REVIEW ARTICLE

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Dental Pulp Banks – A Promising Tool For Tooth Tissue Engineering

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ABSTRACT:

Stem cells which are primitive cells have an ability to differentiate and regenerate the deteriorating cells in different parts of heart, muscles, bones and even the nervous system. Stem cells derived from the teeth are derived using minimally invasive method with little or no trauma. They have a potential to differentiate into a variety of cells which have applications in tissue engineering. They provide the biggest benefit of being autologous i.e. they are obtained from patient's own vital pulp of 30 deciduous and 32 permanent teeth. Thus, banking the pulpal stem cells instead of discarding them provides an excellent opportunity for treatment of future diseases using regenerative medicine.

Keywords: Dental Pulp Bank, Dental Stem Cells, DPSC, SHED.

INTRODUCTION

Stem cells are undifferentiated immaturecells that have the potential to divide and multiply for an extended period, differentiating into specific types of cells and tissues. For a cell to be called as a 'stem cell' it must self-replicate and differentiate into at least two different cell types. Various discoveries in stem cell research presents an opportunity for scientific evidence that stem cells, whether derived from adult tissues or the earliest cellular forms, hold great promise that goes far beyond regenerative medicine.[1] They sort of serve as a self-healing or internal repair system.

The following three important characteristics distinguish stem cells from other cell types:

1. They are unspecialized
2. They have the capability to divide and renew themselves for long periods
3. Under certain physiologic and experimental conditions, they can be induced to grow into tissue or organ specific cells with specialized functions.

Sources Of Stem Cells:

Commonly there are two main sources for deriving stem cells:

1. Embryos formed during the blastocyst phase of embryological development (human embryonic stem cells)[hESC]
2. Adult tissue such as bone marrow, teeth, blood, etc. known as adult stem cells (somatic stem cells) [2]

Embryonic cells are classified as pluripotent since they can differentiate into any body cell. However, hESC are not considered totipotent as they lack the capability of producing all of the extra embryonic tissues that is required for mammalian development. There are various ethical and legal barriers in isolation and use of hESC. Hence, post-natal stem cells are indicated more for tooth-related tissue engineering. Post-natal stem cells can be isolated avoiding immunological reactions from the individual itself who requires treatment. Thus, post-natal stem cells constitute an attractive source of cells for regenerative therapies as they exihibit

remarkable plasticity when they are exposed to foreign microenvironments.[3]

Among the adult tissues, dental pulp which is the soft connective tissue entrapped within the crown, serves as an extremely rich site for stem cell collection. The credit attributed to its peculiar formation and the pulp chamber acts as a sort of a ‘‘sealed niche’’ which allows aggregation of large number of stem cells.

During the sixth week of embryogenesis, ectoderm covering the stomodeum begins to proliferate under the influence of neural crest cells, and gives rise to the dental laminae. Reciprocal interactions between ectoderm and mesoderm layers lead to placode formation. One of these thick, ovoid ectodermal structures develops into tooth germs, where cells, belonging to the neural crest, differentiate into the tooth germ, containing both the dental papilla and dental follicle. Therefore, dental pulp is made of ecto-mesenchymal components, containing neural crest-derived cells, which display plasticity and multipotential capabilities.[4]

These adult stem cells are called as Dental Pulp Stem Cells (DPSCs), when found in permanent teeth, and Stem Cells from Human Exfoliated Deciduous Teeth (SHEDs), when found in the deciduous teeth.[4]

1. Dental Pulp Stem Cells (DPSC)

Dental pulp stem cells (DPSCs) which are isolated from the dentalpulp of third molars, pulpectomised teeth and apical papilla, can regenerate new stem cells or differentiate into different kinds of cells and tissuesunder specific environmental signals. The multipotential capacities of DPSCs are comparable to that of bone marrow stem cells (BMSC). Infact it has been demonstrated that the availability, cell number and proliferation of DPSCs are greater than BMSCs. In the year 2000, the National Institute of Health mentioned the discovery of adult stem cells in the impacted third molars.[5,6,7]

2. Stem Cells From Human Exfoliated Deciduous Teeth (SHED)

Human exfoliated deciduous teeth are a relatively easily accessible source of adult stem cells as SHEDs can be isolated fromthe coronal pulp of the exfoliated deciduous teeth. In addition to their role in the eruption of permanent teeth, it is assumed that they also influence the osteogenesis associated with the same. *In vitro*, depending on different conditions,they can differentiate into cells of odontogenic, osteogenic, adipogenic, chondrogenic, or neurogenic lineage. *In vivo*, these multipotent stem cells have the potential to differentiate into odontoblasts, adipocytes, neurons, and osteoinductive and endotheloid cells.[4,7] In 2003, Miura et al., isolated cells from the deciduous dental pulp, which were highly proliferative and clonogenic.[1,8] Abbas et al., 2008, investigated that SHED Teeth were of neural crest origin.

Tooth Eligibility Criteria For Banking :

Different teeth have different regenerative potential. The primary incisors and canines with no pathology, and, at least, one third of the root left, contain these cells in sufficient number. Primary teeth distal to the canine which are retained in the oral cavity for a longer time are generally not recommended for sampling. However, early removal of deciduous molars for orthodontic treatment (e.g. early intervention for space maintenance, etc.) will present an opportunity to recover these teeth for stem cell banking.[9,10]

Stem Cell Banking - Tooth Collection, Stem Cell Isolation And Storage[10]

The tooth exfoliated should have pulp red in color, which is indicative of cell viability. Teeth that become extremely mobile either due to trauma or periodontal disease often have a severed blood supply, and,therefore,are not suitable for stem cell recovery.

<u>At the clinic :</u>

Collection of the tooth

↓

Transfer in hypotonic phosphate buffered saline solution vial (provides nutrients and prevents the tissue from drying during transport)

↓

Vial placed in thermette(a temperature phase change carrier)

↓

Entire assembly placed in insulated metal transport vessel

The time elapse between harvesting of the tooth and arrival at the processing storage facility should not exceed 40 hours.[10]

At the laboratory :

Tooth surface washed with dubecco's phosphate buffered saline (PBSA)

↓

Disinfected withprovidone iodine
Again washed with PBSA

↓

Pulp tissue isolated with sterile forceps or flushed out with salt water

↓

Tissue placed in sterile petri dish

↓

Tissue digested with collagenase Type I and Dispase for 1 hour at 37°C

↓

Isolated cells are passed through a 70 um filter to obtain single cell suspensions

↓

The cells are cultured in a Mesenchymal Stem Cell Medium (MSC) medium which consists of alpha modified minimal essential medium with 2mM glutamine and supplemented with 15% fetal bovine serum (FBS),0.1Mm L-ascorbic acid phosphate, 100U/ml penicillin and 100ug/ml streptomycin at 37°C and 5% CO2 in air

↓

Isolated colonies are visible after 24 hrs

STEM CELL STORAGE

Stem cells can be stored either by cryopreservation or magnetic freezing.

Cryopreservation is the process of preserving cells or whole tissues by cooling them to sub-zero temperatures in liquid nitrogen because at these freezing temperatures of less than -150°C, biological activity is stopped, as are any cellular processes that lead to cell death.[10,11] Papaccio G *et al* (2006) studied the differentiation and morpho-functional properties of cells derived from stem cells after long-term cryopreservation concluded that dental pulp stem cells and their osteoblast-derived cells can be long-term cryopreserved and may prove beneficial for clinical applications.[10,12] **Magnetic freezing** is the Cell Alive System (CAS)which is based on the phenomena that the freezing point of water or cell tissue can be lowered upto 6 – 7 degree celsius by application of a weak magnetic field. The object snap freezes when the magnetic field is turned off. The Hiroshima University company is the first expression of this new technology. Using CAS, it claims that it can increase the cell survival rate in teeth to as high as 83% when compared to 63% for liquid nitrogen (-196 degrees C), 45% for ultra-cold freezing (-80 degrees C), and just 21.5% for a household freezer (-20 degrees C).[10,13]

Applications

1. Tooth reconstruction:

Tooth development takes place by a series of epithelial – mesenchymal interactions which involves the epithelium providing initial instructional signals which are critical for tooth reconstruction. DPSCs are mesenchymal cells which are committed to differentiate in vivo into dental pulp cells and odontoblast-like cells inorder to form a dentin-pulp complex, cannot form a tooth like structure unless they receive some signals from the epithelium.[14]Yan et. al. proposed a futuristic strategy to form a biotooth by co-culturing induced pluripotent stem cells (iPS) from autologous DPSCs with original DPSC. The iPSC could generate dental epithelial cells with embryonic-like properties.[15]Lee et. al. illustrated a strategy for regeneration of multiphase periodontal tissues in which DPSC yielded aligned PDL-like collagen fibres that inserted into bone sialoprotein positive bone like tissue and putative dentin/cementum by spatially released bioactive cues.[16]

2. Dentin/ odontoblasts :
Dental pulp cells, especially DPSC when cultured on dentin disks differentiated into odontoblast like cells within a cytoplasmic process extending into the dentinal tubules. Similar results were seen when SHEDs were cultured in horizontally sliced tooth roots.[14] Batouli et al demonstrated the tissue regeneration capacity of bone marrow mesenchymal structures (BMMSC) and DOSC by transplanting them using dentin as a carrier. It was seen that DPSC generated a reparative dentin like structure directly on the surface of the human dentin while BMMSC failed to form any mineralized tissue.[17] Porcine deciduous pulp stem cells mixed with a beta tricalcium phosphate scaffold as direct pulp capping regenerated dentin like structures nearly completely restoring the pulp chamber roof defects demonstrating the usefulness of DPSC for in situ dentin regeneration.[18]

3. Dental pulp :
Autogenous transplantation of DPSC $CD105^{+}$SP (side population) cells with stromal cell-derived factor- 1 (SDF-1) in a pulpectomized adult canine model caused complete pulp regeneration with neurogenesis, vasculogenesis and complete apical closure in 14 days.[19] Iohara et al developed a new method to obtain DPSC from the dental pulp based on Good Manufacturing Practice (GMP guidelines). Transplantation of these with granulocyte colony stimulating factor (G-CSF) enhanced pulp regeneration and created favourable environment for migration of cells, inhibition of apoptosis, suppression of inflammation and induction of angiogenesis and neurogenesis.[20] Huang G T el.al. showed the evidence of regenerating pulp like tissue de novo in the emptied root canal space by stem cells from apical papilla and DPSC onto a synthetic scaffold of poly – D, L- lactide/ glycolide, into human tooth fragments and transplanted into an immunocompromised mouse.[21]

4. Bone / cartilage[14, 22]
The following table shows the various studies done to show the osteogenic potential of the pulpal stem cells.

Year	Authors	Cell source	Host	Scaffolds	Outcome
2008	Graziano et.al.	DPSC	Rats	PLGA	Bone nodule formation
	De Mendonca et.al.	SHED	Rat	Collagen membrane	Dense and mature bone formation
	Abe s. et.al.	SCAP	Rat	Hydroxyapeptite(HA) – TricalciumPhoaphate (TCP)	Formation of bone like tissues contatining osteocytes
	Seo et.al.	SHED	Mouse	HA/TCP	Repair of defects and substantial bone formation
	Zhang et.al.	Rat DPSC hDPSC	Mouse	HA/TCP	No bone formation
2009	D' Aquino et.al.	DPSC	Human extracted socket of	Collagen sponge	Formation of bone with restoration of PDL tissue

			third molar		
	Yang et.al.	STRO-1 + rat DPSC	Nude mice	HA/TCP	High rate of hard tissue formation
	Zheng et.al.	Procine deciduous teeth stem cells	Minipig	Beta- TCP	More efficient regeneration of critical size mandibular bone defects
2010	Yamada et.al	Canine DPSC & SHED	Canine mandible	Platelet rich plasma (PRP)	Induction of mature bone formation, neovascularization and enhanced bone-implant contact
	Yu et.al.	Rat DPSC	Rat	Absorbable gelatin sponges	Early passage cells: Differentiation into dentine (9/28), bone (28/28) and cartilage structures (2/28) Late passage cells: bone (28/28)
2011	Chan et.al.	DPSC	Nude mice	Non-calcium based biomaterial self Assembling peptide nanofibre hydrogel	Mineralized tissue formation
	Ikeda et.al	DPSC	Nude mice	HA granule	Mineralized tissue formation
	Liu et.al.	Rabbit DPSC	Rabbit (alveolar defects)	nanoHA/ PLA + rhBMP2	Large volume of bone formation in BMP2 plus group
	Ito et.al.	Canine DPSC	Dog	PRP	High osteogenic potential of DPSCs contributed to dental implant integration
2012	Yang et.al.	DPSC	Nude mice	Chitosan/ collagen scaffold	Mineralized tissue formation

				containing BMP7	
	Riccio et.al.	DPSC	Rat	Fibroin scaffolds	Mature bone formation and defect correction

5. Hepatic cells:

Nikolay Ishkitiev et al showed that hydrogen sulfide increases hepatic differentiation in tooth-pulp stem cells.[23]

6. Muscles:

Many studies have evaluated the myogenic potential of DPSCs. Kerkis et al systemically transplanted hDPSCs by arterial or muscular injections in golden retriever dogs suffering from muscular dystrophy showed significant engraftment with improved clinical symptoms. Gandia et al used hDPSCs to treat myocardial infarction in a rat model and observed repair of the infarcted myocardium, reduction in size of the infarct and increased blood vessels.[14,15]

Other applications of dental pulp stem cells are based on the potential of the cells to differentiate into hair follicular cells, corneal cells (corneal reconstruction), neuronal cells (Alzheimer's, Parkinson's disease, cerebral palsy), endothelial cells, melanocytes, islet-like aggregates (diabetes type 1) and sperm producing cells (infertility).[14,15,22,24]

Thus, these stem cells derived from the pulp have high plasticity as they can be differentiated into many cell types.

Advantages of Banking Pulp Cells[25]

• It provides an autologous transplant which has many advantages including; no immune reaction and tissue rejection of the cells, no immunosuppressive therapy needed, and significantly reduced risk of communicable diseases.

• It is a simple and a painless procedure which is less than 1/3rd the cost of cord blood storage.

• As these are adult stem cells there are no ethical concerns as embryonic stem cells.

• They may also be useful for close relatives of the donor such as grandparents, parents, uncles, and siblings.

Dental Pulp Banks:

Tooth banking is based on the firm belief that personalized medicine is the most promising avenue for treating challenging diseases and injuries that would occur throughout life.[9]

CONCLUSION

Stem cells obtained from the dental pulp, albeit into experimental phase, provides a window into the fascinating world of regenerative and personalized medicine. Thus, banking stem cells at the right time and at the right age can help us with a promising future.

REFERENCES

1. Rai S, Kaur M, Kaur S. Applications of Stem Cells in InterdisciplinaryDentistry and Beyond: An Overview. Annals of Medical and Health Sciences Research 2013(3)2.

2. Durgasreenivas S, Rao AS, Satyavani SS, Reddy BH, Vasudevan S. Where Will The Stem Cells Lead Us? Prospects For Dentistry In The 21st Century. Journal of Indian Society of Periodontology 201;15(3).

3. Demarco FF, Conde MCM, Cavalcanti BN, Casagrande L, Sakai VT, Nör JE. Dental Pulp Tissue Engineering. Braz Dent J 2011;22(1): 3-14.

4. D'aquino R, De Rosa A, Laino G, Caruso F, Guida L, Rullo R, Checchi V, Laino L, Tirino V, Papaccio G. Human Dental Pulp Stem Cells: From Biology to Clinical Applications. Journal of Experimental Zoology 310b (2008).

5. Elluru V, Ramesh DNSV. Stem Cell Therapy: Dental Aspects. Journal of Indian Academy of Oral Medicine and Radiology 2012;24(1):45-50.

6. Ulmer FL, Winkel A, Kohorst P, Strech M. Stem cells– prospects in dentistry. Schweiz Monatsschr Zahnmed 2010;120:860-72.

7. Demarco FF, Casagrande L, Zhang Z, et al. Effects of morphogen and scaffold porogen on

the differentiation of dental pulp stem cells. J Endod 2010;36:1805-11.
8. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG. SHED: Stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci USA 2003;100:5807-12.
9. Jay B. Reznick. Continuing Education: Stem Cells: Emerging Medical And Dental Therapies For The Dental Professional. Dentaltown Magazine 2008;42-53.
10. D'Souza CM, Hegde MN, Hegde P. Tooth Stem Cell Banking-A Review. International Journal of Research and Reviews in Pharmacy and Applied science 2(2):423-8.
11. Oh YH, Che ZM, Hong JC, Lee EJ, Lee SJ, Kim J. Cryopreservation Of Human Teeth For Future Organization of A Tooth Bank—A Preliminary Study. Cryobiology 51(3):322-9, Epub 2005.
12. Papaccio G, Graziano A, d'Aquino R, Graziano MF, Pirozzi G, Men-Ditti D, De Rosa A, Carinci F, Laino G. Long-Term Cryopreservation of Dental Pulp Stem Cells (SBP-Dpscs) and their differentiated Osteoblasts: A Cell Source For Tissue Repair. J Cell Physiol 2006;208(2):319-25.
13. TT-450—Stem Cells and Teeth Banks, ebiz news from Japan http://www.japaninc.com/tt450.
14. Kawashima N. Characterisation of dental pulp stem cells: a new horizon for tissue regeneration? Arch Oral Biol 2012;57:1439-58.
15. Tatullo M, Marrelli M, Shakesheff K, White L. Dental Pulp Stem Cells: Function, Isolation and Applications in Regenerative Medicine. J Tissue Eng Regen Med 2014;21. Doi:10.1002/Term 1899.
16. Lee CH, Hajibendeh J, Suzuki T et al. 3D printed multiphase scaffolds for regeneration of periodontium complex. Tissue Eng Part A 2014;20(8):1342–51.
17. Batouli S, Miura M, Brahim J et al, Comparison of stem cell mediated osteogenesis and dentinogenesis. J Dent Res 2003;82:976–81.
18. Zheng Y, Wang XY, Wang YM et al. Dentin regeneration using deciduous pulp stem/progenitor cells. J Dent Res 2012;91: 676–82.
19. Iohara K, Imabayashi K, Ishizaka R et al. Complete pulp regeneration after pulpectomy by transplantation of CD105+ stem cells with stromal cell-derived factor-1. Tissue Eng A 2011;17:1911–20.
20. Iohara K, Murakami M, Takeuchi N et al. A novel combinatorial therapy with pulp stem cells and granulocyte colony-stimulating factor for total pulp regeneration. Stem Cells Transl Med 2013;2:521–33.
21. Huang GTJ, Yamaza T, Shea LD et al. Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue Eng A 2009;16:605–15.
22. Daltoé FP, Mendonça PP, Mantesso A, Deboni MCZ. Can SHED or DPSCs be used to repair/regenerate non-dental tissues? A systematic review of in vivo studies. Braz Oral Res (São Paulo) 2014;28(1):1-7.
23. Ishkitiev N, Calenic B, Aoyama I, Hisataka Ii, Yaegaki K, Imai T. Hydrogen Sulfide Increases Hepatic Differentiation In Tooth-Pulp Stem Cells. J Breath Res (2012) 017103 (7pp).
24. Verma K, Bains R, Bains VK, Rawtiya M, Loomba K, Srivastava SC. Therapeutic potential of dental pulp stem cells in regenerative medicine: An overview. Dent Res J (Isfahan) 2014;11(3):302–8.
25. Arora V, Arora P, Munshi AK. Banking Stem Cells From Human Exfoliated Deciduous Teeth (SHED): Saving For The Future. The Journal Of Clinical Pediatric Dentistry2009;33(4).
26. Rai S, Kaur M, Kaur S, Arora SP. Redefining the potential applications of dental stem cells: An asset for future, Indian Journal of Human Genetics 2012;18(3).

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