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Review Article

Stem cells in regenerative dentistry- A comprehensive review

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Abstract

Human teeth are most non-invasive and natural sources of stem cells. The undifferentiated body tissue precursors that are capable of differentiating into specialized cells are called as stem cells; these cells have the capacity to divide through mitosis to produce more of stem cells. These stem cells are said to be highly potential in regenerating tissues that are lost. The Tissue regeneration potentiality of stem cells in the field of medicine hopes to repair heart damage, baldness, deafness, blindness, vision impairment, muscle damage, diabetes and other related disorders etc. whereas in dentistry, the dental stem cells [stem cells obtained from intraoral region] have potential to repair bone loss or any soft tissue loss and it can be effectively used in maxillofacial reconstruction since it provides better physiological structure and functions. Nowadays these dental stem cells are studied to be used for whole tooth regeneration and even for treatment of mucosal lesions.

Keywords: Dental stem cell, Regenerative dentistry, Tissue engineering, Tooth banks.

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1. Introduction

Regenerative medicine, the emerging branch of medical science, deals with functional restoration of specific tissue and/or organ of the patients suffering with severe injuries or chronic disease conditions, in the state where bodies own regenerative responses do not suffice [1]. This idea of regeneration in medical field is not new, but it has significantly advanced after the of innovation of stem cells and recently after the discovery of dental stem cells, this idea of regenerative medicine has found its way into dentistry.

Body tissues namely hematopoietic system, gastrointestinal system, layers of skin etc. has high proliferative capacity and turnover rate and these tissues were found to have more of regenerative potential [2]. This concept enlightens the fact that tissues which have regenerating capability may contain cells that are responsible for the replacement of original tissue frame. These types of cells are the said as presumptive "stem cells."[2]

The undifferentiated body tissue precursors that are capable of differentiating into specialized cells are called as stem cells, these cells have the capacity to divide through mitosis to produce more of stem cells [3] . They are also defined as immature or undifferentiated cells and they are said to be efficient of generating daughter cells identical to themselves or of differentiating into diverse cellular phenotypes [3]. Stem cells are used as three elements [cells, scaffolds, and signaling molecules] which are the most critical components for regeneration and play a pivotal role in tissue engineering and regenerative medicine [4].

As far as dental tissues concerned, they have a limited potential to regenerate when comparing whole body, but after the discovery of dental stem cells obtained from oral tissues, it paved new ideologies in regenerative dentistry.

2. History

The regenerative capability in a living creature was observed as early as 330 BC, when Aristotle noticed that a lizard could grow back the lost tip of its tail. Since then, there have been many attempts to understand regenerative capacity of human being and it is only in the last few decades that we have seen informative researches in the field of stem cell[5].

The term "stem cell' appeared first time in the works of German biologist Haeckel. in the year 1868.[6] Alexander Maksimov [Russian histologist], postulated the existence of hematopoietic stem cells in in Berlin, 1908 during the congress of hematologic society meeting [6].

Feldman showed the regenerative potential of dental pulp under optimal biological conditions in the year 1932[7]. The first paper published regarding the accidentally discovery of self-renewing' cells was done by Till and McCulloch in the year 1961, as they were studying the radiation sensitivity of mouse bone marrow, they found out some cells which were capable of differentiating from the same original cell. Since then, the field of stem cell research has attained popularity [8].

Major breakthrough in history of dental science was achieved in the year 2000 when Gronthos *et al* identified and isolated odontogenic precursor cells in adult dental pulp. These were concluded as dental pulp stem cells [9].

3. Classification of stem cells

Based on their origin [10]

3.1 Embryonic stem cells

These cells are derived from four to five days old embryo or early morula-stage embryo. During this stage, the embryos are spherical and are called as blastocysts. Each blastocyst is made of 50 to 150 cells and includes three structures: an outer layer of cells, a fluid filled cavity and a group of approximately 30 pluripotent cells at one end of the cavity which is known as inner cell mass [2].

3.2 Embryonic germ cells or foetal germ cells

These germ cells are basically pluripotent stem cells derived from the primordial germ cells, which are responsible for formation of gametes [sperm and eggs] in adults.

These cells can be isolated from the precursor to the gonads in aborted tissues in adult Female [10].

3.3 Adult stem cells [11]

Adult stem cells can be categorized as hematopoietic or mesenchymal, Hematopoietic stem cells have potency to form all types of white blood cells and red blood cells. Sources of Haematopoeitic stem cells are Umbilical cord, Umbilical cord blood, peripheral blood and bone marrow. Hematopoietic stem cells are widely used to treat blood cancers such as leukaemia and other blood disorders.

Mesenchymal stem cells are capable of forming a connective tissue such as bone, muscle, cartilage, fat, tendons but not internal organs or skin. [12] The Sources of Non-haematopoietic Stem Cells/Mesenchymal Stem Cells are bone marrow, adipose tissue, and almost all body tissues including the dental pulp tissue[11].

Bone marrow: - Bone marrow stem cells [BMSCs] are obtained from sternum or iliac crest. It has both hematopoietic stem cells and mesenchymal stem cells [MSCs]. The advantage of bone marrow is that it has a large quantity of stem cells and can be differentiated into wide group of cells [13].

Adipose tissue: - These cells can be obtained from the lipectomy or liposuction aspirate. Adipose derived stem cells [ADSCs] constitute a group of pluripotent mesenchymal stem cells. Advantage of obtaining adipose tissue stem cell is that it is easily accessible and plenteous in many individuals. [14]

3.4 Induced pluripotent stem cells

Induced pluripotent stem cells [iPSCs] are adult cells that had genetically remodelled to an embryonic stem cell-like state by being compelled to express genes and factors important for maintaining the defining characteristics of embryonic stem cells. They also express stem cell markers and has potency to generate cells containing all three germ layers. iPSCs are useful tools for drug development and modelling of diseases and also in transplantation medicine [2].

3.5 Dental stem cells

Dental stem cells may be either

- Dental mesenchymal stem cells that are derived from pulp. deciduous teeth, periodontal ligament and dental follicle or
- Dental epithelial stem cells derived from the incisors and molars[15].

3.5.1 Dental pulp derived stem cells [DPSC]

This is the most common source of dental tissue derived stem cells that are obtained from the pulp tissue of permanent teeth. They are multipotent and express markers such as STRO-I, CD 44 and CD 146 MSC. '7 DPSC were first isolated from teeth in the year 2000 and they have capacity to differentiate into osteogenic, odontogenic, myogenic, adipogenic and neurogenic components both *in-vitro* and *in-vivo* and it can produce pulp dentin complex *in-vivo* [16].

3.5.2 Stem cells derived from human exfoliated deciduous teeth [SHED]

They are derived from exfoliated teeth and have more proliferative capacity than DPSC. They can differentiate into neurogenic, adipogenic and odontogenic components and are useful for tissue regeneration involving orofacial bony structures [15]. They contain MSC markers like STRO-1 and CD 146 and neuronal and glial markers like Nestin and ß III Tubulin. These markers have the capacity to produce bone and dentin *in-vivo* [17].

3.5.3 Periodontal ligament stem cells [PDLSC]

They are derived from separated periodontal ligaments of third molars and contain progenitors for self-renewal structures like cementum and bone[18]. They were first isolated by Seo *et al* where he founded that they were multipotent[19]. They also contain MSC markers such as STRO-1, Muc 18, CD 44 and CD 146 and can differentiate

into adipogenic, chondrogenic and osteogenic components *in-vitro* and cementum and periodontal ligament *in-vivo*.[17,18]

3.5.4 Dental follicle stem cells [DESC]

They are derived from the follicle surrounding third molars and are pluripotent in nature. They are composed of ectomesenchyme component and contain markers such as Notch 1, STRO-I and Nestin. They have potential to differentiate into osteoblasts, adipocytes and neuroblasts *in-vitro* and periodontal ligament *in-vivo* under optimum conditions. [15,18]

3.5.5 Stem Cells from Apical Papilla [SCAP]

They are derived from the apical part of a developing tooth and have high proliferation, migration and regeneration capacity. They are composed of fibroblast-like and odontoblast like cells with MSC markers such as STRO-I. CD 24, CD 146 and CD 44. They differentiate into pulp-dentin complex *in vivo* under optimum conditions [17,18].

3.5.6 Oral mucosa derived stem cells

These cells are either being oral epithelial stem cells or gingival stem cells. The epithelial stem cells are unipotent in nature and develop only into epithelial cells *in-vivo*. However, when used *in-vitro*, they develop a well stratified oral mucosal graft and are used for clinical applications such as grafting procedures involving the oral structures. Whereas the Gingival stem cells are multipotent in nature and have reprogramming capabilities, the advantages of these cells are they are more abundant and are easy to isolate and have a rapid proliferation in *in-vitro* that makes their use clinically viable. [20].

3.5.7 Human dental epithelial stem cells [hDESC]

These stem cells are derived either from third molars or from epithelial sheaths that disintegrate into cell rests of Malassez. These cells express epithelial stem cell markers like p75, E-CAM and BMI-l and also embryonic stem cell markers like Nanog and Oct4 [17].

3.5.8 Periosteum derived stem cells

Human periosteum derived stem cells are multipotent in nature and has properties like odontogenic, chondrogenic, adipogenic and myogenic potential both *in-vitro* and *in-vivo*. They produce cortical bone and can be used for regenerative management of large defects in the orofacial region. [20]

3.5.9 Salivary gland derived stem cells

The stem cells obtained from salivary gland has the potential to form duct cells and acinar cells *in-vitro* and these cells has capacity to produce both mucin and amylase. They can therefore be useful in rehabilitation of patients who has reduced salivary gland function following irradiation procedures [20].

4. Stem Cell Properties

A classic stem cell should possess two properties namely self-renewal and potency. [13,21,22]

Self-renewal - the capacity of the cell to undergo numerous cycles of cell division maintaining the undifferentiated state. An ideal stem cell should have the capacity of self-renewal [the ability of the cell to proliferate to about 40-60 population doublings].

Potency - Means the differentiation capacity of the stem cells.

5. Characteristics of stem cell [5]

Totipotency: Potency to generate all types of cells.

Pluripotency: Potency to generate all types of cells excluding cells of the embryonic membrane.

Multipotency: Potency to differentiate into more than one mature cell.

***Self-renewal*:** These cells have potency to divide without differentiation.

Plasticity: MSCs has plasticity and can undergo differentiation. The trigger for this feature is stress / tissue injury which causes stimulation of stem cells and releases mediators such as chemo attractants and growth factors.

6. Dental stem cells suitable for regenerative dentistry [23]

Stem cells suitable for regenerative medicine/ dentistry must be subject to the complete control of cell fate in the body to ensure the safety of the patient. In this regard, only adult MCs currently have realistic clinical potential.

Suitable stem cells for dental tissue engineering should be able to differentiate into target tissue or organ and should be possible to collect easily and prepared, and possible immunomodulatory properties which can be used to provide a further benefit.

6.1 Differentiation capacity

Bone Marrow derived Stem Cells, particularly those obtained from the orofacial bone marrow, and periosteum-derived stem cells may be suitable for alveolar bone regeneration because of the compatibility of the cell source and the target tissue [23].

Similarly, MSCs derived from dental tissue may be appropriate for regeneration of dental Mesenchyme derived tissue such as dentin, pulp and periodontal tissues. But, the differentiation potentiality of the adult MSCs is restricted only to mesenchymal lineages, which hinders their application for the regeneration of complex oral organs, such as teeth and salivary glands, which are formed as a result of interaction of epithelial and mesenchymal tissues.[23]

6.2 Accessibility

On the subject of accessibility, bone marrow aspiration from the iliac crest or liposuction from extra-oral tissue is not an easy deed for dentists as the limitations of the dental license and the dental specialization barriers. On the contrary, orofacial bone marrow, periosteum. Salivary glands are easy source of stem cell for dentists; however,

the isolation of these dental stem cells may still not be convenient as it requires surgical procedures and tooth or pulps require the extraction of the wisdom teeth. On top of that, these adult stem cells are present in minimal quantities and can be difficult to isolate, cleanse and expand harmoniously. [23]

On the other hand, the gingival tissue that is easily obtainable by dentists and whose cells can be easily extracted from patients with minimal discomfort, seems to be a promising source of adult stem cells in dentistry [24,35].

6.3 Immunomodulation

In addition to tissue repair and regeneration capacity of stem cells, immunomodulatory properties have also been identified for MSCs in humans that may be associated with therapeutic effects such as angiogenesis, anti-inflammation and antiapoptosis [26].

7. Tooth banking

7.1 Collection and transport of extracted teeth

7.1.1 Tooth Collection

The tooth exfoliated should have pulp red in colour, which is indicative of cell viability. Teeth that become very mobile either through trauma or disease [e.g., Class III or IV mobility] often have a severed blood supply, and are not candidates for stem cell recovery [27].

Extracted human teeth [deciduous teeth or permanent third molars] are placed into sterile chilled vials containing 20 mL of one of three collection/transport solutions: Hypo Thermosol, MesenCult basal medium or phosphate-buffered saline. [28]

Teeth were transported on ice to the laboratory, where they were processed. The time from harvesting to arrival at the processing storage facility should not exceed 40 hours [29].

7.1.2 Processing and digestion of dental pulp, and initial culture of DPSC [28]

According to Perry *et al* Teeth were externally sterilized briefly and teeth are subjected into several washes in sterile Phosphate buffered saline [PBS] followed by immersion in 1% povidone iodine [PVP-I] for 2 min. immersion in 0.1% sodium thiosulfate in PBS for 1 min and another wash in sterile PBS. The roots of cleaned teeth were separated from the crown to reveal the dental pulp, and the pulp was placed into an enzymatic bath consisting of type I and type Il collagenase with thermolysis as the neutral protease. Pulps were allowed to incubate at 378 degrees Celsius for 40 min to digest the tissue and liberate the cells. Once digestion was complete, Mesencult complete medium [e.g., basal medium containing MSC stimulatory supplements] was added to a final volume to neutralize the digestion enzymes.

This mixture was centrifuged at 500 g for 5 min, and the supernatant aspirated. The cell pellet was resuspended in fresh Mesencult complete medium plus 0.25 mg/mL amphotericin B, 100 IU/mL penicillin-G, and 100 mg/mL streptomycin. Cells were plated at an initial concentration of one tooth digest per 25 cm^2 flask. Culture flasks were monitored daily, and any contaminated flasks removed immediately and recorded. Non-contaminated flasks were monitored for cell growth, with medium changes taking place three times per week.

After 14 days of growth, DPSC were detached using 0.25% trypsin/ 1 mm EDTA, cell counts and viability were assessed using a standard trypan blue dye exclusion assay [Sigma] and hemacytometer, and the DPSC divided equally between two 75 cm^2 flasks. After this, DPSC cultures were split when they reached 70% confluence.

7.2 Storage of stem cells

Cryopreservation and Magnetic Freezing. [30]

7.2.1 Cryopreservation of cultured DPSC and whole teeth

Cryopreservation of cultured DPSC: It is performed using standard cell freezing methods[30]. 10% dimethyl concentration will be added drop-wise to DPSC suspensions containing 0.5-1.5x 106 cells. DPSC suspensions were then cooled at 18C/min to 858C followed by plunging into liquid nitrogen. All cells were frozen in 2 mL cryovials [28].

Cryopreservation of whole teeth: These same general freezing procedures were followed for the freezing of whole teeth, with two exceptions. Whole teeth were frozen in 15 mL cryogenic vials and allowed to sit in cryoprotectant solution for 1 hour at 48 prior to freezing to aid in penetration of the cryoprotectant into the tissue. [28]

7.2.3 Magnetic freezing

It is the Cell Alive System [CAS]. Under the condition of CAS magnetic field energy, water clusters do not accumulate but remain in smaller groups, thus minimizing restraining the expansion of the water. This technology is called CAS and uses the phenomena that applying even a weak magnetic field to water or cell tissue will lower the freezing point of that body by up to 6-7 degrees Celsius. Once the object is uniformly chilled, the magnetic field is turned off and the object snaps freezes. The Hiroshima University company is the first expression of this new technology. Using CAS, Hiroshima University claims that it can increase the cell survival rate in teeth to as high as 83%. This 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]. Maintaining a CAS system is a lot cheaper than cryogenics and more reliable as well [27].

7.2.4 Thawing of cultured DPSC and whole teeth

According to Perry *et al* all cells and whole teeth were frozen for at least 1 month before thawing. Vials of DPSC were retrieved from liquid nitrogen and plunged into a 37^0C water bath. Upon thawing, a slow addition of Mesencult complete medium was performed, followed by an equilibration step at 37^0 Celsius for 5 min, and

centrifugation. The supernatant was discarded. the pellet resuspended in MesenCult complete medium, and a count and viability stain with trypan blue was conducted. Cells were plated in 25 cm^2 flasks and observed for growth. Whole teeth were also thawed in a 378 Celsius water bath followed by a wash in sterile saline. Dental pulp was obtained and digested to make single-cell suspensions and cultured in the same manner as described above. [28]

7.3 Tooth eligibility criteria for banking

Not all teeth hold the same regenerative potential. The teeth especially primary incisors and canines with no pathology and at least one third of root left contain these unique types of cells in sufficient number. Primary teeth distal to the canine are generally not recommended for sampling. Primary molars have a broader root base, and therefore, are retained in the mouth for a longer period of time than anterior teeth. In some instances, early removal of deciduous molars for orthodontic considerations [e.g., early intervention for space maintenance] will present an opportunity to recover these teeth for stem cell banking[31].

7.4 Tooth banks

These cells can be best utilized by the donor in future and to a certain extent their immediate family and blood relatives. The key to successful stem cell therapy lies in being able to harvest the cells at the right point of development and to safely store them until accident or disease requires their usage. They can be potentially stored for decades [31].

Till date, tooth banking is not very popular but the trend is catching up mainly in the developed countries. In India, as of now, dental stem cell banking in India is offered by a select few companies, like Stemade and Store Your Cells. The procedure and then preservation of the stem cells can cost around Rs. 100,000 for a period of 21 years.

8. Clinical applications in medicine

DPSCs were proven to differentiate into functionally active neurons, and when implanted DPSCs induce endogenous axon guidance, proving their potential as cellular therapy for neuronal disorder. based on these findings, stem cell research has spurred hope in the field of medicine directed towards treating brain damage, spinal cord injury, Parkinson's disease, Alzheimer's disease, amyotrophic lateral sclerosis, multiple sclerosis and other neurodegenerative disorders[34]. The Tissue regeneration potentiality of stem cells hopes to repair heart damage, baldness, deafness, blindness, vision impairment, muscle damage, diabetes and other related disorders etc. Though not an established treatment yet, autologous hematopoietic stem cell therapy induces remissions and even cure some selected patients with therapy refractory autoimmune diseases [2].

9. Clinical applications in dentistry

Stem cell therapy has become a promising alternative in dentistry and maxillofacial rehabilitation since it could provide better physiological structure and functions [35]. Jeremy Mao, director of the Tissue Engineering and Regenerative Medicine Lab used tooth shaped scaffolds and added growth factors and proteins to attract stem cells from the body and grow the appropriate bone in place in just 9 weeks"[32].

In orofacial region these stem cell therapies are being used for tooth and periodontal regeneration, temporomandibular joint reconstruction, alveolar bone regeneration [36]. Cell-based therapy by intravenous injection and cell encapsulation system also has implications in dentistry, and stem cell research is directed towards achieving the following: Experiments *in-vitro* and a few on animal models have regeneration of damaged coronal dentine and pulp, regeneration of resorbed root, cervical or apical dentin and perforations ,periodontal regeneration, craniofacial defects by osteogenesis, whole tooth regeneration and treatment of oral mucosal lesions [oral submucous fibrosis, oral lichen plans, dyskeratosis congenita, premalignant lesions such as leukoplakia, recurrent oral ulcers, graft versus host disease and oral cancers [3].

10. Conclusion

The oral and maxillofacial regions have been described as a promising source of adult stem cells. Dental clinicians should recognize the evolution of the regenerative dentistry field and take into consideration the possibility of acquiring stem cells during dental treatments [from deciduous teeth, third molars, and the gingiva], which can be stored for future autologous therapeutics. Stem cells derived from all sources hold immense medical promises. Stem cell therapies have virtually unlimited medical and dental applications. Stem cell therapy has brought in a lot of optimistic hope amongst researchers, doctors, and not to forget the patients who are the chief supportive and beneficiary of this innovation

References

[1]. Mahla RS. Stem Cells Applications in Regenerative Medicine and Disease Therapeutics. *Int J Cell Biol*. 2016; 2016:6940283. Doi: 10.1155/2016/6940283.

[2]. K. Saraswathi Gopal, E. Madhubala, “Stem cell and the regenerative dentist,” *Int J Contemp Dent Med Rev*, Vol. 2015. Article ID: 060215, 2015

[3]. Saraswathi Gopal. K and Padma. M. Stem cell regenerative therapy in oral and maxillofacial region: a systematic review. *Int. J. Of Adv. Res*. 2017; 1631-16431.

[4]. Yamada, Y., Nakamura-Yamada, S., Konoki, R. *et al*. Promising advances in clinical trials of dental tissue-derived cell-based regenerative medicine. *Stem Cell Res Ther* 2020; 11: 175.

[5]. Gopal.S K. and Arathy Manohar Lankupalli. Stem Cell Therapy: A Newhope for Dentist. *Journal of Clinical and Diagnostic Research* 2012; 6(1):142-144 [2012].

[6]. Bansal R, Jain A. Current overview on dental stem cells applications in regenerative dentistry. *J Nat Sci Biol Med*. 2015; 6 (1): 29–34.

[7]. Satpute TS, Verma JS, Shah JR, *et al*. Pulp revascularisation of a non-vital immature young permanent tooth - a case report and literature review. *J Evolution Med Dent Sci* 2021:10(11): 845-848.

[8]. The potential impact of stem cells on modern medicine by Shiffa Shahid, research paper based on pathology lectures at medlink 2011

[9]. Gronthos S, Mankani M. Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells [DPSES] in vitro and *in vivo*. *Proc Natl Acad Sci USA* 2000: 97: 13625-30

[10]. Sumit Narang and Nidhi Sehgal Stem cells: A potential regenerative future in dentistry: *Indian I Hum Genet*. 2012 May-Aug; 18[2]: 150-154.

[11]. Singh H, Bhaskar DJ, Rehman R, Jain CD, Khan M. Stem cells: An Emerging future in dentistry. *Int J Adv Health Sci.* 2014; 1(2):17-23.

[12]. Ullah I, Subbarao RB, Rho GJ. Human mesenchymal stem cells - current trends and future prospective. *Biosci Rep.* 2015 Apr 28; 35(2): e00191.

[13]. Mouli PC, Kumar SM, Senthil B, Parthiban S, Priya R, Subha R. stem cells in dentistry-A review. *Journal of Pharmaceutical Sciences and Research*. 2012; 4(7): 1872.

[14]. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, *et al*. Multilineage cells from human adipose tissue: Implications for cell-based therapies. *Tissue Eng* 2001; 7: 211-28.

[15]. Huang YH, Yang JC, Wang CW, Lee SY. Dental stem cells and tooth banking for regenerative medicine. *J Exp Clin Med* 2010;2(3):111-117

[16]. Madiyal A, Babu S, Bhat S, Hegde P, Shetty A. Applications of stem cells in dentistry: A review. *Gülhane Tip Dergisi*. 2018; 60(1): 26.

[17]. Mitsiadis TA, Orsini G, Jimenez-Rojo L. Stem cell-based approaches in dentistry. *Eur cell mater* 2015; 30:248-257

[18]. Rai S, Kaur M, Kaur S. Applications of stem cells in interdisciplinary dentistry and beyond: An overview. *Annals of medical and health sciences research* 2013; 3(2):245-524

[19]. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, Young M, Robey PG, Wang CY, Shi S. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004: 364:149-155.

[20]. Majid S, Fry RR, Goyal S, Chawla JPS. Stem cells in dentistry: A boon to oral and maxillofacial surgery. *IOSR-JDMS* 2016; 15[1]:62-68.

[21]. Chotkowski G. Stem Cells: Emerging Medical and Dental Therapies and the Dental Professional. Friends of hu-friedy academy. Oct 2008. Available from: http://www.friends of hu-friedy.com

[22]. Gardner RL. Stem cells: Potency, plasticity and public perception. *J Anat* 2002; 200:277-82

[23]. Egusa H, Sonoyama W, Nishimura M, Atsuta I, Akiyama K. Stem cells in dentistry–part I: stem cell sources. *Journal of prosthodontic research.* 2012; 56(3):151-65.

[24]. Zhang Q, Shi S, Liu Y, Uyanne J, Shi Y, Shi S, *et al*. Mesenchymal stem cells derived from human gingiva are capable of immunomodulatory functions and ameliorate inflammation-related tissue destruction in experimental colitis. *J Immunol* 2009; 183:7787-98.

[25]. Tomar GB, Srivastava R, Gupta N, Barhanpurkar AP, Pote ST, Jhaveri HM, *et al*. Human gingiva-derived mesenchymal stem cells are superior to bone marrow-derived mesenchymal stem cells for cell therapy in regenerative medicine. *Biochem Biophys Res Commun* 2010; 393:377-83.

[26]. Nauta Al, Fibbe WE. Immunomodulatory properties of mesenchymal stromal cells. *Blood* 2007; 110:3499-506.

[27]. Hegde MN, Hegde P, D'Souza CM. Tooth stem cell banking-A review. Conservative and Endodontic Society of Kerala. *International Journal of Research and Reviews in Pharmacy and Applied science* 2012; 45:423-8.

[28]. Perry, B. C., Zhou, D., Wu, X., Yang, F.-C., Byers, M. A., Chu, T.-M. G., Goebel, W. S. Collection, Cryopreservation, and Characterization of Human Dental Pulp-Derived Mesenchymal Stem Cells for Banking and Clinical Use. *Tissue Engineering Part C: Methods*. 2008; 14(2): 149-156.

[29]. Freshney Lan R *et al*. Culture of human stem cells. Chapter 8: 187-207, 2007.

[30]. Woods, E.J., Benson, J.D., Agca, Y., and Critser, J.K. Fundamental cryobiology of reproductive cells and tissues. *Cryobiology* 2004; 48: 146.

[31]. Jay B. Reznick. Continuing Education: Stem Cells: Emerging Medical and Dental Therapies for the Dental Professional. *Dentaltown magazine,* 2008; 42-53.

[32]. Singularity Hub. Body's own stem cells used to grow teeth in mouth by Aaron Saenz 2010.

[33]. Subha R. Stem Cells in Dentistry- A Review. *J. Pharm. Sci. & Res*. 2012; 4(7): 1872-1876

[34]. Ueda T, Inden M, Ito T, Kurita H, Hozumi I. Characteristics and Therapeutic Potential of Dental Pulp Stem Cells on Neurodegenerative Diseases. *Front Neurosci.* 2020 May 7; 14:407. doi: 10.3389/fnins.2020.00407.

[35]. Yang B. Qiu Y, Zhou N. Ouyang H. Ding J, Cheng B and Sun J. Application of Stem Cells in Oral Disease Therapy: Progresses and Perspectives. *Front. Physiol*. 2017; 8:197. Doi 10.3389/fphys.2017.00197

[36]. Suma GN, Arora MP, Lakhanpal M. Stem cell therapy: A novel treatment approach for oral mucosal lesions. *J Pharm Bioallied Sci.* 2015 Jan-Mar; 7(1): 2-8.