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Evaluation of vitamin D supplementation in osteogenic differentiation potential of diabetic and non-diabetic dental pulp stem cells

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Abstract

AIM. The aim of the present study was to assess the influence of vitamin D supplementation on the osteogenic differentiation potential of Dental Pulp Stem Cells (DPSC) in diabetic and non-diabetic subjects.

MATERIALS AND METHODS. The experimental study was conducted using oral mesenchymal stem cells (MSC) derived from adult dental pulp extirpated from extracted permanent premolar and third molar teeth of healthy patients and diabetic patients. Pulp tissue was extirpated, sectioned, and cultured in T25 flasks with Minimum essential medium-Alpha (MEM- α), fetal bovine serum (FBS) and antibiotic-antimycotic reagents. Stem cells were isolated, characterized via flow cytometry for specific markers, and subjected to tri-lineage differentiation which was confirmed through staining reagents such as Alizarin Red for osteogenesis, Safranin O for chondrogenesis, and Oil Red O for adipogenesis. Subsequently, influence of Vitamin D3 on the DPSCs viability was assessed employing MTT assays across varying concentrations.

RESULTS. Findings of the present study show that Vitamin D3 plays a significant role in enhancing osteogenic differentiation of DPSCs. Diabetic groups showed poor ability for bone regeneration as compared to the control group, with considerable disparity regarding biomechanical properties as well as decreased levels of hypoxia-inducible factor (HIF-1α) and vascular endothelial growth factor (VEGF). Subsequently, the study highlighted the significance of optimal vitamin D levels aiding in bone regeneration.

CONCLUSIONS. Vitamin D supplementation has shown to have a positive effect on the osteogenic differentiation of DPSCs, especially under diabetic conditions. The outcomes of this study infer the therapeutic potential of vitamin D in bone regeneration.

Keywords: dental pulp, mesenchymal stem cell, bone regeneration, diabetic, in vitro

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Оценка влияния добавок витамина D на остеогенную дифференцировку стволовых клеток пульпы зуба у пациентов с сахарным диабетом и без него

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Резюме

ЦЕЛЬ. Целью настоящего исследования было оценить влияние добавок витамина D на остеогенный потенциал дифференцировки стволовых клеток пульпы зуба (DPSC) у пациентов с сахарным диабетом и без него.

МАТЕРИАЛЫ И МЕТОДЫ. Экспериментальное исследование было проведено с использованием мезенхимальных стволовых клеток полости рта (MSC), полученных из пульпы постоянных премоляров и третьих моляров, удаленных у взрослых здоровых пациентов и пациентов с сахарным диабетом.

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Пульповая ткань была экстерпирована, секционирована и культивировалась во флаконах T25 с минимальной основной средой α (MEM-α), фетальной бычьей сывороткой (FBS) и антибиотико-противогрибковыми реагентами. Стволовые клетки были выделены, охарактеризованы методом проточной цитометрии по экспрессии специфических маркеров и подвергнуты трилинейной дифференцировке, подтвержденной окрашиванием: Alizarin Red для остеогенеза, Safranin O для хондрогенеза и Oil Red O для адипогенеза. Влияние витамина D3 на жизнеспособность DPSCs оценивалось с использованием MTT-тестов при различных концентрациях.

РЕЗУЛЬТАТЫ. Полученные данные свидетельствуют о значительной роли витамина D3 в усилении остеогенной дифференцировки DPSCs. В группах с сахарным диабетом наблюдалась сниженная способность к костной регенерации по сравнению с контрольной группой, а также выраженные различия в биомеханических свойствах, сниженные уровни фактора, индуцируемого гипоксией (HIF-1α), и сосудистого эндотелиального фактора роста (VEGF). Исследование подчеркивает важность поддержания оптимального уровня витамина D для процессов регенерации костной ткани.

ВЫВОДЫ. Добавки витамина D оказывают положительное влияние на остеогенную дифференцировку DPSCs, особенно в условиях сахарного диабета. Результаты данного исследования указывают на терапевтический потенциал витамина D в костной регенерации.

Ключевые слова: пульпа зуба, мезенхимальные стволовые клетки, костная регенерация, сахарный диабет, *in vitro*

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INTRODUCTION

DPSCs have gained popularity as a valuable source of MSCs owing to their unique characteristics along with therapeutic applications [1; 2]. These cells are sourced from the dental pulp tissue and possess properties similar to bone marrow derived MSCs, such as the capability of the multilineage differentiation, including the potential to transform into osteoblasts, chondrocytes, and adipocytes [3–5]. The non-invasive method of extraction and immunomodulatory capabilities further enhance their appeal for therapeutic use [6].

The osteogenic differentiation of DPSCs is of particular significance for use in bone repair and regeneration [7; 8]. DPSCs have proven to differentiate effectively into osteoblasts, which contribute to the establishment of mineralized matrices, and exhibit a range of osteogenic markers [8; 9]. However, the osteogenic potential is influenced by several factors, including the cellular microenvironment, growth factors, and external stimuli [10; 11]. Among these, Vitamin D is known to play a pivotal role in regulating calcium metabolism and cellular differentiation [12].

In recent years, role of vitamin D in the metabolic processes of MSCs has been extensively studied. It has been shown that the differentiation of MSCs into osteoblasts is increased by the presence of 1,25-dihydroxyvitamin D3 (1,25(OH)2D3), an active metabolite of vitamin D3. Additionally, MSCs, especially DPSCs have been known to possess proteins responsible for Vitamin D3 metabolism, catalyzing the conversion and activation of Vitamin D3 [13]. These established facts, direct towards the potential of vitamin D supplementation in enhancing the osteogenic capacity of DPSCs, especially in immunocompromised conditions such as in Diabetes, a condition that is well known to impair stem cell functionality [14–16]. The adverse effect that diabetes has on the stem cell behaviour necessitates further research on the effects vitamin D in mitigating these difficulties. Hence, this study focuses to evaluate the osteogenic differentiation potential of vitamin D supplementation on both diabetic and non-diabetic DPSCs, thereby addressing a significant gap in the current literature.

This research seeks to provide insights on the rationale of using vitamin D3 supplementation to enhance therapeutic outcomes in bone modifying conditions that require the augmentation of host regenerative capabilities. Considering the chronic burden that diabetes and its associated conditions provide on the population, finding therapeutic strategies that contribute to minimizing the systemic effects of diabetes, like the use of Vitamin D3 supplementation to boost regenerative potential of DPSCs is pragmatic. There has been extensive research on the effect of vitamin D on the osteogenic potential of MSCs, but only a few studies have been carried out on dental stem cells [17; 18]. Also, the combination of diabetic influence on the MSCs and the enhancing effect of vitamin D on the osteogenesis of the MSCs have never been addressed with respect to dental MSCs. Hence, the present study aims at assessing the osteogenic potential of DPSCs when treated with diabetic and healthy serum.

MATERIALS AND METHODS

Study Design and Ethical Approval

This experimental study was conducted in the Regenerative Medicine Laboratory at Dr. D.Y. Patil Dental College and Hospital, Pune, India, following approval from the Institutional Ethics Committee. Informed consent was obtained from all participants whose biological specimens were utilized in the research.

Sample Collection

DPSCs were isolated from the extracted permanent teeth (premolar and third molar) of both diabetic and non-diabetic patients. The selection criteria for participants included age, health status, and the absence of systemic diseases that could affect bone metabolism. Teeth were extracted for orthodontic or disimpaction purposes, without damaging the pulp and collected under sterile conditions.

Tooth Preparation and Pulp Extraction

Each tooth included in the study were cleansed using a 5% solution of sodium hypochlorite, followed by three rinses with Phosphate buffered saline (PBS) to avoid any microbiological contamination. Further, pulp was extirpated by drilling utilizing an airotor and straight fissure burs followed by transferring them in a sterile petri dish filled with PBS (Fig. 1).

Explant Culture Methodology

The pulp tissue which was first rinsed with PBS and antibiotic solution several times was then excised into miniscule pieces, approximately 1–2 mm in size. Simultaneously, T25 flask surface was coated with FBS and placed into a CO_2 incubator at 37°C. Once the pulp tissue fragmentation was complete it was transferred into the pre-incubated T25 flask, then a drop of FBS containing 1% antibiotic-antimycotic (Ab-Am) solution was introduced and the flask was incubated for 24 hours.

After 24 hours flask was enriched with MEM-α and combination of ten percentage of FBS with 1% Ab-Am solution (Fig. 2). Then the flask was further incubated for 4 days and after every four days it was subjected to examination to assess for cellular expansion and proliferation, employing phase contrast microscope (Fig. 3). Culture Media in the flask was replaced biweekly.

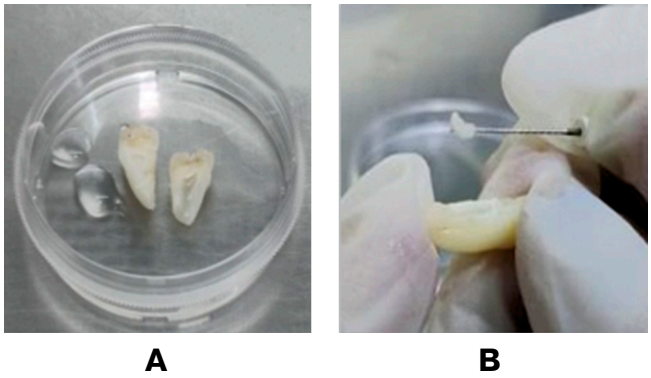


Fig. 1. Teeth specimen after extirpation of pulp (*A*); sterile extraction of pulp (*B*)

Рис. 1. Зубной образец после экстерпации пульпы (*A*); стерильное извлечение пульпы (*B*)

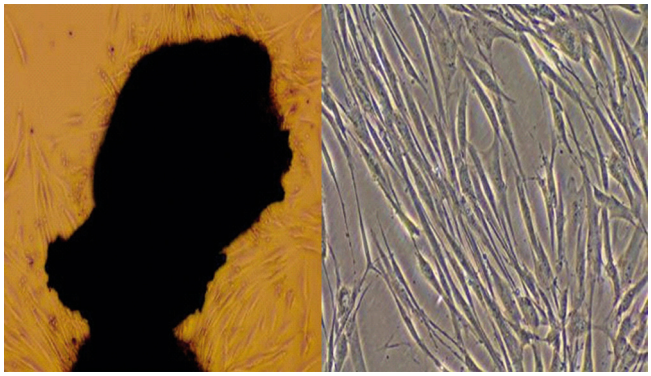
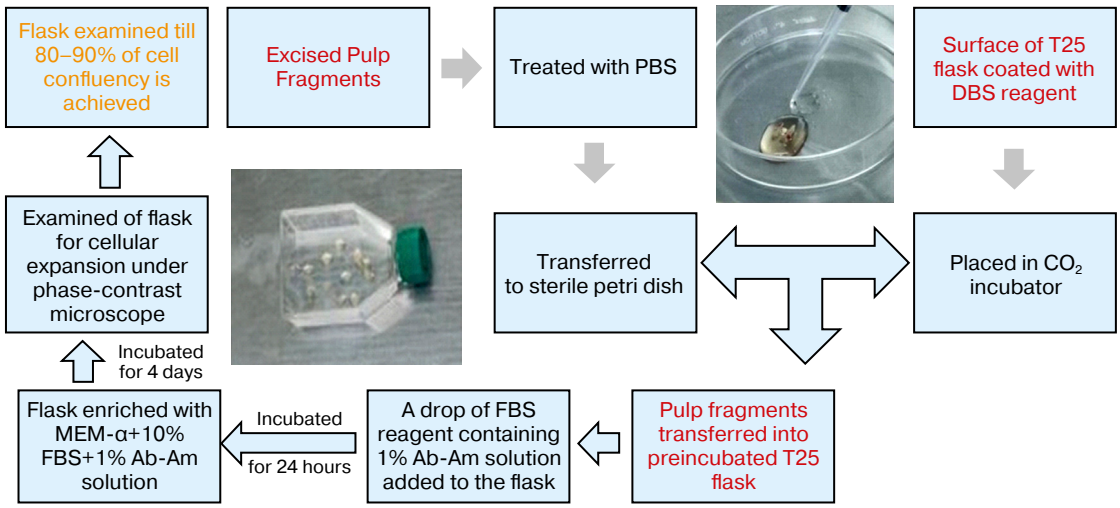


Fig. 3. Cultured cells under Phase-contrast microscope

Рис. 3. Культивированные клетки под фазово-контрастным микроскопом



Note. PBS – phosphate buffered solution, FBS – fetal bovine serum, MEM-α – minimum essential medium–Alpha, Ab–Am solution – antibiotic-antimycotic solution

Fig. 2. Flowchart depicting procedure to culture DPSCs

Рис. 2. Блок-схема, демонстрирующая процедуру культивирования стволовых клеток пульпы зуба (DPSC)

Cell passaging

The cultured stem cells were then passaged as they achieved 80–90% confluence within the flask, following which they underwent trypsinization process. The process involved removing the liquid, washing with PBS, and adding 2 microliters of trypsin after which the mix was incubated for a minute. After incubation, trypsin activity was halted with complete media, and non-adherent cells were removed. The suspension was then centrifuged, yielding a cell pellet, which were then resuspended in complete media and shifted to a new T25 flask (passage zero, P0). This process was repeated every 2 days until cells reached passage 4.

Characterization of DPSCs

The MSCs derived from dental pulp were characterized for their stemness properties using flow cytometry. This technique involved the use of specific cell surface markers, including CD34, CD44, CD45, CD73, CD90(PE-labeled), and HLA-DR(FITC-labeled). Subsequently, the process of trypsinization and collection of cells in the log- growth phase was performed, specimens further fixed with 4% paraformaldehyde, following a rinse using PBS solution comprising of 0.5% bovine serum albumin (BSA) the cells further incubated with fluorescently labelled antibodies specific to the markers of interest. Data analysis was conducted with the 'Cell Quest Pro program.

Tri-lineage differentiation

The DPSCs were further induced for a tri-lineage differentiation:

Osteogenic Differentiation: DPSCs were induced to undergo osteogenic differentiation using a medium supplemented with dexamethasone, ascorbic acid-2-phosphate, and β-glycerophosphate. The medium was replaced every three days for 21 days. Confirmation of differentiation was achieved with Alizarin Red staining, which detected calcium deposits in the cells.

Chondrogenic Differentiation: DPSCs were induced to undergo chondrogenic differentiation using a medium containing sodium pyruvate, dexamethasone, ascorbate-2-phosphate, TGF-b3, and L-proline. The culture medium was changed every three days for 21 days. Chondrogenic differentiation was validated using Safranin O staining, which detected glycosaminoglycan synthesis.

Adipogenic Differentiation: Adipogenic differentiation of the DPSCS was achieved by using a medium consisting of dexamethasone, indomethacin, 3-isobutyl-1-methylxanthine, and insulin. Induction medium was changed every three days for 21 days. Adipogenic differentiation was assessed using Oil Red O staining, which detected lipid droplets in cells.

Treatment of DPSCs with Vitamin D and assessment of post-treatment cellular viability

DPSCs were treated with variable concentrations of Vitamin D3 (0-200 nM) for 48 hours. Cellular viability were assessed utilizing the MTT test. The assay evaluated the viability of DPSCs treated with Vitamin D extract for 48 hours. After treatment, cells rinsed with PBS, detached using 0.25% Trypsin-EDTA, and centrifuged. The pellet was resuspended in complete media (DMEM media in combination with high glucose supplemented with 10% FBS and 1% Ab-Am), and 100 µl of this suspension was seeded into 12 wells of a 96-well plate. Plate was incubated for twenty-four hours at 37°C under 5% CO_2 and 95% humidity. Media was then replaced with 100 µl of varying Vitamin D concentrations (prepared in complete media) added to triplicate wells, followed by another 48-hour incubation. To assess cytotoxicity, each well was covered with 50 µl of MTT reagent, following which plate was incubated for three hours. The reaction was stopped by using 100 µl dimethyl sulfoxide (DMSO), dissolving formazan crystals, and absorbance at 570 nm was measured using a microplate spectrophotometer (SkanIt Software 5.0). Blank-adjusted absorbance (using DMSO-only controls) was calculated as mean ± standard error of the mean, and results were graphically represented as absorbance A570 versus extract concentration.

Preparation of Human Blood Serum

Selection criteria: To acquire human blood sera, blood samples were collected from healthy and diabetic participants. Certain criteria were established to include and exclude participants to avoid bias.

In Diabetic participants, age, gender and period of type 2 diabetes mellitus (T2DM) was matched. To prevent bias from bone modifying drugs, any diabetic participants who had undergone thiazolidinedione (rosiglitazone or pioglitazone) treatment and individuals with osteoporotic fracture were excluded from the study.

Patients having normal glucometabolic characteristics with HbA1c (Glycated hemoglobin test) levels below 6.5 percent were considered as non-diabetic/healthy individuals and were matched for age, and gender

Serum Processing: The collected blood was allowed to clot, and serum was separated by the process of centrifugation. Serum was then heat-processed at 56°C for thirty minutes to inactivate any potential pathogens. Following this, the serum underwent filtration through a 0.22 µm membrane to ensure sterility. The treated serum was cryopreserved at –20°C until needed for cell culture.

Osteogenic differentiation of DPSCs

Cells were placed in a 24-well plate at a cell concentration of 8×10^4 cells/ml and cultivated under standard conditions using 10% α-MEM and 1% Ab-Am solution. After 24 hours, cells were evaluated for 80% confluency. From that point onwards the cells were evaluated for osteogenic differential potential. That point was considered as day zero and the osteogenic differentiation was executed for 21 days. Following the induction process, the medium was substituted after every three days for 21 days. Subsequently, confirmation of osteogenic differentiation was achieved via alizarin red staining.

Groups established for assessment

A total of six groups were established for assessment (Fig. 4).

Statistical Analysis

The analysis was performed in IBM SPSS statistics version 20 software. P-value < 0.05 was considered statistically significant. A quantitative assessment of the osteogenic differentiation of DPSCs, when stained with alizarin red, was performed. Data analysis was conducted employing an Image J analyzer. Data were presented as mean ± SD. Error bars indicate SD.

RESULTS

Isolation of MSCs

The isolated MSCs sourced from the dental pulp exhibited spindle-shaped morphology under the microscope.

Surface marker characterization

When the immunophenotypic characteristics were assessed to express particular cluster differentiation (CD) markers utilizing flow cytometry, DPSCs showed net positivity for CD90, CD105, CD73. MSCs showed negative results for expression of CD34, CD45, and Human Leukocyte Antigen-DR isotype (HLA-DR). DPSCs expressed positive for CD90 (97.920%), CD105 (99.114%), and CD73 (99.128%). Expressed negative for CD34 (1.181%), CD45 (0.313%), and HLA-DR (0.531%) (Fig. 5).

In vitro tri-lineage differentiation

DPSCs were characterized by their tri-lineage differentiation potential, such as osteogenic, chondrogenic, and adipogenic lineages. Osteogenic differentiation was assessed using Alizarin Red staining, which demonstrated a prominent bright orange-red coloration along with the presence of extracellular calcium deposits. In contrast, the undifferentiated control cells displayed only a faint reddish hue and lacked any detectable calcium deposition, indicating the absence of osteogenic activity (Fig. 6, *A*). Chondrogenic differentiation was verified through the deposition of a bright red colour, indicative of extracellular matrix proteoglycan production, a hallmark of chondrocyte activity and cartilage matrix synthesis (Fig. 6, *B*). For adipogenic differentiation, intracellular lipid droplet formation was observed following Oil Red O staining, validating the capability of MSCs to transform into adipocytes. These lipid droplets, stained in red, were clearly visible within the cytoplasm of the differentiated cells, while undifferentiated cells lacked such features (Fig. 6, *C*).

Cellular viability

The survivability of DPSCs was first assessed by subjecting them to various concentrations of Vitamin D (10 nM, 20 nM, 30 nM, 40 nM, 50 nM, 100 nM, and 200 nM) for 48 hours. The MTT assay's findings demonstrated that Vitamin D enhances the proliferation of DPMSCs; and exhibits no cytotoxic effect (Fig. 7).

Influence of Vitamin D on osteogenic differentiation Capability of DPSCs

Impact of Vitamin D on osteogenic differentiation of DPSCs was evaluated using Alizarin Red staining. The data that were obtained are displayed in Fig. 8 A & B revealed a significant enhancement of osteogenesis of DPSCs when cultivated in an osteogenic medium supplemented with vitamin D3 (50 nM). Vitamin D promotes the growth of bone in DPMSCs, whereas diabetic serum hinders the growth of bone in DPMSCs. Vitamin D, when present with diabetic serum, demonstrates a modest level of osteogenesis.

Alizarin red staining is used to see matrix mineralization, which indicates osteogenic differentiation. Six groups: Undifferentiated DPSCs as negative control showing no calcium deposits, Differentiated DPSCs as positive control exhibiting moderate calcium deposition, Differentiated DPSCs and Vitamin D showing the highest amount of calcium deposition, Differentiated

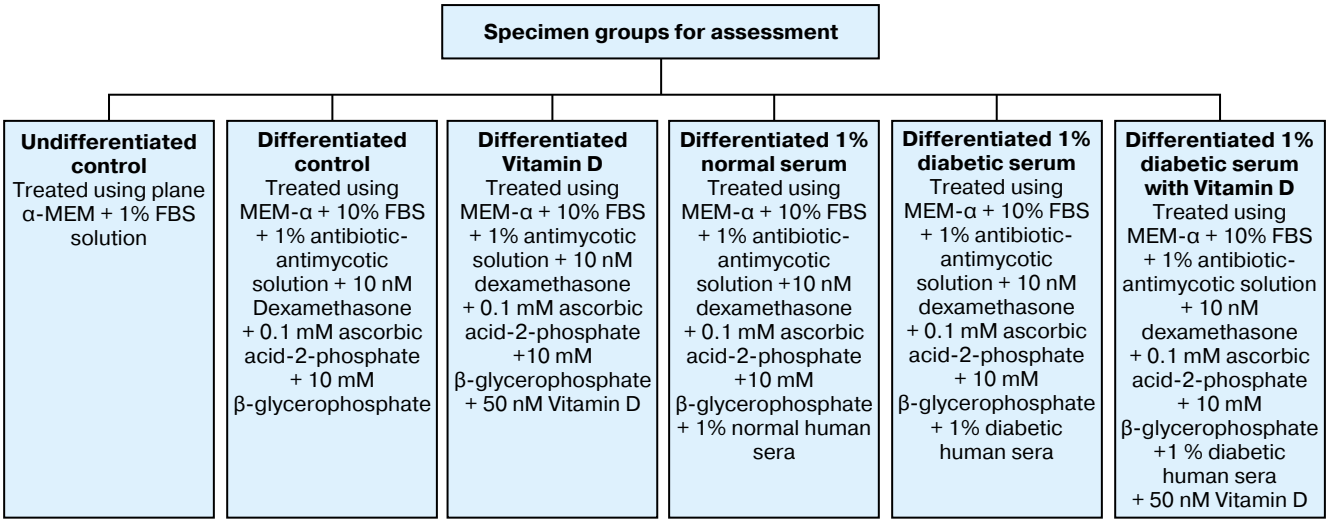


Fig. 4. Groups for Assessment

Рис. 4. Группы для оценки

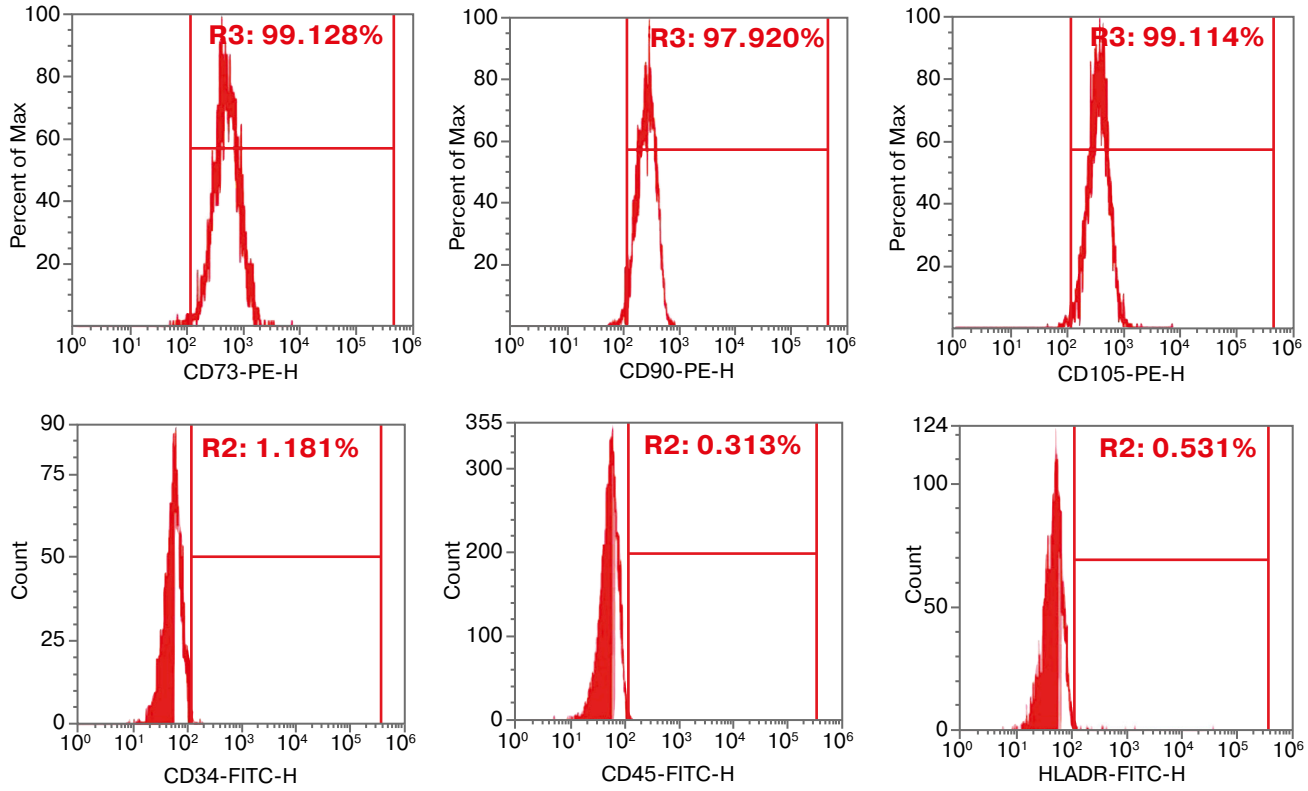


Fig. 5. Immunophenotypic characteristic expression through flow cytometric analysis for MSCs derived from dental pulp

Рис. 5. Иммунофенотипическая характеристика MSC, полученных из пульпы зуба (анализ методом проточной цитометрии)

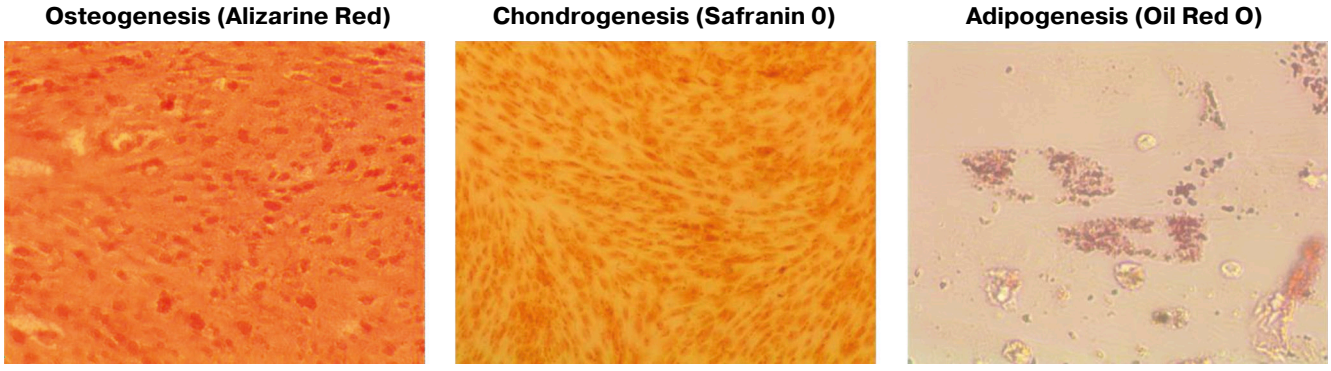


Fig. 6. In-vitro differentiation of DPSCs for osteogenic (*A*), chondrogenic (*B*), and adipogenic (*C*) purposes. The control group consisted of MSCs that were not inducted. DPSCs were cultivated In-vitro using chondrogenic, osteogenic, and adipogenic induction process for durations of 21, 18, and 15 days, respectively. Alizarin red staining is used to see matrix mineralization, which indicates osteogenic differentiation. Safranin o staining is used to visualize the cartilage matrix, which indicates chondrogenic differentiation. Moreover, oil red staining is used to see lipid- containing adipocytes, indicating their adipogenic differentiation at a magnification 200

Рис. 6. In vitro дифференцировка стволовых клеток пульпы зуба (DPSC) по остеогенному (*A*), хондрогенному (*B*) и адипогенному (*C*) направлениям. Контрольная группа состояла из MSC, не подвергавшихся индукции. DPSC культивировали *in vitro* с применением хондрогенной, остеогенной и адипогенной индукции в течение 21, 18 и 15 дней соответственно. Окрашивание Alizarin Red применяли для выявления минерализованного матрикса, указывающего на остеогенную дифференцировку. Окрашивание Safranin O использовали для визуализации хрящевого матрикса, что свидетельствует о хондрогенной дифференцировке. Окрашивание Oil Red применяли для выявления адипоцитов, содержащих липиды, что указывает на адипогенную дифференцировку. Увеличение ×200

DPSCs and 1% Normal Serum showing mild to moderate calcium deposition, Differentiated DPSCs and 1% Diabetic Serum showing no calcium deposition, and lastly Differentiated DPSCs containing both Vitamin D and 1% Diabetic Serum showing slightly higher calcium deposits compared to the positive control group (Fig. 8).

The osteogenesis of DPSCs is greatly improved when grown in an osteogenic medium containing vitamin D3 (50 nM). Vitamin D stimulates bone growth in DPMSCs, while diabetic serum inhibits bone growth in DPMSCs. When vitamin D is present in diabetic serum, it shows an increased level of osteogenesis (Fig. 9).

DISCUSSION

MSCs, particularly those derived from the oral cavity are indispensable to regenerative medicine owing to their ability to differentiate into many cell types and their simplicity of isolation process [19; 20]. Oral cavity derived MSCs such as DPSCs [21–24], gingival mesenchymal stem cells (GMSCs) [25], and periodontal ligament-derived MSCs (PDLSCs) [26] also possess high angiogenic potential and greater differentiation capacity, thus qualifying as ideal candidates for cell-based therapeutic interventions [27; 28]. This paper is particularly concerned with the role of vitamin D3 in increasing

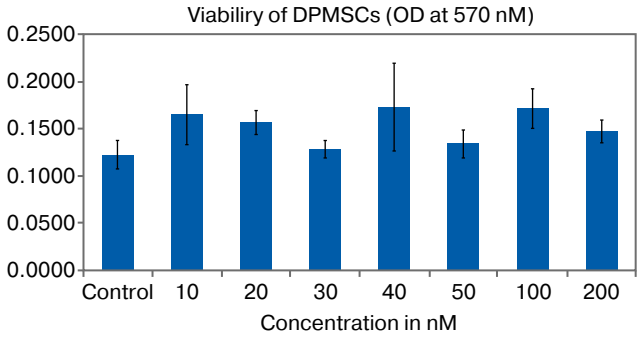


Fig. 7. The Proliferation-Boosting Effects of Vit-D on DPMSCs with no Cytotoxicity

Рис. 7. Стимулирующее влияние витамина D на пролиферацию мезенхимальных стволовых клеток пульпы зуба без проявления цитотоксичности

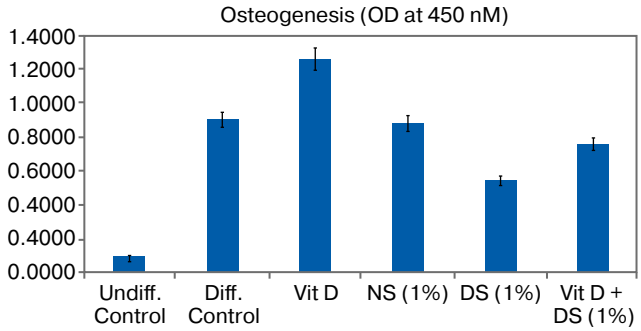


Fig. 9. Quantitative assessment of osteogenic differentiation of DPSCs when stained with alizarin red

Рис. 9. Количественная оценка остеогенной дифференцировки стволовых клеток пульпы зуба (DPSC) при окрашивании Alizarin Red

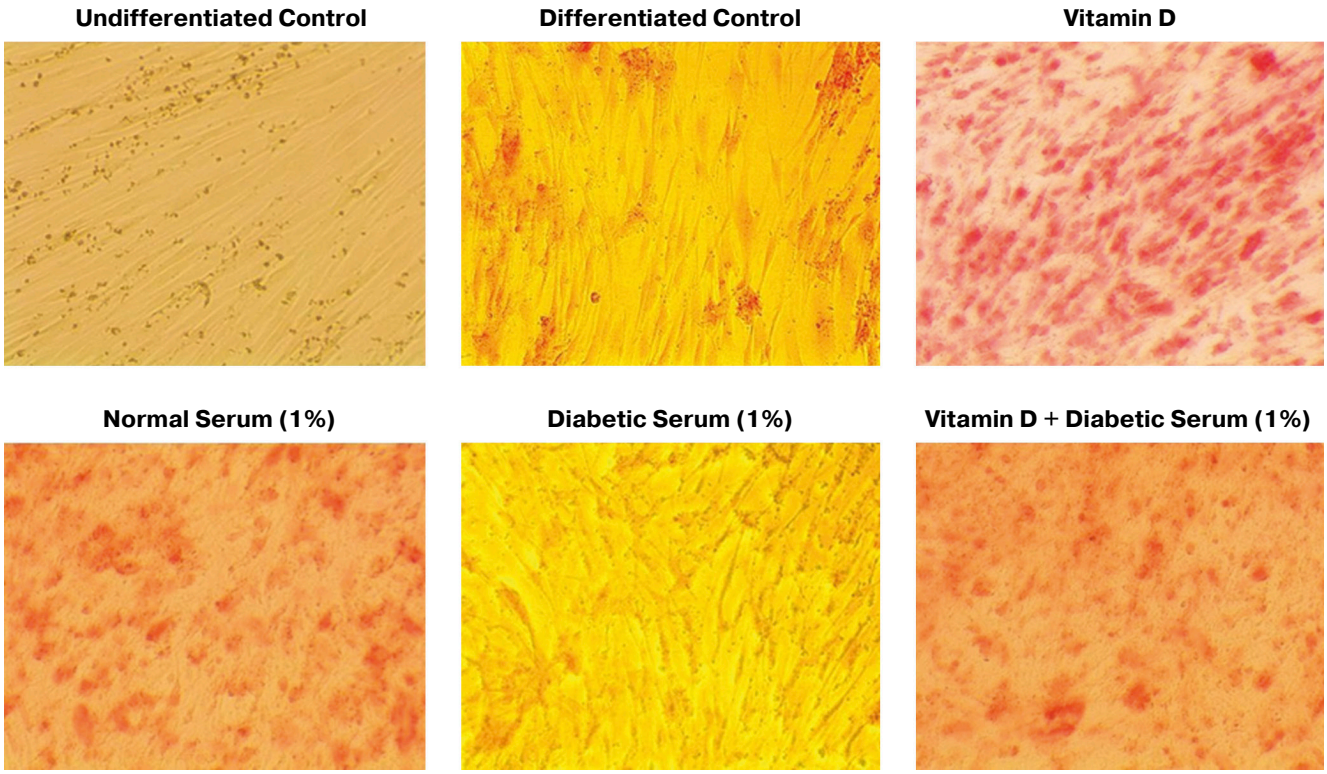


Fig. 8. Osteogenic differentiation of DPSCs

Рис. 8. Остеогенная дифференцировка стволовых клеток пульпы зуба (DPSC)

the osteogenic differentiation of DPSCs, its potential in bone regeneration, and its implications in treating diseases such as osteoporosis and diabetic complications related to bone metabolism [29].

Studies have shown that Vitamin D3 may promote the growth of bone-forming cells (MSCs) derived from diverse oral tissues in laboratory settings. Khanna-Jain et al. [30] found that the presence of osteogenic supplements enhances the mineralization of human dermal fibroblast cells (hDFCs) and human dermal papilla cells (hDPCs) via the action of 1,25-dihydroxyvitamin D3 [1,25(OH)2D3] and 25-hydroxyvitamin D [25(OH) D3], similar results were obtained in the present study. However, these substances do not stimulate mineralization when osteogenic supplements are not present [31].

Even though, the present study offers significant findings on the osteogenic activity of vitamin D3 on DPSCs, one must acknowledge some limitations of this study. The study was in vitro, which may not exactly reflect the dynamics of the intricate in vivo environment. Further studies are necessary to confirm these findings in animal models and human clinical trials. The study was also specific to DPSCs, and more studies are necessary to establish if vitamin D3 has the same effect on other OC-MSC subtypes. Additionally, the study did not address the synergistic interactions of vitamin D3 with other osteogenic agents or biomaterials, which can further unlock its therapeutic potential. Lastly, the study did not address the long-term effects of vitamin D3 on DPSC differentiation and mineralization, which is necessary for evaluating its potential as a sustainable regenerative therapy.

Future studies need to overcome these limitations by implementation of animal studies and clinical trials. Future studies should also evaluate the effect of vitamin d3 on other OC-MSCs along with comparative evaluation to assess if the effect is cell-type dependent. Additionally, studies analysing the concomitant use of vitamin d3 with other osteogenic agents or biomaterials will help optimize the therapeutic benefits. Finally, long-term studies need to be conducted to determine the long-term maintenance of vitamin D3-induced osteogenesis and its applicability to clinical use in regenerative medicine. Elucidation of the underlying molecular mechanisms by which vitamin D3 influences DPSCs and other OC-MSCs will provide further insight into its therapeutic potential.

The current study focused on the potential ability of vitamin D3 on osteogenic differentiation, which revealed that its metabolites facilitate mineralization and marker expression associated with osteogenesis under specific osteogenic prone conditions. Furthermore, the current research pointed out that 1,25(OH)2D3 initiates extracellular signal-regulated kinases (ERKs), thereby further facilitating mineralization processes. These observations confirm the basic function of vitamin D3 in enhancing DPSCs differentiation into calcified tissues like dentin and cementum by inducing dentin sialo phosphoprotein (DSPP) and dentin matrix protein 1 (DMP-1) expression. With the outcomes highlighted above, vitamin D3 appears to be a viable therapeutic option, particularly with the increasing prevalence of diabetes mellitus (DM) and its related skeletal complications, which comprise increased bone fragility and defective fracture healing [32]. Patients with DM usually have low bone mineral density (BMD) and enhanced vulnerability to fractures related to osteoporosis [1], calling for interventions that enhance bone metabolism and fracture healing among this group of patients.

This study is novel in its specific investigation of DPSCs' osteogenic potential in the context of vitamin D3 metabolites. While previous studies have established vitamin D3's role in promoting mineralization and osteogenic marker expression in various MSC subtypes, this study is novel in highlighting its impact on DPSCs of oral tissue–a rapid and readily available source for regenerative medicine. Furthermore, it bridges the gap between the biological actions of vitamin D3 on MSCs and its therapeutic potential for diabetes-induced skeletal complications. Through the demonstration of these mechanisms, this study offers insightful knowledge toward the development of targeted therapies for bone regeneration and systemic diseases like diabetes mellitus. By highlighting the therapeutic potential of vitamin D3 in promoting DPSC-mediated bone regeneration, this study opens doors to the development of novel treatments for skeletal defects, osteoporosis, and bone complications in diabetes.

CONCLUSION

In conclusion, the present research work adds strength to the already emerging body of evidence favouring the use of DPSCs as a potential regenerative therapy tool in dentistry and orthopaedics. The results of this study indicate that Vitamin D supplementation, especially if supplemented with some growth factors, may be a potential way of enhancing the therapeutic efficacy of DPSCs in bone regeneration therapy. With the progress in regenerative medicine, the comprehension of the interplay between the various factors regulating stem cell differentiation will be instrumental in the establishment of effective therapeutic approaches.

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