Original Article

In *Vitro* Evaluation of Odontogenic Differentiation Potential of Adipose Tissue-Derived Stem Cells in Presence and Absence of Nano-Hydroxyapatite Crystals

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ABSTRACT

Aim of the Study: Tissue regeneration therapy using stem cells is becoming the state of the art for dental treatment. The present study aimed to use adipose tissue-derived stem cells for odontogenic differentiation, as a more abundant replacement for dental pulp stem cells, using 3 different media.

Material and Methods: Cryopreserved cell lines of adipose tissue-derived stem cells (ATDSCs) at 3rd passage were used in the study. These cells were characterized using flow cytometric analysis for CD90, CD105, CD34 and CD45 and MTT assay was used to determine the best concentration of hydroxyapatite nanoparticles should be used in the study. Then, these cells were grouped into 3 groups: Group 1 the cells incubated in growth medium (served as a control negative), Group 2 the cells incubated in odontogenic medium, while group 3 was grown in odontogenic medium modified with Hydroxyapatite nanoparticles powder. The difference in cells differentiation and production of calcified materials was measured after one and two weeks between the three groups. This was achieved by using mineralization test and RT-PCR for odontogenic differentiation genes which are dentin sialophosphoprotein (DSPP), alkaline phosphatase (ALP) and dentin matrix phosphoprotein1 (DMP1).

Results: Group 3 had the better odontogenic differentiation capacity among the other groups as it showed a significant increase in both production of mineralized nodules and odontogenic genes expression.

Conclusion: Adipose tissue-derived stem cells had an odontogenic differentiation capacity especially in presence of Hydroxyapatite nanoparticles and can be used as an easy alternative and more abundant source for dental pulp stem cells.

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Key Words: Adipose tissue-derived stem cells, dental pulp stem cells, hydroxyapatite nanoparticles and odontogenic differentiation media, odontogenic differentiation.

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INTRODUCTION

The majority of contemporary medical research has focused on the regenerative potential of human body stem cells as a source of tissue regeneration and "spare parts" for organs.

Despite the fact that modern root canal therapies have solved the difficulties of previous methods, they cannot generate a structure that is morphologically and functionally identical to the dentin-pulp complex[1].

A new era in restorative dentistry has begun thanks to advances in basic scientific research in the domains of stem cells, biomaterials, and odontogenesis. Some restorative materials, such as mineral trioxide aggregates (MTA), calcium hydroxide, and hydroxyapatite nanoparticles (HANPs), have been required for spontaneous hard tissue regeneration rather than artificial restoration[2].

To improve dental therapeutic procedures, researchers are using mesenchymal stem cells (MSCs) in combination with scaffolds and/or biomaterials to regenerate dentin-pulp. A three-dimensional culture of MSCs at a specific region, in particular, has the ability to form a dentin/pulp-like structure. These bioengineered tissues will produce a functionally dynamic tissue[3].

Nanohydroxyapatite (NHA) is a bioactive substance with a particle size that is close to that of apatite crystals found in human calcified tissue. As a result, when employed as a scaffold material in tissue engineering, it is beneficial. As a result, in the field of periodontology, it plays a significant role in directed tissue regeneration to promote bone regeneration.[4] It is employed as a bioactive material in many endodontic applications because of its propensity to generate calcium and phosphate ions when hydrated[5]. Furthermore, HANPs have been shown to have a clear effect on the differentiation of mesenchymal stem cells[6].

Dental stem cells (DSCs) are post-natal stem cell populations that originate from the neural crest and have mesenchymal stem cell (MSC)-like features, such as the ability to self-renew and differentiate into many lineages[7]. DSCs are capable of producing dentin-like tissue and developing into osteoblast-like cells in *vitro* when given the right stimulation[8].

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DPSCs are a type of DSCS generated from the pulp of impacted wisdom teeth or exfoliated deciduous teeth. The morphology of these multipotent cells is similar to that of fibroblasts[9]. Differentiation medium were used to cultivate isolated DPSCs. Adipogenic, neurogenic, chondrogenic, and myogenic differentiation were confirmed in addition to their dentinogenic and osteogenic potentials[10,11].

Because DPSCs can differentiate into osteoblasts and/or odontoblasts, both of which can produce mineralized matrix, Tecles *et al* concentrated on growing these cells in various culture media to restore missing dental structures[12].

DPSCs are stimulated to differentiate into odontoblasts when the right differentiation media are used. Odontoblasts, in turn, have the ability to synthesize dentin[13]. The first step involves the deposition of an organic matrix called predentin, which is primarily composed of collagenous fibers organized in bundles. The predentin works as a scaffold for calcium deposition, eventually transforming into mature dentin[14]. Odontoblasts secrete various proteins that contribute to the dentin matrix, such as osteocalcin (OC) and DMP-1, in addition to collagen. These proteins, however, are not unique to dentin; they are also found in bone[15]. Odontoblasts, on the other hand, secrete additional matrix proteins that are specific to dentin development, such as dentin phosphoprotein (DPP) and dentin sialoprotein (DSP)[16].

However, a major disadvantage of DPSCs is that they are difficult to harvest in the clinic for use in tooth regeneration[17]. Furthermore, DPSCs must be extracted from a vital pulp, which necessitates the loss of a healthy tooth[18].

As a result, researchers looked for alternative stem cells that were easier to collect and plentiful in the body[19]. ATDSCs (adipose tissue-derived stem cells) have been considered as a potential substitute for DPSCs since they are pluripotent cells with the ability to differentiate into a variety of tissue-forming cells. They also have the advantage of not losing their potential as other stem cells do with age[20]. Jing *et al*. (2008) postulated that ATDSCs can develop into odontoblasts and be used in dental tissue engineering and construction, similar to how they have been used in the regeneration of other bodily tissues[21].

As a result of the surge in obesity, medical procedures such as tummy tucks and liposuction have increased. Despite the fact that adipose tissue is frequently discarded after surgery, researchers have identified it as a rich source of multipotent stromal cells for regenerative medicine[22] due to its ease of separation, culture expansion, differentiation capabilities, and immunomodulatory properties. This tissue is extracted in a less invasive manner than bone marrow and yields a larger volume of material[23].

Some study mixes stem cells with biomaterials to increase their usage. When ATDSCs are coated in calcium alginate, they can grow into osteoblasts, as stated by Song *et al*. (2016)[24]. While Godoy Zanicotti *et al*. (2017) investigated the osteogenic potential of ATDSCs grown on various titanium surfaces in serum-free conditions and they observed no significant differences in bone formation[25].

On the other hand Babaki *et al*.(2020) demonstrated that MTA-conditioned media can induce ATDSC proliferation and differentiation into osteo/odontoblast-like cells[26].

As a result, we will investigate the odontogenic differentiation potential of ATDSCs in the presence of hydroxy apatite nanoparticles (HANPs) as an odontogenic differentiation stimulus. Then apply their usage as a source of stem cells that is more plentiful and easier to access and harvest than DPSCs.

MATERIAL AND METHODS

Stem Cells Preparation and Characterization

All the experiment procedures were performed in Nile Center for Experimental Researches, Mansoura, Egypt. Adipose tissue derived stem cells (ATDSCs) used in this study were cryopreserved cell line at 3rd passage. The cells were purchased from biochemistry department, Faculty of Medicine, Cairo University, Egypt. The experiment procedures, cells recovery and all other manipulations, were performed under complete aseptic condition in a laminar flow hood (BIOLOGICAL SAFETY CABINET CLASS II A2, UNIL@B)). The frozen cells cryovial was thawed in a water path at 37 °C for 60 sec. In the biosafety hood, cells were transferred into a sterile 15-mL conical tube containing 5 mL growth media (GM); Dulbecco's modified Eagle's medium (DMEM) (lonza, Belgium) supplied with 10% fetal bovine serum (FBS) (Gibco, USA) and 1% penicillin and streptomycin (lonza, Belgium). Then they were centrifuged for 3 min at 2000/prm and the supernatant was discarded. The cell pellet was resuspended by 7ml fresh GM and transferred into 75 cm2 tissue culture flask for incubation in a humidified incubator at 5% CO2 and 37 °C. The GM was exchanged every 2-3 days. After 70-80% of confluence, the cells were isolated from the flask by trypsinization and the cell pellet was resuspended and used for odontogenic induction and further investigations.

Flow cytometric analysis

ATDSCs were characterized by flow cytometric analysis to confirm their phenotypic characteristics. The expression of cell-surface markers in ATDSCs was determined by Fluorescein activated cell sorting (FACS) analysis. ATDSCs were resuspended in phosphate-buffered saline (PBS) supplied with 3% FBS and saturated concentration of 4 fluorescein isothiocyanate-conjugated monoclonal antibodies ; anti-CD105, anti-CD90, anti-CD34 and anti-CD45 (eBioscience, company). They were incubated with isotype controls, human IgG peridinin chlorophyll protein complex, at 28°C for 30 min in the dark to differentiate between the signals of specific antibody from the signals of nonspecific background. The cells were washed twice with PBS containing 2% Bovine serum albumin (BSA) then

analyzed immediately using BD Accuri C6 programme software.

MTT assay

MTT assay, cytotoxicity test, was performed to estimate the suitable concentration of hydroxy apatite nanoparticles (HANPs) that have the least cytotoxic effect on ATDSCs. This assay display cell viability, proliferation, and cytotoxicity through measuring the cellular metabolic activity. ATDSCs were seeded in 96 well plates at a density of 5x103 cells/well and cultured in DMEM with 10% FBS. After 24h of seeding, the medium was replaced by DMEM/F12 (mixture of DMEM and Ham's F-12 Medium) containing HANPs at different concentrations (10 µg / mL, 20 µg /mL, 30 µg /mL, 40 µg /mL and 50 µg /mL) and cultured for 48h. After 24h and 48h, 10 µl of MTT dye (Vybrant®), of a concentration 5 mg/ml PBS, was added to each well in the dark then incubated for 3 h at 37 ◦C. The optical density of each well was measured by spectrophotometry after dissolution in dimethyl sulfoxide (DMSO). The metabolically active cells were quantified to be compared with negative control, cells supplied with DMEM/F12 alone

Induction of odontogenic differentiation

Stem cells were counted and planted in a 6-well plate at a density of 5x104 cell/well. Then they were divided into 3 groups (2 wells for each group) according to the type of culture media:

Group 1: the cells were grown in GM only (served as negative control).

Group 2: the cells were grown in odontogenic differentiation medium (ODM) which is GM supplemented with 15% FBS, 10 mM β-glycerophosphate, 0.2 mM /L ascorbate-2-phosphate, and 100 nM dexamethasone (Sigma-Aldrich, USA).

Group 3: the cells were grown in ODM supplemented with HANPs (of the selected concentration according to MTT assay).

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

- Subgroup a: the cells were incubated for 7 days.
- Subgroup b: the cells were incubated for 14 days.

For 2 weeks, the media were changed every 3 days with the respective culture media for each plate.

Odontogenic differentiation was confirmed by

Mineralization test

After 7 and 14 days, Alizarin red stain (Sigma Aldrich) was used to stain the monolayer cells in each well to detect mineralization nodules; the cells were washed twice with PBS and fixed with 4% paraformaldehyde (Sigma- Aldrich) for 1 hour at room temperature. Then they were washed with deionized H2O and stained with 40 mM Alizarin Red (Sigma-Aldrich) at pH 4.1 for 20 minutes. The mineralized nodules were detected by examination of the stained monolayers cells under an inverted phase contrast light microscope (by ×4 and ×10 objective lenses).

To quantify the degree of mineralized nodules, 400 µL 10% acetic acid was added to each well, of a 6-well plate, and incubate for 30 minutes with shaking. Then the cells were scraped gently from the plate and transferred with the acetic acid to a 1.5 mL microcentrifuge tube and vortexed vigorously for 30 seconds. Then tube was sealed with parafilm and heated to 85°C for 10 minutes. The tube was transferred to ice for 5 minutes and left until fully cooled. The tube was centrifuged at 20,000xg for 15 minutes. 400 µL of the supernatant was aspirated and transferred to a new 1.5 mL microcentrifuge tube. ~150 µL 10% Ammonium hydroxide was added to the tube to neutralize the pH. About 150 µL of the sample was added to an opaque-walled, transparent bottom 96-well plate and read at optical density 405 (OD405).

RT-PCR analysis for odontogenic genes expression

At day 7 and 14, the mRNA level of odontogenic differentiation genes were detected by quantitative Real-time reverse transcription polymerase chain reaction (RT-PCR). The odontogenic differentiation genes used in the present study were dentin sialophosphoprotein (DSPP), alkaline phosphatase (ALP) and dentin matrix phosphoprotein1 (DMP1).

Cells were detached by trypsinization. The cell pellet was re-suspended by GM followed by adding 600 ml of Trizol (Qiagen, USA) and 200ml of chloroform then mixed for 5 min by vortex. The mixture was incubated for 2 min at room temperature then centrifuged for 15 min at 13,000 rpm. 700ml of 70% ethanol was added to the pellet and centrifuged for 5 min again at 9000 rpm. The supernatant was removed and pellet was kept for air dry.

The extracted RNA was converted to complementary DNA (cDNA) using a High-Capacity cDNA Reverse Transcription Kit. qPCR reaction mixture, performed triple, contained 2µl (10 pmol/µl) of forward and reverse primers of studied genes, 2µl of cDNA template, and 10 µl SYBR green PCR master mix (Thermo Scientific, Lithuania). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) was used to normalize the cycle threshold (Ct) values for each target gene. Primer sequences of the studied genes are presented in (Table 1). Each reaction was amplified for 40 cycles. The Expression of studied genes was determined using the comparative ΔΔCT method.

Table 1: Primer sequences for quantitative real-time PCR analysis. (GAPDH; Glyceraldehyde-3-phosphate dehydrogenase which was considered the housekeeping gene to normalize the RNA expression levels in all the experimental samples.)

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

Statistical Analysis

All data were collected and tabulated in Excel sheets and exported to IBM SPSS software, version 22 for analysis. Data were presented as the mean and standard deviation. Paired sample t-test was used to compare between different groups.

RESULTS

Flow cytometric analysis

The flow cytometric analysis of ATDSCs revealed 81.6% expression of CD90 and 81.7% expression of CD105. In contrast, it revealed –ve expression of CD34 and CD45; 11% and 7.4% respectively (Figure 1).

MTT assay

This assay depends on mitochondrial activity of the living cells; The viable cells mitochondria contains NAD(P) H-dependent oxidoreductase enzymes. This enzyme reduces the yellow tetrazolium salt {3-(4,5-dimethylthiazol-2-yl) -2,5-diphenyltetrazolium bromide or MTT} to purple formazan crystals[27]. So, the darker the solution, the greater number of viable metabolically active cells. The optical density of the solution in the wells was measured by the spectrophotometry which revealed the best concentration of HANPs used, in the well, was 10 µg /mL; the percentage of viable cells was 80.2% after 24 h and increased to 84.6% after 48 h (Figure 2).

Mineralization test

The inverted microscope examination of the monolayer cells revealed presence of red stained mineralized nodules on extracellular matrix in all experimental plates, at day 7 and 14, except the negative controls (group 1) (Figure 3). Gr.3, Plates contained HANPs + ODM, revealed more numerous and highly positive stained mineralized nodules than group 2 which contained ODM alone. For all +ve groups, the positively stained nodules were increased on day 14 than day 7. Means and standard deviation of each group were summarized in (Table 2).

The statistical results, of mineralized nodules quantification, revealed a statistical significance difference between control –ve group (group 1) and all other groups that contained ODM (Table 3). Also there were statistical differences between group 2 and 3 at day 7 and 14 (*P values* were 0.017 and 0.030 respectively) but there was no statistical difference between group 3 at day 7 and day 14.

Real time RT-PCR analysis

Quantitative RT-PCR displayed that the mRNAs expression of DSPP, BMP1 and ALP increased in day 14 more than day 7 in all groups except control –ve group (group 1) (Table 4, Figure 4). Cells in all groups showed a significant higher expression of the 3 odontogenic differentiation genes than the -ve control group. Regarding the comparison between presence and absence of HANPs in the media, group 3 recorded a statistically significant higher mean value of genes expression than that of group 2 at days 7 and 14. There was also a statistically significant increase in all gene expressions of subgroup b (at day14) over subgroup a (at day7).

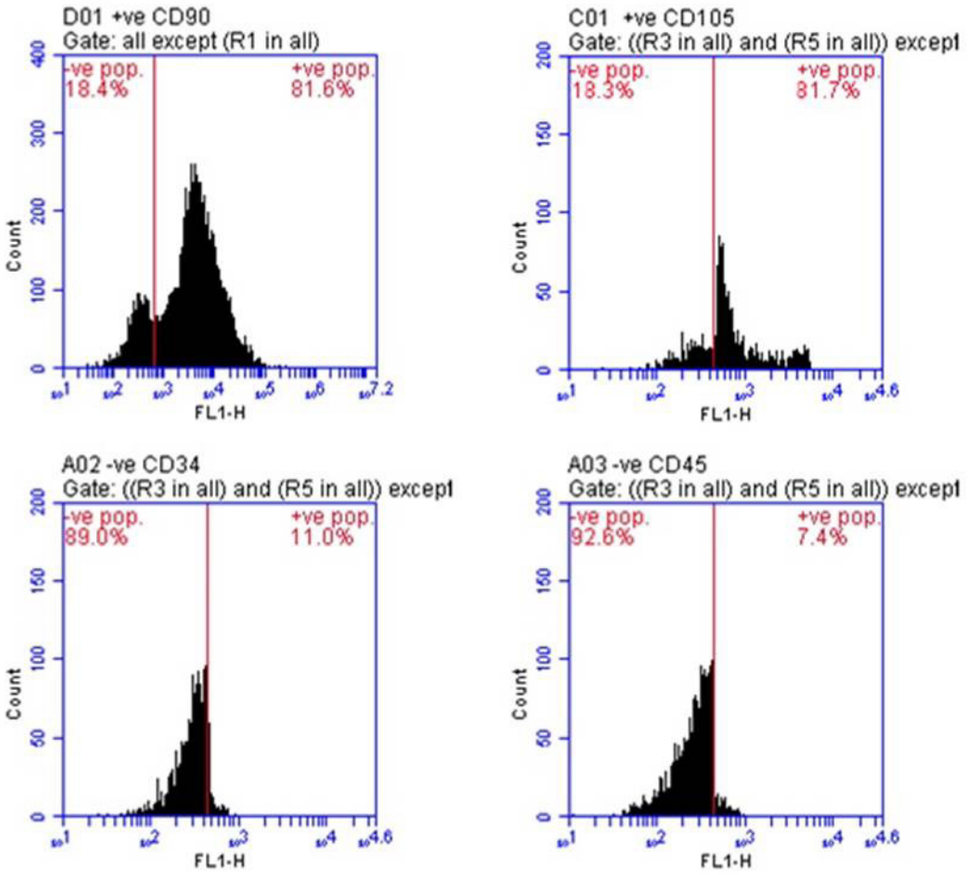

Fig. 1: Flow cytometric analysis of ATDSCs demonstrate +ve reaction to CD90, CD105 and –ve reaction to CD34, CD45

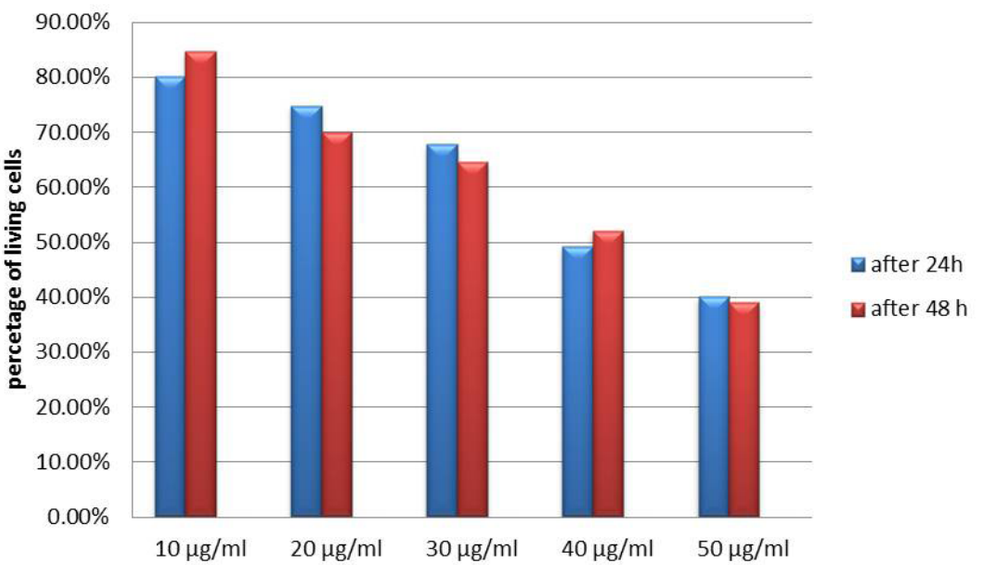

Fig. 2: Graph demonstrates MTT assay results to determine the best concentration of HANPs used

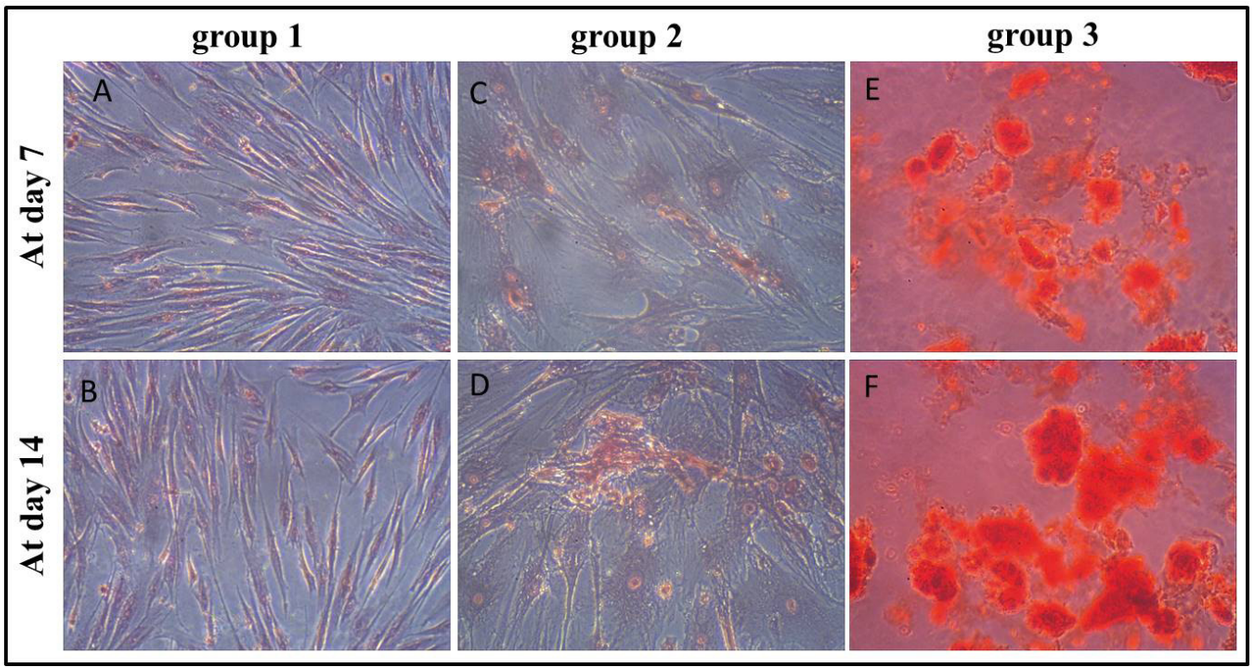

Fig. 3: photomicrograph demonstrates the mineralized nodules (red color) stained with Alizarin Red present in each group; A and B: control –ve (gr. 1), C and D: cells in ODM (gr. 2) and E and F: cells in ODM + HANPs (gr. 3)

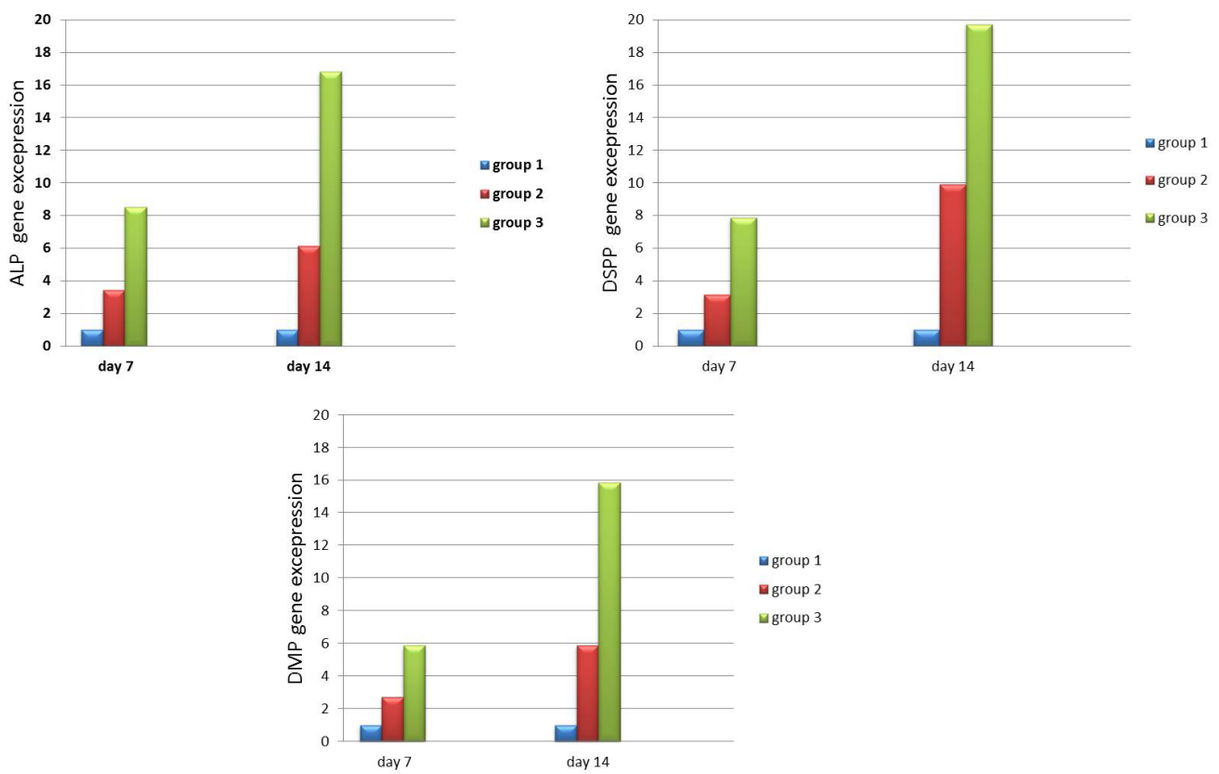

Fig. 4: graph demonstrates the differences between odontogenic differentiation genes expression (ALP, DSPP and DMP1) at day 7 and 14

Table 2: means and std. deviation of mineralized nodules in each group at day 7 and 14

		Mean	Std. Deviation
At 7 days	gr.1.a	.1280	.00529
	gr.2.a	.3977	.05701
	gr.3.a	.7767	.04539
At 14 day	gr.1.b	.1930	.00794
	gr.2.b	.5673	.09817
	gr.3.b	.8990	.07077

Table 3: Comparison between mineralized nodules formation of adipose tissue stem cells for all groups (the difference is considered statistically significant if *P value* is less than 0.05)

	Paired Differences					t	df	Sig. (2-tailed)
	Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference				
				Lower	Upper			
group.1.a - group.2.a	-.26967	.05835	.03369	-.41461	-.12473	-8.005	3	.015
group.1.a - group.3.a	-.64867	.04028	.02325	-.74872	-.54861	-27.894	3	.001
group.2.a - group.3.a	-.37900	.08711	.05029	-.59539	-.16261	-7.536	3	.017
group1.b - group.2.b	-.37433	.09745	.05626	-.61641	-.13226	-6.653	3	.022
group1.b - group.3.b	-.70600	.06298	.03636	-.86246	-.54954	-19.415	3	.003
group.2.b - group.3.b	-.33167	.10153	.05862	-.58389	-.07944	-5.658	3	.030
group.2.a - group.2.b	-.16967	.06608	.03815	-.33381	-.00552	-4.447	3	.047
group.3.a - group.3.b	-.12233	.08048	.04646	-.32225	.07758	-2.633	3	.119

Table 4: mean values of odontogenic differentiation genes (DMP1, DSPP and ALP)

		DMP	DSPP	ALP
At day 7	gr.1.a	1.008685	1.000727	1.009149
	gr.2.a	2.717381	3.129357	3.447509
	gr.3.a	5.876992	7.869692	8.507993
At day 14	gr.1.b	1.002403	1.020271	1.014454
	gr.2.b	5.897048	9.890532	6.136749
	gr.3.b	15.83688	19.7002	16.80364

DISCUSSION

The purpose of traditional endodontic regeneration techniques such apexogenesis, apexification, and revascularization is to repair the dentin–pulp complex at the lesion site utilizing biomaterials and stimulating cells, especially dental stem cells[28,29].

According to the International Society for Cellular Therapy's (ISCT) minimal criteria, human MSCs are defined by a combination of significant CD105, CD73, and CD90 expression and extremely low/no CD34, CD45, CD11a, CD19, and HLA-DR expression. Currently, there is no single cell marker that can be used to isolate and characterize MSCs[30,31].

As a result, we employed flow cytometry to validate the phenotypic of the stem cell line used in our experiment. CD-90 and CD105 immune expression were positive, but CD34 and CD45 immune expression were negative.

Several researches[32] have looked at the bioactivity and biocompatibility of dental biomaterials using dental stem cells derived from the pulp or the periodontium. The use of nano-sized particles for biomaterials has recently gained popularity in dentistry[33]. A variety of laboratory assays can be used to detect cytotoxic activity. Because of its sensitivity, the MTT test has been employed as a screening assay and is considered the gold standard of cytotoxicity assays[34].

The MTT assay revealed that HANPs at low concentrations (10 g/mL) are not only biocompatible with ATDSCs but also do not impede their growth in the current investigation. Remya *et al*., 2014 demonstrated that low concentrations of HANPs, less than 500 g/mL, are non-toxic and harmless to BMSCs at the molecular level[35]. Hii *et al*. also discovered that reducing the quantity of Nano-Hydroxyapatite-Silica Glass Ionomer cement boosted the cell survival of dental pulp stem cells[36]. Babaki *et al* investigated MTA's biocompatibility and found that at low concentrations, MTA not only demonstrated biocompatibility but also induced ATDSC proliferation[26].

The mineralized nodules of the induced cells were compared among the three groups of cells in this study. At days 7 and 14, cells incubated in odontogenic-HANPs medium produced the most mineralization nodules, followed by cells in odontogenic media alone. Non-induced cells produced no mineralized material in growth medium alone. Previous studies on DPSCs[37,38,39] or ATDSCs in the presence of MTA[40] revealed the similar outcome.

Dentin sialophosphoprotein (DSPP) and dentin matrix protein-1 are two non-collagenous proteins secreted by odontoblasts (DMP-1). Dentin sialophosphoprotein is a precursor protein for other proteins present in teeth and, to a lesser extent, in bone tissues[41]. It is broken down into three proteins during tooth development: dentin sialoprotein (DSP), dentin glycoprotein (DGP), and dentin phosphoprotein (DPP)[42]. DSPP gene transcription is regulated by DMP-1 during early odontoblast differentiation[43]. It has two functions: it causes odontoblastic differentiation and it is required for biomineralization[44].

ALP is a critical component of dentin biomineralization. It helps to promote mineralization by providing phosphate and allowing crystal formation to proceed. The rise in extracellular matrix mineralization globules at later stages is responsible for the late increase in ALP expression[45,46].

Because of its high sensitivity and precise genetic quantification, this method is regarded as the gold standard in gene detection [50]. As a result, the present investigation used qRT PCR to validate and quantify odontogenic differentiation in ATDSCs by quantifying the RNA levels of odontogenic differentiation genes (DMP, DSPP, and ALP)[37,47,48,49,50].

Furthermore, the stimulated cells' genotype revealed a considerable increase in odontogenic genes (DMP 1, DSPP, ALP). Within a 7-day and 14-day period, they rose in group 2. Within the three groups, group 3, which was cultured in odontogenic medium containing HANPs, demonstrated the most significant rise of the aforementioned three genes.

This finding could be explained by the fact that ATDSCs receiving dual odontogenic differentiation signals from HANPs (due to their hydrophilicity in combination with the alkaline pH and calcium ion released by hydration[51,6]) and odontogenic induction media promoted more odontogenic differentiation than those cells receiving only a single differentiation signal[37].

Our findings significantly support the use of adipose tissue stem cells for dental tissue regeneration, which was previously advocated. This is consistent with the findings of surgical studies of bone abnormalities, which showed that ATSCs result in bone regeneration. ATDSCs transplants in reconstructive medicine were used to repair a 7-year-old girl's cranial vault after a traumatic accident caused bone resorption, necessitating a decompressive craniotomy, which resulted in a 120-cm2 cranial defect[52].

The results of all of these studies clearly support the hypothesis that the ATDSCS can be employed as a more plentiful, easier-to-obtain alternative to DPSCs in the dental tissue regeneration process.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

التقييم المختبري لإمكانات التمايز السني للخلايا الجذعية المشتقة من الأنسجة الدهنية في وجود وغياب بلورات الهيدروكسيباتيت النانوية

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الخلفية: أصبح علاج تجديد الأنسجة باستخدام الخلايا الجذعية أحدث ما توصلت إليه دراسة الأسنان.
الهدف من الدراسة: تهدف الدراسة الحالية إلى استخدام الخلايا الجذعية المشتقة من الأنسجة الدهنية للتمايز السني، كبديل أكثر وفرة للخلايا الجذعية المشتقة من لب الأسنان ، باستخدام ٣ وسائط مختلفة.
المواد والطرق: تم استخدام خطوط الخلايا المحفوظة بالتبريد للخلايا الجذعية المشتقة من الأنسجة الدهنية في المرحلة الثالثة. تم توصيف هذه الخلايا باستخدام تحليل التدفق الخلوي ل CD٩٠ و CD١٠٥ و CD٣٤ و CD٤٥ و تم استخدام فحص سمية الخلايا لتحديد أفضل تركيز لجسيمات هيدروكسيباتيت النانوية التي يجب استخدامها في الدراسة. تم تجميع الخلايا في ٣ مجموعات: المجموعة ١ الخلايا المحتضنة في وسط النمو (كانت بمثابة المجموعة الحاكمة سلبي) ، المجموعة ٢ الخلايا المحتضنة في وسط سني المنشأ ، بينما نمت المجموعة ٣ في وسط سني المنشأ معدل باستخدام الجسيمات النانوية لهيدروكسيباتيت. تم قياس الفرق في تمايز الخلايا وإنتاج المواد المتكلسة بعد اسبوع وأسبوعان من بداية التجربة وقد تم تحقيق ذلك باستخدام اختبار التمعدن و تفاعل البلمرة المتسلسل لجينات التمايز السني المنشأ التي هي بروتين العاج سيالوفوسفوسفوبروتين (DSPP) ، الفوسفاتيز القلوي (ALP) والبروتين الفوسفوبروتين مصفوفة العاج ١ (DMP١).
النتائج: كان لدى المجموعة ٣ قدرة تمايز سنية أفضل بين المجموعات الأخرى حيث أظهرت زيادة معنوية في كل من إنتاج العقيدات المعدنية والتعبير عن جينات التمايز السنيي.
الخلاصة: كان للخلايا الجذعية المشتقة من الأنسجة الدهنية قدرة تمايز سنية المنشأ خاصة في وجود جسيمات هيدروكسيباتيت النانوية ويمكن استخدامها كبديل سهل ومصدر أكثر وفرة للخلايا الجذعية المشتقة من لب الأسنان.