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Effect of two different concentrations of 1α,25-dihydroxyvitamin D3 on odontogenic differentiation of stem cells from human exfoliated deciduous teeth

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ABSTRACT

Background: Stem cells from human exfoliated deciduous teeth (SHEDs) can transform into odontoblasts *in vitro* and *in vivo*. The role of 1α,25-dihydroxyvitamin D3 (1α,25 vitD3) has been reported in the mineralization of hard tissues and teeth, as well as osteoblastic differentiation. This study aimed to assess the effect of different concentrations of 1α,25 vitD3 on odontogenic differentiation of SHEDs.
Materials and Methods: In this experimental study, second-passage SHEDs were exposed to odontogenic medium along with 0, 10, 50, 100, and 150 nmol concentrations of in 1α,25 vitD3 to determine its optimal concentration for odontogenic differentiation. The methyl thiazolyl tetrazolium (MTT) assay was performed. Odontogenic differentiation was evaluated by QRT- polymerase chain reaction for dentin matrix protein (DMP1) and dentin sialophosphoprotein (DSPP) genes. Morphology of differentiated cells was studied by Scanning Electron Microscopy. Data were analyzed using the Kruskal–Wallis, Mann–Whitney, Friedman, and Chi-square test. $P < 0.05$ is considered statistically significant.
Results: MTT test result showed the two groups of odontogenic medium + 10 nm 1α,25 vitD3 and odontogenic medium + 150 nm 1α,25 vitD3 provided the most suitable conditions for cell viability at 72 h. Expression of both genes significantly increased in the presence of 1α,25 vitD3 ($P < 0.001$). Expression of both genes was significantly higher at 14 days compared with 7 days ($P < 0.01$). At both time points, expression of both genes was significantly higher in the presence of 150 nm 1α,25 vitD3 compared with 10 nm ($P < 0.01$). The accumulation of cells with odontoblastic morphology, cell interactions, and calcifications were evident.
Conclusion: 1α,25 vitD3 upregulates DMP1 and DSPP and results in odontogenic differentiation of SHEDs in odontogenic medium. This upregulation increases with time and by an increase in concentration of 1α,25 vitD3.

Key Words: Deciduous teeth, dental pulp, stem cells

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INTRODUCTION

Dental pulp contains stem cells with high potential for proliferation and differentiation to osteoblasts and odontoblasts.[1] Stem cells have unique properties, which differentiate them from other human cells. The

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most important characteristic of stem cells is their self-renewal capacity and the ability to differentiate into different cell lines.[2,3]

Regenerative dentistry is now considered a facile therapy to efficiently restore tooth function. After a tooth injury, dental pulp is involved in reparative dentinogenesis, where the cells elaborate microenvironments and deposit a new dentin matrix to repair the injured site. This is possible because of the presence of stem cells in the adult dental pulp, which are able to form odontoblasts under appropriate environments. Conventional endodontic surgery depends largely on the therapeutic treatment of disease and injured sites and/or the removal of the tissue and subsequent restoration with inert filling materials.[3,4]

Stem cells are a rich source for regeneration of dental tissue.[5] Gronthos *et al*.[6] were the first to confirm the presence of stem cells for the first time in the year 2000. Different types of stem cells are commonly used in tissue engineering. Stem cells from human exfoliated deciduous teeth (SHEDs) were first isolated by Miura *et al*.,[7] in 2003. These cells can differentiate into odontoblasts *in vitro* and *in vivo* and can be directly used for the treatment of teeth or for the fabrication of bioactive therapeutic agents *in vitro*.[2,8] Knowledge about the factors that regulate cell differentiation can greatly help in finding new, conservative treatment strategies to preserve the pulp tissue and restore the tooth structure.[4,8,9] Proliferation, chemotaxis, and differentiation of SHEDs to odontoblasts play a fundamental role in regeneration, formation of reparative dentin, and healing of pulp tissue following trauma.[10]

An interesting topic of research is to find effective and efficient materials to add to cell culture to enhance the proliferation of cells and their differentiation into different cell lines.[4,9] 1α,25-dihydroxyvitamin D3 (1α,25 vitD3) plays a fundamental role in the formation of dentin.[6] Its role in mineralization of hard tissue and teeth and osteoblastic differentiation has been previously confirmed *in vitro* and *in vivo*.[2,6,11,12] The shortage of 1α,25 vitD3 can cause enamel hypocalcification and result in insufficient mineralization of dentin structure.[11,12] Some studies have evaluated its effect on odontogenic differentiation of dental pulp stem cells of permanent teeth.[9,13] Evidence shows that 1α,25 vitD3 metabolites in 10, 100, and 350 nmol concentrations can cause osteogenic differentiation of dental pulp stem cells of permanent teeth.[11,12,14] Furthermore, it has been demonstrated that 100 nmol concentration of 1α,25 vitD3 enhances osteoblastic differentiation of SHEDs.[15] The role of 1α,25 vitD3 in tooth formation and is well known based on clinical and *in vivo* studies[6,11,12] However, the effect of 1α,25 vitD3 on odontogenic differentiation of SHEDs has not been previously. Since odontogenic differentiation can be evaluated by quantifying the expression of some related genes and level of mineralization of tissue,[16,17] this study aimed to assess the effect of 1α,25 vitD3 on odontogenic differentiation of SHEDs by quantifying the expression of dentin.

MATERIALS AND METHODS

In this experimental study, which was performed in Tissue Engineering and Regenerative Medicine Institute (Tehran, Islamic Azad University, Central Branch), SHEDs were obtained from Tabriz University of Medical Science[18] in cryovials in liquid nitrogen flask at −196°C. Sample size was calculated to be 3 wells in each group (a total of 24).

Preparation of cells

In order to defrost the cells, 4.5 mL of Dulbecco's modified Eagle's medium (Gibco, Munich, Germany) supplemented with 100 U/mL penicillin (Merck, Darmstadt, Germany) and 100 μg/mL streptomycin (Merck, Darmstadt, Germany) was transferred to a 15 mL Falcon tube and 0.5 mL of fetal bovine serum (Gibco, Munich, Germany) was added to it. The bottom of the cryovial containing frozen cells was placed in a bain-marie bath on a rotary shaker. As soon as the frozen crystal was detached from the internal wall of the vial, the vial was transferred under a hood.[19] The cell suspension was dripped into a Falcon tube containing complete culture medium and pipetted. The internal wall of the cryovial was rinsed with 1 mL of culture medium and the solution was transferred to the tube. The tube containing cells was then centrifuged at 130 g for 5 min.

After removing the supernatant, complete culture medium containing 20% fetal bovine serum was added to the cells. The cell suspension was transferred into a 25 mL flask and incubated at 37°C and 5% CO_2. The cells then started to proliferate in the flask.[20] The culture medium was replaced two to three times a week. After reaching 80% to 90% confluence in each flask, the cells were passaged.[7]

Preparation of 1α,25-dihydroxyvitamin D3

The 1α,25 vitD3 powder (Cat. No. 679101; Merck, Darmstadt, Germany) was maintained in 0.01M stock solutions in dimethyl sulfoxide (DMSO) and treated in culture medium with 10 nM and 100 nM concentration.[11,12]

Preparation of odontogenic medium

Odontogenic medium containing Dulbecco's modified Eagle's medium, 10% fetal bovine serum, 50 mmol beta-glycerophosphate, 50 µg/mL ascorbic acid 2 phosphate and 0.1 µmol dexamethasone[15,21] was purchased from Idea Zist NoTarikib Company (Tehran, Iran) and used in this study.

Methyl thiazolyl tetrazolium assay

To determine the optimal concentration of 1α,25 vitD3 to induce odontogenic differentiation of cells, 12.5 µL of the second-passage cell suspension containing 10,000 cells[18] was transferred into each well of a 96-well plate. The wells were divided into six groups of three; 200 µL of the odontogenic medium and 0, 10, 50, 100, and 150 nmol 1α,25 vitD3 were added to the wells in groups 1–5. The basic culture medium was added to the wells in group 6. The effect of 1α,25 vitD3 on stem cells was evaluated at 24, 48, and 72 h using the methyl thiazolyl tetrazolium (MTT) assay.[22] A 96-well plate was considered for each time point. The cells were seeded simultaneously in the three plates for assessment at 24, 48, and 72 h and the plates were then incubated for 24 h. After 24 h, the MTT powder (Merck, Darmstadt, Germany) was dissolved in phosphate buffered saline to obtain 0.5 mg/mL MTT solution. The tube was wrapped with aluminum foil. The respective plate was then removed from the incubator and cell density was evaluated under a microscope. The overlaying medium was discarded and 200 µL of MTT solution was added to each well and incubated at 37°C in the presence of CO2 for 3 h. Next, the MTT solution was replaced with 100 µL of DMSO. Colorimetry was performed using an ELISA Reader at 570 nm wavelength. The same was repeated at 48 and 72 h. This test was repeated for each concentration of 1α,25 vitD3 in triplicate to minimize errors. The graphs were drawn by ELISA Reader. The amount of generated color has a direct relationship with the number of metabolically active cells.

Induction of odontogenic differentiation

The cells were randomly divided into four groups and transferred to a 6-well plate for polymerase chain reaction (PCR). Two plates were considered for each time point; 100,000 cells (125 µL of cell suspension) and 3 mL of complete culture medium were added to each well. The following groups were evaluated:

- Group 1: Cells cultured in odontogenic medium (positive control)
- Group 2: Cells cultured in odontogenic medium containing 10 mmol 1α,25 vitD3
- Group 3: Cells cultured in odontogenic medium containing 150 mmol 1α,25 vitD3
- Group 4: Cells cultured in basic culture medium (negative control)

Odontogenic differentiation of cells was evaluated by quantifying the expression of dentin matrix protein (DMP1) and dentin sialophosphoprotein (DSPP) at 7 and 14 days using real-time RT-PCR.

Real time reverse transcription-polymerase chain reaction

In order to assess the expression of genes at the mRNA level, RNA was extracted from the cells. For this purpose, the cell suspension was mixed and homogenized with 1 mL of Tripure (Roche, Bassel, Switzerland) according to the manufacturer's instructions and then 200 µL of chloroform was added and the samples were centrifuged at 12,000 rpm at 4°C. The clear supernatant was then mixed with equal volume of isopropanol and the samples were stored at room temperature for 10 min. They were then centrifuged again at 12,000 rpm at 4°C for 10 min. Next, 1 mL of 75% ethanol was added to the RNA sediments and they were centrifuged at 7,500 rpm at 4°C for 8 min. The sediments were then exposed to DEPC-treated water. The quality of RNA samples was evaluated by 1% agarose gel electrophoresis and their quantity was determined using a NanoDrop.[11] To eliminate possible genomic contamination, the extracted RNA was treated with 1 U/µL of DNase I. For this purpose, 1 µL of DNase I buffer (Thermo Fisher, MA, USA) (x10) containing 0.5 µL MgCl2, 40 U/µL RiboLock (RNase inhibitor) and 1 µL DNase I enzyme (1 U/µL) was mixed with DEPC-treated water (Sigma, St. Louis, USA) and the final volume was reached to 10 µL. The reaction solution was incubated at 37°C for 30 min. Next, 1 µL of EDTA (50 mmol) was added to the solution and the mixture was incubated at 65°C for 10 min (to deactivate the enzyme). In order to synthesize cDNA, 1 µg of extracted RNA treated with 1 µL of DNase I was mixed with 1 µL of primer (0.5 µg/

μL) and the final volume was reached to 12 μL using DEPC-treated water. The mixture was incubated at 65°C for 5 min. The samples were then transferred on ice and 4 μL of buffer (×5), 0.5 μL of RiboLock, 2 μL of dNTPs mixture (10 mmol) and 1 μL of M-MuLV reverse transcriptase (20 u/μL) were added to the reaction solution. The final volume was reached to 20 μL using DEPC-treated distilled water. The reaction solution was incubated at 42°C for 1 h and then at 70°C for 5 min. The synthesized cDNAs were stored at −20°C. All procedures were performed according to the instructions provided in the cDNA synthesis kit (ThermoFisher Science, MA, USA).

Polymerase chain reaction

PCR was performed to assess the changes in expression of DMP1 and DSPP genes according to GeneRunner program. The synthesized cDNA was used as a pattern. The beta-2 macroglobulin gene was used as the reference gene. The PCR protocol included one cycle of primary denaturation at 94°C for 7 min followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 58°C for 45 s and extension at 72°C for 30 s and one cycle of final extension at 72°C for 10 min. Table 1 shows the sequence of primers used.

Scanning electron microscopy

For Scanning Electron Microscopy (SEM) assessment, 125 μL of cell suspension containing 100,000 cells was transferred to two 6-well plates as described earlier. A microscopic slide was placed at the bottom of the plates and 3 mL of odontogenic culture medium and 10 and 150 nm concentrations of 1α,25 vitD3 were added to the wells of each group. After 14 days, the plates underwent SEM assessment and micrographs were obtained.

Statistical analysis

Data were analyzed using the Kruskal–Wallis test, Mann–Whitney test, Friedman test, and Chi-square test.

RESULTS

As mentioned earlier, in order to determine the optimal concentration of 1α,25 vitD3 to induce odontogenic differentiation, the MTT assay was performed at 24, 48, and 72 h for all 6 groups. Table 2 shows the results of the MTT assay at 24, 48, and 72 h. As shown, the two groups of odontogenic medium + 10 nm 1α,25 vitD3 and odontogenic medium + 150 nm 1α,25 vitD3 provided the most suitable conditions for odontogenic differentiation of cells at 72 h.

Table 1: Sequence of primers used in this study

Primer name	Primer sequence
Human DSPP F	AAGCAATAACAGTACAGACAATG
Human DSPP R	TCTTGCTGTTGATCTGAGGTG
Human DMP1 F	TCATAGCAGAAGACATTGGAG
Human DMP1 R	TCTTATCCAAAACAACTCACTG
Human B2M F	TGCTGTCTCCATGTTTGATGTATC
Human B2M R	TCTCTGCTCCCCACCTCTAAGT

DSPP: Dentin sialophosphoprotein, DMPI: Dentin matrix protein

Tables 3 and 4 show the expression of DMP1 and DSPP genes in the 8 groups (basic culture medium, odontogenic medium, odontogenic medium + 10 nm 1α,25 vitD3, and odontogenic medium + 150 nm 1α,25 vitD3 at 7 and 14 days).

Expression of dentin matrix protein 1

Minimum expression of DMP1 was noted in group 1 followed by group 3 at 7 days. The Kruskal–Wallis test showed a significant difference in expression of DMP1 among the groups ($P < 0.001$). At 7 and 14 days, the expression of DMP1 was the same in groups 1 and 2 but expression of this gene increased over time in groups 3 and 4. The Kruskal–Wallis test showed that the increase in expression of DMP1 in groups 3 and 4 was significant over time ($P < 0.01$). Also, the expression of DMP1 gene in group 4 was significantly greater than that in group 3 at 7 and 14 days ($P < 0.001$). The Friedman test showed maximum gene expression in group 4 on day 14 ($P < 0.001$). The difference in gene expression between groups 3 and 4 at 14 days was smaller than that at 7 days. The Chi-square test showed that the reduction in this difference had a significant association with time ($P < 0.05$).

Expression of dentin sialophosphoprotein

Maximum expression of DSPP was noted in group 4 at 14 days while minimum expression was noted in group 1 followed by group 2. The Kruskal–Wallis test showed a significant difference in expression of DSPP among the groups ($P < 0.001$). At 7 and 14 days, the expression of DSPP was the same in groups 1 and 2 but increased in groups 3 and 4 over time. The Kruskal–Wallis test showed that the increase in expression of DSPP in groups 3 and 4 was significant over time ($P < 0.01$).

The Mann–Whitney test revealed significantly higher expression of DSPP in group 4 compared with group 3 at both 7 and 14 days ($P < 0.001$). The Friedman test showed maximum expression of DSPP on day 14 in group 4 ($P < 0.001$). The difference in expression

Table 2: Results of the methyl thiazolyl tetrazolium assay at 24, 48 and 72 h (*n*=3 wells in each group)

Time point	Groups	Minimum	Maximum	Mean	SD
24 h	Basic culture medium	0.118	0.233	0.174	0.037
	Odontogenic medium	0.138	0.268	0.183	0.074
	Odontogenic medium+10 nmol D3	0.199	0.250	0.228	0.021
	Odontogenic medium+50 nmol D3	0.189	0.224	0.214	0.022
	Odontogenic medium+100 nmol D3	0.152	0.302	0.223	0.075
	Odontogenic medium+150 nmol D3	0.194	0.250	0.231	0.032
48 h	Basic culture medium	0.182	0.305	0.254	0.064
	Odontogenic medium	0.167	0.240	0.213	0.041
	Odontogenic medium+10 nmol D3	0.197	0.234	0.212	0.021
	Odontogenic medium+50 nmol D3	0.226	0.268	0.254	0.024
	Odontogenic medium+100 nmol D3	0.234	0.245	0.241	0.006
	Odontogenic medium+150 nmol D3	0.174	0.279	0.223	0.053
72 h	Basic culture medium	0.266	0.379	0.307	0.006
	Odontogenic medium	0.269	0.293	0.282	0.007
	Odontogenic medium+10 nmol D3	0.221	0.289	0.252	0.034
	Odontogenic medium+50 nmol D3	0.184	0.2250–	0.198	0.023
	Odontogenic medium+100 nmol D3	0.104	0.240	0.179	0.092
	Odontogenic medium+150 nmol D3	0.199	0.267	0.234	0.034

SD: Standard deviation

Table 3: Expression of dentin matrix protein in the 8 groups

Groups	Time point (days)	Expression of DMP1
Basic culture medium	7	0
	14	0
Odontogenic medium	7	1
	14	1
Odontogenic medium+10 nmol 1α,25-dihydroxy Vitamin D3	7	0.46±0.32
	14	12.82±4.23
Odontogenic medium+150 nmol 1α,25-dihydroxy Vitamin D3	7	13.66±4.02
	14	20.39±1.02

DMPI: Dentin matrix protein

Table 4: Expression of dentin sialophosphoprotein in the 8 groups

Groups	Time point (days)	Expression of DSPP
Basic culture medium	7	0
	14	0
Odontogenic medium	7	1
	14	1
Odontogenic medium+10 nmol 1α,25-dihydroxy Vitamin D3	7	10.54±1.37
	14	21.68±4.43
Odontogenic medium+150 nmol 1α,25-dihydroxy Vitamin D3	7	25.57±4.05
	14	30.93±4.23

DSPP: Dentin sialophosphoprotein

of DSPP between groups 3 and 4 was smaller at 14 days compared with 7 days. The Chi-square test demonstrated that this reduction in difference was significant over time ($P < 0.05$).

Figures 1 and 2 show SEM micrographs of 10 nm and 150 nm 1α,25 vitD3 groups at 14 days, revealing accumulation of cells, their odontoblastic morphology and interactions.

DISCUSSION

This study assessed the effect of 1α,25 vitD3 on odontogenic differentiation of SHEDs quantified by the expression of DMP1 and DSPP genes. DMP1 and DSPP genes encode proteins of the small integrin-binding ligand N-linked glycoprotein family. They are both present on chromosome 4 and produce two main non-collagenous dentin proteins,[23] which are

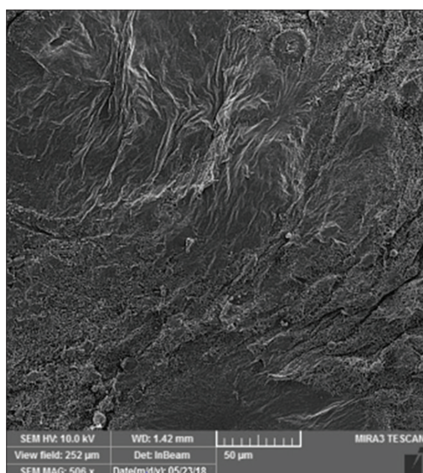

Figure 1: Scanning Electron Microscopy micrograph of 10 nmol 1α,25-dihydroxyvitamin D3 group at 14 days, revealing accumulation of cells, their odontoblastic morphology and interactions

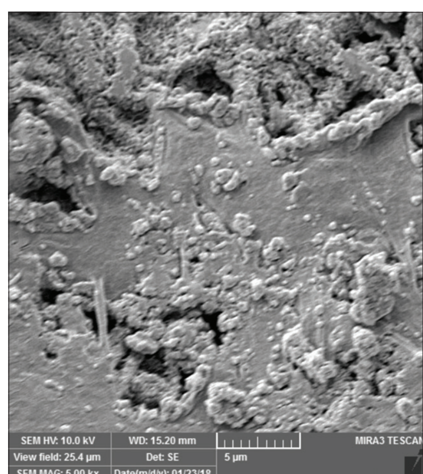

Figure 2: Scanning Electron Microscopy micrograph of 150 nm 1α,25-dihydroxyvitamin D3 group at 14 days, revealing accumulation of cells, their odontoblastic morphology and interactions. Calcifications can be seen (arrows) confirming odontogenic differentiation of cells

necessary for dentin formation and play a fundamental role in the process of dentin mineralization. Defects of these genes are responsible for the occurrence of dentinogenesis imperfecta and dentin dysplasia. The expression of these genes is correlated with each other.[11] Gibson *et al*.[1] reported that expression of DMP1 controls the expression of DSPP. They demonstrated that suppressing the expression of DMP1 gene in rats decreased the expression of DSPP. However, no change in expression of DMP1 was noted in rats when DSPP gene was inactivated.[1]

We assessed the expression of these genes in eight groups of basic culture medium, odontogenic medium, odontogenic medium + 10 nm 1α,25 vitD3, and odontogenic medium + 150 nm 1α,25 vitD3 at 7 and 14 days. The results showed that expression of both genes in 1α,25 vitD3 groups was significantly higher than that in the control group. The expression of both genes increased with time. In addition, in the presence of 150 nmol concentration of 1α,25 vitD3, the expression of both genes was higher at 7 and 14 days when compared with the presence of 10 nmol concentration of 1α,25 vitD3. However, the difference in gene expression in presence of 150 nmol concentration of 1α,25 vitD3 on day 14 was smaller than that on day 7 when compared with the presence of 10 nmol 1α,25 vitD3.

Woo *et al*.[9] demonstrated that the expression of protein and mRNA related to DSPP and DMP1 genes are increased in permanent dental pulp stem cells on days 2 and 7. They reported that the expression of DSPP in the presence of 100 nmol 1α,25 vitD3 was greater than that in presence of 10 nmol 1α,25 vitD3 at both 2 and 7 days. The expression of DMP1 on day 2 was greater in presence of 100 nmol 1α,25 vitD3 compared with 10 nmol 1α,25 vitD3; but the difference in this regard was not significant at 7 days. Similar to our study, they indicated that the difference in gene expression between the two concentrations of 1α,25 vitD3 became smaller over that in groups containing 1α,25 vitD3, the production of DSP was higher than that in the control group and this production had a dose-dependent fashion. This finding was in line with our result. Mucuk *et al*.[13] reported that the number of DSP + cells increased in a dose-dependent manner in the presence of 1α,25 vitD3 in the pulp stem cells of permanent teeth. The expression of DSP was evaluated after 7 days by measuring the protein in cells using immunohistochemistry.

Tonomura *et al*.[24] demonstrated that 1α,25 vitD3 induced the formation of DSP by dental pulp stem cells of permanent teeth cultured in presence of dexamethasone and beta glycerophosphate. They demonstrated the production of DSP only in presence of all three factors of dexamethasone, beta glycerophosphate and 1α,25 vitD3. They used 1000 nmol concentration of 1α,25 vitD3, which was much higher than the concentrations used in our study.

Almost all previous studies unanimously believe that 1α,25 vitD3 increases the expression of DSPP. However, the results regarding the effect of 1α,25 vitD3 on expression of DMP1 are controversial. In contrast to the results of Woo *et al*.,[9] Mucuk

et al.,[13] and our study, Nociti *et al*.[23] evaluated murine osteoblasts and cementoblasts and showed that 1α,25 vitD3 down-regulated the DMP1 gene. They used 0.01, 10, and 100 nmol concentrations of 1α,25 vitD3, extracted the mRNA after 24 h and assessed the expression of DMP1 by PCR. Both osteoblasts and cementoblasts showed dose-dependent down-regulation of DMP1 following exposure to 1α,25 vitD3. They also showed down-regulation of DMP1 in murine cementoblasts over time (from 3 to 72 h). However, it should be noted that they evaluated murine cells and different cell types. Furthermore, their extraction time was shorter than that in our study, which could have affected the results.

Similar to the results of Nociti *et al*.,[23] Wang *et al*.[25] used RNA sequencing and showed that parathormone and 1α,25 vitD3 synergistically upregulated 36 genes and down-regulated 27 genes in murine cementoblasts; these genes were related to bone/tooth homeostasis, cell differentiation and calcium signaling. They also showed decreased expression of DMP1.

Further studies are required to assess the effect of 1α,25 vitD3 alone and in combination with other odontogenic materials in basic culture medium on odontogenic differentiation of SHEDs. Also, higher concentrations of 1α,25 vitD3 should be evaluated in future studies to find the most optimal concentration for this purpose without cytotoxic effects. Last but not least, future studies should employ tests such as Western Blotting and ELISA instead of MTT assay to find more accurate results.

CONCLUSION

The results showed that 1α,25 vitD3 upregulated DMP1 and DSPP genes and caused odontogenic differentiation of SHEDs in an odontogenic medium. This upregulation increased with time and by an increase in concentration of 1α,25 vitD3.

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Conflicts of interest

The authors of this manuscript declare that they have no conflicts of interest, real or perceived, financial or non-financial in this article.

REFERENCES

1. Gibson MP, Zhu Q, Wang S, Liu Q, Liu Y, Wang X, *et al*. The rescue of dentin matrix protein 1 (DMP1)-deficient tooth defects by the transgenic expression of dentin sialophosphoprotein (DSPP) indicates that DSPP is a downstream effector molecule of DMP1 in dentinogenesis. J Biol Chem 2013;288:7204-14.
2. Potdar PD, Jethmalani YD. Human dental pulp stem cells: Applications in future regenerative medicine. World J Stem Cells 2015;7:839-51.
3. Soares DG, Rosseto HL, Basso FG, Scheffel DS, Hebling J, Costa CA. Chitosan-collagen biomembrane embedded with calcium-aluminate enhances dentinogenic potential of pulp cells. Braz Oral Res 2016;30:e54.
4. Teti G, Salvatore V, Focaroli S, Durante S, Mazzotti A, Dicarlo M, *et al*. *In vitro* osteogenic and odontogenic differentiation of human dental pulp stem cells seeded on carboxymethyl cellulose-hydroxyapatite hybrid hydrogel. Front Physiol 2015;6:297.
5. Paduano F, Marrelli M, White LJ, Shakesheff KM, Tatullo M. Odontogenic differentiation of human dental pulp stem cells on hydrogel scaffolds derived from decellularized bone extracellular matrix and collagen Type I. PLoS One 2016;11:e0148225.
6. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. Proc Natl Acad Sci U S A 2000;97:13625-30.
7. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, *et al*. SHED: Stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 2003;100:5807-12.
8. Annibali S, Cristalli MP, Tonoli F, Polimeni A. Stem cells derived from human exfoliated deciduous teeth: A narrative synthesis of literature. Eur Rev Med Pharmacol Sci 2014;18:2863-81.
9. Woo SM, Lim HS, Jeong KY, Kim SM, Kim WJ, Jung JY. Vitamin D promotes odontogenic differentiation of human dental pulp cells via ERK activation. Mol Cells 2015;38:604-9.
10. Qin W, Huang QT, Weir MD, Song Z, Fouad AF, Lin ZM, *et al*. Alcohol inhibits odontogenic differentiation of human dental pulp cells by activating mTOR signaling. Stem Cells Int 2017;2017:8717454.
11. Lou YR, Toh TC, Tee YH, Yu H. 25-Hydroxyvitamin D 3 induces osteogenic differentiation of human mesenchymal stem cells. Sci Rep 2017;7:42816.
12. Posa F, Di Benedetto A, Colaianni G, Cavalcanti-Adam EA, Brunetti G, Porro C, *et al*. Vitamin D effects on osteoblastic differentiation of mesenchymal stem cells from dental tissues. Stem Cells Int 2016;2016:9150819.
13. Mucuk G, Sepet E, Erguven M, Ekmekcı O, Bılır A. 1,25-Dihydroxyvitamin D3 stimulates odontoblastic differentiation of human dental pulp-stem cells *in vitro*. Connect Tissue Res 2017;58:531-41.
14. Khanna-Jain R, Vuorinen A, Sándor GK, Suuronen R, Miettinen S. Vitamin D(3) metabolites induce osteogenic differentiation in human dental pulp and human dental follicle cells. J Steroid Biochem Mol Biol 2010;122:133-41.
15. Mojarad F, Amiri I, Rafatjou R, Janeshin A, Farhadian M. The Effect of 1α,25 (OH) 2D3 on Osteogenic Differentiation of Stem Cells from Dental Pulp of Exfoliated Deciduous Teeth. J Dent (Shiraz) 2016;17:348-53.
16. Ching HS, Luddin N, Rahman IA, Ponnuraj KT. Expression

of odontogenic and osteogenic markers in DPSCs and SHED: A review. Curr Stem Cell Res Ther 2017;12:71-9.

17. Yang X, Song Z, Chen L, Wang R, Huang S, Qin W, *et al*. Role of transient receptor potential channel 6 in the odontogenic differentiation of human dental pulp cells. Exp Ther Med 2017;14:73-8.
18. Samiei M, Aghazadeh M, Movassaghpour AA, Fallah A, Aminabadi NA, Pakdel SM, *et al*. Isolation and Characterization of Dental Pulp Stem Cells from Primary and Permanent Teeth. J American Sci 2013;9:123-33.
19. Terry C, Dhawan A, Mitry RR, Lehec SC, Hughes RD. Optimization of the cryopreservation and thawing protocol for human hepatocytes for use in cell transplantation. Liver Transpl 2010;16:229-37.
20. Lian M, Zhang Y, Shen Q, Xing J, Lu X, Huang D, *et al*. JAB1 accelerates odontogenic differentiation of dental pulp stem cells. J Mol Histol 2016;47:317-24.
21. Zhu L, Ma J, Mu R, Zhu R, Chen F, Wei X, *et al*. Bone morphogenetic protein 7 promotes odontogenic differentiation of dental pulp stem cells *in vitro*. Life Sci 2018;202:175-81.
22. Kim SM, Lee BN, Koh JT, Chang HS, Hwang IN, Oh WM, *et al*. The effect of chlormadinone acetate on odontogenic differentiation of human dental pulp cells: *In vitro* study. BMC Oral Health 2017;17:89.
23. Nociti FH Jr, Foster BL, Tran AB, Dunn D, Presland RB, Wang L, *et al*. Vitamin D represses dentin matrix protein 1 in cementoblasts and osteocytes. J Dent Res 2014;93:148-54.
24. Tonomura A, Sumita Y, Ando Y, Iejima D, Kagami H, Honda MJ, *et al*. Differential inducibility of human and porcine dental pulp-derived cells into odontoblasts. Connect Tissue Res 2007;48:229-38.
25. Wang L, Tran AB, Nociti FH Jr, Thumbigere-Math V, Foster BL, Krieger CC, *et al*. PTH and Vitamin D Repress DMP1 in Cementoblasts. J Dent Res 2015;94:1408-16.