



International Journal of Medical Research & Health Sciences

www.ijmrhs.com

Volume 3 Issue 4

Coden: IJMRHS

Copyright @2014

ISSN: 2319-5886

Received: 6th June 2014

Revised: 15th July 2014

Accepted: 5th Aug 2014

Review article

STEM CELLS IN ENDODONTIC THERAPY

*Sita Rama Kumar M¹, Madhu Varma K², Kalyan Satish R³, Manikya kumar Nanduri.R⁴, Murali Krishnam Raju S⁵, Mohan rao⁶

¹Senior Lecturer, ^{2,3}Professor, Department of Conservative Dentistry and Endodontics, Vishnu dental college, Bhimavaram, Andhra Pradesh, India

⁴Senior Lecturer, Department of Pedodontics, Lenora Institute of Dental Sciences, Rajahmundry, Andhra Pradesh, India

⁵Senior Lecturer, Department of Conservative Dentistry and Endodontics, GSL Dental College, Rajahmundry, Andhra Pradesh, India

⁶Senior Lecturer, Department of Conservative Dentistry and Endodontics, Anil Neerukonda Institute of Dental sciences, Vishakapatnam, Andhra Pradesh, India

*Corresponding author email: sitaramrajubds@gmail.com.

ABSTRACT

Stem cells have the remarkable potential to develop into many different cell types in the body. Serving as a sort of repair system for the body, they can theoretically divide without limit to replenish other cells as long as the person or animal is still alive. However, progress in stem cell biology and tissue engineering may present new options for replacing heavily damaged or lost teeth, or even individual tooth structures. The goal of this review is to discuss the potential impact of dental pulp stem cells on regenerative endodontics.

Keywords: Dental pulp complex stem cells, Periodontal ligament stem cells, Stem cells from human-exfoliated deciduous teeth, Stem Cells from apical papilla.

INTRODUCTION

The complex structural composition of teeth ensures both hardness and durability. These structures are vulnerable to trauma and bacterial infections. As ameloblasts are lost during eruption and odontoblasts can create new dentine only on a dentine-pulp border, a damaged tooth cannot self-repair. However, teeth show a degree of reparative processes such as a tertiary dentine formation. Once the odontoblast layer is damaged, odontoblast-like cells are recruited from somewhere within the pulp. Loss of the tooth, jawbone or both, due to periodontal disease, dental caries, trauma or some genetic disorders, affects not only basic mouth functions but aesthetic appearance and quality of life. Current dentistry resolves these problems using autologous tissue grafts or metallic implants. These

treatments have some limitations such as an adjoining tooth damage, bone resorption etc. The stem cell bioengineered tooth is a promising way of single tooth restoration. Some studies have reported that after dental pulp necrosis, dental pulp complex stem cells (DPSCs) can be used for the creation of dental pulp, which after implantation into the shaped root canals has affinity for the dentine¹. More recently the potential use of stem cells in dental pulp tissue engineering has boosted much interest in the field of Regenerative Endodontics.

Stem cells are defined as clonogenic cells capable of both self-renewal and multilineage differentiation since they are thought to be undifferentiated cells with varying degrees of potency and plasticity². They

differentiate into one daughter stem cell and one progenitor cell.

Classification of stem cells:

I. Stem cells can be classified according to their plasticity: i) Totipotent stem cell ii) Pluripotent stem cell. iii) Multipotent stem cell.

II. Stem cells can be classified according to their growth stage³:

a) **Embryonic stem cells** - located within the inner cell mass of the blastocyst stage of development. These stem cells have the highest potential to regenerate and repair diseased tissue and organs in the body.

b) **Postnatal stem cells/ Adult stem cells** - that have been isolated from various tissues including bone marrow, neural tissue, dental pulp and periodontal ligament. These are multipotent stem cells capable of differentiating into more than one cell type, but not all cell types.

III. Stem cells often categorized by their source.

a) **Autologous stem cells** - are obtained from the same individual to whom they will be implanted.

b) **Allogeneic stem cells** - originate from a donor of the same species.

c) **Xenogenic cells** - are those isolated from individuals of another species.

Characteristics of stem cells:

1. Totipotency: generate all types of cells, including germ cells (ESCs).

2. Pluripotency: generate all types of cells except cells of the embryonic membrane.

Induced pluripotent stem cells (IPS) are an evolving concept in which 3-4 genes found in the stem cells are transfected into the donor cells using appropriate vectors. The stem cells, thus derived by culturing will have properties almost like embryonic stem cells.

3. Multipotency: differentiate into more than one mature cell (MSC).

4. Self-renewal: divide without differentiation and create everlasting supply.

5. Plasticity: MSCs have plasticity and can undergo differentiation. The trigger for plasticity is stress or tissue injury which up regulates the stem cells and releases chemo attractants and growth factors.

Various sources for postnatal dental stem cells have been successfully studied:

• Permanent teeth - Dental pulp stem cells (DPSC): derived from third molar⁴.

• Deciduous teeth - Stem cells from human-exfoliated deciduous teeth (SHED): stem cells are present within the pulp tissue of deciduous teeth⁶.

• Periodontal ligament - Periodontal ligament stem cells⁶ (PDLSC).

• Stem Cells from apical papilla⁷ (SCAP).

• Stem cells from supernumerary tooth – Mesiodens⁸.

• Stem cells from teeth extracted for orthodontic purposes⁹.

• Dental follicle progenitor cells¹⁰.

• Stem cells from human natal dental pulp¹¹ (hNDP).

The Stem Cells that are found in the pulp of deciduous and permanent teeth are adult multipotent mesenchymal Stem Cells. The central region of the pulp contains large nerve trunks and blood vessels. This area is lined peripherally by a specialized odontogenic area which has **three layers** (from innermost to outermost)

1. **Cell rich zone**; innermost pulp layer which contains fibroblasts and undifferentiated mesenchymal Stem Cells.

2. **Cell free zone (zone of Weil)** which is rich in both capillaries and nerve networks. The nerve plexus of Rashkow are located in this zone.

3. **Odontoblastic layer**; outermost layer which contains odontoblasts and lies next to the predentin and mature dentin.

Dental pulp stem cells (DPSC): Mesenchymal stem cells that are isolated from the dental pulp of permanent teeth, are termed as Dental Pulp Stem Cells¹² (DPSC).

Dental follicle stem cells (DFSCs) are isolated from mesenchymal tissue localized around developing tooth germ. This source of stem cells can be easily obtained from follicles of impacted third molars¹³.

DFSCs are recognized as progenitors for cementoblasts, PDL stem cells, osteoblasts as well as neural cells. DFSCs have the capacity to induce calcification processes *in vitro* and *in vivo*.

Experiments undertaken with DFSCs revealed their potential for use in tissue engineering applications, including periodontal and bone regeneration.

DFSCs are recognized as osteogenesis and dentinogenesis inducers, but have not shown ability to produce dentin-pulp complex formation^{14, 15}.

DPSCs have three advantages over other more widely researched stem cell sources. The first is that they are possibly more prone to forming neurons than other stem cells.

The second advantage is that there are fewer ethical consideration than those which shroud other stem cells. Thirdly, they are more easily isolated than other stem cells, such as MSCs from the bone marrow and NSCs from cadavers.

The factors which make dental stem cells unique are:

- They are plentiful and easy to collect. Unlike harvesting bone marrow stem cells, which requires invasive surgery and cord blood stem cells, which are only available at birth, dental stem cells can be collected from baby teeth and wisdom teeth which would otherwise be discarded.
- Dental stem cells are highly proliferative, growing better in culture than many other types of adult stem cells.
- Dental stem cells have been reported to be more immature than other sources of mesenchymal stem cells (MSCs), thus may offer greater potential.
- Dental stem cells are adult stem cells and are not the subject of the same ethical concerns as embryonic stem cells. (<http://www.store-a-tooth.com/>).

Stem cells from Human Exfoliated Deciduous teeth (SHED):

Mesenchymal stem cells that are isolated from the dental pulp of exfoliated deciduous teeth, are termed as Stem cells from Human Exfoliated Deciduous teeth⁵ (SHED). SHED were identified to be a population of highly proliferative clonogenic cells capable of differentiating into a variety of cell types including neural cells, adipocytes and odontoblasts⁵. Deciduous teeth are the ideal resource of stem cells to repair damaged tooth structures, induce bone regeneration and possibly treat neuronal tissue injury or degenerative diseases. The difference between dental stem cells and cord blood stem cells are dental pulp contains mostly mesenchymal stem cells while cord blood consists predominantly of hematopoietic stem cells; bone marrow contains both types of stem cells. While cord blood stem cells have proven valuable in the regeneration of blood cell types, dental stem cells are able to regenerate solid tissue types that cord blood are less well suited for - such as potentially repairing connective tissues, dental tissues, neuronal tissue and bone. (<http://www.store-a-tooth.com/>).

SHED are distinct from DPSC with respect to their higher proliferation rate, increased cell population doublings, viability, osteoinductive capacities and failure to reconstitute a dentin pulp like complex.

Types of Stem Cells in Human Exfoliated Deciduous teeth (SHED):

Adipocytes; Adipocytes have successfully been used to treat cardiovascular disease, spine and orthopedic conditions, congestive heart failure, Crohn's disease, and to be used in plastic surgery^{16,17}.

Chondrocytes and Osteoblasts: Chondrocytes and Osteoblasts have successfully been used to grow bone

and cartilage suitable for transplant. They have also been used to grow intact teeth in animals^{5,18-20}.

Mesenchymal; Mesenchymal stem cells have the potential to treat neuronal degenerative disorders such as Alzheimer's and Parkinson's diseases, cerebral palsy, as well as a host of other disorders^{5,17,20-23}. Mesenchymal stem cells have more therapeutic potential than other type of adult stem cells^{5,20,23}.

Periodontal ligament stem cells (PDLSC):

The PDL is a specialized tissue located between the cementum and the alveolar bone and has as a role the maintenance and support of the teeth. Periodontal ligament stem cells (PDLSC), are isolated from the root surface of extracted teeth. These cells could be isolated as plastic-adherent, colony-forming cells, but display a low potential for osteo-genic differentiation under in vitro conditions. PDL stem cells differentiate into cells or tissues very similar to periodontium. Moreover, PDL stem cells transplanted into immune

Compromised mice and rats demonstrated the capacity for tissue regeneration and periodontal repair²⁴. It has been shown that a functional periodontium could successfully be established using PDL stem cells²⁵. Once the cells have been processed and stored in freezers, all biological activity has stopped, as a result, cells that have been properly banked can be stored almost indefinitely. Human cells have been effectively stored for up to 50 years.

Stem Cells from the apical papilla (SCAP):

Mesenchymal stem cells that are isolated from the apical end of developing tooth roots, are termed as Stem Cells from the Apical Papilla (SCAP). Stem cells from the apical part of the human dental papilla (SCAP) have been isolated and their potential to differentiate into odontoblasts was compared to that of the periodontal ligament stem cells²⁶ (PDLSC). SCAP exhibit a higher proliferative rate and appears more effective than PDLSC for tooth formation. Importantly, SCAP are easily accessible since they can be isolated from human third molars.

Stem cells from the dental follicle (DFSCs): The dental follicle is a mesenchymal tissue that surrounds the developing tooth germ. During tooth root formation, periodontal components, such as cementum, periodontal ligament (PDL), and alveolar bone, are created by dental follicle progenitors²⁷. DFSCs were found to be able to differentiate into osteoblasts / cementoblasts, adipocytes, and neurons. In addition, immortalized dental follicle cells were transplanted into

immunodeficient mice and were able to recreate a new periodontal ligament (PDL)-like tissue after 4 weeks²⁷.

Sources of dental stem cells: In a child the most accessible stem cells are from the oral cavity. For deciduous teeth, the best candidates are moderately resorbed canine and incisors with the presence of healthy pulp. Most Deciduous Molars are not candidates because of their resorption pattern. In children, other sources of easily accessible stem cells are supernumerary teeth, mesiodens and over retained deciduous teeth associated with congenitally missing permanent teeth.

Potential applications of stem cells in dentistry²⁸

The regenerative potential of adult stem cells obtained from various sources, including dental tissues has been of interest for clinicians over the past years and most research is directed toward achieving the following:

- Regeneration of damaged coronal dentin and pulp
- Regeneration of resorbed root, cervical or apical dentin, and repair perforations
- Periodontal regeneration
- Repair and replacement of bone in craniofacial defects
- Whole tooth regeneration.

Role of dental stem cells in regenerative medicine²⁹

Regenerative medicine is to regenerate fully functional tissues or organs that can replace lost or damaged ones occurred during diseases, injury and aging. The dynamic features of isolated dental stem cells revealed much potential for their use in regenerative medicine and tissue engineering.

1. Dental Pulp Regeneration.
2. Bio-Root Engineering.
3. Neural Regeneration.

Dental stem cell banking: Dental stem cell banking i.e., the process of storing stem cells obtained from patients deciduous teeth and wisdom teeth, may be one strategy to realize the potential of dental-stem-cell-based regenerative therapy³⁰⁻³². Recently, cell/tissue banks in the dental field has been planned and placed into practice in several countries, e.g.,

1. Advanced Center for Tissue Engineering Ltd., Tokyo, Japan (http://www.acte_group.com/).
2. Teeth Bank Co., Ltd., Hiroshima, Japan (<http://www.teethbank.jp/>).
3. Store-A-Tooth TM, Lexington, USA (<http://www.store-a-tooth.com/>).
4. BioEDEN, Austin, USA (<http://www.bioeden.com/>).
5. Stemade Biotech Pvt. Ltd., Mumbai, India (<http://www.stemade.com/>).

We can collect baby teeth for dental stem cell banking at home, with the following restrictions: (<http://www.store-a-tooth.com/>).

1. There must be a blood supply to the tooth when it is removed – that is, the tooth should bleed slightly when removed.
2. The tooth must be banked using our Cultured Cell Service, so lab tests can be performed to confirm the presence of stem cells prior to cryopreservation.

The cost to process and store stem cells for 20 years - \$1,250, Annually- \$95.00, Monthly- \$9.50.

Stem cells in endodontic therapy

Stem cells in the dental pulp: The fraction of multipotent stem cells in the dental pulp is small³³ and the location of these cells are not clearly known, but their phenotype is suggestive of their presence in perivascular niches³⁴. Both DPSC and SHED cells are originated from the dental pulp, they exhibit significant differences. For example, during osteogenic differentiation, SHED present higher levels of alkaline phosphatase activity and osteocalcin production, and higher proliferative rate than DPSC^{5,35,36}. SHED and DPSC cells are capable of regenerating dentin and pulp-like tissues in vivo^{2,5,37,38}.

Stem cells and caries-induced dentinogenesis: The dental pulp is a highly vascularized and innervated connective tissue responsible for maintaining the tooth vitality and able to respond to injuries. Dentinogenesis is a unique process, which involves the interaction between odontoblasts, endothelial cells, and nerves³⁹. The odontoblasts, ecto-mesenchymal derived cells, are the first cells to respond to the injury caused by bacterial invasion during caries progression⁴⁰. The endothelial cells and nerve cells located in the vicinity of the carious lesion modulate the odontoblastic response⁴¹⁻⁴³. Primary odontoblasts are induced to secrete a dentin matrix that mineralizes as reactionary dentin in response to shallow caries^{44,45}. This type of tertiary dentin protects the dental pulp from irritants and maintains dental pulp integrity.

Stem cells and pulp angiogenesis: Vascular endothelial growth factor (VEGF) is a potent inducer of endothelial cell differentiation and survival, and it is the most effective angiogenic factor⁴⁶⁻⁴⁸. VEGF also plays a critical role on the control of vascular permeability during physiological and pathological events⁴⁸. VEGF is strongly expressed by odontoblasts and in the sub-odontoblastic layer in vivo⁴⁹⁻⁵¹. VEGF is potently expressed in dental pulp tissues of teeth undergoing

caries-induced pulpitis, by immunohistochemical studies⁵².

Application of stem cells in regenerative endodontics:

Implantation: In pulp implantation, replacement pulp tissue is transplanted into clean and shaped root canal systems. The source of pulp tissue may be a purified pulp stem cell line that is disease or pathogen-free or is created from cells taken from a biopsy, that has been grown in the laboratory. Stem cell treatment is not dangerous.

Pulp revascularization: Pulp necrosis of an immature tooth as a result of caries or trauma could arrest further development of the root, leaving the tooth with thin root canal walls and blunderbuss apices. Regeneration of the pulpal tissue of an infected immature tooth might take place if suitable environment is possible with absence of intrapulpal infection. The pulpal space might become repopulated with mesenchymal cells arising from dental papilla or apical periodontium^{53,54}.

Whole tooth regeneration: Tooth-like tissues have been generated by the seeding of different cell types on biodegradable scaffolds. A common methodology is to harvest cells, expand and differentiate cells in vitro, seed cells onto scaffolds, and implant them in vivo, in some cases, the scaffolds are re-implanted into an extracted tooth socket or the jaw. Ikeda *et al*, 2009 reported a successful fully functioning tooth replacement in an adult mouse achieved through the transplantation of bioengineered tooth germ into the alveolar bone in the lost tooth region⁵⁵. This technology was proposed as a model for future organ replacement therapies. In many cases teeth with cavities works. However, teeth with extensive decay, or where there is reason to believe that the pulp has been compromised, should be discarded.

A possible risk of some stem cell treatments may be the development of tumors or cancers. For example, when cells are grown in culture (a process called expansion), the cells may lose the normal mechanisms that control growth or may lose the ability to specialize into the cell types you need. Also, embryonic stem cells will need to be directed into more mature cell types or they may form tumors called teratomas. Other possible risks include infection, tissue rejection, complications arising from the medical procedure itself and many unforeseen risks.

FUTURE CHALLENGES:

1. A major challenge facing regenerative techniques is the ability to obtain a sufficient number of autogenous cells for scaffold seeding.

2. For regeneration of the dental pulp, fabrication of vascularized scaffolds is likely a key requirement.
3. Advances in growth factors or drugs to control the activity of cells must be sought out.

The understanding of the mechanisms underlying pulp angiogenic responses is critical for the development of new, targeted therapies that aim at the conservation of dental pulp viability. However, developments in this area have the potential to revolutionize the way that we practice clinical Endodontics in the future.

CONCLUSION

Stem cells are critical for the physiology of the dental pulp and for the response of this tissue to injury. Recent findings have unveiled dental pulp stem cells as potential therapeutic targets in cases of reversible pulpitis. Importantly, these cells may become the primary strategy for the revitalization of necrotic immature permanent teeth. Such discoveries have the potential to fundamentally change the paradigms of conservative vital pulp and root canal therapy, and perhaps allow for the treatment in the future of conditions that are presently untreatable in Dentistry. Therefore, endodontist should recognize the potential of the emerging field of regenerative endodontics and the possibility of obtaining stem cells during conventional dental treatments that can be banked for autologous therapeutic use in the future.

Acknowledgment: The authors would like to thank the Vishnu dental college.

Conflict of interest: No

REFERENCES

1. Gotlieb EL, Murray PE, Namerow KN, Kuttler S, Garcia-Godoy F. An ultrastructural investigation of tissue-engineered pulp constructs implanted within endodontically treated teeth. *JADA* 2008; 139: 457-65
2. Gronthos S, Brahimi J, Li W, Fisher LW, Cherman N, Boyde A. Stem cell properties of human dental pulp stem cells *J Dent Res* 2002;81:531-5
3. Fortier LA. Stem cells: classifications, controversies, and clinical applications. *Vet Surg* 2005;34:415-23
4. Langer R, Vacanti JP. Tissue engineering. *Science* 1993;260:920-6
5. Miura M, Gronthos S, Zhao M, Fisher LW, Robey PG, Shi S. SHED: stem cells from human exfoliated

- deciduous teeth. *Proc Natl Acad Sci USA* 2003;100:5807-12.
6. Ballini A, De Frenza G, Cantore S, Papa F, Grano M, Mastrangelo F et al. In vitro stem cell cultures from human dental pulp and periodontal ligament: new prospects in dentistry. *Int J Immunopathol Pharmacol* 2007;20:9-16
 7. Sonoyama W, Liu Y, Yamaza T, Tuan RS, Wang S, Shi S, et al. Characterization of the apical papilla and its residing stem cells from human immature permanent teeth: a pilot study. *J Endod* 2008;34: 166-71.
 8. Huang AH, Chen YK, Lin LM, Shieh TY, Chan AW. Isolation and characterization of dental pulp stem cells from a supernumerary tooth. *J Oral Pathol Med* 2008;39:571-4
 9. Yang XC, Fan MW. Identification and isolation of human dental pulp stem cells. *Zhonghua Kou Qiang Yi Xue Za Zhi* 2005;40:244-7.
 10. Huang GT, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs those from other sources: Their biology and role in regenerative medicine. *J Dent Res* 2009;88:792-806.
 11. Karaöz E, Do an BN, Aksoy A, Gacar G, Akyüz S, Ayhan S. Isolation and *in vitro* characterisation of dental pulp stem cells from natal teeth. *Histochem Cell Biol* 2010;133:95-112.
 12. Gronthos S, Mankani M, Brahimi J. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. *Proc Natl Acad Sci U S A* 2000;97:13625–30.
 13. Volponi AA, Pang Y, Sharpe PT. Stem cell-based biological tooth repair and regeneration. *Trends Cell Biol* 2010;20: 715-22.
 14. Sandhu SS, Nair M. Stem cells: Potential implications for tooth regeneration and tissue engineering in dental science. *People J Scien Res* 2009;2: 41-45.
 15. Honda MJ, Imaizumi M, Tsuchiya S, Morsczech C. Dental follicle stem cells and tissue engineering. *J Oral Sci* 2010; 52: 541-552.
 16. Gandia C, Armiñan A, García-Verdugo JM, Lledó E, Ruiz A, Miñana MD et al. Human dental pulp stem cells improve left ventricular function, induce angiogenesis, and reduce infarct size in rats with acute myocardial infarction. *Stem Cells* 2007;26(3):638–45.
 17. Perry BC, Zhou D, Wu X, Yang FC, Byers MA, Chu TM, Hockema JJ, Woods EJ, Goebel WS. Collection, cryopreservation, and characterization of human dental pulp-derived mesenchymal stem cells for banking and clinical use. *Tissue Eng Part C Methods* 2008;14(2):149–56.
 18. De Mendonça Costa A, Bueno DF, Martins MT, Kerkis I, Kerkis A, Fanganiello RD, Cerruti H, Alonso N, Passos-Bueno MR. Reconstruction of large cranial defects in nonimmunosuppressed experimental design with human dental pulp stem cells. *J Craniofac Surg* 2008;19(1):204–10.
 19. Seo BM, Sonoyama W, Yamaza T, Coppe C, Kikuri T, Akiyama K, Lee JS, Shi S. SHED repair critical-size calvarial defects in mice. *Oral Dis* 2008;14(5):428–34.
 20. Shi S, Bartold PM, Miura M, Seo BM, Robey PG, Gronthos S. The efficacy of mesenchymal stem cells to regenerate and repair dental structures. *Orthod Craniofac Res* 2005; 8(3):191–99
 21. Irina Kerkis, Carlos E Ambrosio, Alexandre Kerkis, Daniele SM, Eder Zucconi, Simone AS Fonseca, et al., Early transplantation of human immature dental pulp stem cells from baby teeth to golden retriever muscular dystrophy (GRMD) dogs: Local or systemic? *J Transl Med* 2008; 6: 35.
 22. Arthur A, Rychkov G, Shi S, Koblar SA, Gronthos S. Adult human dental pulp stem cells differentiate toward functionally active neurons under appropriate environmental cues. *Stem Cells* 2008; 26(7):1787–95.
 23. Jeremy J. Mao. Stem Cells and the Future of Dental Care. *New York State Dental Journal* 2008;74(2): 21–24.
 24. Seo BM et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. *Lancet* 2004; 364:149–55.
 25. G.Bluteau et al .Stem cells for tooth engineering. *European Cells and Materials* 2008;16: 1-9.
 26. Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, Liu H, et al., Mesenchymal stem cell-mediated functional tooth regeneration in swine. *PLoS One* 2006;20(1):79.
 27. Li Peng, Ling Ye, Xue-dong Zhou. Mesenchymal Stem Cells and Tooth Engineering. *International Journal of Oral Science* 2009;1(1):6–12.
 28. Saraswathi Gopal, Arathy Manohar L. Stem cell therapy: A new hope for dentist. *Journal of Clinical and Diagnostic Research* 2012; 6(1): 142-44.
 29. Mohamed Jamal, Sami Chogle, Harold Goodis and Sherif M. Karam. Dental Stem Cells and Their Potential Role in Regenerative Medicine. *Journal of Medical Sciences* 2011; 4(2): 53-61.

30. Abedini S, Kaku M, Kawata T, Koseki H, Kojima S, SumiH et al. Effects of cryopreservation with a newly-developed magnetic field programmed freezer on periodontal ligament cells and pulp tissues. *Cryobiology* 2011; 62:181–7.
31. Arora V, Arora P, Munshi AK. Banking stem cells from human exfoliated deciduous teeth (SHED): saving for the future. *J Clin Pediatr Dent* 2009; 33:289–94.
32. Kaku M, Kamada H, Kawata T, Koseki H, Abedini S, Kojima S, et al. Cryopreservation of periodontal ligament cells with magnetic field for tooth banking. *Cryobiology* 2010; 61:73–8.
33. Balic A, Aguila HL, Caimano MJ. Characterization of stem and progenitor cells in the dental pulp of erupted and unerupted murine molars. *Bone* 2010; 46:1639–51.
34. Shi S, Gronthos S. Perivascular niche of postnatal mesenchymal stem cells in human bone marrow and dental pulp. *J Bone Miner Res* 2003; 18:696–704.
35. Koyama N, Okubo Y, Nakao K, et al. Evaluation of pluripotency in human dental pulp cells. *J Oral Maxillofac Surg* 2009; 67:501–506.
36. Nakamura S, Yamada Y, Katagiri W. Stem cell proliferation pathways comparison between human exfoliated deciduous teeth and dental pulp stem cells by gene expression profile from promising dental pulp. *J Endod* 2009; 35:1536–42.
37. Sakai VT, Zhang Z, Dong Z. SHED differentiate into functional odontoblast and endothelium. *J Dent Res* 2010; 89:791–96.
38. Huang G, 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 Part A* 2010; 16:605–15.
39. Linde A, Goldberg M. Dentinogenesis. *Crit Rev Oral Biol Med* 1993; 5:679–728.
40. Ruch JV, Lesot H, Bègue-Kirn C. Odontoblast differentiation. *Int J Dev Biol* 1995; 39:51–68.
41. Takahashi K. Pulpal vascular changes in inflammation. *Proc Finn Dent Soc.* 1992; 88(S1): 381–85.
42. Avery, JK. Cox, CF. Chiego, DJ. Structural and physiologic aspects of dentin innervation. In: Linde, A., editor. *Dentin and Dentinogenesis*. CRC Press; Boca Raton: 1984;1:19-46.
43. Kramer IR. The vascular architecture of the human dental pulp. *Arch Oral Biol* 1960; 2:177–89.
44. Linde A, Lundgren T. From serum to the mineral phase. The role of the odontoblast in calcium transport and mineral formation. *Int J Dev Biol* 1995; 39:213–22.
45. Smith AJ, Cassidy N, Perry H. Reactionary dentinogenesis. *Int J Dev Biol* 1995; 39:273–280.
46. Leung DW, Cachianes G, Kuang WJ. Vascular endothelial growth factor is a secreted angiogenic mitogen. *Science* 1989; 246:1306–09.
47. Nor JE, Christensen J, Mooney DJ, et al. Vascular endothelial growth factor (VEGF)-mediated angiogenesis is associated with enhanced endothelial cell survival and induction of Bcl-2 expression. *Am J Pathol* 1999; 154:375–84.
48. Ferrara N, Gerber HP, LeCouter J. The biology of VEGF and its receptors. *Nat Med* 2003; 9:669–76.
49. Telles PD, Hanks CT, Machado MAAM, Nor JE. Lipoteichoic acid upregulates VEGF expression in macrophages and pulp cells. *J Dent Res* 2003; 82:466–70.
50. Botero TM, Mantellini MG, Song W, et al. Effect of lipopolysaccharides on vascular endothelial growth factor expression in mouse pulp cells and macrophages. *Eur J Oral Sci* 2003; 111:228–34.
51. Botero TM, Son JS, Vodopyanov D. MAPK signaling is required for LPS-induced VEGF in pulp stem cells. *J Dent Res* 2010; 89:264–69.
52. Guven G, Altun C, Günhan O. Co-expression of cyclooxygenase-2 and vascular endothelial growth factor in inflamed human pulp: an immune histochemical study. *J Endod* 2007; 33:18–20
53. Ding RY, Cheung GS, Chen J, Yin XZ, Wang QQ, Zhang CF. Pulp revascularization of immature teeth with apical periodontitis: a clinical study. *J Endod* 2009;35(5):745-9.
54. Torabinejad M, Chivian N. Clinical applications of mineral trioxide aggregate. *J Endod* 1999 Mar; 25(3):197-205.
55. Ikeda E, Morita R, Nakao K, Ishida K, Nakamura T, Takano-Yamamoto T et al. Fully functional bioengineered tooth replacement as an organ replacement therapy. *Proc Natl Acad Sci USA* 2009;106(32):13475-80.