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ODONTOGENIC DIFFERENTIATION POTENTIAL OF ADIPOSE TISSUE STEM CELLS IN THE PRESENCE OF ODONTOGENIC STIMULI COMPARED TO DENTAL PULP STEM CELLS [AN IN-VITRO STUDY]

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ABSTRACT

Objectives: The current study aimed to compare between the odontogenic differentiation potential of adipose tissue stem cells (ATSCs) and dental pulp stem cells (DPSCs) in presence of odontogenic differentiation medium (ODM) and nano-hydroxy apatite crystals (NHA)

Material and methods: Cryopreserved cell line of DPSCs and ATDSCs were used in the study. The cells were characterized by flow cytometric analysis. Odontogenic induction was performed by culturing the cells in odontogenic differentiation medium (ODM) and nano-hydroxy apatite crystals (NHA). Now, the cells were grouped into 2 groups: DPSCs group and ATSCs group. Each group was subdivided into 2 subgroups according to the incubation period; Subgroup a: 7 days and Subgroup b: 14 days. Odontogenic differentiation potential of each group was evaluated by; viability test, qualitative and quantitative analysis of calcified nodules formed, and rt-PCR for odontogenic genes; dentin sialophosphoprotein (DSPP), alkaline phosphatase (ALP) and dentin matrix phosphoprotein1 (DMP).

Results: DPSCs Group had better odontogenic differentiation capacity than ATSCs group which was manifested by decrease its proliferation curve at 2nd week, significant increase in the odontogenic differentiation genes expression and mineralized nodules formed.

Conclusion: according to the results of the present study, ATSCs displayed an odontogenic differentiation potential but this potential is significantly less than DPSCs in vitro.

KEY WORDS: odontogenic differentiation potential, dental pulp stem cells, adipose tissue stem cells, nano-hydroxyapatite, odontogenic differentiation media and viability test.

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INTRODUCTION

Stem cells are divided into four categories based on their origin: embryonic, fetal, adult, and induced pluripotency. The division between embryonic and adult mesenchymal cells can be simplified [1]. Because of their pluripotency, human embryonic stem cells (ES cells) are collected from the inner layer of the blastocyst and are employed in tissue engineering and regenerative medicine. Human fetal mesenchymal stem cells (hfMSCs) can be extracted from amniotic fluid or the umbilical cord stem cells. They are multipotent, yet, like embryonic cells, they are difficult to get due to restricted availability and ethical concerns. The recovery of induced pluripotent stem cells (iPSCs) is not restricted, and the primary challenge with their clinical use is the laboratory induction procedures used to differentiate them into particular cells needed for the treatment of certain disorders [2]. Adult stem cells, as a result, have the best chance of being used in therapeutic treatment and research into the fundamental elements of this cellular compartment.

Adipose tissue stem cells (ATSCs) have several benefits, including simpler access and collection through subcutaneous lipo-aspiration, which is significantly less painful than collecting bone marrow stem cells, and their usage is less morally contentious because they are derived from autologous fat rather than ES cells [3]. Due to its endocrine activity, which reveals itself in the release of adipocytokines, cytokines, transcription factors, and growth factors, adipose tissue has been one of the most investigated tissues in the last decade [4]. Adipose tissue is now recognised for its role in a complex network of interactions with the endocrine, neurological, and cardiovascular systems, rather than only as an energy storage, thermal insulator, or mechanical buffer. Adipocytes and a percentage of stromal cells such as vascular smooth muscle cells, endothelial cells, fibroblasts, monocytes, macrophages, preadipocyte lymphocytes, and ATSCs make up this mesoderm-derived tissue. ATSCs can develop into mesodermal or trans-mesodermal lineages, resulting in ectodermal-derived cells [1].

The greatest challenge for the application of these cells in future cell replacement therapies is to be able to control their differentiation in certain tissues. In this sense, there is a multitude of cultivation methods, reprogramming strategies, genetic manipulation, epigenetic modulation, organisation in three-dimensional matrices, and the directing of stem cells to the areas where they are needed [5]. Biomaterials are important in the culture of SCs as they're three-dimensional polymeric structures in order to achieve a cell growth organisation closer to that of tissues [6]. Among the materials to be assembled into the three-dimensional biomatrices in order that the stem cells can embed, we will include silica, collagen, and mucopolysaccharide that interact with the SCs through integrin-like proteins. The biomatrices formed by collagen increase the range of differentiation possibilities in several cell lineages like cartilage, bone, skin, and lung, while others formed by polysaccharides that aren't found within the extracellular matrix limit their possibilities of differentiation [7]. The dimensions of the particles or the density of the networks that are developed by various biomaterials like silica, influence the expansion and differentiation potential of ATSCs. It's been proven that kinase systems are involved in transducing signals generated by cell contact with the biomatrices.

Several experimental studies were conducted using ATSCs in the regeneration of various body tissues. Rodriguez et al. (2005) described a study on rats in which cells differentiated from ATSCs were injected into the injured tibialis anterior muscle to see if muscle tissue regeneration occurred; after 60 days, the cross-section of muscle and maximal contraction force increased in the treated group compared to the untreated control group. Meanwhile,

another study observed that when ATSCs were transplanted into mice, they produced dystrophin in a model of Duchenne muscular dystrophy [8].

The therapeutic benefits of ATSC transplants from mice of the same line (syngenic) and BALB/c (allogenic) animals were examined in a model of autoimmune thyroiditis in C57B/6 mice. In both cases, allogeneic and syngeneic ATSCs reduced the number of antithyroglobulin autoantibodies as well as the inflammatory response, and the Th1/Th2 balance was restored [9]. In addition, syngenic and allogenic transplantation of mouse or human ATSCs resulted in reduced cartilage degradation and lower antibodies against mouse collagen II, as well as interleukin 6 in the treated groups in a rat model of rheumatoid arthritis [10]. In research on murine models has been reported the repopulation of the pancreas with cell aggregates identical to the islets of Langerhans, which were produced from ATSCs are capable of secreting insulin, [11, 12]. Furthermore, ATSCs can differentiate into the cell lines required for the regenerative treatment of various conditions, such as osteoporosis, bone regeneration of the jaw and the vestibular table, amyotrophic lateral sclerosis, osteogenesis imperfecta [13, 14].

Some authors studied the influence of culture media utilized on the osteogenic differentiation ability of ATSCs. The cells were grown on three different types of basal medium: DMEM + Low [Glucose] (LG), DMEM + High [Glucose] (HG), and DMEM + F12, and the benefits and limits of each type were compared. Fibroblast Growth Factors (FGF) Supplementation is also an asset in terms of osteogenic differentiation potential. Medium with a high concentration of glucose plus FGF yields better outcomes. Cells grown in media with a low concentration of glucose, on the other hand, have an advantage in terms of cell proliferation potential [15].

In recent years, dental tissue regeneration using stem cells has attracted the interest of researchers.

Many protocols had been used to increase the odontogenic differentiation potential of stem cells either by; using odontogenic differentiation media (ODM) alone [3, 16], growth factors as insulin-like growth factors [17] or BMP and VEGF [18], different bioactive material alone [19] [20], ODM with dentin extract [21] or using, the most popular protocol, ODM combined with different bioactive materials [22] [20].

In the presence of odontogenic media, both MTA and nanohydroxyapatite were found to improve DPSC (dental pulp stem cells) odontogenic differentiation. It was also obvious that nanohydroxyapatite had greater odontogenic differentiation capability than MTA. These findings are relevant in research to stimulate odontogenic differentiation of DPSCs and the development of dentin regeneration treatments [23]. In our previous work we investigated the odontogenic differentiation potential of ATSCs in presence of 3 different types of media. [24]. So, the current study aimed to compare between the odontogenic differentiation potential of adipose tissue stem cells (ATSCs) and dental pulp stem cells (DPSCs) in presence of odontogenic differentiation medium (ODM) and nano-hydroxy apatite crystals (NHA)

MATERIAL AND METHODS

Stem cells preparation and characterization

3rd passage Human dental pulp stem cells (DPSCs) were obtained from the Nile Center of Experimental Research, Mansoura, Egypt. Adipose tissue stem cells (ATSCs) were obtained from the biochemistry department, faculty of medicine, Cairo University, Egypt. All treatment procedures of the cells were performed under a septic condition in a biosafety laminar flow hood. First, the cells were placed into a 75 cm^2 tissue culture flask with pre-warmed 5 mL of growth media (GM) [which is Dulbecco's modified Eagle's medium (DMEM) (lonza, Belgium) supplied with 10% foetal bovine serum (FBS) (Gibco, USA) and 1% penicillin and streptomycin (lonza, Belgium)]. and incubated

in a humidified incubator at 5% CO_2 and 37°C for subculture. The GM was exchanged every 2-3 days. The culture flask was regularly observed for any contamination or change in the colour of the media. After 70-80% of confluence, the cells were trypsinated for 3-4 min, centrifuged for 3 min at 2000 rpm, the supernatant was discarded, and the cell pellet was resuspended with 7 ml of fresh GM and used for odontogenic induction and further investigations. all the procedures of the study were performed in triplicate.

To identify the surface markers of stem cells, DPSCs and ATSCs were washed twice with phosphate-buffered saline (PBS). 2 X 10^6 cells from each type were incubated with different fluorescinated antibodies (anti-CD105, anti-CD90, anti-CD34, and anti-CD45) against their isotype controls (human IgG peridinin chlorophyll protein complex) for half an hour at room temp. The expression profile was analysed immediately by a fluorescein activated cell sorter (FACS) using BD Accuri C6 programme software.

Odontogenic Induction:

For odontogenic induction, the cells were incubated in an odontogenic differentiation medium (ODM) [20, 23] mixed with NHA crystals [23, 24, 25]

The cells were placed in 6-well plate at a density of 5x10^4 cell/well with ODM [which is GM supplemented with 15% FBS, 10 mM -glycerophosphate, 0.2 mM/L ascorbate-2-phosphate, and 100 nM dexamethasone (Sigma-Aldrich, USA)] supplemented with 10 g/mL NHA crystals for 14 days for better odontogenic induction. The cytotoxicity test of this concentration of NHA was previously tested on BMSCs [26] and ATSCs [24].

DPSCs and ADCs were seeded into 6-well plates at a density of 5x104/well. The media was exchanged every 3 days. So, the cells were divided into 2 groups:

1) DPSCs group; for dental pulp stem cells group

2) ATSCs group; for adipose tissue stem cells group

Each group was subdivided into 2 subgroups according to the induction period:

Subgroup a: the cells were incubated for 7 days.

Subgroup b: the cells were incubated for 14 days.

Viability test:

A cell viability test was performed on each type of cell [16] to compare their proliferation rates in the presence of ODM + NHA. At the beginning of the experiment, the cells were seeded in 6-well plates at a density of 50x10^3 /well in the previously mentioned media. After 7 and 14 days of incubation with ODM and NHA, the cells were detached from the plates by trypsinization, and 10 µL of trypan blue was added to 10 µL of cell suspensions. The viable cells in 10 µL of this mixture were placed in a haemocytometer chamber, examined under an Inverted Phase Contrast Microscope (Cambridge Instruments, Buffalo, NY, United States) and the viable cells were counted.

Evaluation of odontogenesis by

1. Alizarin red mineralization assay

To assess in vitro mineralization, cells were washed twice with PBS and fixed with 4% paraformaldehyde (Sigma- Aldrich) for 1 hour, washed with deionized H2O and finally stained with 1% Alizarin Red Stain (Sigma-Aldrich) for 20 minutes. They were then rinsed three times with deionized water. The stained mineralized nodules were observed under an inverted phase contrast light microscope [27] [16].

Quantification of mineralized nodules was performed by adding 400 μL 10% acetic acid to each well and incubating it for 30 minutes with shaking. Then the Mixture of cells and acetic acid

TABLE (1): Primer sequences for quantitative real-time PCR analysis. (GAPDH; the housekeeping gene to normalise the RNA expression levels in all the experimental samples.)

Gene	Primer sequence	
	Forward	Reverse
DSPP	5'-TTAAATGCCAGTGGAACCAT-3'	5'-ATTCCCTTCTCCCTTGTGAC-3'
DMP1	5'-CCCAAGATACCACCAGTGAG-3'	5'-CACCCAGTGCTCTTCACTCT-3'
ALP	5'-GGACCATTCCCACGTCTTCAC -3'	5'-CCTTGTAGCCAGGCCCATTG-3'
GAPDH	5'-TGCACCACCAACTGCTTAGC-3'	5'-GGCATGGACTGTGGTCATGAG-3'

was vigorously centrifuged for 30 seconds. The tube was heated to 85°C for 10 minutes and then left to cool completely. It was centrifuged again at 20,000xg for 15 minutes, and 400 μL of the supernatant was aspirated and transferred to a new 1.5 mL microcentrifuge tube. pH was neutralised by adding 10% ammonium hydroxide to the tube. The samples were read from opaque-walled, transparent bottom well plates [16]

2. RT-PCR analysis for odontogenic genes expression

The odontogenic differentiation genes chosen in the present study were dentin sialophosphoprotein (DSPP), dentin matrix phosphoprotein1 (DMP1), and alkaline phosphatase (ALP).

After culturing the cells for 7 and 14 days, the total RNA was extracted from each sample using a Qiagen RNeasy Mini kit (Qiagen, USA), and then converted to complementary DNA (cDNA) with a High-Capacity cDNA Reverse Transcription Kit. A reverse transcriptase (RT) reaction was carried out using 2 µg aliquot of the total RNA. The differentiation of DPSCs was monitored based on differences in the differentiation markers, including dentin sialophosphoprotein (DSPP), dentin matrix phosphoprotein1 (DMP1), and alkaline phosphatase (ALP).

Glyceraldehyde-phosphate-dehydrogenase (GAPDH) was used as a standard housekeeping gene [28,29] for normalising mRNA levels as presented in Table 1. Accumulation of the RT-PCR products was monitored and quantified using a SYBR green PCR master mix (Thermo Scientific, Lithuania), which was carried out in a spectrofluorometric thermal cycler (Rotor-Gene 3000, Corbett Research, Korea). After the rt-PCR run, the amplification efficiency of all the studied genes was analysed using the comparative Ct method [30, 31]. Each measurement was assessed in triplicate.

Statistical Analysis

The collected data, which were tabulated in Excel sheets, were analysed using a paired sample t-test in IBM SPSS software.The results of the viability test, quantified mineralized nodules, and RT-PCR of odontogenic gene expression were presented as a mean and standard deviation.The results were considered statistically significant if the P-value was less than or equal to 0.05.

RESULTS

Flowcytometric analysis

The flowcytometric analysis of both cells showed -ve expression of CD34 and CD45 and +ve expression of CD105 and CD90, which confirms their phenotypic characteristics as mesenchymal stem cells of origin (fig 1).

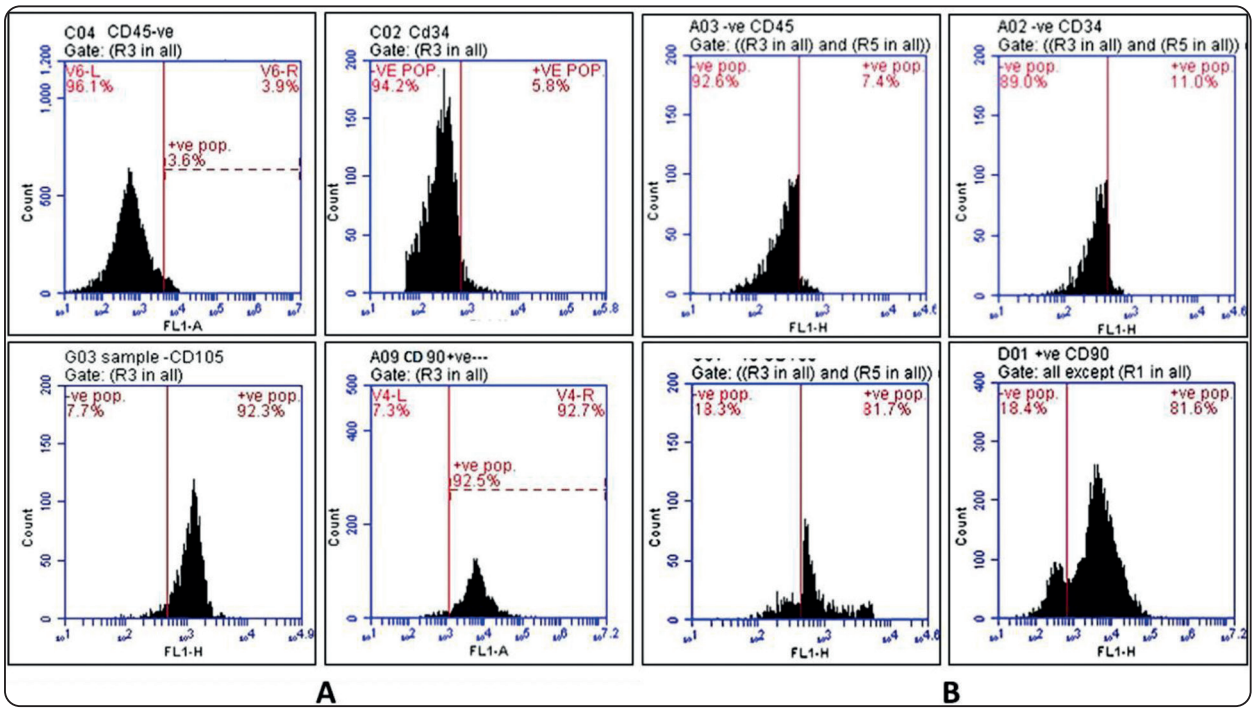

Fig. (1) Shows the flow cytometric analysis of: dental pulp stem cells (A) and adipose tissue stem cells (B).

Viability test

The viable cells were counted using Trypan blue stain, which is actively excluded by the vital cells, unlike the dead cells that allow its entrance and retention in the cells due to its non-intact cell membrane. So, the viable cell appears clear, but the dead cells are deeply blue stained. The cell count at the 7th day for DPSCs and ATSCs was nearly the same (75100 and 76400, respectively), while at the 14th day, the difference between the cells started to increase (85200 and 90800, respectively). A decline in the proliferation curve was observed at the 2nd week in both cells and especially in the DPSC group (fig. 2).

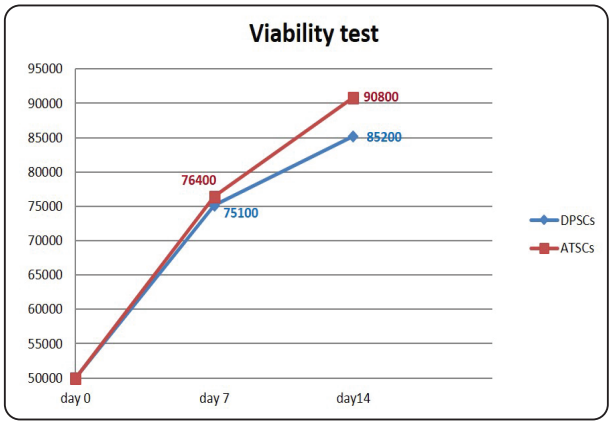

Fig. (2): Line chart showing the number of viable cells count at day 7 and 14 starting from 50x 103 cells at day zero

Mineralization assay

Mineralized nodule formation was observed in both types of cells, but it was greater in the DPSC group than in the ATSC group at days 7 and 14, and this difference was statistically significant. The pattern of nodules that appeared was different in both groups; in ATSCs they appeared as small, separated granules, while in the DPSCs group the mineralized nodules appeared larger and more coherent with each other, forming a continuous layer (fig. 3).

Also, the quantitative analysis of mineralized nodules (table 2, 3) revealed a statistically significant increase in mineralized nodule formation in the DPSCs group at day 14 than that at day 7 unlike in the ATSCs group as the increase was non-significant (0.119 p value).

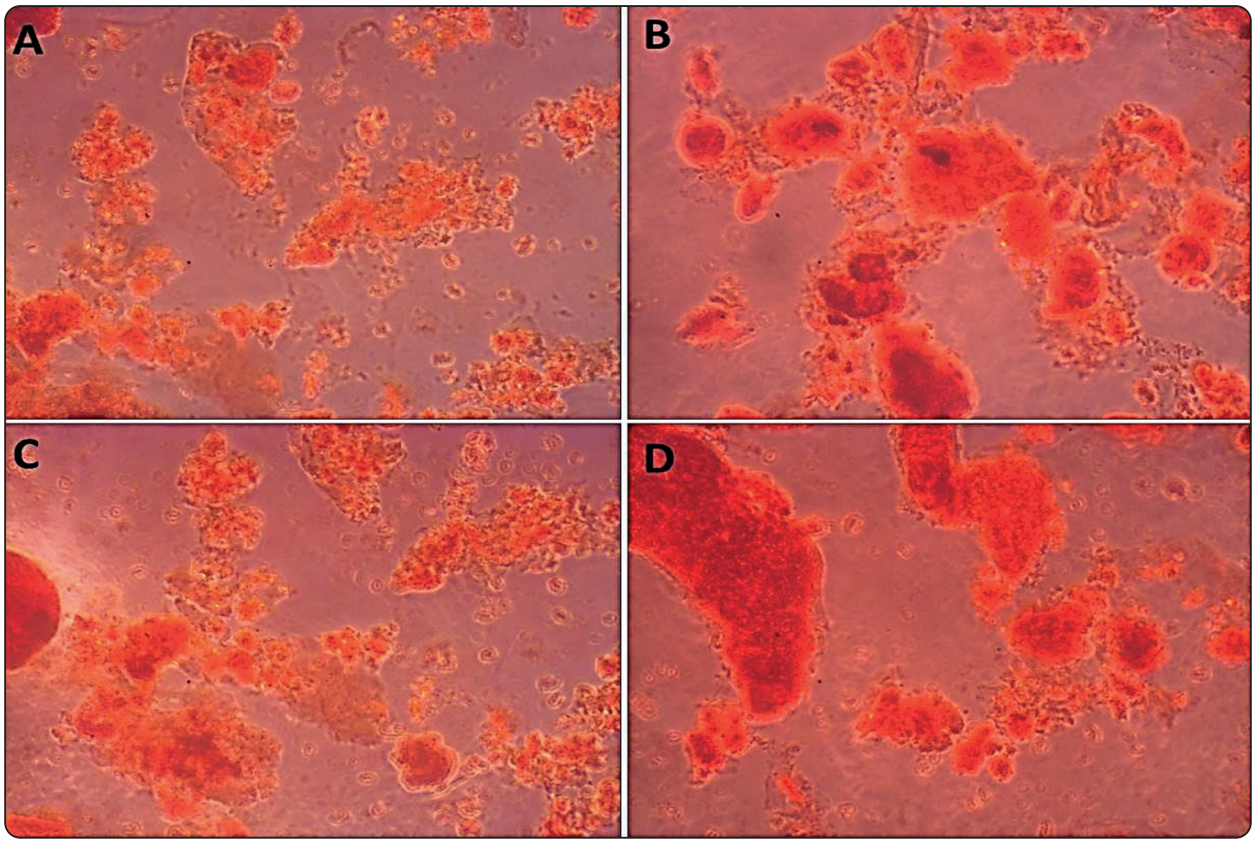

Fig. (3): Showing the shape of mineralized nodules stained with Alizarin red stain for; ATSCs at day 7 (A) and day 14 (C). DPSCs on days 7 (B) and 14 (D)

TABLE (2): Means and standard deviation of mineralized nodules at 7 and 14 days

Time	Group	Mean	Std. Deviation	Std. Error Mean
day 7	DPSCs. A	1.4307	.12854	0.07421
	ATSCs. A	0.7767	0.04539	0.02621
day 14	DPSCs. B	1.9860	0.01323	0.00764
	ATSCs. B	0.8990	0.07077	0.04086

rt-PCR:

The statistical analysis revealed a significant increase in the expression of all odontogenic differentiation genes used (DMP, DSPP, and ALP) on day 14 compared to day 7 in both groups. On the same day, there was an increase in the expression of all odontogenic differentiation genes in the DPSCs group compared to the ATSCs group, and all of these increases were significant except for ALP on

TABLE (3): Comparison of mineralized nodule formation in all groups

Paired Samples Test								
	Paired Differences							
				95% Confidence Interval of the Difference				
	Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
DPSCs.7 - ATSCs.7	.65400	.08316	.04801	.44741	.86059	13.621	2	.005
DPSCs.14 - ATSCs.14	1.08700	.06295	.03635	.93062	1.24338	29.907	2	.001
DPSCs.7 - DPSCs.14	-.55533	.13789	.07961	-.89786	-.21281	-6.976	2	.020
ATSCs.7 - ATSCs.14	-.12233	.08048	.04646	-.32225	.07758	-2.633	2	.119

Statistical significance at p-value ≤ 0.05.

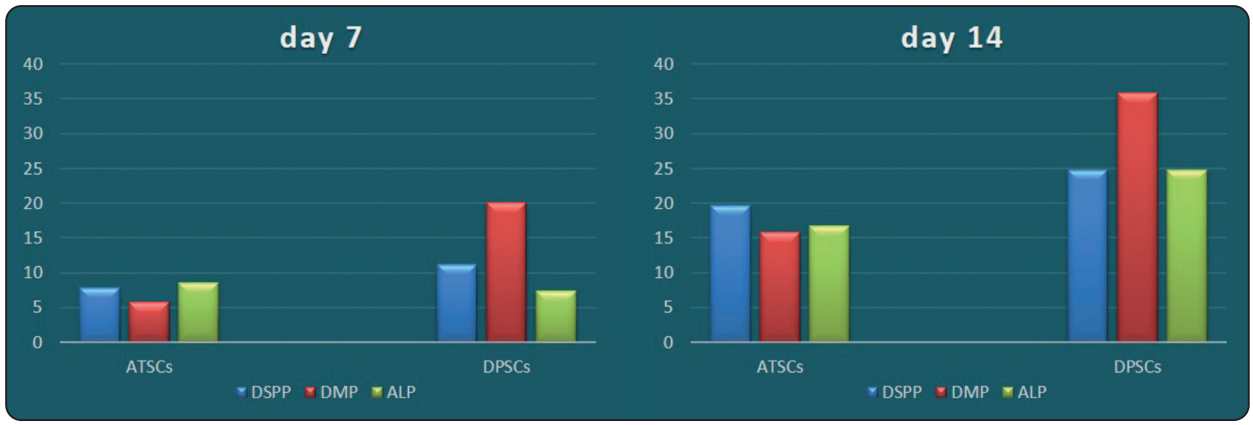

Fig. (4): Bar chart showing the differences between DSPP, DMP, and ALP gene expression for DPSCs and ATSCs at days 7 and 14.

Day 7 (where the ALP expression of ATSCs group (8.5) showed a non-significant increase compared to DPSCs group (7.4), as P value = 0.106). The mean values of rt-PCR expression of odontogenic differentiation genes were summarized in table 4 and figure 4.

TABLE (4): Shows mean values of rt-PCR expression of DMP, DSSP, and ALP at days 7, and 14.

Time	Group	DSPP	DMP	ALP
day 7	DPSCs. a	11.19729	20.08134	7.4014371
	ATSCs. a	7.869692	5.876992	8.507993
day 14	DPSCs. b	24.76238	35.88076	24.767645
	ATSCs. b	19.7002	15.83688	16.80364

DISCUSSION

Generally, stem cell therapy offers immense potential for dental tissue regeneration.

However, many authors have studied the ability of ATSCs to differentiate into mineralizing cells for hard tissue regeneration such as bone [3] and dentine [24, 32, 33].

Some of these studies have revealed that ATSCs display an odontogenic potential in vitro [34] [35], but the inductive stimulus required for odontogenic differentiation induction of ATSCs has not been fully explained.

Many studies have assessed the odontogenic differentiation potential of DPSCs in vitro for regenerative endodontic therapies. Some of them evaluated the odontogenic induction potential of un-induced cells with different biomaterials, such as calcium hydroxide, hydroxyapatite, nanohydroxyapatite (NHA) or mineral trioxide aggregates (MTA), to detect their effect, without any additional stimulus, and used the odontogenic differentiation medium as a positive control [19] for regenerative endodontic treatments. While the others preferred to use the bioactive materials with the induction medium on the odontogenically induced cells to mimic the clinical situation [22, 36]. Some of these studies have proved that the addition of growth factors or inductive biomaterials to odontogenic induction media effectively stimulates odontogenic differentiation of DPSCs [20, 21, 37]. However, the effect of these stimuli on ATSC culture has not been elucidated.

In our previous study, it was proved that the addition of nanohydroxyapatite (NHA) to the odontogenic differentiation medium (ODM) significantly increased the odontogenic differentiation potential of ATSCs more than culturing them in growth media or ODM alone [24].

So, in the current study, the odontogenic differentiation potential of ATSCs has been compared to that of DPSCs in vitro in the presence of

ODM and NH as a proven successful odontogenic differentiation stimulus for both types of cells.

ATSCs have been chosen in these studies due to their ease of harvesting, availability in a substantial number, and rapid in vitro expansion. In addition, some authors suggested that the osteogenic potential of ATCs could be maintained by ageing cells, which supports the possibility of using ATSCs for hard tissue regeneration in elderly people [38] [39].

In normal circumstances and by culturing the cells in DMEM without any odontogenic stimulus, DPSCs proliferate more rapidly than ATSCs [3] .

But the present study revealed a slower growth curve in DPSCs than in ATSCs, especially at the second week, indicating a higher rate of odontogenic differentiation in DPSCs than in ATSCs.

The relationship between proliferating cells and differentiated cells has been explained by Strehl et al., 2002 [40]. They reported that, the dividing cell can only maintain a minimal degree of differentiation to a specific tissue, and this differentiation depends on the length of the interphase needed for each tissue. In the case of tissue engineering, during cell differentiation, the phase of mitotic stimulation must be switched from proliferation to differentiation depending on the interphase in which differentiation is maintained. And this switch passes through 3 phases; 1) expansion of the cell number, 2) decrease in the proliferation rate and induction of tissue-specific differentiation, and 3) tissue differentiation is stabilized to maintain the specific characteristics of the tissue.

Many factors, in addition to differentiation, may influence stem cell growth rate. Bakopoulou et al. (2011) [16] discovered that DPSCs have a lower growth rate than dental papilla stem cells in the presence of odontogenic differentiation medium, and they attributed this to the smaller size of dental papilla stem cells, which allows for more cell growth before the culture reaches confluence.

In the present study, mineralized nodules displayed by Alizarin Red staining, revealed a statistically significant increase in the DPSCs group than the ATSCs group on days 7 and 14. In addition, the ATSCs nodules appeared small and separated, while in DPSCs the mineralized nodules appeared as a continuous calcified layer. This pattern of mineralization is exactly consistent with previous studies by Balic and Mina (2010) [41] and Stanko et al. (2014) [42], who found the mineralized deposition of ATSCs and BMSCs produced characteristic small mineralized nodules, while DPSCs nodules appeared as a mineralized sheet.

Conversely, Jine et al., 2019 found that ATSCs exhibited greater osteogenic differentiation potential and greater mineral deposition than DPSCs [3].

Both types of cells displayed an increase in odontogenic differentiation genes expression of DMP, DSSP, and ALP, indicating the ability of ODM in the presence of NHA to differentiate DPSCs and ATSCs into odontogenic-like cells. And this agrees with several studies which examined the effect of NHA on odontogenic differentiation potential either on DPSCs [23, 20] or ATSCs [24].

In accordance with this result, Yousefi et al. (2014) regarded the significant increase in odontogenic gene expression and intense mineralized nodule deposition of mesenchymal stem cells, cultured in odontogenic induced biomaterial and ODM, to the dual differentiation signals (synergistic effect) on the stem cells from both materials. which stimulates more odontogenic differentiation than those cells that received only one differentiation signal from ODM or tested biomaterial [43]

The NHA used in this study resembles the natural mineralization phase in dentin building units, calcium, and phosphates [43,44]. Besides, by hydration of NHA with fluids, a proper Ca2+ concentration and alkaline pH (10–12) are formed, which have been shown to promote cell proliferation and differentiation to form a strong mineralized interface [43, 45, 46].

The ODM used in the present study contains ascorbic acid, which is proven to promote cell viability [47] and the formation of collagen fibre type I by odontogenic cells and extracellular matrix. In addition, β-glycerophosphate has been shown to stimulate the formation and mineralization of nodules in tissue cultures [48]. Finally, dexamethasone was shown to stimulate MSCs' terminal differentiation towards osteogenic or odontogenic lineages in vitro [47].

The special low concentration of DSPP mRNA expression observed in ATSCs compared to DPSCs cultures is consistent with the lower mineralization rate observed in ATSCs during the first week. DSPP is an early odontogenic marker and plays a critical role in dentin mineralization during early dentinogenesis. Importantly, it is profusely expressed in dentin and only small expressions were detected in bone [49].

It has been obvious that DPSCs have higher odontogenic differentiation potential than ATSCs, which is manifested by significant elevation of odontogenic differentiation genes and greater mineral deposition. These results are exactly consistent with Stanko et al. (2014) who compared diverse types of MSCs (dental pulp, bone marrow, adipose tissue, and umbilical cord) and found that DPSCs had the superior odontogenic differentiation potential, regarding nodule formation and odontogenic genes, among the other stem cells. In addition, they concluded that ATSCs had a significantly higher adipogenic potential than BMSCs or DPSCs. In contrast to BMSCs and especially DPSCs, the differentiation potential toward mineralizing cells was significantly lower [42].

Several assumptions may explain the potential odontogenic superiority of DPSCs over ATSCs. According to some authors, tooth-resident stem cells are the better options for odontogenic induction [50].

The origin of DPSCs may also explain their high odontogenic potential. Stanko et al. (2014) discovered that DPSCs had significantly higher levels of pleuripotent protein products than other mesenchymal stem cells, including ATSCs [42]

These differences in expression of pluripotent stem cell genes in DPSCs reflect their embryonic origin, which is formed of ectomesenchymal cells containing neural crest cells, which display high plasticity and multipotential abilities [51]. Also, this pleuripotent potential may give the DPSCs a sensitivity toward external stimuli, which are NHA and ODM in the present study, more than ATSCs.

Furthermore, the response of MSCs to different stimuli may change from one type to another, This may be due to the differences in surface receptor profiles of MSCs isolated from different tissues. For instance, ATSCs have been revealed to have altered bone morphogenetic protein (BMP) and TGF-b receptors that may decrease their capacity to form mineralized tissue when compared to DPSCs or BMSCs [42] [52] Finally, ATSCs have displayed a gradual loss of their oddontogenic differentiation potential by increasing the number of passages, unlike DPSCs, which displayed an increase in their potential by increasing the passages [42]. Confirming this assumption, many authors attributed the reparative function of DPSCs to a higher expression of E-cadherin and lower expression of Snail of DPSCs among the other MSCs [42] [53] [54]. These are epithelial-mesenchymal transition (EMT) markers that regulate embryonic mesoderm maintenance, growth arrest, and cell migration [55, 56].

CONCLUSION

Although the previous study revealed that ATSCs represented an easily available source of mesenchymal stem cells that primarily have best odontogenic differentiation potential in presence of ODM and NHA. But, according to the results of the present study, this potential is significantly less than DPSCs in vitro.

Recommendations:

More studies are needed to improve the odontogenic differentiation ability of ATSCs either by increase the number of the cells or use another odontogenic induction material

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