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Overexpression of Lentiviral Vector-Mediated Human BMP2 Gene in Mesenchymal Dental Pulp Stem Cells (DPSCs): An *In Vitro* Study

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Abstract

Background: Cell based gene therapy is an efficient technique for the cure of inherited diseases as well as for tissue engineering. Human bone morphogenetic protein 2 (hBMP2) has been shown to play a significant role in regulating the odontogenic differentiation of dental tissue-derived stem cells. However, there have been few studies on the effects of the BMP2 gene on the proliferation and odontogenic differentiation of human dental pulp stem cells (hDPSCs).

Methods: This study evaluates the overexpression of BMP2 gene transfected in DPSCs infected by lentivirus. DPSCs were transduced with a lentiviral vector to the secretion of BMP2 along with green fluorescent proteins (GFP). The transduced cells were analysed for BMP2 secretion with RT PCR.

Results: Almost 70% of hDPSCs could express GFP marker following transduction. Graphical analysis and GFP assay confirmed that the BMP2 expression level in transduced DPSCs was ten times more than native DPSCs.

Conclusions: DPSCs could be considered as a promising source for tissue engineering. Moreover, lentiviral-mediated BMP2 gene transfection in DPSCs could enhance the osteogenesis or odontogenesis capacity of these cells.

Keywords: *Bone Morphogenetic Protein (BMP2); Dental Pulp Stem Cells (DPSCs); Lentiviral Vector*

Introduction

Regenerative medicine holds the promise of tissue and functional organ regeneration. Science has been looking for ways to replace old and disabled organs, in order to repair damaged tissues using modern methods of gene therapy, cell therapy, and tissue engineering [1]. Meanwhile, the role of tissue engineering in repairing various injuries has been evaluated to a great extent in recent years. Regenerative medicine plays a significant role in research and development of new technologies related to the production and repair of tissues and organs [2]. Due to the dynamic potency of bone tissue, it has been a major concern to find efficient methods for repairing bone defects. More than 2.2 million grafting operations take place annually worldwide for different bone deficiency cases [3].

Although bone grafting is the most common method to replace the missing bone tissue, complications such as surgical removal of donor site recovery period, and difficult surgical procedures are the problems. Therefore, different processes are developed for dental bone regeneration, including guided tissue regeneration (GTR)/guided bone regeneration (GBR), platelet rich plasma (PRP), and recombinant

BMP2 proteins (rBMP2). However, clinical data obtained from these techniques are contradictory and a standard clinical protocol has yet to be established. Temporary and short-term presence of triggers and growth factors are the main issues faced by researchers and clinicians [4-7]. Nowadays, cell-based gene therapy attracts a great deal of attention for bone tissue engineering, by continuous and gradual availability of efficient growth factors [8]. On the other hand, transferring of genes has various advantages in comparison to the delivery of proteins such as extended capability of local and systemic protein expression and fewer side effects [9,10].

Between hormones and cytokines, BMPs, which belong to the superfamily of TGF-β and are considered as external osteogenesis stimulators, are one of the most powerful cytokines for the formation of external bones. They highly increase the rate of mesenchymal cell differentiation into osteoblasts and play a central role in signalling networks and the processes associated with osteogenesis [11,12]. Since the discovery of BMPs as bone-inducing proteins by Orist, these proteins have been widely used in bone tissue engineering to perform craniofacial and dental/periodontal surgeries [13-19]. Also, many research groups have shown that BMPs can increase the osteogenic differentiation of mesenchymal stem cells (MSCs) [20-22]. There is considerable evidence to suggest that among 20 known amino acids [23,24], BMP-2, 4, 6, 7, and 9 can supply the original signal for inducing bone cell differentiation to osteoblasts, thus forming the extracellular bone matrix [25-27].

Many studies have shown that BMP-2 is one of the most effective proteins to induce spontaneous osteogenesis or even induce BMPs by prompting the expression of chemical signals [8,14,28-32]. It is used in bone formation of craniofacial surgeries [33].

Only a few companies produce the recombinant protein of rBMP2 for clinical use. Although the temporary *in vivo* presence of this protein, the lack of clear protocols on dosage and time, and high costs have limited the clinical use of this protein virus-mediated gene transfer therapy is one of the most common and popular methods in bone production and regeneration studies. Viral vectors have accounted for more than 66% of the total clinical trials of gene therapy up to 2010 [34]. Despite limitations, such as limited capacity and immunogenicity, the use of viral vectors in gene transfer and gene therapy is common because of their higher efficiency compared to other non-viral types [35]. Lentiviral technology is mainly used in gene therapy. Many efforts have been made to improve its safety and effectiveness in recent years and several research groups have reported that the lentivirus can play a role in the treatment of several diseases, including thalassemia, sickle-cell anaemia, and haemophilia B. Also, great advances have been reported in the treatment of genetic disorders such as Parkinson's disease, CF and spinal muscular atrophy [36-39]. Lentiviruses are single positive-sense strands belonging to the retroviral family. They can be integrated into the host genome; however, members of the retroviral family have the necessary cellular and molecular components to cross the nuclear pore and infect the cells during mitosis. These viruses can be produced in high titres from the genome after the removal of inflammatory and viral pathogenic parts [40-42]. Lentiviruses also have many advantages, including long-term and stable expression, ability to infect both dividing and non-dividing cells, low immune response, and ability to accommodate the longer transferred genes [43,44]. Thus, in light of the suitability of this viral vector and its advantages over other viral vectors, it was selected as the intended gene vector in the present study. In recent years, much researches have been conducted on stem cells and their use in tissue engineering, because of their potential to help in the treatment of various diseases [45]. These treatments play an important role in regenerative medicine, including the regeneration of bone and dental lesions.

MSCs are multipotent progenitor cells with renewability potential. They can be removed from adult bone marrow, adipose tissue, skin, umbilical cord, and placenta. Therefore, in light of their benefits, including long-term preservation of the differentiation ability, they are considered as an appropriate alternative tissue engineering site [46]. MSCs are usually used as an effective alternative in clinical trials on bone lesions. They also have the ability to be differentiated into osteoblasts, adipocytes and chondrocytes [47,48].

DPSCs in comparison with the other four dental stem cells, stem cells from exfoliated deciduous teeth (SHED), periodontal ligament stem cells (PDLSCs), dental follicle progenitor cells (DFPCs) and stem cells from apical papilla (SCAP) [49] are considered for this study due to ease of access, low side effects arising from the separation, and having properties similar to bone marrow stem cells (BMSCs) [50].

In this study, similar to result of a study on SCAP cells [51], the overexpression of human BMP-2 gene was seen in the DPSCs using the lentivirus vector. The results could improve regenerating of the mineral part of tooth and bone and improve the osteointegration around implants.

Materials and Methods

DPSCs was prepared same as previous study [52]. Both permanent and deciduous teeth, which were extracted for orthodontic treatments, were used in the isolation of DPSCs. The study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (TUMS) which was in compliance with the Helsinki declaration.

The teeth were extracted transferred to the laboratory in the transfer solution containing streptomycin antibiotic in DMEM and 1X/1X penicillin and amphotericin B (Gibco 250 µg/ml). After scrubbing the surface of the teeth, a 0.5 - 1.0 mm deep groove was made using sterile low-speed manual disk. The teeth were broken from the grooved region with a hatchet and dental pulp was extracted using endo file or forceps. The extracted pulp tissues were later cut into small pieces using surgical scissors and were subsequently digested with Collagenase type I (3 mg/ml), *Invitrogen* and dispase (4 mg/ml, *Invitrogen*) for 40 minutes at 37°C. The resulting cell suspension from each tooth was centrifuged for five minutes at 1200 rpm with a Sigma 2-16PK device. Single-cell suspensions were cultured in DPSC medium containing Dulbecco's Modified Eagle's Medium (GIBCO 1g/l glucose, pyruvate +), 10% bovine serum (Hyclone), 100 units/ml of penicillin (Sigma), and 100 µg/ml streptomycin (*Invitrogen*).

The DPSCs were in contact with 5% CO_2 cultured at 37°C for 10 days. Then the medium was switched every three days. To perform FCM evaluation, the DPSCs were divided into six round-bottom tubes (Becton Dickinson Falcon, Sunnyvale, CA) at a rate of 2 - 10^5 cells per tube and were later stained using G fluorescent Isothiocyanate-conjugated with immunoglobulin or conjugated phycoerythrin.

Known positive and negative markers for MSCs were assessed in these cells, such as CD105, CD90, CD166, CD73, CD11b CD34, CD133, CD64, CD106, CD31, and CD45 markers (All the antibodies were prepared by ABCAM (ABCAM, UK). After 20 minutes of incubation at room temperature in the dark, the cells were washed twice using 2 ml of fluorescence-activated cell. Phosphate buffer saline (PBS) solution containing 0.1% FBS and 0.1% NAN3 was centrifuged for five minutes at 230g. The supernatant was discarded and cells were fixed using 1% formaldehyde (in PBS). Corresponding immunoglobulin G (Beckman Coulter) was used as negative control. All data was obtained by using COULTER® EPICS® XL (Beckman Coulter) software and EXPO32 ADC (Beckman Coulter) and WinMDI version 2.8 (Windows Multiple Document Interface for FCM; freeware developed by Joe Trotter, downloadable at methods. info/software/flow/winmdi. htm).

Human BMP-2 gene with extracted genome length of 1191bp and specified restriction sites was prepared using Oligo 7.0 (Molecular Biology Insights, Inc., Plymouth, MN, USA) according to NCBI GeneBank no. KC294426.1. The plasmid PCDH-hBMP2-EF-cGFP-Pur was amplified by using DH5α vector. PCDH; (System Biosciences, Mountain View, CA, USA). The cells were later cultured in lysogeny broth (LB) medium, after transferring 100λ of the remaining micro tube contents to LB agar plate containing 100 mg/ml of ampicillin (1λ per ml). A single colony containing pCDH plasmid and BMP-2 gene grew overnight. During the transformation process, a single colony was cultured in LB broth agar medium containing 5 cc of ampicillin. The colony was later placed in a shaker incubator and shaken at 125 rpm for about 16 hours at 37°C in order to reach the intended optical density (OD) of about 600 nm. Plasmid purification was later performed to separate the bacterial plasmid using the QIAGEN Kits and protocols. After double-digestion using specific two enzymes, electrophoresis was performed on the agarose gel to confirm the presence of hBMP-2 gene in the pCDH plasmid.

Transfection was performed in HEK293T cells in the DMEM medium (*Invitrogen*), containing 10% FBS and 1% (100λ) of streptomycin-penicillin (Pen-Strep) from the Pasteur Institute of Iran. HEK293T cells have an epithelial cell morphology and are considered to be the best cells for gene transfer and the production of false type viruses. HEK cells are the most appropriate alternative for the packaging

and production of lentiviruses in the Laboratory of molecular biology and belong to the human embryonic kidney cell lining [54,55]. For the packaging and transfection processes, the cells were cultured in order to reach 70% confluency.

The testing method used for Preparation of recombinant viruses carrying BMP-2 gene along with GFP marker was changed a little according to the Tronolab protocol (Tronolab, Lausanne, Switzerland) [9,56]. In fact, in this method, transfection is done using calcium phosphate. Recombinant pCDH- hBMP2-EF-cGFP-Puro plasmid, psPAX2 packaging plasmid containing the gag-pol-tat-rev, and an envelope pMD2-G plasmid were packaged using calcium chloride and a GFP marker. Meanwhile, the transfection was done according to the protocol and OD of psPax and pMD2 which were read before starting this stage and the mixture was added to the medium containing HEK293T cells (Complete DMEM,10% FBS and pen/strep antibiotics) with confluency over 70%.To determine the transfection rate of the HEK293T cells, they were analysed via fluorescence microscopy.

After 24 hours, the viruses could be collected and the supernatant containing the virus could be collected after switching the medium containing HEK293T cell thrice. The supernatant was later filtered by a 0.22 μm syringe filter to obtain the purified virus. The virus titration was transfected by the Pasteur Institute of Iran at this stage.

After thawing in 10 ml of DMEM medium (*Invitrogen*), containing 10% FBS and 1% (100λ) streptomycin-penicillin antibiotic, DPSCs were cultured in the conical tube. The mixture was later centrifuged at 1200 rpm for five minutes and the supernatant was discarded. The deposit was dissolved with 1cc DMEM medium and the resulting solution was poured into the T25 flasks, to which 5cc DMEM medium was later added and was incubated at 37°C in 90% humidity and 5% CO_2, so that the cells would continue to grow. The number of pCDH/BMP2 viruses added to DPSCs at optimum multiplicity of infection (MOI) with the ratio of 10.

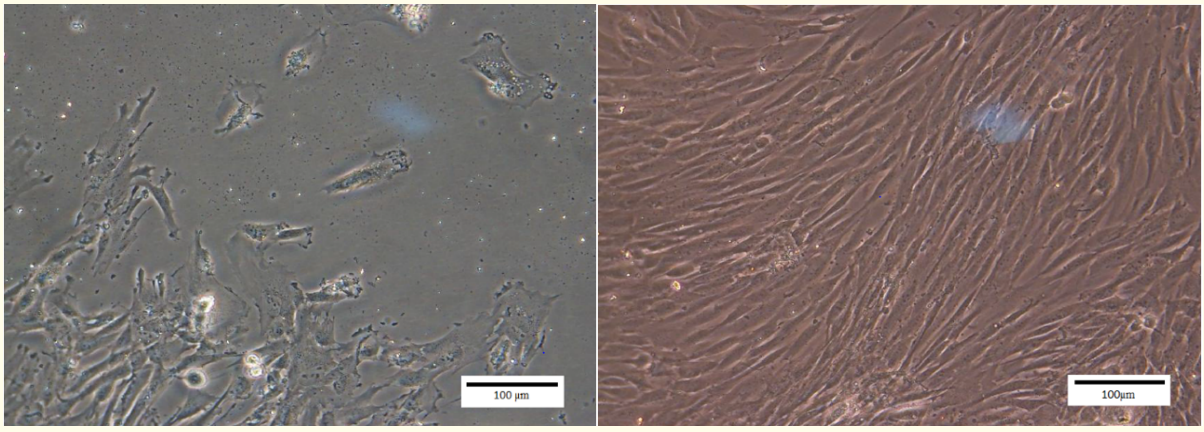
The viruses were later transferred to the incubator after being added to the medium.

The number of transduced DPSCs/BMP2 cells can be determined using fluorescence microscopy about 48 - 72 hours after adding pCDH/BMP2 to DPSCs. In this method, recombinant transfected cells are screened using puromycin as the selected antibiotic marker, so that DPSCs, that have not received the recombinant virus, can be removed from the medium. Later, 25 mg/ml puromycin in distilled water was added to the medium in order to culture the non-transduced cells and enable DPSCs/BMP2 to survive in the medium.

Then they were passed through the 0.22 μm filter. The RNA extraction and purification was performed based on Qiagen RNeasy kit protocols (Qiagen, Valencia, CA), and the RNA concentration was later determined using the spectrophotometry device. cDNA was produced from 2 μg/μl of RNA samples using the reverse transcriptase enzyme, and PCR was later performed on it. The accuracy of the reaction was studied on the 1.5% gel electrophoresis, as follows: A total amount of 12.5λ of Master Mix PCR, 1λ of forward primer, 1λ of reverse primer, 1λ of cDNA sample, and 25λ of water were added into the thermocycler, on which PCR was performed in three stages as discussed below: denaturation at 94°C for five minutes, annealing at 94°C for 30 seconds, annealing at 60°C for 30 seconds, elongation or extension at 72°C for 30 seconds for 30 rounds, and finally electrophoresis at 72°C for seven minutes to reach final elongation and PCR.

Results

As described in our previous study [52], DPSCs of both permanent and deciduous teeth were quickly proliferated while they were attached to the bottom of the flask with their fusiform morphology. After the third passage, the homogeneous population of deciduous and permanent teeth was determined (Figure 1).



(A) ***(B)***

Figure 1: *Culture of DPSCs; (A) Morphology of DPSCs after 5 days Scale 100 µm, (B) Morphology of DPSCs after 14 days the cells reached 70% confluency.*

More than 80% of the cell population representing expression of surface antigen was identified in the mesenchymal lineage, such as CD90, CD166, CD105, and CD73, while only less than 4% of endothelial progenitor epitopes included CD11b, CD34, CD133, CD64, CD106, CD31, and CD45 [52].

For Transformation of PCDH carrying BMP-2 gene into the dh5-alpha bacterium for plasmid cloning, the transformation process was done in the medium plates containing LB with ampicillin markers. Single colonies grew overnight.

In the stage of Plasmid purification and plasmid test verification using electrophoresis, plasmid extraction was done using kit. Electrophoresis was used to confirm the complete vector separation. Afterwards, two BMP-2 bands were detected. The heavier band belonged to pCDH-EF-cGFP-Puro plasmid while the lighter band-placed at the bottom-related to the hBMP2 gene (Figure 2).

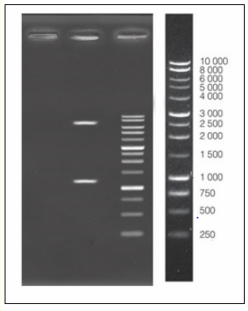


Figure 2: *Enzyme-digesting gel.*

After completing co-transport by virus-transformed cells, HEK293T cells were prepared (Figure 3).

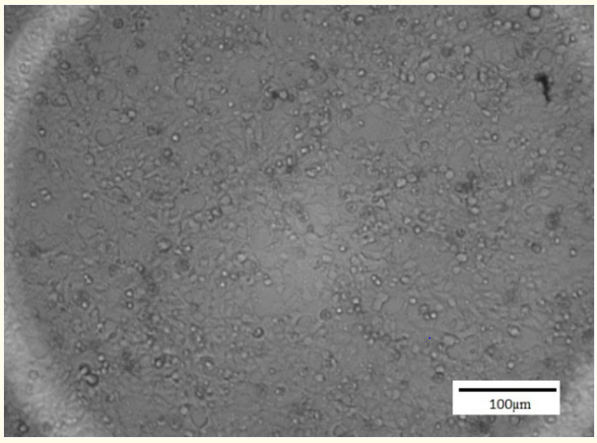


Figure 3: *HEK293T cells before transfection with magnification of 100.*

Fluorescence microscope was used to perform the test and study the transferred transgenic viruses. This process was performed at wavelengths that excited GFP protein. As the figure shows, HEK293 cells were demonstrated in green after 48 hours, indicating GFP expression and the production of the recombinant virus (Figure 4).

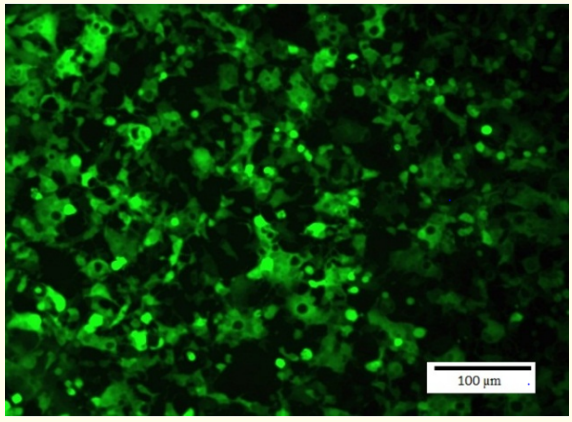
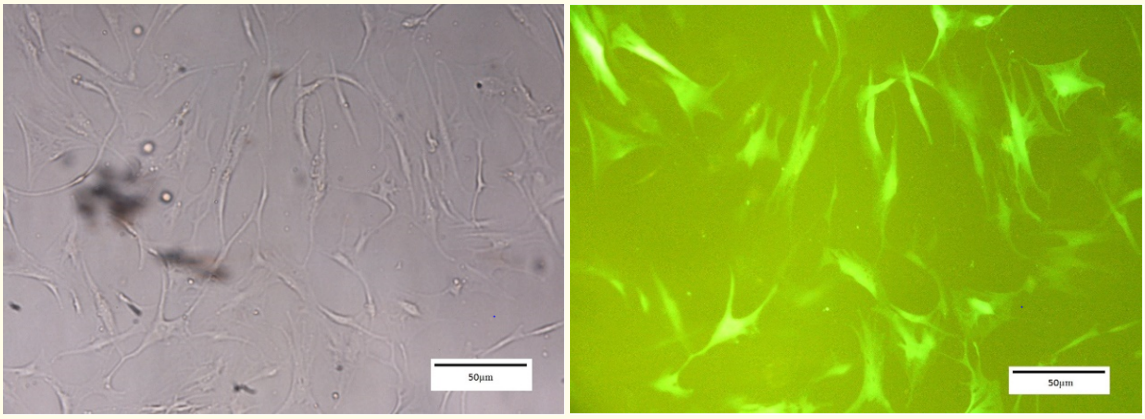


Figure 4: *HEK293 cells after transfection under fluorescent radiation with magnification of 100.*

Transduction of DPSCs occurred by using virus carrying the gene producing the hBMP- 2 protein along with GFP marker. DPSCs were infected by the recombinant virus by replacing the medium collected from HEK 293T cell medium. The fluorescent microscopic observation indicates expression of hBMP-2 gene and Copa-GFP protein on the day of infection leading to the formation of colonies over time (Figure 5A and 5B).



(A) ***(B)***

Figure 5: *(A) Recombinant hDPSCs before exposure to fluorescent, (B) Recombinant hDPSCs after exposure to fluorescent light under magnification of 50.*

RT-PCR technique was used for better investigation and approval of hBMP-2 gene expression in DPSCs that have been infected with the recombinant virus containing the desired gene. The size of the resulting product correlated strongly with the human BMP-2 gene using specific primers. -Actinβ was used to control the expression rate. In this stage, it was expected to obtain a product with 275bp size which would be visible on agarose gel due to the ladder and size of the BMP-2 gene (Figure 6 and 7A, 7B).

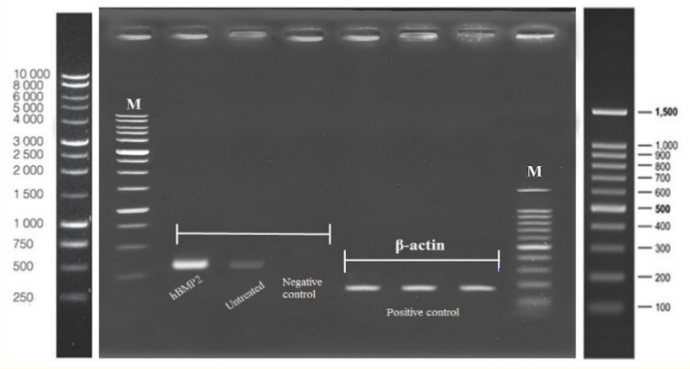
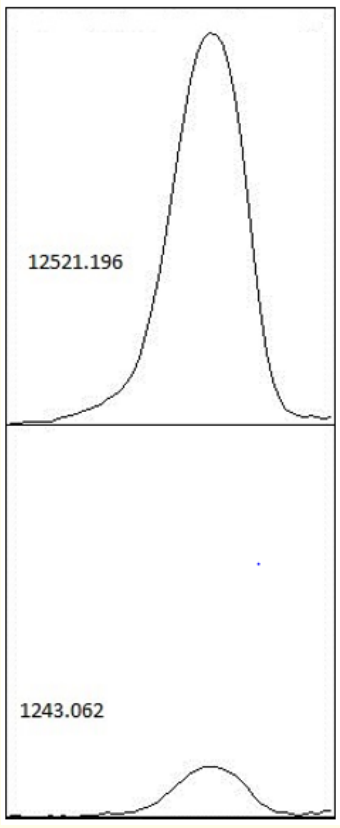
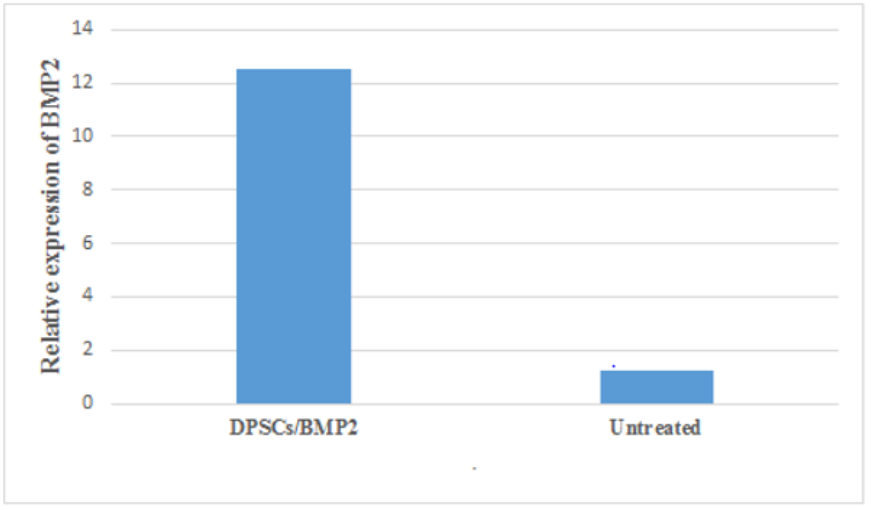


Figure 6: *Agarose gel showing RT-PCR product of the hBMP-2 and -actinβ gene.*



(A)



(B)

Figure 7: *(A) Comparative diagram of BMP-2 gene overexpression in hDPSCs compared to non-recombinant stem cells by ImageJ software (B) Diagram BMP-2 gene overexpression in DPSCs.*

Discussion

Nowadays, gene therapy and genome editing is a considerable and reliable method for transferring genes for the targeted, selective, and specific treatment of damaged or non-functional tissue [9]. Bone defect occurs in the maxillofacial region for various reasons, leading to the failure of numerous treatment strategies including dental implants. In this regard, several methods have been used for repairing bones in recent years. The use of BMPs, as an osteogenic stimulating protein is one of the options. This protein regulates the differentiation of mesenchymal cells by stimulating cellular signalling pathways. In fact, BMPs are one of the most powerful cytokines that can stimulate external bone formation and increase the differentiation of mesenchymal cells into osteoblasts drastically. Among the BMP groups, BMP-2 is one of the most effective forms of the protein to play this role [57,58].

Studies on transferring and expression of adenoviral vector-mediated BMP7 *in vivo* and *ex vivo* on dental pulp cells revealed that the transfer of BMP7 into the inflamed dental pulp in *ex vivo* conditions induces and increases the expression of restorative dentinogenesis through the reconstruction of dentin pulp complex. However, it does not lead to the restoral production of dental tissues *in vivo* in all cases [59]. Sonoporation was also used to boost the transferal of the BMP11 gene into the DPSCs. In this study, the expression of dental sialoprotein (Dsp) and other odontoblastic differentiating markers was increased and lead to dentin formation [60].

Furthermore, in another experiment the same group analysed expression rate of this factor and its effectiveness in the osteogenesis process using the electroporation method. In this study, an approximate viability of 85% and efficiency of 70% were measured using the FCM technology and the expression rate of Collagen types 1 and 3 increased after 10 days. Moreover, the expression rate of odontoblastic osteogenic differentiation markers such as alkaline phosphatase and dental matrix protein (Dmp1), dentin sialo-phosphoprotein and enamelysin were increased after 14 days. Finally, dentin formation was observed as an *in vivo* study [61].

Yang., *et al.* used adenoviral vectors to transfer BMP-2 to rat and mice DPSCs selected by STRO-1. They used real time PCR to investigate the mineralization rate for osteocalcin, bone sialoprotein, dentin sialophosphoprotein *in vitro* and *ex vivo* after 1, 4 and 12 weeks. In this experiment, mineralization was increased around the implant (33 ± 7.3% of implant pore area), which occurred at a high rate in both time periods. Thus, DPSCs were considered as appropriate cells in transferring the BMP2 gene. DPSCs were also known as an important component in the effective mineralization process of dental implants in comparison to non-transgenic cells even without the use of materials such as dexamethasone. The *in vivo* analysis showed no symptom of inflammation in any mice or fibrous tissue implants after one week, but after 4 weeks mineralized tissue formation occurred. After 12 weeks however, an improvement of mineralization was observed [62].

Wen Zhang., *et al.* (2014), used a lentiviral vector containing BMP-2 to differentiate stem cells of apical papilla (SCAP). The experiment showed that the BMP-2 gene was successfully transferred through the lentivirus vector. The results also showed more odontogenic differentiation more alkaline phosphatase (ALP) granules were released, and more mineral reserves (about six times) were formed. According to this manuscript, transferring of a homologous gene may be an effective strategy to develop functional methods for tissue engineering [51].

As mentioned above, DPSCs can be a good choice for gene therapy and tissue engineering research because of their ease of access and similarity with bone marrow mesenchymal cells (BMMSCs). However, unlike BMMSCs which lack the ability to mineralize dentin and tooth surfaces, these cells have the ability to mineralize and thus can be used in dental repairs and treatment [63]. Positive effects of using DPSCs in tissue regeneration and bone research can be an incentive to further usage of these cells in tissue engineering [64].

Lentiviral vector is widely used in different cases due to its high capacity and efficiency. The capacity to be integrated in the host genome, persistent expression compared to other similar types [65] and possibility to create new generations are the other positive points of this vector. The experiment showed that the expression of BMP-2 in DPSCs was increased approximately ten times, more than the results obtained in Zhang's study on SCAP cells in 2014 [51]. Therefore, considering the aforementioned reasons, DPSCs are preferred over other dental stem cells as a good alternative to promote osteogenesis process in jaws and relevant treatments.

In this experiment, cells were transfected with GFP expression level of more than 70% after the BMP-2 gene was transferred into DPSCs. Considering that DPSCs are available and appropriate cells, in terms of their better efficiency, ease of isolation and similarities with bone marrow stem cells, they can be studied in osteogenesis and tissue engineering processes, and are to be favoured over other stem cell alternatives. DPSCs can also be used around the implants, maxillofacial surgeries, and bone recession caused by different factors and thus can be used in bone loss and bone remodelling. In this experiment, the BMP2 expression was approximately ten times higher than untreated. In our recent studies we assessed the effect of different stimulator on DPSCs.

We evaluated the osteogenesis and angiogenesis ability at these cells by adding local inducing factors in media or scaffolds. The results showed increase of expression of osteogenesis such as BMP2, DSPP, Osteonectin and Osteocalcin by using NaF, MSA, SIM, HA. Angiogenesis markers in surface of cells such as CD31 and VEOFR2 was over expressed in DPSCs faces to PRP, VEGF and BEGF. Despite the positive results of using stimulators in front of minerals of proteins, it is necessary to use genes to external the duration and amount of the inducers in target tissues and regions [66-69].

Conclusion

Although several methods are used in surgeries, especially maxillofacial surgeries to improve tissue regeneration and osteogenesis, it is essential to access methods derived from gene therapy to overcome the limitations of tissue engineering. DPSCs were selected as the optimal source of differentiation, because they have considerable potential to differentiate to other cells and are very similar to bone marrow stem cells. Also, viral lentivirus-mediated of the BMP2 gene showed considerable over expression of this gene.

Therefore, DPSCs can be considered as a good resource for bone regeneration and gene therapy, and can be further investigated in experiments in the field of tissue engineering. DPSCs can be investigated not only in the maxillofacial region, but also in different experiments on other body regions in future experiments on tissue engineering, because of their structural similarities with BMMSCs, appropriate results and their ease of isolation.

Competing Interests

The authors have no financial conflicts of interest.

Bibliography

1. Kaur S., *et al*. "Tissue engineering and its future perspective in therapeutic medicine-A brief review". *Journal of Advanced Medical and Dental Sciences Research* 4.4 (2016): 159.
2. Oryan A., *et al*. "Bone regenerative medicine: classic options, novel strategies, and future directions". *Journal of Orthopaedic Surgery and Research* 9.1 (2014): 18.
3. Ozdemir T., *et al*. "Osteoinductive biomaterial geometries for bone regenerative engineering". *Current Pharmaceutical Design* 19.19 (2013): 3446-3455.
4. M Murray PE., *et al*. "Regenerative endodontics: a review of current status and a call for action". *Journal of Endodontics* 33.4 (2007): 377-390.
5. Jimi E., *et al*. "The current and future therapies of bone regeneration to repair bone defects". *International Journal of Dentistry* (2012): 148261.
6. Tonelli P., *et al*. "Bone regeneration in dentistry". *Clinical Cases in Mineral and Bone Metabolism* 8.3 (2011): 24-28.
7. Sheikh Z., *et al*. "Bone replacement materials and techniques used for achieving vertical alveolar bone augmentation". *Materials* 8.6 (2015): 2953-2993.
8. Bleich NK., *et al*. "Gene therapy approaches to regenerating bone". *Advanced Drug Delivery Reviews* 64.12 (2012): 1320-1330.
9. Balmayor ER and van Griensven M. "Gene therapy for bone engineering". *Frontiers in Bioengineering and Biotechnology* 3 (2015): 9.
10. Evans CH. "Gene delivery to bone". *Advanced Drug Delivery Reviews* 64.12 (2012): 1331-1340.
11. Jimi E., *et al*. "Molecular mechanisms of BMP-induced bone formation: cross-talk between BMP and NF-κB signaling pathways in osteoblastogenesis". *Japanese Dental Science Review* 46.1 (2010): 33-42.
12. Xiao YT., *et al*. "Bone morphogenetic protein". *Biochemical and Biophysical Research Communications* 362.3 (2007): 550-553.
13. Bragdon B., *et al*. "Bone morphogenetic proteins: a critical review". *Cellular Signalling* 23.4 (2011): 609-620.

14. Carreira AC., *et al*. "Bone morphogenetic proteins: Facts, challenges, and future perspectives". *Journal of Dental Research* 93.4 (2014): 335-345.

15. Skogh AC and Engstrand T. "Bone morphogenetic proteins in cranial reconstructions: clinical evaluation of heparin-chitosan as a carrier for BMP-2". *Plastic and Reconstructive Surgery* 123.6 (2009): 192e-193e.

16. Chenard KE., *et al*. "Bone morphogenetic proteins in craniofacial surgery: current techniques, clinical experiences, and the future of personalized stem cell therapy". *Journal of Biomedicine and Biotechnology* (2012): 601549.

17. Argintar E., *et al*. "Bone morphogenetic proteins in orthopaedic trauma surgery". *Injury* 42.8 (2011): 730-734.

18. Rao SM., *et al*. "Bone morphogenetic proteins: periodontal regeneration". *North American Journal of Medical Sciences* 5.3 (2013): 161-168.

19. Carreira AC., *et al*. "Bone morphogenetic proteins: structure, biological function and therapeutic applications". *Archives of Biochemistry and Biophysics* 561 (2014): 64-73.

20. Granjeiro JM., *et al*. "Bone morphogenetic proteins: from structure to clinical use". *Brazilian Journal of Medical and Biological Research* 38.10 (2005): 1463-1473.

21. Myllylä RM., *et al*. "Bone morphogenetic proteins 4 and 2/7 induce osteogenic differentiation of mouse skin derived fibroblast and dermal papilla cells". *Cell and Tissue Research* 355.2 (2014): 463-470.

22. Zhang X., *et al*. "The roles of bone morphogenetic proteins and their signaling in the osteogenesis of adipose-derived stem cells". *Tissue Engineering Part B: Reviews* 20.1 (2013): 84-92.

23. Ducy P and Karsenty G. "The family of bone morphogenetic proteins". *Kidney International* 57.6 (2000): 2207-2214.

24. Huse K., *et al*. "Role of Smad proteins in resistance to BMP-induced growth inhibition in B-cell lymphoma". *PLoS One* 7.10 (2012): e46117.

25. Wang RN., *et al*. "Bone Morphogenetic Protein (BMP) signaling in development and human diseases". *Genes and Diseases* 1.1 (2014): 87-105.

26. Yamaguchi A., *et al*. "Recombinant human bone morphogenetic protein-2 stimulates osteoblastic maturation and inhibits myogenic differentiation in vitro". *The Journal of Cell Biology* 113.3 (1991): 681-687.

27. Cheng H., *et al*. "Osteogenic activity of the fourteen types of human bone morphogenetic proteins (BMPs)". *Journal of Bone and Joint Surgery* 85.8A (2003): 1544-1552.

28. Jung UW., *et al*. "The efficacy of BMP-2 preloaded on bone substitute or hydrogel for bone regeneration at peri-implant defects in dogs". *Clinical Oral Implants Research* 26.12 (2015): 1456-1465.

29. Kim SE., *et al*. "The effect of immobilization of heparin and bone morphogenic protein-2 (BMP-2) to titanium surfaces on inflammation and osteoblast function". *Biomaterials* 32.2 (2011): 366-373.

30. Yang W., *et al*. "Bmp2 is required for odontoblast differentiation and pulp vasculogenesis". *Journal of Dental Research* 91.1 (2012): 58-64.

31. Wang Y., *et al*. "BMP activity is required for tooth development from the lamina to bud stage". *Journal of Dental Research* 91.7 (2012): 690-695.

32. Rakian A., *et al*. "Bone morphogenetic protein-2 gene controls tooth root development in coordination with formation of the periodontium". *International Journal of Oral Science* 5.2 (2013): 75-84.

33. Smith DM., *et al*. "Bone morphogenetic protein 2 therapy for craniofacial surgery". *Journal of Craniofacial Surgery* 19.5 (2008): 1244-1259.

34. Gene Therapy Clinical Trials Worldwide (2010).

35. Li SD and Huang L. "Non-viral is superior to viral gene delivery". *Journal of Controlled Release* 123.3 (2007): 181-183.

36. Malik P., *et al*. "Successful Correction of the Human Cooley's Anemia β-Thalassemia Major Phenotype Using a Lentiviral Vector Flanked by the Chicken Hypersensitive Site 4 Chromatin Insulator". *Annals of the New York Academy of Sciences* 1054.1 (2005): 238-249.

37. Brown BD., *et al*. "A microRNA-regulated lentiviral vector mediates stable correction of hemophilia B mice". *Blood* 110.13 (2007): 4144-4152.

38. Bianco CL., *et al*. "Lentiviral vector delivery of parkin prevents dopaminergic degeneration in an α-synuclein rat model of Parkinson's disease". *Proceedings of the National Academy of Sciences of the United States of America* 101.50 (2004): 17510-17515.

39. Azzouz Mimoun., *et al*. "Lentivector-mediated SMN replacement in a mouse model of spinal muscular atrophy". *The Journal of Clinical Investigation* 114.12 (2004): 1726-1731.

40. Anson DS. "The use of retroviral vectors for gene therapy-what are the risks? A review of retroviral pathogenesis and its relevance to retroviral vector-mediated gene delivery". *Genetic Vaccines and Therapy* 2.1 (2004): 9.

41. Bushman FD. "Retroviral integration and human gene therapy". *The Journal of Clinical Investigation* 117.8 (2007): 2083-2086.

42. Martin-Rendon E., *et al*. "Lentiviral vectors for the treatment of neurodegenerative diseases". *Current Opinion in Molecular Therapeutics* 3.5 (2001): 476-481.

43. Goss JR., *et al*. "Antinociceptive effect of a genomic herpes simplex virus-based vector expressing human proenkephalin in rat dorsal root ganglion". *Gene Therapy* 8.7 (2001): 551-556.

44. Lachmann RH and Efstathiou S. "The use of herpes simplex virus-based vectors for gene delivery to the nervous system". *Molecular Medicine Today* 3.9 (1997): 404-411.

45. Damda A., *et al*. "Tissue engineering in endodontics".

46. Patel DM., *et al*. "Therapeutic potential of mesenchymal stem cells in regenerative medicine". *Stem Cells International* (2013): 496218.

47. R Marx RE. "Platelet-rich plasma (PRP): what is PRP and what is not PRP?" *Implant Dentistry* 10.4 (2001): 225-228.

48. Dominici ML., *et al*. "Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement". *Cytotherapy* 8.4 (2006): 315-317.

49. Huang GJ., *et al*. "Mesenchymal stem cells derived from dental tissues vs. those from other sources: their biology and role in regenerative medicine". *Journal of Dental Research* 88.9 (2009): 792-806.

50. Bansal R and Jain A. "Current overview on dental stem cells applications in regenerative dentistry". *Journal of Natural Science, Biology, and Medicine* 6.1 (2015): 29-34.

51. Zhang W., *et al*. "Proliferation and odontogenic differentiation of BMP2 gene transfected stem cells from human tooth apical papilla: An in vitro study". *International Journal of Molecular Medicine* 34.4 (2014): 1004-1012.

52. Samiei M., *et al*. "Isolation and Characterization of Dental Pulp Stem Cells from Primary and Permanent Teeth". *Journal of American Science* 9.12 (2013): 153-157.

53. Dalton AC and Barton WA. "Over-expression of secreted proteins from mammalian cell lines". *Protein Science* 23.5 (2014): 517-525.

54. Barde I., *et al*. "Production and titration of lentiviral vectors". *Current Protocols in Neuroscience* 23 (2010): 4-21.

55. Zufferey R and Trono D. "Production of High-Titer Lentiviral Vectors". *Current Protocols in Human Genetics* 12.1 (2002): .

56. Salmon P and Trono D. "Production and titration of lentiviral vectors". *Current Protocols in Human Genetics* 24 (2007): 12.

57. Ylä-Herttuala S and Alitalo K. "Gene transfer as a tool to induce therapeutic vascular growth". *Nature Medicine* 9.6 (2003): 694-701.

58. Yamaguchi A., *et al*. "Recombinant human bone morphogenetic protein-2 stimulates osteoblastic maturation and inhibits myogenic differentiation in vitro". *The Journal of Cell Biology* 113.3 (1991): 681-687.

59. Rutherford RB. "BMP-7 gene transfer to inflamed ferret dental pulps". *European Journal of Oral Sciences* 109.6 (2001): 422-424.

60. Nakashima M., *et al*. "Induction of reparative dentin formation by ultrasound-mediated gene delivery of growth/differentiation factor 11". *Human Gene Therapy* 14.6 (2003): 591-597.

61. Nakashima M., *et al*. "Stimulation of reparative dentin formation by ex vivo gene therapy using dental pulp stem cells electrotransfected with growth/differentiation factor 11 (Gdf11)". *Human Gene Therapy* 15.11 (2004): 1045-1053.

62. Yang X., *et al*. "Mineralized tissue formation by BMP2-transfected pulp stem cells". *Journal of Dental Research* 88.11 (2009): 1020-1025.

63. Tatullo M., *et al*. "Dental pulp stem cells: function, isolation and applications in regenerative medicine". *Journal of Tissue Engineering and Regenerative Medicine* 9.11 (2015): 1205-1216.

64. Turgeman G., *et al*. "Engineered human mesenchymal stem cells: a novel platform for skeletal cell mediated gene therapy". *The Journal of Gene Medicine* 3.3 (2001): 240-251.

65. Stathopulos PB. "Taking the good out of the bad: lentiviral-based gene therapy of the hemoglobinopathies". *Biotechnology Advances* 21.6 (2003): 513-526.

66. Salehi R., *et al*. "Bioengineering of dental pulp stem cells in a microporous PNIPAAm-PLGA scaffold". *International Journal of Polymeric Materials and Polymeric Biomaterials* 63.15 (2014): 767-776.

67. Samiei M., *et al*. "Osteogenic/odontogenic bioengineering with co-administration of simvastatin and hydroxyapatite on poly caprolactone based nanofibrous scaffold". *Advanced Pharmaceutical Bulletin* 6.3 (2016): 353-365.

68. Zijah V., *et al*. "Towards optimization of odonto/osteogenic bioengineering: in vitro comparison of simvastatin, sodium fluoride, melanocyte-stimulating hormone". *In Vitro Cellular and Developmental Biology-Animal* 53.6 (2017): 502-512.

69. Aghazadeh M., *et al*. "Towards osteogenic bioengineering of dental pulp stem induced by sodium fluoride on hydroxyapatite based biodegradable polymeric scaffold". *Fibers and Polymers* 18.8 (2017): 1468-1477.

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