacturer's instructions (Roche Applied Science, Mannheim, Germany) [Roche Diagnostics, 2008]. The E-plate 96 was connected to the xCELLigence system and verified in a cell culture incubator to ensure that proper electrical contacts were established. The background impedance was measured. Subsequently, 100 μL of MSC culture medium containing different concentrations of *H. perforatum* L. were added into each well of the E-plate 96, and the cells were resuspended (5,000 cells/cm^2) in MSC culture medium containing the appropriate concentration of *H. perforatum* L. Cell growth and proliferation were monitored every 30 min for up to 314 h for DP-MSCs, and 290 h for BM-MSCs.

Effect of H. perforatum *L. on MSC Differentiation*

The concentration that decreased the doubling time (DT) and increased the proliferation was selected based on the results of the xCELLigence system analysis. The selected concentration was added to the osteogenic and adipogenic differentiation media [Pittenger et al., 1999], and the secreted osteocalcin (OCN) and osteonectin (ON) levels in the supernatants were assessed using an ELISA kit, in line with the manufacturer's instructions (R&D Systems, Inc. Minneapolis, USA). The limits of detection for the ELISA were 1.2–75 ng/mL for OCN and 1.56–50 ng/mL for ON. The calcium ion concentration in the differentiation medium was measured using a QuantiChrom calcium assay kit according to the manufacturer's instructions (DICA 500, BioAssay Systems, Hayward, CA, USA).

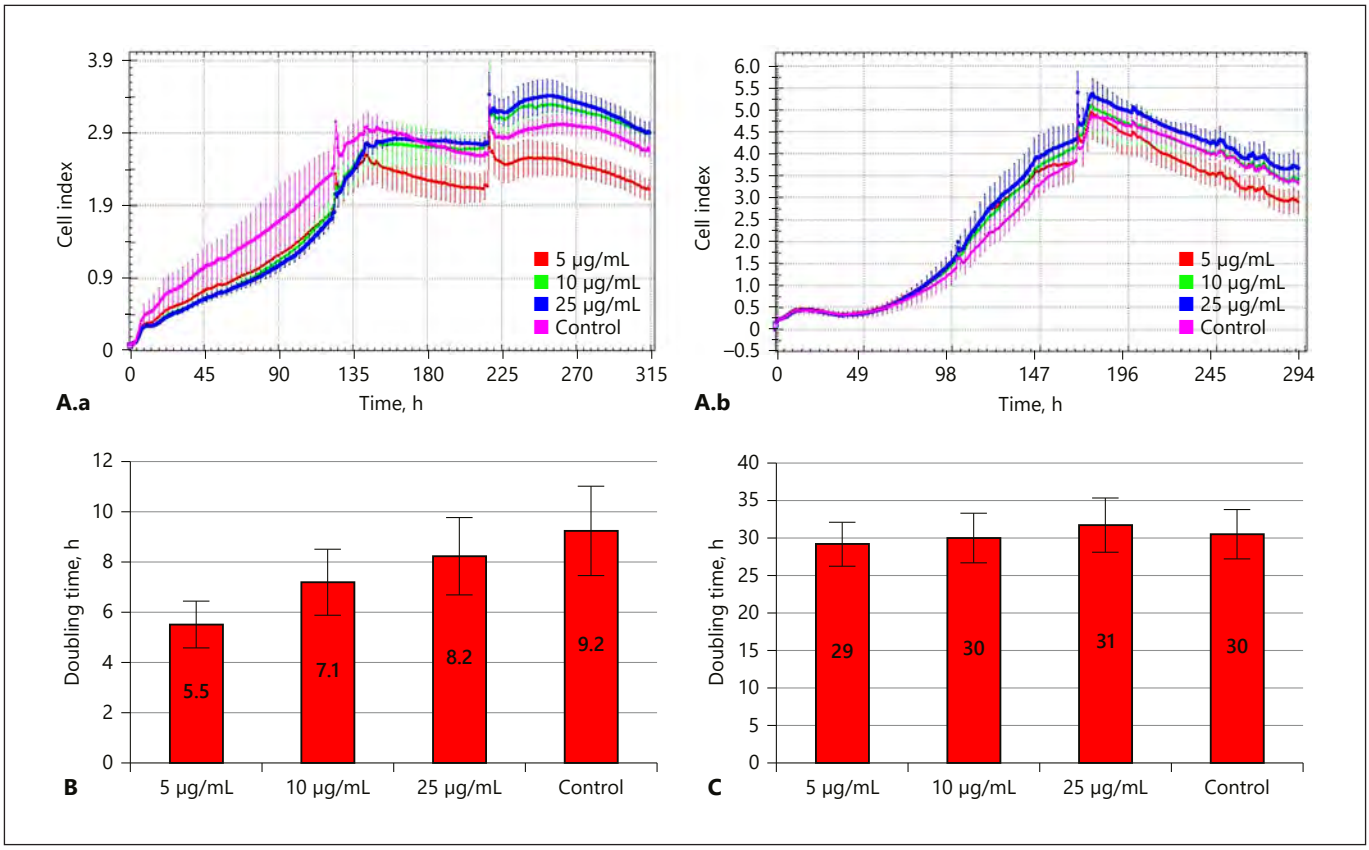

Fig. 2. Growth curve (**A**) and doubling time (**B**, **C**) graphs for DP-MSCs and BM-MSCs. **A.a** DP-MSCs showed a rapid logarithmic phase and *H. perforatum* L. increased proliferation. **A.b** BM-MSCs showed a slow proliferation rate. **B** Population doubling time was decreased with *H. perforatum* in DP-MSCs. **C** *H. perforatum* L. showed no effect on the population doubling time of the BM-MSCs.

Effect of H. perforatum *L. on MSC Migration*

To investigate the migration of DP-MSCs and BM-MSCs in response to TNF-α and the *H. perforatum* L. extract, we used a Transwell chamber assay in a 6-well microchemotaxis chamber with 8-µm pores (Corning Costar, USA). The upper chambers were loaded with 5×10^4 MSCs in 500 µL of MSC culture medium, and the MSC culture medium containing 10 µg/mL *H. perforatum* L. extract was placed in the upper chamber. After 48 h of incubation at 37 °C in 5% CO_2, the migrated cells were counted using a Trypan blue cell viability assay. Each experiment was performed in duplicate, and the means of the garnered data were recorded for statistical analysis. We used 10 ng/mL TNF-α (Invitrogen/GIBCO) as the control [Lopez-Ponte et al., 2007].

Effect of H. perforatum *L. on MSC Immunomodulatory Activities*

DP-MSCs and BM-MSCs were plated at a density of 5,000 cell/cm^2 on 96-well culture plates and allowed to attach overnight. The cells were pretreated with 10 µg/mL *H. perforatum* L. extract for 1 h, after which 10 ng/mL TNF-α (Invitrogen/GIBCO) was added. After 24 h, the cell culture supernatants were collected and stored at −80 °C for use in the IL-6 and IL-10 ELISAs, according to the manufacturer's instructions, and medium alone, with TNF-α and *H. perforatum* L. were included as controls.

Statistical Analysis

All calculations were carried out using the RTCA integrated software of the xCELLigence system, which fits the curve of the selected sigmoidal dose response equations to the experimental data points. Data are presented as mean (µg/mL) ± SD ($n = 4$). For the proliferation experiments, a statistical analysis was performed using ANOVA ($p < 0.05$).

Results

Identification of MSCs

The common MSC markers (CD90, CD44, CD29, and CD73) were constitutively positive (>92%) and the hematopoietic markers (CD14, CD45, and CD34) were negative (>97.9) in the tested BM-MSC samples, indicating a mesenchymal origin of the cells (Fig. 1). Interestingly, 2

Table 1. *H. perforatum* L. effect on DP-MSCs and BM-MSCs

	Group	DP-MSCs[a]	BM-MSCs[b]
Osteogenic differentiation			
ON, ng/mL	*H. perforatum*	82.11±0.02	89.11±0.01
	Culture medium	286.83±0.02	369.16±0.02↑
OCN, ng/mL	*H. perforatum*	20.24±0.06↑	89.11±0.03↑
	Culture medium	16.54±0.02	10.22±0.01
Calcium granule concentration, mg/dL	*H. perforatum*	13.57±0.02↑	7.83±0.02
	Culture medium	13.32±0.02↑	11.39±0.02
Migration			
Cells/well	*H. perforatum*	120,000±0.02↑	75,000±0.01
	TNF-α	18,000±0.01	18,000±0.01
	Culture medium	75,000±0.01	11,250±0.01
Immune response			
IL-6, pg/mL	*H. perforatum*	6.8±0.02	116.1±0.02↑
	H. perforatum + TNF-α	8.1±0.03	60.5±0.03
	TNF-α	37.3±0.03	137.1±0.03
	Culture medium	17.4±0.03	7.5±0.03
IL-10, pg/mL	*H. perforatum*	9.2±0.01	5.9±0.02
	H. perforatum + TNF-α	73.78±0.02↑	11.4±0.02
	TNF-α	8.0±0.01	7.2±0.01
	Culture medium	3.6±0.02	5.8±0.02

DM-MSCs, dental pulp-derived mesenchymal stem cells; BM-MSCs, bone marrow-derived MSCs; ON, osteonectin; OCN, osteocalcin; ↑, increased.
[a] IC50 value: 250 μg/mL; [b] IC50 value: 1,000 μg/mL.

subpopulations were identified in the flow cytometer analysis, suggesting that the DP has multiple stem cell niches.

xCELLigence Assays

The Trypan blue assay showed that concentrations of 1–25 μg/mL increased cell viability, while 50–100 μg/mL reduced viability, and 10 μg/mL was found to be the effective concentration (data not shown). Based on these findings, 5, 10, and 25 μg/mL were selected for the xCELLigence analysis system, and a cell proliferation graph, cell index, and DT values for DP-MSCs and BM-MSCs were produced (Fig. 2). The IC50 value was 250 μg/mL for DP-MSCs and 1,000 μg/mL for BM-MSCs (Table 1).

Differentiation Assay

The DP-MSCs did not undergo an adipogenic differentiation (Fig. 3A.a1, b1). Approximately 20% of the cells became rounder, but no lipid droplets were observed. In contrast to the adipogenic differentiation, the DP-MSCs underwent rapid osteogenic differentiation, with small, dark deposits becoming visible after 7–10 days, and increasing over the next several days of culture. Calcium mineralization was confirmed from positive Alizarin Red S staining on day 21 of the culture. The DP-MSCs that were treated with 10 μg/mL *H. perforatum* L. extract for 21 days exhibited calcium mineralization (Fig. 3B.a2, b2). Although ON and OCN levels were found in *H. perforatum* L.-treated BM-MSCs, the calcium mineralization was found to be reduced (Table 1). The levels of the ON and OCN osteogenic markers were also determined (Fig. 3B), and the data showed that the level of the early osteogenic marker (ON) decreased in the *H. perforatum* L.-treated group, whilst the levels of the late osteogenic marker (OCN) increased when compared to the control group.

Migration Assay

The spontaneous migration capacity of DP-MSCs and BM-MSCs in the presence of medium alone (without TNF-α or *H. perforatum* L.) was low, but it increased in the presence of TNF-α (Table 1), and *H. perforatum* L.

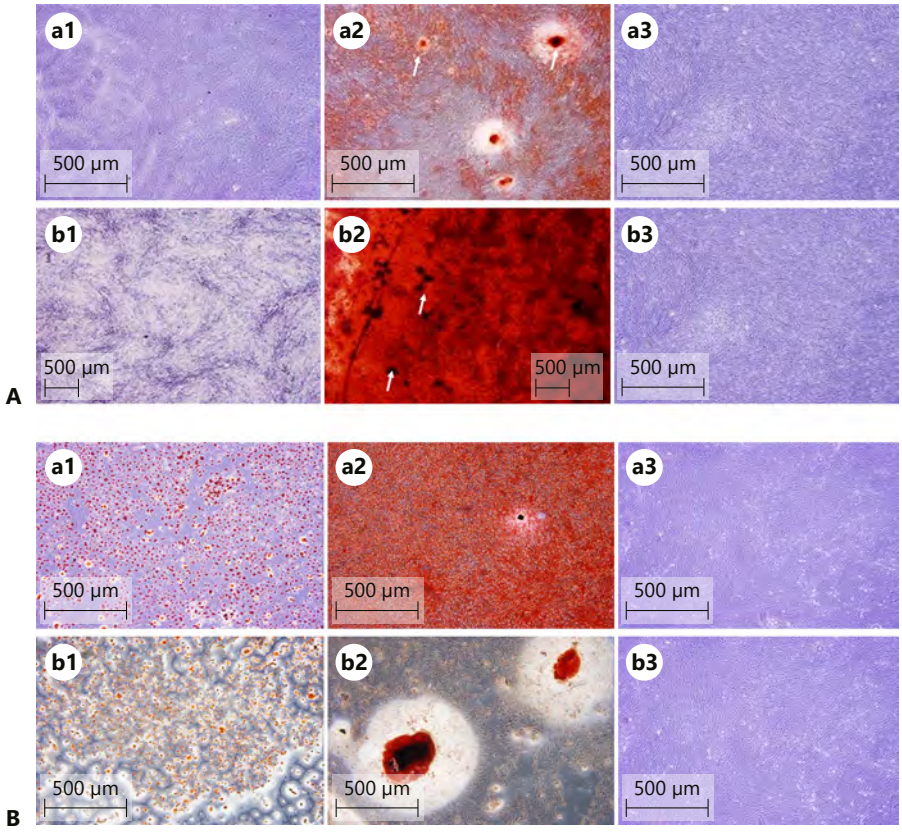

Fig. 3. I. Differentiation potential of DP-MSCs (**A**) and BM-MSCs (**B**). **A.a1**, **b1** Adipogenic differentiation was not seen in either the controls or in *H. perforatum* L.-treated DP-MSCs. The osteogenic differentiation of the DP-MSCs increased in the *H. perforatum* L.-treated group (**A.b**2) when compared to the control group (**A.a2**). ×4 (Olympus CKX41). White arrows show the calcium granules in the culture. **B.a1** BM-MSCs were well-differentiated into adipocytes in the control group. **B.b1** However, this was found to be decreased by *H. perforatum* L. **B.b2** Osteogenic differentiation was also decreased by *H. perforatum* L. **B.a1** However, large calcium granules were observed in the control group.

increased the migration of DP-MSCs 2-fold when compared to the control and the BM-MSCs.

Determining the Preventive Effect of H. perforatum *L. on the Inflammatory Response of MSCs*

Both IL-6 and IL-10 were present in the DP-MSC and BM-MSC cell culture supernatants (Table 1). Our findings showed that when used alone, *H. perforatum* L. reduced the IL-6 level in DP-MSCs, and, as expected, TNF-α increased the IL-6 level. *H. perforatum* L., was able to reduce the IL-6 level in DP-MSCs; however, in BM-MSCs, it increased the IL-6 level both when used alone and with TNF-α stimulation.

Discussion

Research into osteoinductive agents for bone tissue engineering is more likely focused on promoting cell proliferation, differentiation, and migration, and determining immune response. This study has shown that the functions of MSCs may vary according to their site of origin, even when they are treated with the same osteoinductive agent.

We demonstrated that DP-MSCs and BM-MSCs showed different population characteristics when the BM-MSC surface markers were taken as a reference. Interestingly, we identified 2 subpopulations of DP-MSCs in the flow cytometry analysis, which suggests the presence of multiple stem cell niches in the DP. This finding concurs with that of Pisciota et al. [2015], showing that the heterogeneity of the stem cell population within human DP, particularly its peculiar embryological origin, may explain the existence of 2 different subpopulations. Of course, further studies could better sort and analyze subpopulations to identify their differences and proliferation potencies.

To determine the *H. perforatum* L. effect, we first sought to define effective concentrations of *H. perforatum* L., selecting 5, 10, and 25 μg/mL for the xCELLigence analysis. This analysis allowed real-time cell proliferation monitoring, leading to a robust cell count, and we were also able to obtain and compare the growth curves of 2 cell types. Growth curves provide information about 3

parameters: the lag phase before cell proliferation is initiated after subculture, the DT in the middle of the exponential growth phase, and the terminal density. It was clear from the curves that the log phase of DP-MSCs was shorter than that of BM-MSCs. Cell indexes and proliferation graphs revealed that DP-MSCs entered the log phase earlier than BM-MSCs, while BM-MSCs showed increased proliferation at 180 h when compared with DP-MSCs, although the DP-MSCs were still alive in stationary phase at 314 h. We also calculated the DT at the mid-log phase of the cells. A shortened DT with *H. perforatum* L. (10 μg/mL) in DP-MSCs suggests a more rapid increase in cell proliferation, and the healing fracture or implant region could be shortened.

Oriental medicine practices are based primarily on personal experience, but often rely on unknown mechanisms, leading to difficulties in dose specification. The xCELLigence system is a sophisticated cell-based assay. It offers an alternative to commercialized conventional cell analyses, in which expensive agents are used, by simultaneous monitoring of cell proliferation and viability as well as label-free cytotoxicity [Hensten-Pettersen, 1988]. A lack of determination of concentrations can lead to conflicting reports. Demiroğlu et al. [2005] reported a rare case of BM necrosis in a patient who had been taking *H. perforatum* L. for the treatment of depression at a dose of approximately 1,000 mg/day for 3 weeks; this could be attributed to the usage of high concentrations of *H. perforatum* L. Since BM-MSCs line the marrow and are involved in hematopoiesis, high doses of extracts could lead to tissue necrosis. On the other hand, Knüppel and Linde [2004] reported that the available evidence suggests that *H. perforatum* L. is well-tolerated and safe if taken under the guidance of a physician who is aware of the potential risks under specific circumstances. In addition, Bray et al. [2002] administered extract of St John's wort at a dose of 140 mg/kg in their animal study. There is no recommended concentration of *H. perforatum* L., but we suggest that there should be a unique concentration if a clear conclusion is to be reached. We can conclude with confidence, based on the data obtained, that 10 μg/mL of *H. perforatum* L. protects cell vitality; accordingly, this concentration has been used in subsequent studies.

To demonstrate whether the extracts induce a differentiation of MSCs, we constructed an in vitro model in which lipid-laden adipocytes, calcium granules, and early and late markers of osteogenesis were examined after 21 days. No adipogenic differentiation was seen in DP-MSCs, while BM-MSCs were well-differentiated. Our findings concur with those of Gronthos et al. [2000], who expanded DP-MSCs from single-cell clones and demonstrated that these cells exhibit osteogenic differentiation, but do not form lipid-laden adipocytes. A reduced adipogenic differentiation was seen in a microscopic analysis of *H. perforatum* L.-treated BM-MSCs. A few clones of DP-MSCs with *H. perforatum* L. tried to differentiate into adipocytes, but they could not be determined. The osteogenic differentiation potentials of DP-MSCs in vitro and in vivo have been well-documented in several studies [D'aquino et al., 2007]. During osteogenic differentiation, markers of undifferentiated cells are gradually turned off, and differentiation markers are then expressed sequentially [Huang et al., 2007]. Levels of ON (an early osteogenic marker) and OCN (a late osteogenic marker) levels were determined, and *H. perforatum* L.-treated DP- and BM-MSCs showed reduced ON secretion compared to controls. ON is synthesized by preosteoblasts and has less affinity to collagen. The ON transcript is quite stable, with a half-life of >24 h under conditions of transcription arrest [Dole et al., 2015], and it can thus be concluded that the earlier synthesized ON, via the *H. perforatum* L.-treated DP-MSCs and BM-MSCs, accelerated osteogenic differentiation. The preosteoblasts matured on approximately day 14, and OCN was synthesized by these mature osteoblasts and marked the late phase of osteogenic differentiation. OCN synthesis leads to calcium deposits in the bone, has an affinity for collagen, and plays an important role in the mineralization and formation of bone [Ram et al., 2015]. The *H. perforatum* L.-treated DP-MSCs and BM-MSCs exhibited higher OCN levels than the untreated cells; however, it is notable that calcium concentrations were found to be low in *H. perforatum* L.-treated BM-MSCs, despite the reduced ON and increased OCN levels. We suggest *H. perforatum* L. may disturb the calcium deposit development of the BM-MSCs.

Similarly, but using a different plant extract, Anpo et al. [2011] and our previous study [Mendi et al., 2017] provide evidence that eugenol/*Syzygium aromaticum* reduces the synthesis of collagen, which plays a critical role in osteogenesis. This is an interesting result, since the *H. perforatum* L. effect changes according to the origin of the cells. *H. perforatum* L. could be used safely to induce and accelerate osteogenic differentiation for DP-MSCs, but not for BM-MSCs.

Evidence suggests that MSCs can target injured or ischemic tissues, which involves migration across layers of endothelial cells, and Lopez-Ponte et al. [2007] showed increased migration in response to TNF-α preincubation. In this study, we demonstrated that *H. perforatum* L. can stimulate greater DP-MSC migration than cells stimulat-

ed with TNF-α and BM-MSCs in vitro. Recruitment is important for tissue regeneration, and this is the first study to show the migration effect of *H. perforatum* L. on MSCs.

Next, we determined the response of DP-MSCs and BM-MSCs to *H. perforatum* L. and TNF-α-induced inflammation. Both IL-6 and IL-10 were present in the cell culture supernatants, consistent with the findings of Egermann et al. [2005]. Our results show that when the extract alone is used, the IL-6 levels increased in BM-MSCs but decreased in DP-MSCs. In contrast with this result, in the cells that were pretreated with *H. perforatum* L., before TNF-α stimulation, the IL-6 level was found to be decreased at a ratio of 20 and 60% in DP-MSCs and BM-MSCs, respectively. Pricola et al. [2009] showed that IL-6 is both necessary and sufficient for enhanced MSC proliferation, in that it protects MSCs from apoptosis, inhibits the adipogenic and chondrogenic differentiation of MSCs, and increases the rate of in vitro wound-healing of MSCs. Based on these findings, we suggest that *H. perforatum* L. may lead to a direct increase of IL-6 and a reduced adipogenic differentiation indirectly in BM-MSCs.

The need for bone regeneration in cranial, oral, maxillofacial, and orthopedic surgery is one of the central clinical issues in regenerative and rehabilitation medicine. We conclude that *H. perforatum* L. differentially affected DP-MSCs and BM-MSCs, especially with regard to IC50 values, osteogenic differentiation, and IL-6 secretion. There is emerging evidence that MSCs and their niches are critically important for tissue regeneration, based on the secretion of interleukins in response to different stimulants, such as *H. perforatum* L., as shown in this study. We propose that therapeutic efforts to regenerate the stem cell niche are important for tissue engineering. In this regard, *H. perforatum* L. was found to be more efficient in DP-MSCs than BM-MSCs, suggesting that it can be used for topical and oromaxillofacial regions. Tissue-specific osteoinductive agents should be examined in future studies.

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Statement of Ethics

The research was approved by the Gazi University Ethics Committee of Clinical Research.

Disclosure Statement

The authors declare no conflict of interests related to this study.

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