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Comparative Analysis of Mesenchymal Progenitor Cells from Dental Pulp and Cord Tissue and their Potentiality Towards Trans-Differentiation

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Abstract

Objective: The objective of our study was to successfully isolate progenitor cells from dental pulp and umbilical tissue and perform a comparative investigation of their potential transdifferentiation into osteo and neuronal-like cells. **Methods:** Progenitor cells were harvested from dental pulp tissue as well as cord tissue and cultured through explant culture method over the span of 4 weeks. Image-based cytometric analysis was done to determine the cell viability along with phenotypic analysis to validate the occurrence of stem cell surface markers such as CD13, CD29, CD31, CD34, CD45, CD73, CD90, CD105, HLA-DR, and HLA-ABC. After culturing mesenchymal progenitor cells, osteogenic and neurogenic differentiation potential of both tissue sources was studied. The cells were seeded in two different surfaces tissue culture treated dishes and titanium sheets and cultured along with osteogenic differentiation medium (for 28 days) and neurogenic differentiation medium (for 5 days). The osteogenic potential of the progenitor cells were checked with the detection of calcium deposits by Vonn kossa staining and PCR studies were done to confirm the presence of osteogenic genes like BMP2, HDAC1, HNF1A. The neurogenic potential of the progenitors were phenotypically determined by the observation of neuronal cells in the culture medium. Post differentiation PCR studies were done to confirm the presence of neuronal genes like NESTIN, AGRIN, MAG, DAPDH, NF-M. **Findings:** Progenitor cells extracted from cord tissue and dental pulp were positive for markers such as CD13, CD29, CD73, CD90, and CD105 and were found negative for markers such CD31, CD34, CD45, and HLA-DR. Progenitors obtained from dental pulp tissue showed a higher expression of cell surface markers indicating a stronger mesenchymal lineage. After culturing progenitor cells in osteogenic differentiation specific medium, these cells were successfully differentiated into cells of osteogenic lineage. Within 28 days of culture calcium deposits were detected by Von kossa staining. The differentiated cells were also found positive for osteogenic markers such as BMP2, HDAC1, HNF1A.In neural differentiation, post day 5 of culture neurospheres of varying sizes were observed floating in the culture medium. The fraction of the cells differentiated into osteogenic and neurogenic lineages were higher in progenitor cells derived from dental pulp in comparison with umbilical cord tissue. The higher potentiality of progenitor cells derived from dental pulp for neurogenic trans-differentiation could be explained by the fact that human adult dental pulp stem cells residing within the perivascular niche are thought to originate from the migrating cranial neural crest cells. There was no difference observed in the osteogenic and neurogenic differentiation capabilities of mesenchymal cells when plated in a plastic dish or titanium surface. **Improvement:** In-depth studies needs to be carried out on progenitor cells from dental pulp tissues in order to enhance the clinical efficacy of stem cells based therapies.

Keywords: Dental Pulp, Mesenchymal Progenitor Cells, Neurons, Osteocyte, Umbilical Cord Tissue Stem Cells

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1. Introduction

Mesenchymal progenitor cells are an unique population of stem cells, mostly found in major organ systems of the body. Some of the characteristic features of these cells are: they are easy to isolate, they have the ability to self-renew, amplify in numbers, their multi-lineage differentiation capabilities and they possess immunomodulatory abilities[1]. Hence, it seems to be a fascinating source of stem cells for research and therapeutic purposes. Mesenchymal progenitor cells have been harvested from numerous sources, such as cord tissue, peripheral blood[2-4], bone marrow, adipose tissue[5], placenta[6,7], dermis, heart skeletal muscle[8], synovium, periosteum, amniotic fluid[9], skeletal muscle[10] and dental pulp[11]. These cells are characterized for positive markers such as CD90+, CD73+, CD105+ with the additional expression of stage specific embryonic antigen (SSEA-4) and low affinity nerve growth factor receptor (LNGFR) and are negative markers for CD34, CD45 and HLA DR[12]. Mesenchymal stem cells are known to be multipotent in nature; they predominantly differentiate into adipo, osteo and chondrogenic lineages[13]. Certain studies on mesenchymal progenitor cells have shown their ability to differentiate into neuroglial, hepatocyte like cells[14] and also possess an affinity for endothelial differentiation[15]. Another outstanding feature of MSC's is immune-modulation and immunoprivilege. In immunomodulation MSC's are able to suppress several functions of the immune system such as proliferation of immune cells, production of cytokines and prevent cellular toxicity of immunological cells like T, B and NK cells.

Several studies have been done on these progenitor cells from the different sources, which showed that cord tissue was an ideal source for isolation of mesenchymal progenitor cells, it is rich in number, can be obtained non-invasively with minimum risk to the donors and can be cultured easily without ethical controversy[16]. Till today UC tissue is inevitably discarded even though it is an excellent source for progenitor cells. In *in-vitro* cultures, comparison with BM MSC and Adipose-derived MSC, UC tissue derived -MSCs displayed higher frequency in forming of colony forming units[12], high proliferative capacity with highest doubling number in all passages[7]. UC- MSCs are positive for cell surface markers such as CD90, CD44, CD29, CD13, and CD10 and negative for CD45, CD34, CD31, CD56, CD33, CD14, and HLA-DR confirming their non-hematopoietic lineage. UC-MSCs show a higher affinity for osteogenic[17] odontoblastic, chondrogenic, adipogenic and neural lineages[18]. The progenitor cells harvested from the cord tissue have shown to trans-differentiate into neuron specific protein NEuN and neurofilament NF-positive neurons[19,20] and hepatocytes[21]. Another advantage of cord tissue derived progenitor cells is that these cells express embryonic stem cell (ESC) markers, and therefore are more primitive in nature as compared to MSC found from other sources[22]. Several studies have been undertaken over the past couple of years asserting the fact that these cells can be used for therapeutic treatment of liver cirrhosis[16].

The dental pulp stem cells (DPSCs) were 1st isolated in the year 2000; these multipotent cells were highly proliferative, capable of regenerating a tissue and showed the affinity for osteogenic differentiation. Gronthos identified human adult progenitor cells from dental pulp tissue and found that they could regenerate a dentin and pulp-like complex, which is composed of a mineralized matrix with tubules lined with odontoblasts, and fibrous tissue containing blood vessels in an arrangement similar to the dentin-pulp complex found in normal human teeth. Adult dental stem cells can differentiate into many dental components, such as dentin, periodontal ligament, cementum and dental pulp tissue, but not into the enamel. DPSCs area also able to adhere and proliferate in scaffolds and they can also differentiate into odontoblastic lineage cells[23]. A remove DPSCs are positive for markers such as β2 integrin, CD13, CD24, CD29, CD44, CD73, CD90, CD105, CD106, CD146, NANOG, OCT4, and STRO-1 and were negative for CD14, CD45, CD34 and HLA-DR, thus affirming that these cells are not derived from a hematopoietic source, but instead have a mesenchymal origin[24].

DPSCs were also found to be capable of reprogramming into multiple cell types such as, odontoblast, osteoblast[25], endotheliocyte, neurons[26], chondrocyte, corneal epithelial cell, myocyte, melanoma cell, neurocyte, adipocyte, iPS cells[27-29], neural crest-derived melanocytes[30] and neural-like cells. DPSCs were capable of forming ectopic dentin and associated pulp tissue *in vivo*[28]. DPSC have shown active potential for cellular migration, organization and mineralization, which could produce 3D mineralized structures[31]. The potential of DPSC in dental tissue engineering is due to the high clonogenic capacity of these cells[28]. Like umbilical cord tissue progenitor cells dental pulp progenitor cells have the following benefits: extraction of stem cells from pulp tissue is difficult but and has a high-efficiency rate. They seem to possess immune privileges, they have an exten-

sive differentiation ability as mentioned above. DPSC in several reports has shown successful interaction with several scaffolds and biomaterials which make them ideal for tissue reconstruction. The availability and ease of culture of dental pulp progenitor cells facilitate a future in clinical trials[32]. Human dental pulp progenitor cells are found in the perivascular niche of tooth and are thought to originate from migrating cranial neural crest cells[33]. In 2008 Arthur stated that, DPSC have shown the ability to differentiate into functionally active neurons *in vitro*, strongly suggested their possible applications in regenerative medicine[34]. Kairy reported that, DPSC were transplanted into the cerebrospinal fluid of rats in which cortical lesion was induced. Then the cells migrated to the injured area and expressed neuron-specific markers indicating definitive spontaneous neuronal differentiation ability[35].

Both dental pulp and cord tissue are better sources for mesenchymal progenitors and are preferred options over other sources because cells derived from these sources is easily available with no ethical concerns. In this current study we aimed at isolating mesenchymal progenitor cells from dental pulp and cord tissue, and perform a comparative analysis of their potential into osteo & neurogenic cells.

2. Materials and Methods

Biological samples of tooth and umbilical cord tissue were collected with prior approval from the Institutional ethics committee. The research significance was informed to the donors and the samples were obtained with their consents.

3. Mesenchymal Stem Cells Harvesting from the Donated Tooth Sample

The human exfoliated tooth (n=2) was collected in phosphate buffered saline (PBS) containing antibiotics from the donor and transported to the laboratory at a temperature between 2- 8°C. Tooth sample was washed twice with antibiotic and anti - mycolytic solution. Tooth pulp was harvested using a clean sterile classic max hard wire cutter (1.5 mm). Harvested pulp was kept in 35 mm tissue culture treated dish and the digestion of tissue was done using 1 ml of 0.25% of trypsin at 37°C for a period of 45 minutes with 5 % CO_2. After 45 minutes the trypsin was neutralized with 1 ml of complete growth media. The pulp was taken out from the growth medium and kept in new 35 mm tissue culture dish along with a drop of complete growth medium and incubated at (37°C, 5 % CO_2, for 1day) After 1day, 3 ml complete culture medium was added to the culture dish and incubated for 3-4 weeks, every alternate day the spent medium was changed. The cells that were found to be adherent were passaged for 3 cycles and cryopreserved for further experiments.

4. Mesenchymal Stem Cells Harvesting from the Umbilical Cord Tissue Sample

The donated umbilical cord tissue was collected along with an informed consent from the donor. Cord tissue sample (approximately – 3cm in size) was collected in an aseptic tube containing phosphate buffer saline. Immediately the tube was transported to the laboratory at temperatures between 2 to 8°C. Umbilical cord tissue was then washed twice with antibiotic and anti mycolytic solution then cord tissue was cut longitudinally. The tissue was digested with trypsin (0.25%) and incubated in humidified chamber at (37°C, 5% CO_2, 30 minutes). After 30 minutes of incubation, trypsin activity was neutralized with 2 ml of complete growth medium. Then the tissue was cut into small pieces close to 0.5 cm^3 length wise. The dissected umbilical cord lining was placed for explanting in 35mm tissue culture coated dish in complete growth medium with the gelatinous side down and cultured in complete growth medium for 4 weeks by changing medium on alternative days. The cells that were found to be adherent were passaged for 3 cycles and cryopreserved for further experiments.

5. Cell Phenotype Determination

Both cord tissue and dental pulp progenitor cell at passage 3 were used for image-based cytometric analysis. For the analysis trypsinized cells were incubated with fluorescein isothiocyanate monoclonal antibodies as per manufacturer instructions. Cells plated in a T-75 cm^2 flask were 1st washed with phosphate buffer saline, and detached with the help of trypsin (0.05%), a final concentration of 1×10^6 cells /ml was prepared in PBS. Then, 100 µl of cell suspension was transferred to each tube and 5 µl of antibody was added, mixed and incubated in dark at room temperature for 30 minutes. Fluorescein isothiocyanate labeled mAb against human

HLA-DR, HLA-ABC, CD 13, CD 29, CD 31, CD 45, CD 34, CD 90, CD 105, CD 73 and CD 146 (AbD Serotec). After incubation, the cells were washed with 2ml of washing buffer twice, centrifuged at 400g for 5 minutes. Discard the supernatant. The cellular pellet was suspended in 200 µl of phosphate buffered saline and glycerol at 1:1 ratio and 25 µl of the cell suspension were loaded on the tali cellular analysis slide by pipetting the sample at sample loading area by capillary action. The slide was inserted into the slide port of the tali image-based cytometer and cells were observed. Data were acquired for 10,000 cells and the desired marker was determined from the given cell populations.

6. Cell Viability

Cell viability and cell number are determined using the tali viability dead cell red kit (Life Technologies, Invitrogen). The tali dead cell red reagent contains a solution of propidium iodide. This is a cell-impermeant fluorescent DNA binding dye which is used to detect the necrotic cells. For live cells this propidium iodide is impermeant, where as for dead cells this will penetrate into nucleic acids and become fluorescent. After trypsinizing, centrifuge the cells and remove the supernatant. To 100 µl of suspended cells add 1 µl of tali propidium iodide (PI, component B) mixed well and kept in the dark at room temperature for 5 minutes. From this 25 µl of sample was loaded on to tali cellular analysis slide by capillary action. The slide is inserted in to the slide port of the tali image-based cytometer and observed for live cells.

7. Osteogenic Trans-Differentiation in Tissue Culture Coated Plastic Surface

Osteogenic differentiation was done in monolayer cultures of both cord tissue and dental pulp progenitors. After the cells attained 80% confluency cells were then cultured in osteogenic differentiation medium (Invitrogen) for a period of 28 days in a humidified chamber at 37°C with 5% CO_2 to determine osteogenesis. Microscopic images were captured and confirmed with von kossa staining by detecting calcium deposits.

8. Von Kossa Staining

For the detection of the calcium deposits, the cultured cells are washed twice with phosphate buffered saline and later fixed with 70% ethanol for 1 hour at room temperature without any disturbance. After fixation, the fixative is washed with double distilled water carefully. After aspirating double distilled water from the fixed cells. Then silver nitrate solution (5%) was added to cells and the cell container was placed under a UV light till the calcium deposits change color to dark brown/black. The cell culture plate was further washed three times with double distilled water by gentle shaking. Then 5% sodium thiosulphate was added at room temperature to remove unreacted silver, followed by another three washes with double distilled water (DDW).

9. Seeding Cells onto the Titanium Sheet

The pre-coated titanium sheets were seeded with both umbilical cord tissue and dental pulp progenitor cells at $3x10^3$ cells per sheet. The sheets kept in 50 ml sterile tube containing complete growth medium and incubated at 37°C with 5% CO_2. Every alternative day the spent medium is replaced with fresh medium for a week. The titanium sheets were utilized for differentiation assay.

10. Osteogenic Trans-Differentiation on Titanium Surface

Both dental pulp and cord tissue progenitor cells were seeded on titanium sheets and then cultured in osteogenic differentiation medium (Invitrogen) for 28 days at 37°C, 5% CO_2. The spent medium was changed twice in a week to determine osteogenesis. After 28 days, RNA extracted from the cells were checked for specific osteo genes like BMP2, HDAC1, HNF1A. Microscopic images were captured to confirm the osteogenesis.

11. Neurogenic Trans-Differentiation on Tissue Culture Coated Plastic Surface

Neurogenesis is a cellular level event where in a cascade of steps occurs in forming a neuron from progenitor cells. Generally neurogenesis occurs during embryogenesis. Neurogenic differentiation was done in a monolayer culture after the cells attained 80% confluency. The cells

were then cultured in neurogenic differentiation medium (Invitrogen) for a period of 5 days by changing medium after 48 hours. Both cord tissue and dental pulp derived mesenchymal cells have been proven to have good neurogenic potential but dental pulp progenitor cells showed a greater potential than cord tissue progenitor cells.

11.1 Neurogenic Trans-Differentiation on Titanium Sheets

Both dental pulp and umbilical cord derived progenitor cells seeded on titanium sheets were then cultured in neurogenic differentiation medium (Invitrogen) for a period of 5 days respectively at 37°C with 5% CO_2. Medium changed after 48 hours. Microscopic images were captured to determine the neurogenesis.

12. RNA Isolation from Stem Cells Ware Carried with Standard Kit (Takara RNA Isolation Kit)

The cells were trypsinized and collected in 1.5 ml eppendorf tube. Now the cells were centrifuged and a pellet was obtained. β mercapto ethanol along with RA1 buffer was added to lyse the cells. Now the lysate was added to a nucleospin filter along with a collection tube and spun for 1 min at 11000 X g. To the homogenized lysate, ETOH was added in order to obtain a visible precipitate. The precipitate obtained was disintegrated again and spun for 30 seconds at 11,000 X g along with a new nucleospin ribonucleic acid column and collection tube. MDM was added in to the column and spun at 11,000 Xg for 1 minute to dry the column. The column was further treated with DNase and Rxn for 15 min at room temperature. To the column RAW 2 buffer was added and spun for 30 seconds at 11,000 X g along with a new 2ml collection tube. The column was further treated with RA3 buffer and spun for 30 seconds at 11,000 g. The flow through was discarded and the column was placed back in a new collection tube. Additional buffer RA 3 was added to the spin column, spun again for 2 minutes at 11,000 x g. The membrane was then allowed to dry for 2 min and the RNA was eluted out and added to RNase-free water.

13. Polymerase Chain Reaction

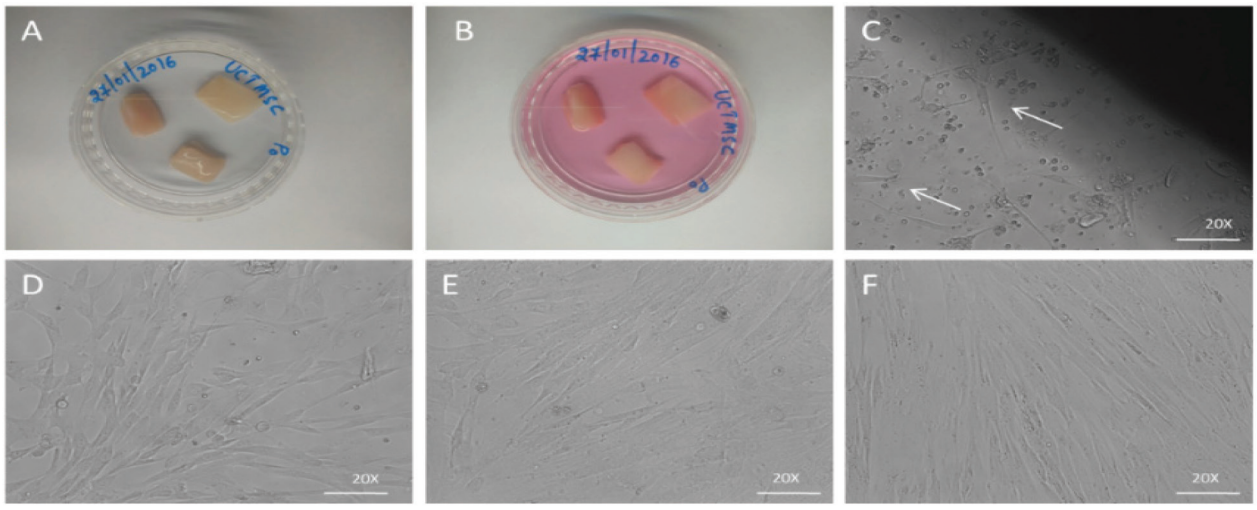
Post RNA extraction c-DNA was prepared for each sample using reverse transcriptase, c-DNA Synthesis kit (TAKARA) as per manufacturer's manual. PCR studies were carried out using standard kit components. A master mix was prepared which consists of nuclease free water, 10 X PCR buffer, dNTPs, and Taq DNA polymerase enzyme. Equal quantities of master mix were added to tubes containing c-DNA template along with forward and reverse primer specific for each gene in separate tubes all the reagents were added with the exception of template DNA which served as a negative control. All the components of the reaction mix were mixed and care was taken to avoid bubbles. The PCR tubes were placed on the thermal cycler for a 45 min long reaction, once the program had finished the products were detected by loading small quantities of each reaction on a agarose gel containing ethidium bromide. If the PCR product is present, the ethidium bromide intercalates with the DNA strands will be visualized with a UV Illuminator. The gel electrophoresis was done in order to visualize the DNA fragments and check for the presence of neural genes such as NESTIN, AGRIN, MAG, DADPH, and osteo genes such as NF-M, BMP2, HDAC1, HNF1A.

14. Results

In the current study, mesenchymal progenitor cells were harvested from two different sources dental pulp and cord tissue. The self-renewal capacities of these cells were observed to be remarkable and the cell culture reached confluency within 20 days. The harvested mesenchymal progenitors were fibroblastic in nature with compact cell body, prominent nucleus, spindle shaped.

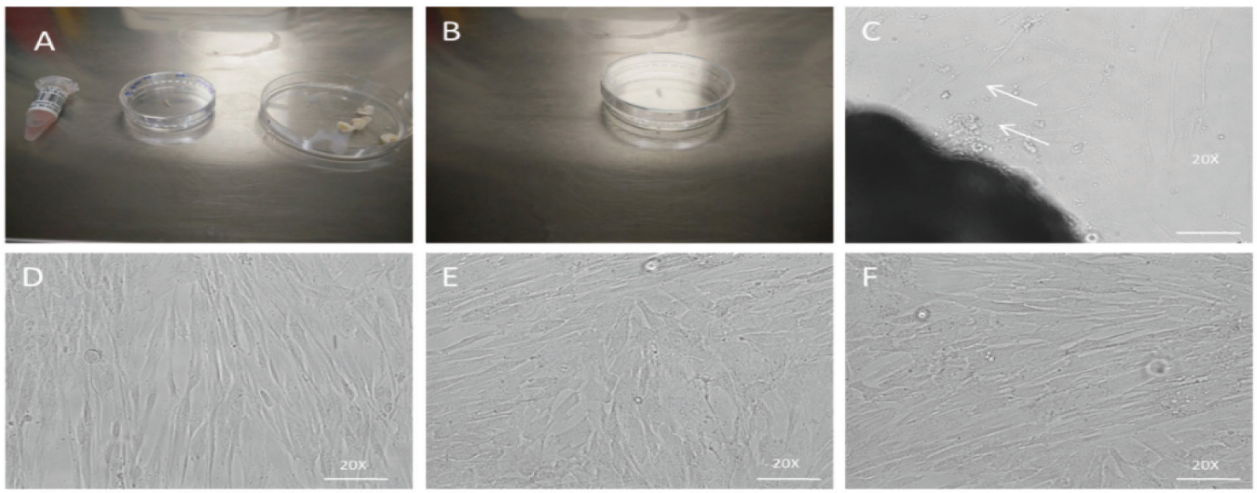
14.1 Mesenchymal Progenitor Cell Viability

Dental pulp and umbilical cord tissue derived mesenchymal progenitor cells were cultured up to P5. These progenitor cells showed fibroblastic morphology and were plastic adherent (Figure 1a-b). The average size of cord and dental pulp derivedcells were 9-15µm and 9-13µm respectively. For the osteo derived cells and neuro differentiation assay passage 3 cells were used after performing the viability assay (Figure 2).



A : Cord tissue explants image; B : Cord tissue explants with complete growth medium
C :Arrows showing mesenchymal stem cells coming from cord tissue explants after a week of incubation; D : Cord tissue derived mesenchymal stem cells passage 2; E: Cord tissue derived mesenchymal stem cells passage 4 ; F : Cord tissue derived mesenchymal stem cells passage 5

Figure 1A. Cord tissue mesenchymal stem cells



A : Exfoliated tooth image; B : Dental Pulp Explant
C :Arrows showing mesenchymal stem cells coming from dental pulp tissue explant after a week of incubation; D : Dental Pulp derived mesenchymal stem cells passage 2; E: Dental Pulp derived mesenchymal stem cells passage 4 ; F : Dental Pulp derived mesenchymal stem cells passage 5

Figure 1B. **Dental pulp mesenchymal stem cells.**

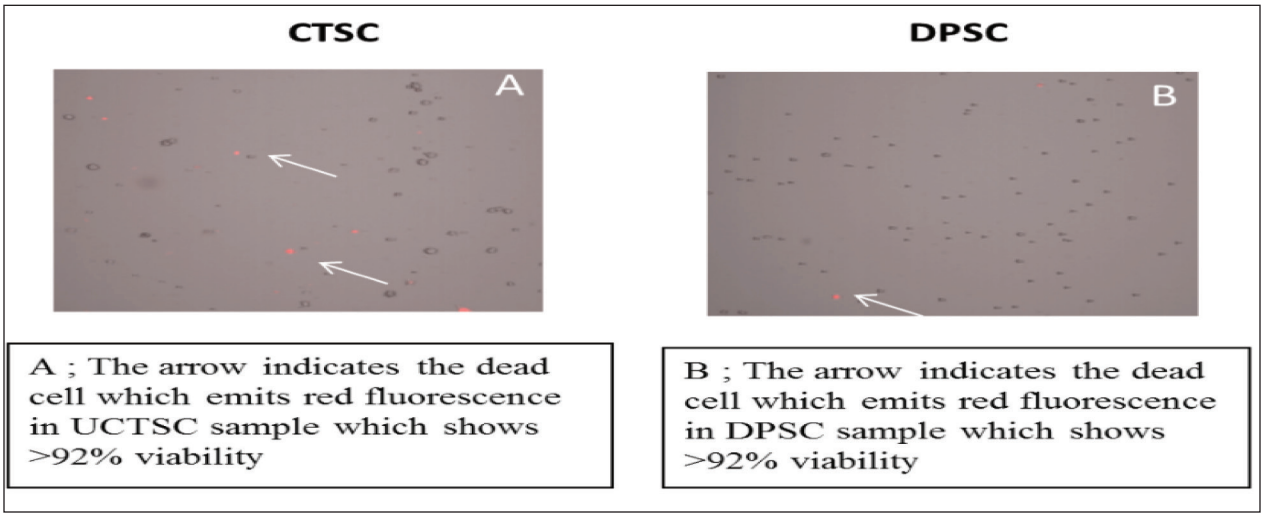


Figure 2. **Mesenchymal progenitor cell viability.**

14.2 Mesenchymal Progenitor Cells Expressed Stem Cell-Specific Phenotype

Mesenchymal cells from the UC and dental pulp sources were positive for standard mesenchymal markers such as HLA-ABC, CD 90, CD105, and CD 73. These cells were also screened for the presence HLA-DR, CD34, CD45, markers and these cell surface antigens were found to be absent. The percentage of expression of all these markers on cells from both the sources was quantified and compared as shown in Figure 3.

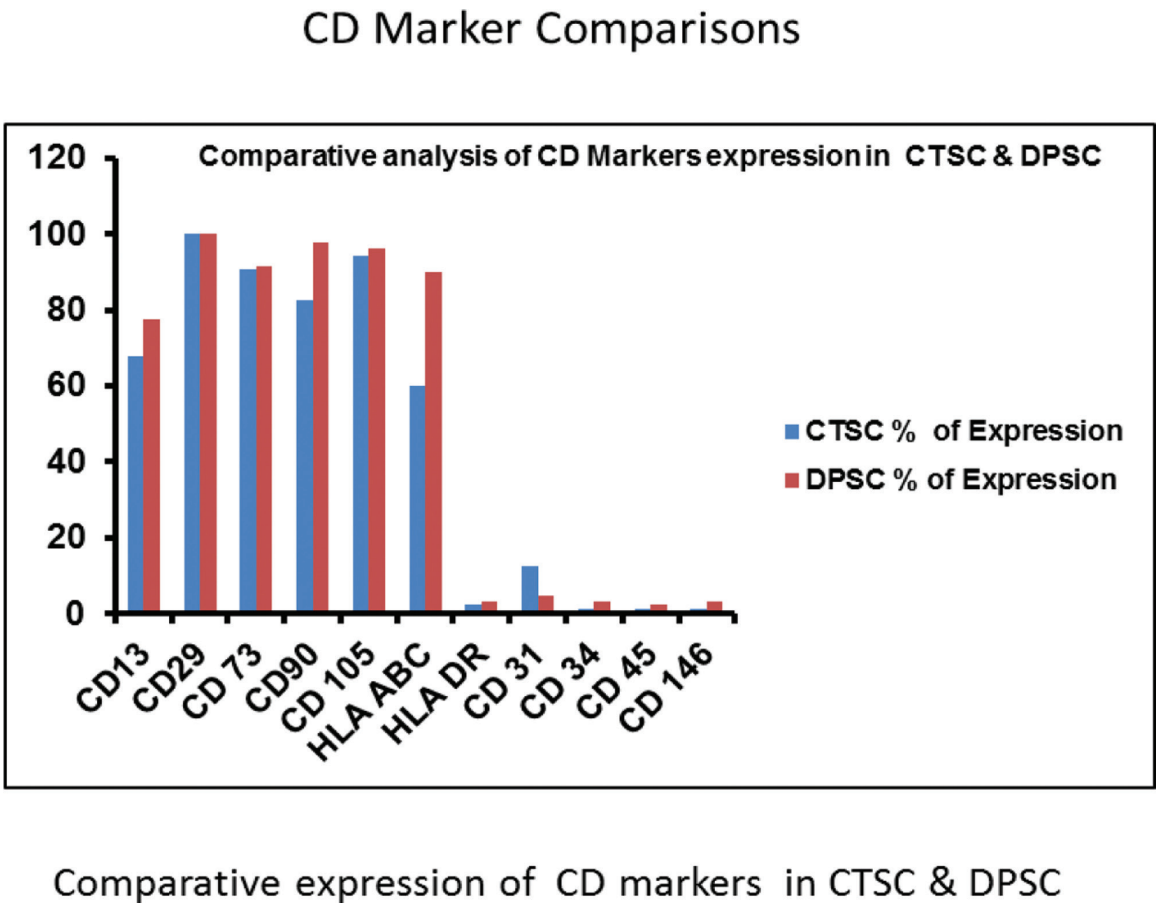


Figure 3. **Mesenchymal progenitor cells expressed stem cell-specific phenotype.**

14.3 Trans-Differentiation Potentiality of Mesenchymal Progenitor Cells from Dental Pulp and Cord Tissue

Cord tissue and dental pulp derived stromal mesenchymal progenitors differentiated into neural cells and osteoblast cells when cultured in lineage specific differentiation medium. After 28 days of induction with osteogenic differentiation media, mesenchymal progenitor cells from both the sources differentiated into osteoblasts-like cells. Significant calcium deposits were detected (Figure 4) and confirmed by von kossa staining (Figure 5). After 5 days

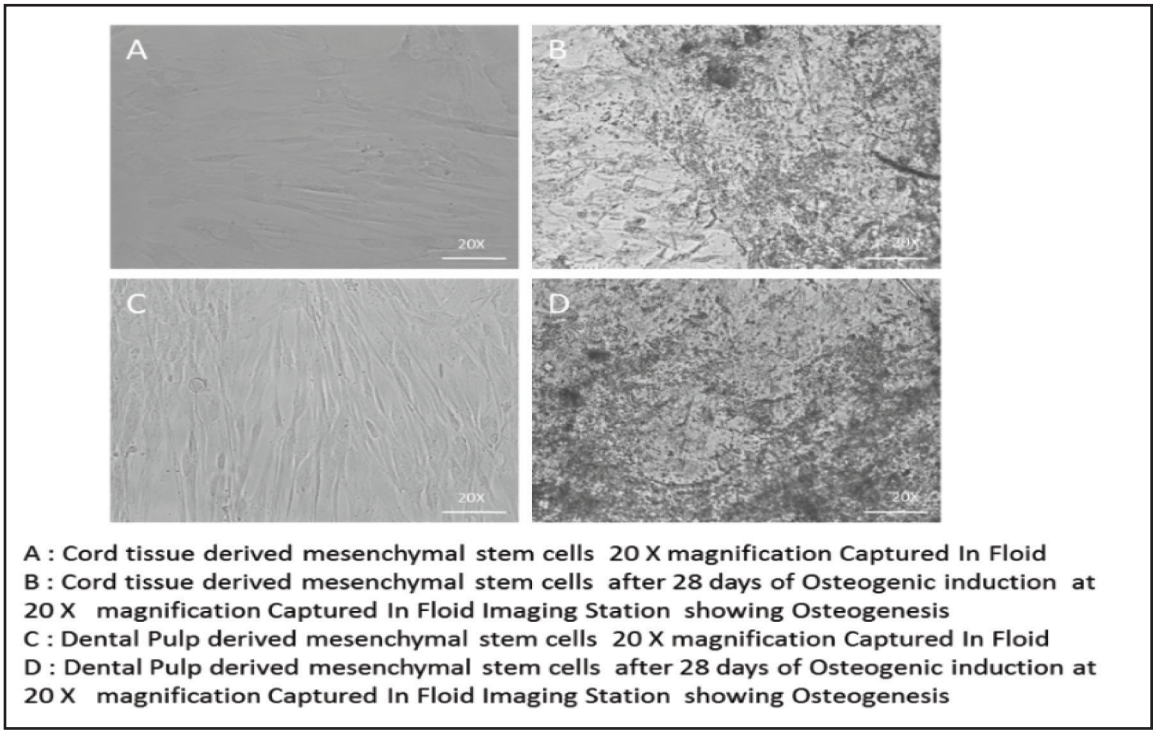


Figure 4. **Comparison of osteogenic differentiation in CTSC & DPSC.**

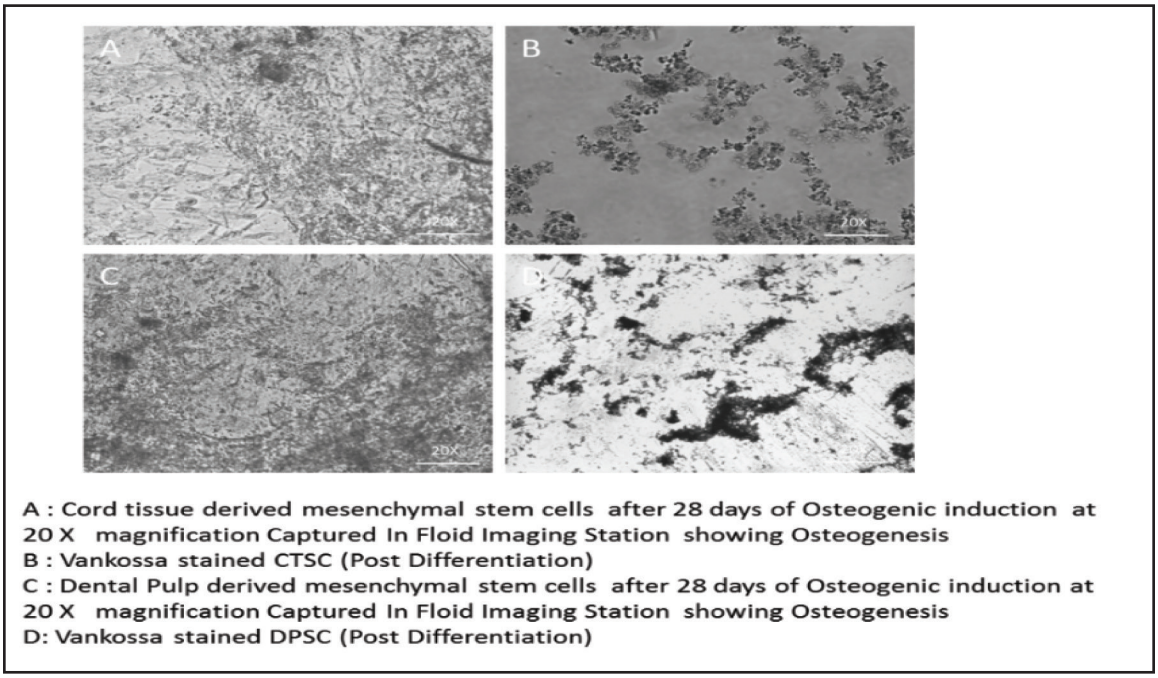


Figure 5. **Vonkossa stained CTSC & DPSC.**

of induction with neurogenic differentiation media, the phenotypic images have depicted neuron like cells (Figure 6). Mesenchymal progenitor cells harvested from dental pulp tissue showed greater affinity towards osteo and neuro differentiation than cord tissue derived progenitors. The untreated mesenchymal progenitor cells served a negative control. Figure 7-8 shows the up regulation of neuro specific markers post neuro differentiation in dental and cord tissue. Figure 9-10 shows the up regulation of osteo specific markers post osteo differentiation in dental and cord tissue.

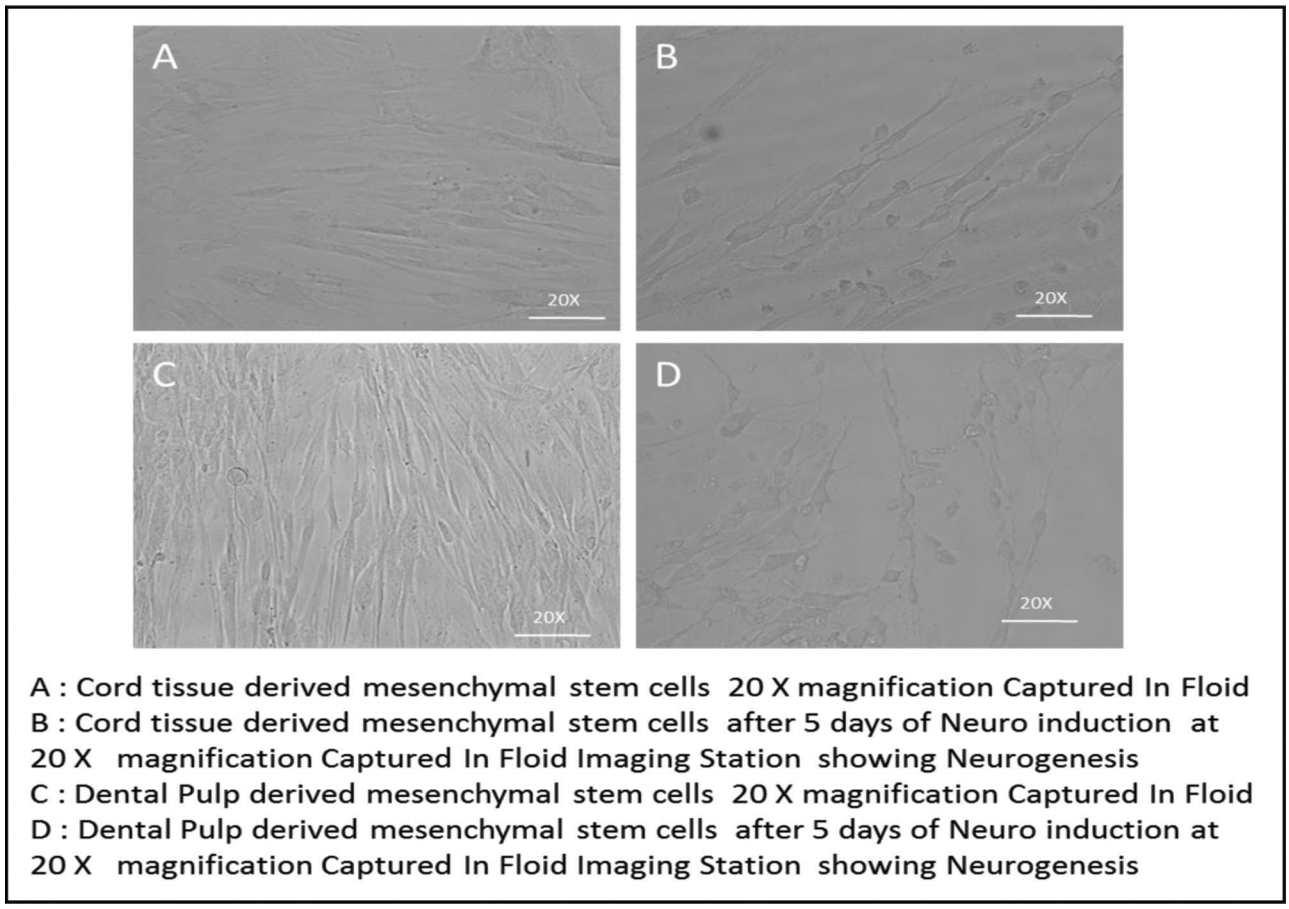


Figure 6. **Comparison of neurogenic differentiation in CTSC & DPSC.**

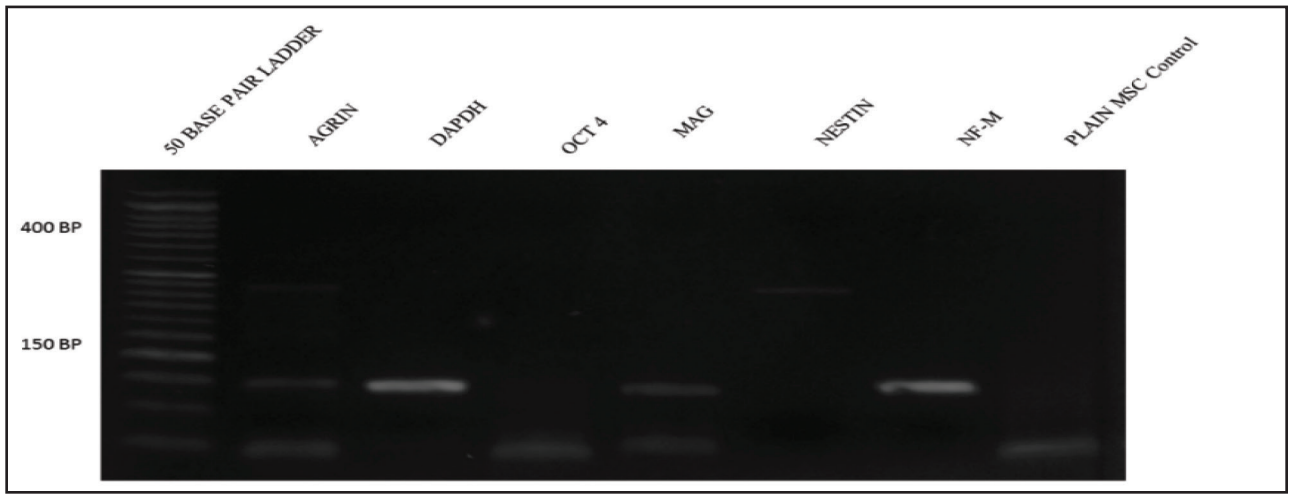


Figure 7. Dental pulp MSC post transdifferentiation showing neuro markers.

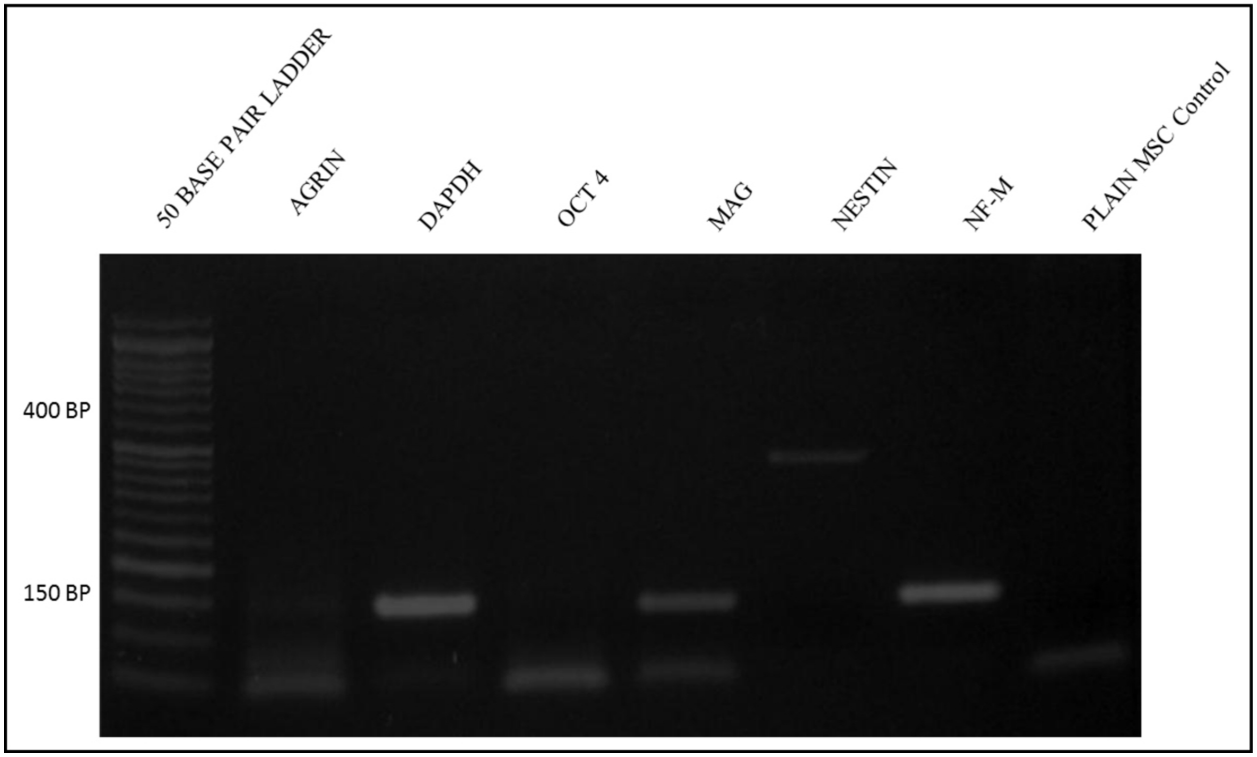


Figure 8. **Cord tissue MSC post transdifferentiation showing neuro markers.**

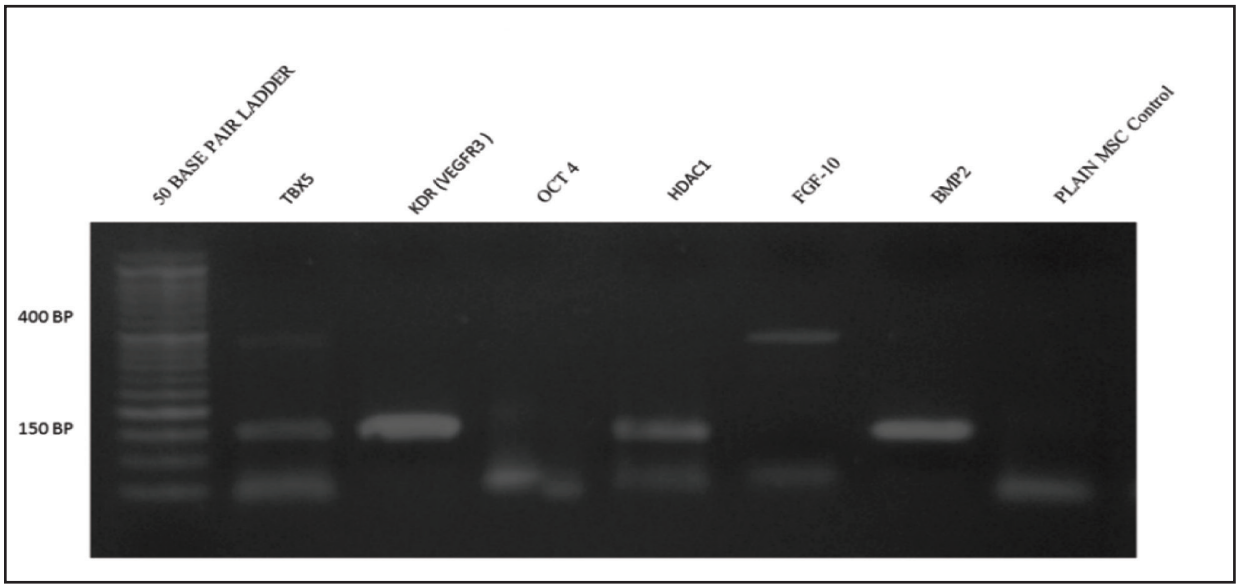


Figure 9. **Dental pulp MSC post transdifferentiation showing osteo markers.**

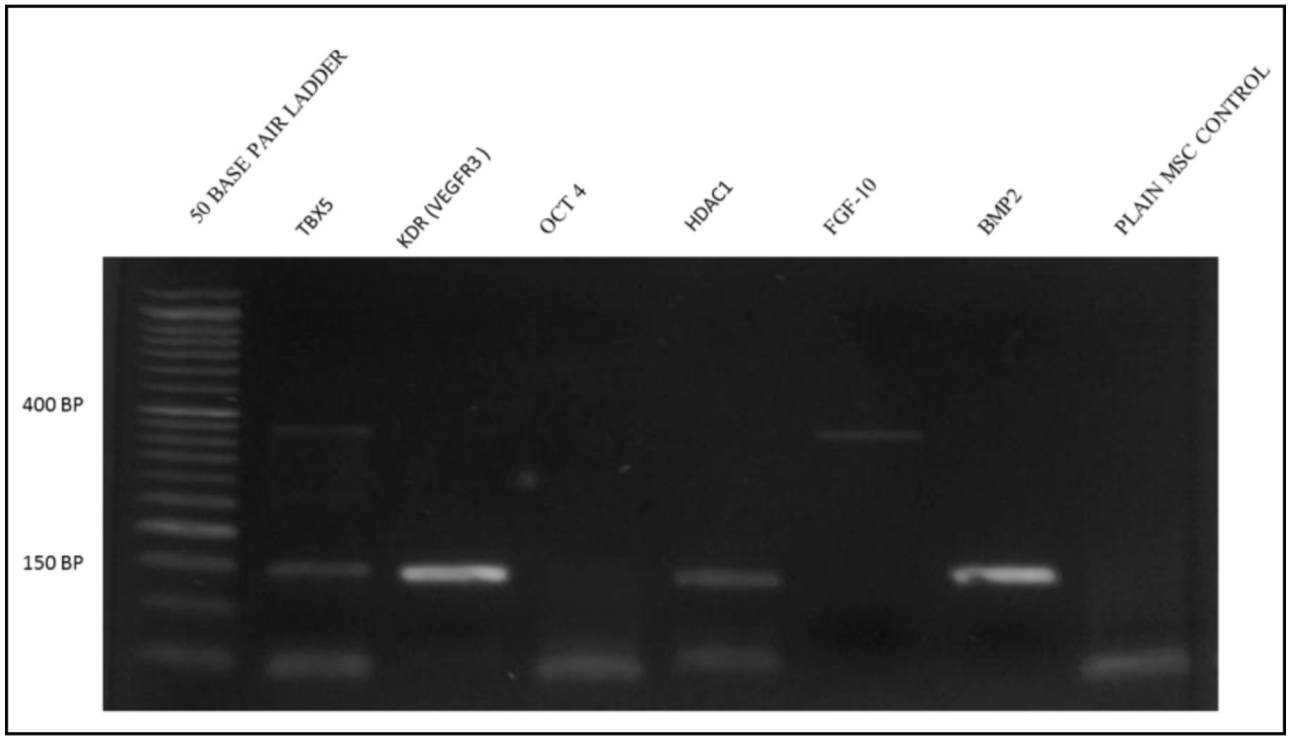


Figure 10. **Cord tissue MSC post transdifferentiation showing osteo markers.**

15. Discussion

Over the last century, extensive research over mesenchymal stem cells has led researchers to identify mesenchymal progenitor cells from major organ system such as brain, spleen, liver, kidney, lung, bone marrow[36], muscle, thymus, pancreas, large blood vessels such as the aorta artery and the vena cava, kidney glomeruli[37] umbilical cord blood[38], placenta[39], umbilical cord tissue[40], amniotic membrane[41], amniotic fluid[42], dental pulp and adipose tissue[43], adipose tissue. Mesenchymal cells isolated from each of these sources have been characterized, and their differentiation potential has been determined. It has been observed that based on the sources from which the mesenchymal stem cells have been derived the differentiation potential, proliferative activity, differentiation efficiency and immuno-suppressive effects differ greatly. Both dental and cord tissue derived mesenchymal progenitor cell population were positive for markers such as CD105, CD90, CD73, CD29, CD13 and negative for CD146, CD45, CD34, CD31 and HLA-DR.

After initiating osteogenic differentiation of cells obtained from dental pulp and cord tissue, capabilities of mesenchymal cells. There was some differences observed in the osteogenic differentiation capabilities of mesenchymal stem cells. The percentage of the cells differentiating into osteo lineage from both sources was compared, and mesenchymal cells derived from dental pulp possessed a higher potential to lineage differentiation, also confirmed by the presence of larger number calcium deposits post-Vonkossa staining. Similarly, for neuro trans-differentiation, mesenchymal progenitor cells from the dental pulp as well as cord tissue were observed to form neurospheres when cultured with the neurogenic medium. The amounts of the cells differentiating into neuron lineage from both sources were compared, dental progenitor showed higher potentiality towards both lineages. (Gronthos *et al.*, 2002). Dental pulp progenitor cells are thought to originate from migrating cranial neural crest (CNC) cells[44] thus explaining their higher potential for neural differentiation.

Mesenchymal progenitor cells for both the sources had an easy induction into neural and osteo lineages. A comparative account of their differentiation potential is done in order to determine which is a better source for future usage of progenitor cells for therapeutic research purposes and can possibly have future clinical applications. From our studies, we have concluded that dental pulp tissue is a more efficient source for the isolation of mesenchymal stem cells, it is easy to obtain and avail-

able at high abundance, extraction of the tissue is easy, low morbidity rate and no ethical concerns. Cells derived from dental pulp showed higher mesenchymal stem cell characteristics, higher values of cell lineage markers and naturally had a higher potency for neural and osteo lineages.

Dental pulp stem cells have proven to be a more superior source for isolating mesenchymal stem cells, along with observed osteogenic and neurogenic potential. And these cells also shown the ability to differentiate into other cell types such as odontoblast, cementobast, myoblast, hepatocyte, melanocyte, chondrocyte, endothelial cells etc[45]. Over the last 10 years there has been extensive research carried out on dental pulp stem cells and the other stem cells from the dentine tissue, several positive reports have already been published on dental pulp stem cells with its potential clinical applications[46]. Isolated dental pulp stem cells and along with a collagen scaffold have used the cells for the regeneration oro-maxillo-facial bone tissue. In this setup dental pulp stem cells along with collagen sponge biocomplexes could completely restore the human mandible bone defects affirming this cell population repair ability. In another study dental pulp stem cells were capable of forming a living autologous fibrous bone (LAB) tissue invitro and post invivo transplantation the lab tissue also showed the ability to form bone containing osteocytes[47]. Several studies have also followed the potential applications dental pulp stem cells for neuro regeneration. A comparative study between bone marrow stem cells, induced pluripotent stem cells and dental pulp stem cells showed that dental pulp stem cells were more effective in repair of spinal cord injury when transected into a rat spinal cord. The dental pulp stem cells facilitated the regeneration of axons, replaced the lost cells and differentiated into adult oligodendrocyte, along with regeneration the dental pulp stem cells inhibited further apoptosis of neural cells, preserved myelin sheaths and neurofilaments and improved locomotor function[48]. In another similar study dental pulp stem cells were transplanted into the spinal cord of a mice, which was subjected to laminectomy which caused compression of the spinal cord, post transplantation the number of oligodendrocytes increased, the number of myelinated axons did not deplete instead remained constant, increased trophic factor expression and overall improvement in locomotor functions was also observed. Thus, proving the fact that dental pulp stem cells can be a highly feasible candidate for therapeutic intervention for bone regeneration, central nervous system repair as well as neuro degenerative disorders such as Parkinson's, Alzheimer's, etc.

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