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Original Research Article

Isolation and Chracterization of Mesenchymal Stem Cells from Dental Pulp at a Tertiary Care Centre

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Abstract

Introduction: *Stem cells have always been a fascinating area in research as they are the building blocks of human body. They have properties like self renewal, proliferation and multi- lineage differentiation. These indispensable properties have always brought a keen interest of researchers all around the world to use them for many diseases. There has always been search for newer and more reliable source to harvest stem cells from various parts of human body.*

Aim: *To study the growth pattern, visual analysis and quantification of mesenchymal stem cells isolated from dental pulp.*

Materials and Methods: *A descriptive type of observational study was done at SMS Medical College, Jaipur. The healthy tooth dental pulp extracted under all aseptic precautions and immediate culture of the pulp tissue done in stem cell laboratory. The culture cells were then evaluated to establish their mesenchymal nature by using immunocytochemistry technique.*

Results: *Total of 10 healthy teeth were extracted and cultured in laboratory and yielded a mean cell count as $1.33X10^3$ cells per D60 culture dish. The mean and median cell viability of the culture dishes came out to be 95%. The mean expression of CD73 in the study subjects was calculated as 94.50% , mean of expression of CD105 was calculated as 94.30%. CD271 was expressed in 42.20% of cells on an average.*

Conclusion: *From our study we concluded that dental pulp is a significant source of stem cells with a mean viability 95%.These cells expressed a high percentage of mesenchymal markers like CD73 and CD105 establishing their mesenchymal nature.*

Keywords: *Mesenchymal stem cells, Dental pulp stem cells , Tissue culture.*

Introduction

The term stem cell was proposed for scientific use by Russian histologist Alexander Maksimov, 1999. He was first to suggest the existence of hematopoietic stem cells (HSC) with the morphological appearance of a lymphocyte,

capable of migrating throughout the blood to micro ecological niches that would allow them to proliferate and differentiate.[1]

Stem cells are building blocks of the body. They have been designated as progenitor or precursor cells. Stem cells can be described as undifferentiated clonigenic cells that are characterized by three fundamental abilities: proliferation, self-renewal and differentiation towards multiple cell lineages.[2]

Stem cells have been identified from three major sources. Embryonic stem cells, derived from the inner cell mass of blastocyst-stage embryos. Adult stem cells, which can be derived from ectodermal, endodermal and mesodermal organs in adults and Induced pluripotent stem cells, generated from neonatal and adult dermal fibroblasts in humans by gene reprogramming.[3]

Adult stem cells have been identified in many organs and tissues, including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium and testis.[4]

Mesenchymal stem cells possess a high self renewal capacity and the potential to differentiate into mesodermal lineages, thus forming cartilage, bone, adipose tissue and skeletal muscle and participate in formation of many craniofacial structures. It can be used autologously without concern of immunorejection, as it can be isolated from patients who need treatment. MSC have been used allogenically to heal the defects.[5]

The dental pulp, a soft connective tissue contained within the pulp chamber of the tooth, is considered an interesting source of adult stem cells due to the high content of cells and to the low invasive procedures required for cell isolation, compared to other adult tissue sources.[6,7,8]

The dental pulp stem cells (DPSCs) were discovered by Dr. Iriana Kerkis as adult stem cells in 2005, and then immature dental pulp stem stem cells (IDPSCs) were discovered through dental pulp organ culture as pleuripotent subpopulation of DPSCs in 2006.[9]

There is a lot of research work going on for the prevention, diagnosis and treatment of devastating human illnesses like heart diseases, diabetes, cancers and diseases of central nervous system like Parkinson's and Alzeimer's disease. Such diseases has lead to discovery of human stem cells.[10]

There has been a tremendous development in the science of tissue engineering and regenerative medicine, that too with a very rapid rate for stem cell research. These tissue engineering approaches require three basic elements for growth and proliferation which are, stem cells, scaffold (matrix on which these cells grow) and growth factors (morphogens, which help in growth and differentiation into specific lineages).[11] Now a days this research in the field of tissue engineering has emerged as one of the most fascinating areas and brings a new hope for improved outcomes by replacing damaged or absent tissues with healthy regenerated tissue.[12]

In the present study, we have isolated these pleuripotent stem cells in the laboratory from the dental pulp. We grew them under ideal conditions and there after characterized their mesenchymal nature. These cells can be a boon to repairative and regenerative medicine.

Materials and Methods

This descriptive observational study on tissue cultured from dental pulp stem cells at the Advanced Stem cell Laboratory of SMS Medical College and Attached Group of Hospitals at Jaipur, Rajasthan was done after approval of ethical committee in May 2018 and continued till November 2019. A total of 10 healthy teeth which were free from dental caries (malaligned or over retained) were extracted from children with mixed dentition period (7-12 years of age) under all aseptic precautions at RUHS College of Dental Sciences, Jaipur, Rajasthan. These teeth were carried immediately in transport media [Dulbacco's modified Eagles's medium (DMEM) with penicillin and streptomycin] immediately to Advanved Stem Cell Laboratory, SMS Medical

College, Jaipur, Rajasthan. All procedures were carried out in a sterile laminar air flow at the Advanced Stem Cell Laboratory which is controlled under the guidelines of GLP and GMP (good laboratory practice and good medical practice). The pulp tissue was isolated and then cultured on D60 petri dishes by both explants method and enzymatic method. The culture dishes were supplemented with DMEM media (Table 1) and placed in incubator at 37 degree Celsius temperature and 5% Carbon di oxide. The culture media was changed every third day and growth was observed. The cells cultured were taken out on eighth day and cell viability was assessed by using trypan blue dye on half of the cells. The other half was processed for immunocyto-chemistry using three cell surface markers, CD73, CD105 and CD271 to assess the mesenchymal nature of these cultured cells. Immunofluorecscence for CD73 was also applied on some the cells.

Results

From the first day of inoculation of explants on the culture dishes containing the dental pulp, following characteristics were observed under a Zeiss Phase Contrast Microscope at 10X magnification. On day 1, no significant growth was seen (Figure-4). We were able to see round shaped cells along the periphery of the expant by day 3, although the nature of the cells could not be confirmed (Figure-5). On day 5, round shaped cells and other cells were observed. The cells seemed to be growing at a steady rate (Figure-6). We could see the full fledged growth of fibroblast like cells and epithelial like cells in the petri dishes (Figure-7).

Now, after a complete proliferation of stem cells, we evaluated the mesenchymal nature of these cells along with testing of live cell viability. The cells counting and viability testing was done on Neubauer's chamber. We used trypan blue dye for assessing cell viability. The cells were stained with trypan blue. The viable cells resist staining and dead cells took the stain (Figure-8). Then the cells were counted on Neubauer's chamber. The mean and median of cell count on 8^{th} day in our study came out to be $1.33X10^3$ cells per D60 dish. The interquartile range is $1.25X10^3$ to $1.40X10^3$. The standard deviation is $0.085X10^3$ (Figure-1 & Table-2). The mean and median cell viability of the culture dishes came out to be 95% with a standard deviation of 1.49%. The interquartile range is 93.75- 96.25% (Figure-2 & Table-3).

The mesenchymal nature of dental pulp stem cells were characterized with the use of immunocytochemistry using cell surface markers CD73, CD105 and CD271. The brown staining was taken as positive as seen for CD73 (Figure-9), CD105 (Figure-10) and CD271 (Figure-11).

The mean expression of CD73 in the study subjects was calculated as 94.50%, mean of expression of CD105 was calculated as 94.30%. CD271 was expressed in 42.20% of cells on an average (Figure-3 & Table-4).

In our study, immunofluorescence was applied on isolated and cultured cells as an qualitative test. Immunofluorescence expression of CD73 (Figure-12), the cells showed a positivity for CD73 which is a mesenchymal stem cell marker.

Based on the above immunofluorescent and immunocytochemistry study, it is clear that the isolated cells have mesenchymal properties. Thus, we could conclude that we were able to isolate mesenchymal stem cells from the pulp of deciduous tooth of children of 7-12 years of age group. As these children are of mixed dentition period, tooth extraction from them is not going to cause any significant morbidity. Tooth being an easily accessible site makes our process of extraction of dental pulp very simple and less technological. The tooth which we have isolated were a kind of therapeutic interventions which needed the removal of unwanted and accessory tooth. Thus, we have not caused any harm to any people in our study. Also we have extracted tooth of children, they gave us a better growth with a good viability and counts with healthy growth of cells in need.

Table 1 Composition of Stem Cell Culture Media (50ml)

COMPONENT	VOLUME
Dulbacco's modified Eagles's medium (DMEM) liquid base	40 ml
Epidermal Growth Factor	25 µl
Basic Fibroblast Growth Factor	25 µl
Serum Fetal Bovine Serum (FBS)	10 ml
Antibiotics (penicillin/ strepyomycin)	50 µl
Insulin	20 µl

Table 2: 8th day Cell counting on Neubauer's Chamber

	8th Day cell counting on D60 dishes
Mean	$1.33X10^3$
Median	$1.33\ X10^3$
IQR	$1.25\text{-}1.40\ X10^3$
Std. Deviation	$.085\ X10^3$
Minimum	$1.20\ X10^3$
Maximum	$1.45\ X10^3$

Table 3: Cell viability

	Cell viability
Mean	95.00%
SD	1.49%
Median	95.00%
IQR	93.75%-96.25%
Minimum	93.00%
Maximum	97.00%

Table 4: Expression of mesenchymal Immunohistochemistry markers

	CD73	**CD271**	**CD105**
Mean	94.50%	42.20%	94.30%
Median	94.50%	42.50%	95.50%
SD	2.46%	5.613%	3.36%
IQR	92.0%-97.0%	40.0%-45.5%	91.75%-97.25%
Minimum	91.00%	32.00%	88.00%
Maximum	98.00%	51.00%	98.00%

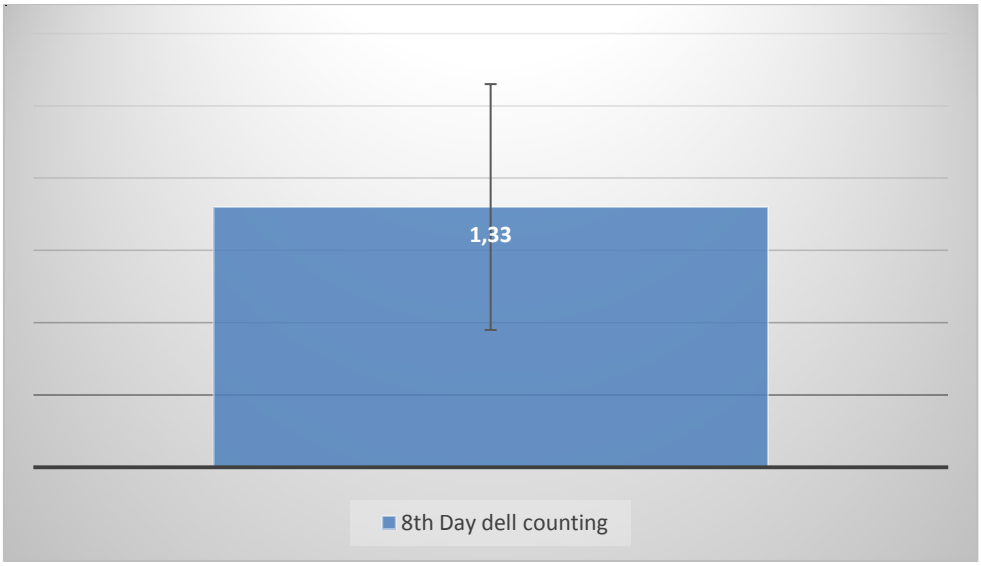

Figure 1 8th Day cell counting ($*10^3$)

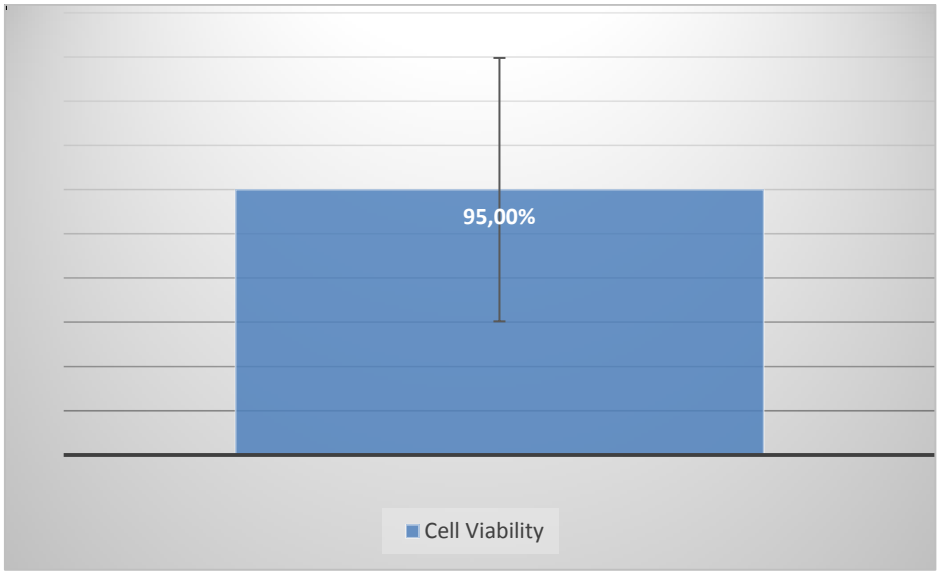

Figure 2- Cell viability

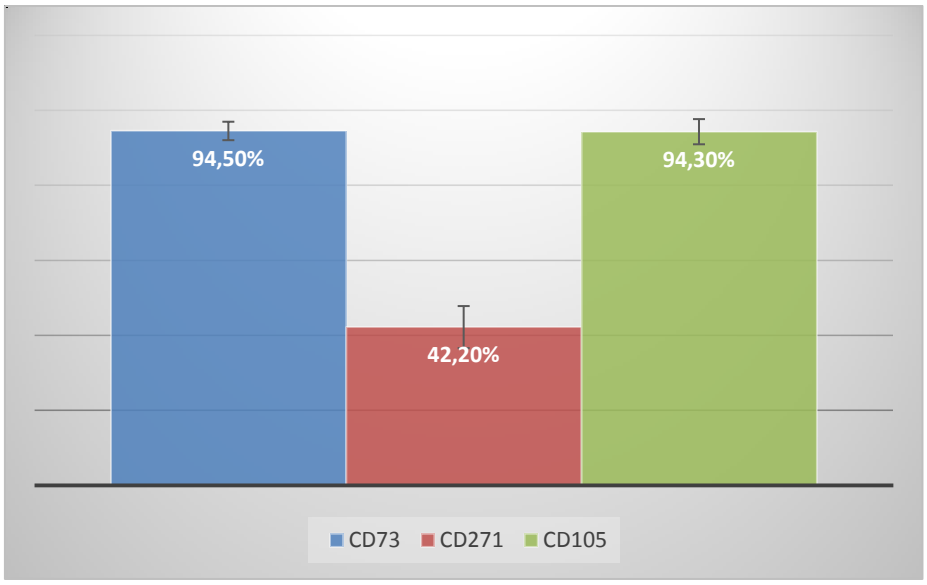

Figure 3- Mean Expression of mesenchymal Immunohistochemistry markers

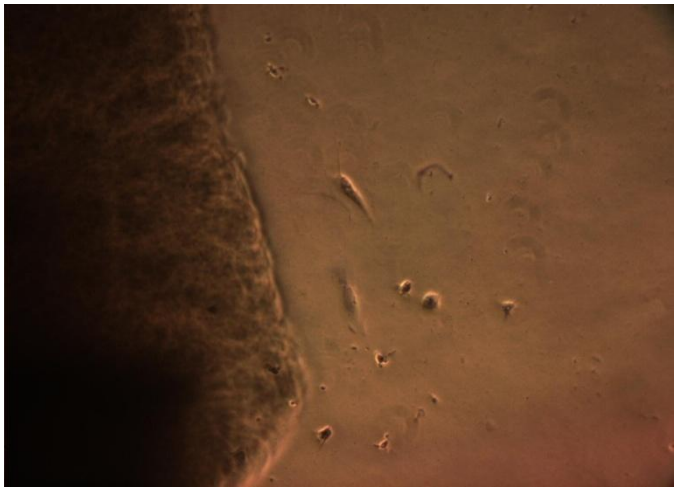

Figure 4- DPSC (100X)- DAY 1- some spindle shaped cells could be appreciated from the explant surface

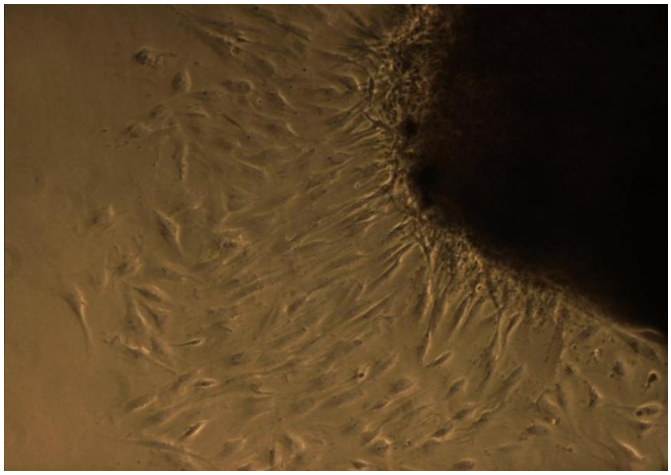

Figure 5- DPSC growth pattern 3rd day (100X) - spindle shaped cells can be seen around the explants

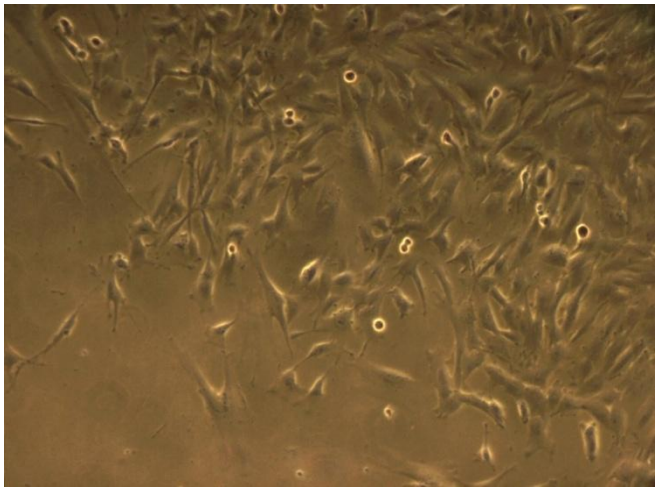

Figure 6- DPSC growth pattern 5th day (100X)- spindle shaped cells showing confluency of around 50%

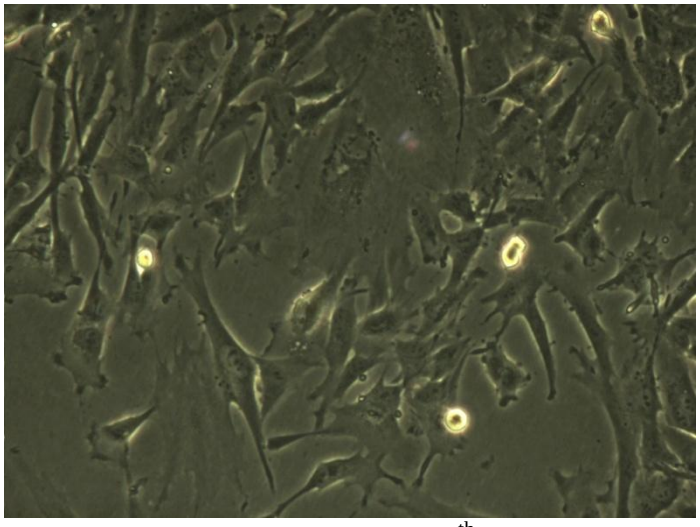

Figure 7- DPSC growth pattern 8th day (100X)- 90-100% confluency. Spindle shaped cell with numerous projections for attachment to surface of petri dishes along with single nucleus are seen.

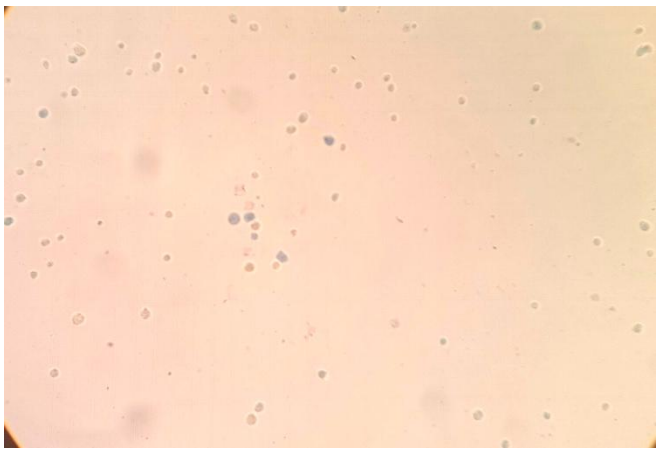

Figure 8- DPSC- (100X)- trypan blue stain- Cell Viability calculation

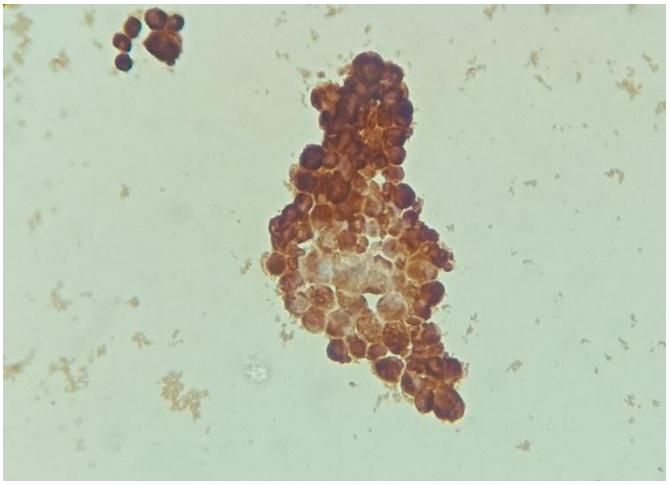

Figure 9- DPSC (400X) CD73 immunocytochemistry- strong positivity

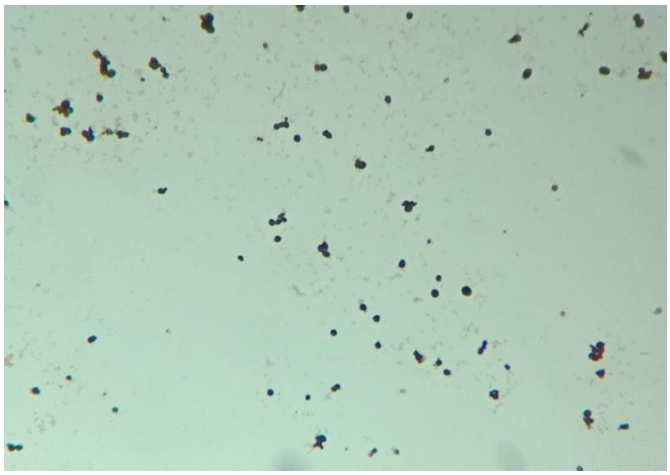

Figure 10- DPSC (100X)- strong positivity for CD105

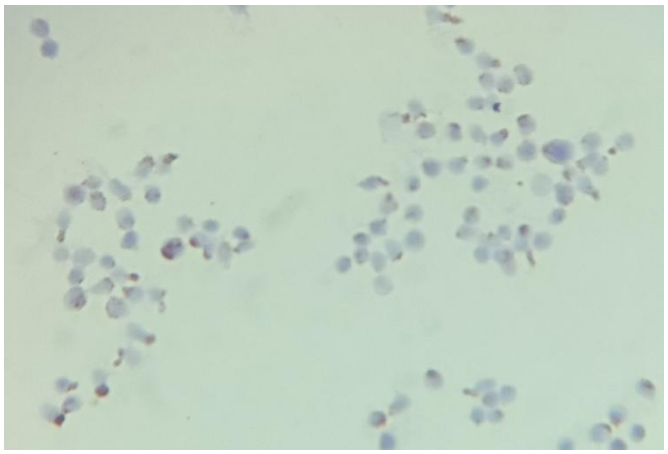

Figure 11- DPSC (400X)- weak positivity in around 40% cells for CD271

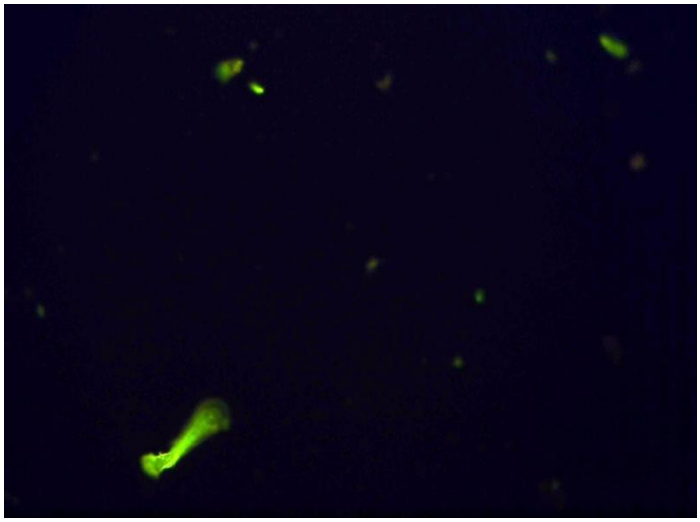

Figure 12- DPSC (100X)- CD73 positive cells on immunofluorescence

Discussion

We have extracted 10 healthy teeth from children of mixed dentition period at RUHS College of Dental Sciences, Jaipur. The isolation of pleuripotent dental pulp stem cells was done in Stem Cell Laboratory of SMS Medical College, Jaipur. We could successfully isolate the viable healthy culture dishes and were able to characterize their mesenchymal nature by taking CD73, CD105 and CD271 as surface markers of mesenchymal cells. The dental pulp tissue appeared to be a promising source of mesenchymal stem cells.

S. Gronthos et al cultured human post natal dental pulp stem cells in vitro and in vivo. They also compared the stem cell property of dental pulp stem cells and bone marrow derived stem cells. They concluded that dental pulp is a better source of stem cells as compared to bone marrow stem cells. Similarly in our study also we could show that dental pulp tissue is a very good source of mesenchymal stem cells.[13]

Similar to study conducted by Tsai et al, we were also able to isolate mesenchymal stem cells from human dental pulp tissue with a success rate of 79.5%, and they found that teeth with caries and pulpitis were relatively poor source for isolation of dental pulp stem cells. In our study, as we have already excluded the teeth with carries and inflammation, so the success rate was almost 100% for isolation of stem cells from dental pulp.[14]

Mohamadreza Baghaban Eslaminejad et al, isolated DPSCs from human third molars and characterized the mesenchymal nature by using CD105, CD90, CD73 and CD44 which showed more than 90% positivity. We in our study used CD73, CD105 and CD271 as mesenchymal markers. Our CD73 and CD105 also showed more than 90% positivity.[15]

Mohammed Samiei et al used both primary and permanent tooth for isolation of dental pulp stem cells whereas in our study we used only primary tooth for isolation of DPSCs. They used CD90, CD73, CD105 and CD166 as mesenchymal markers. They found that these markers are positive in more than 80% of cultured cell population, which is more than 90% in our study with CD73 and CD105 as mesenchymal markers.[16]

In the year 2017, Simone Bonato Luisi et al. were able to isolate the dental pulp stem cells from the third molars. They could isolate the stem cells and then subcultured them in appropriate media for osteogenic, adipogenic and chondrogenic lineages. They found that these cells were more than 95% positive for mesenchymal markers, CD29, CD44, CD73 and CD90 and there was lower positivity for vascular stem cell markers like CD14, CD34, CD45 and HLA-DR. they could also demonstrate the osteogenic and adipogenic differentiation by usage of special stains. Likewise, in our study we could also isolate and characterize the mesenchymal nature of cells isolated from dental pulp and cultured them in laboratory showing the self proliferative capacity of these cells. Thus concluding that these cells are a potential source of tissue engineering studies and therapeutics.[17]

Shagufta Naz et al., isolated and cultured the dental pulp stem cells from permanent and deciduous tooth. They found that average live cell viability was around 99%. The average live cell viability of our cultured cells came out to be 95%.[18]

Conclusion

The stem cell research is an emerging field of modern medicine for newer and better advancement for treatment of many irreversible diseases along with rapid healing of certain wounds. Dental pulp being an easily accessible area with very insignificant ethical issues is easy to retrieve. The property of stemmness of dental pulp is well known because of immense work done by many scholars in this field. Studies are going all around the globe for how and when to use this property of dental pulp stem cells for the mankind. The dental pulp stem cells when grown in vitro under ideal conditions showed multiline age differentiation. They are a huge source of mesenchymal stem cells. These mesenchymal stem cells have property to differentiate into osteogenic, neurogenic, condrogenic, adipogenic, myogenic and dentinogenic tissues. Thus, these cells can be used for repair and regeneration of these tissues in the body.

We concluded following important points in our study-

1. Dental pulp is a significant source of stem cells
2. The cells obtained from dental pulp are highly viable.
3. These cells show a high percentage of mesenchymal markers, CD73 and CD105.

Thus, we can say that these cells derived from dental pulp are a rich source of mesenchymal cells and can be differentiated into different tissues under ideal tissues. These tissues can be mobilized and then be cryopreserved for long term used and can be used therapeutically. Although many therapeutic trials are under study, but this field is still needs to be enhanced further to fulfill the impact that stem cell therapy will have for future healthcare.

References

1. Ramalho SM, Willenbring H. On the origin of the term “stem cell”. Cell stem cell,june 7th 2007;1(1) :35-8.
2. Polak JM, Bishop AE. Stem cells and tissue engineering: past, present, and future. Ann N Y Acad Sci. 2006;1068:352–66.
3. Wada N, Gronthos S, Bartold PM. Immunomodulatory effects of stem cells. Peridontology2000. 2013; 63: 198-216.
4. Bosio A, Bissels U, Miltenyi S. Regenerative medicine: From protocol to patient. 1st ed. Netherlands: Springer, Dordrecht; 2011. Characterization and classification of stem cells. p. 149–67.
5. Bongso A, Lee EH. Stem cells: From bench to bedside. 2nd ed. Singapore: World Scientific; 2005.
6. D’Aquino R, De Rosa A, Laino G, Caruso F, Guida L, Rullo R, et al. Human dental pulp stem cells: From biology to clinical applications. Journal of Experimental Zoology Part B Molecular and Developmental Evolution. July 2009; 312B: 408–15.
7. Goldberg M, Smith AJ. Cells and extracellular matrices of dentin and pulp: A biological basis for repair and tissue engineering. Crit Rev Oral Biol Med. February 2004;15(1):13–27.
8. Tirino V, Paino F, De Rosa A, Papaccio G. Somatic Stem Cells: Methods and Protocols. 1st edition. New Jersey. Humana Press. 2012. Identification, isolation, characterization and banking of human dental pulp stem cells. Methods Mol Biol;879: 443–63.
9. Kerkis I, Kerkis A, Dozortsev D, Stukart-Parsons GC, Gomes Massironi SM, Pereira LV, et al. Isolation and characterization of a population of immature dental pulp stem cells expressing OCT-4 and other embryonic stem cell markers. Cells Tissues Organs. 2006;184 (3-4):105–16.
10. Narang S, Sehgal N. Stem cells: A potential regenerative future in dentistry. Indian J Hum Genet. May 2012; 18(2):150-4.
11. Malhotra N, Mala K. Regenerative endodontics as a tissue engineering approach:

past, current and future. Aust Endod J. 2012; 38(3): 137-48.
12. Sancherz- Lara P A, Zhao H, Bajpai R, Abdelhamid A, Warburton D. Impact of Stem Cells in Craniofacial Regenerative Medicine. Front Physiol. 2012; 3: (188): 1-9.
13. Gronthos S, Mankani MH, Brahim JB, Robey PG, Shi S. Postnatal human dental pulp stem cells in vitro and in vivo. Proc Natl Acad Sci USA. 2000;97(25): 13625-30.
14. Tsai AI, Hong HH, Lin WR, Fu JF, Chang CC, Wang IK, et al. Isolation of mesenchymal stem cells from human deciduous teeth pulp. Biomed Res Int. March 2017; Article ID-2851906, 9 pages.
15. Mohamadreza BE, Nazarian H, Shariati M, Vahabi S, Falahi F. Isolation and in vitro characterization of mesenchymal stem cells derived from the pulp tissue of human third molar tooth. Iran J Med Sci. September 2010; 35(3): 216-25.
16. Samiei M, Aghazadeh M, Movassaghpour AA, Fallah A, Aminabadi NA, Mahdi S et al. Isolation and characterization of dental pulp stem cells from primary and permanent teeth . J Am Sci. 2013;9(12): 153-7.
17. Luisi SB, Sant M, Filho A, Pranke P. Isolation, immunophenotypic characterization and pluripotency of dental pulp stem cells. Dent Oral Craniofac Res. 2017;3(5): 1-3.
18. Naz S, Khan FR, Zohra RR, Lakhindi S, Khan MS, Mohammed N. isolation and culture of dental pulp stem cells from permanent and deciduous teeth. Pak J Med Sci. 2019 August;35(4):997-1002.