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An Overview of Stem Cells in Dentistry

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Abstract:

The stem cells are self renewable ,multipotent cells which have ability to regenerate different types of specialized cells.In dentistry the day to day restorative treatment for variety of cases from normal dental caries restorations to reimplantation of the teeth to socket involves the stem cells.Henceforth to get a better knowledge regarding the stem cells,their functions,clinical applications,processing and storage of these cells are to be reviewed.

Keywords: Dental follicular stem cells Dental pulp stem cells ,Multi potent,pluripotent, periodontal ligament stem cells ,Stem cells from human exfoliated teeth, ,stem cells from apical papilla.

Introduction

Stem cells are present in all complex cellular organism which has capacity to differentiate into specialized cells which are self renewable and can produce more number of cells. There are two types of stem cells embryonic and adult stem cells . The widely used are mesenchymal stem cells in dentistry.(1)

In the text book of The Cell in Development and Inheritance written *by* E. D. Wilson who first named as *stem cell* in 1896.In literature there are many terminologies representing stem cells as "precursor" , "progenitor cell," "stem cell," as an alternative. (2)

Initially stem cells research was initiated in 1953 by Leroy Stevens who noticed teratoma cells in mice breds (3)

Properties of stem cells

When a stem cell divides, each new cell has the potential to either remain a stem cell or become another type of cell with a more specialized function.Stem cells have the following three general properties:

- They are unspecialized cell
- Capable of dividing themselves
- Self renewable for longer period
- They have a nature to develop in to specific cell in body.
- Lineage property- produces a different lineage of cells from that of usual (4)

Specialized cells

Stem cells are self renewable.There are certain cells which cannot replicate as muscle cells,blood cells,nerve cells but stem cells are renewable.Initially these stem cells

proliferates in the laboratory for many days which can produce millions of such cells .If those produced cells are unspecialized like that of parent cells then they have a capacity of longer self renewal period.

Unspecialized cells

When unspecialized cells produces specialized cells then it is called as differentiation.(5)

Stem Cell Potential

- **Totipotent:** Each cell can develop into a new individual. Cells from 1-4 day old embryos. (6,7)
- **Pluripotent EGC:** Cells can form any cell type. Some cells of blastocyst (5-14 days old) (6,8)
- **Multipotent:** Cells differentiated, but can form a number of other tissues. Fetal tissue, cord blood, and adult stem cells.(6,9)

Types of Stem Cells

A. **Embryonic** stem cell (ESC'S)(fig 1)

These embryonic stem cells are obtained from the embryos of 3 or 4 day old from the invitro fertilization which comprises of of 50-150 cells .These cells can be easily grown in culture medium.

These cells have a greater rate of proliferation and hence slightly difficult to control their growth which may some time lead to tumor after they are injected .They may also used in tissue engineering ,These ESC'S have greater ethical and technical issues.

B. **Adult-somatic**

- Found among some differentiated cells in a specific tissue or organ;
- placental cord;
- primary teeth. (6)

Adult stem cells does nt have much potential differentiating ability as ESC'S but when in some cases as hemopoietic cells have less rejection of tissues after transplantation due to which immune suppressants are not required. (6)

Advantages

- They are more stable against immune attack
- specialized partly
- they form other type of cells as being more flexible.(6)

Disadvantage

- The control of growth is difficult
- Ethical issues in case of embryonic stem cells mainly(2)
- They are flexible in their nature(6)

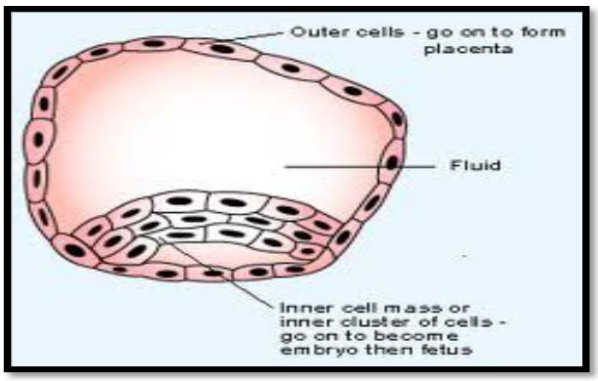

Figure 1.embryonic stem cells (6)

Dental stem cells :-(figure 2)

- There are variety of stem cells derived from teeth .they are:-
- Stem cell from human exfoliated deciduous teeth (SHED'S)-Miura et al 2003(10)
- Dental pulp stem cells-Gronthos et al 2000(11)
- Stem cells from apical papilla (SCAP)-Sonoyama et al 2006(12)
- Periodontal ligament stem cell(PDSLC)-Seo et al 2004(13)
- Dental follicle progenitor stem cells(DFPSC)-Morsczeck et al 2005(14)

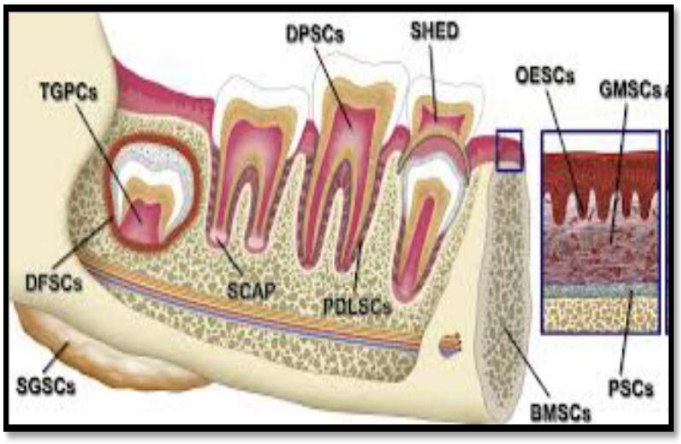

Figure 2-Different types of dental stem cell(6)

shed (stem cells from human exfoliated deciduous teeth)

Also called as "immature stem cells".

According toXi Wang et al 2012,In in vitro it cannot regenerate the dentine pulp complex ,whereas SHED can proliferate and differentiate in higher rate compared to DPSC'S.Also SHED has increased capacity of mineralization .SHED have a better potentiality,ability,accessibility for use in regenerative and therapeutic medicine.(15)

The umbilical cord and the stem cells obtqained from the human exfoliated deciduous teeth are someway similar.The SHED contains marker neural cells which has its origin from neural crest cells.These SHED cells have a greater differentiation potential like chondrocytes,adibocytes ,osteoblasts and odontoblastic cells(16)

Stem cells derived from the wisdom teeth and the teeth extracted for orthodontic treatment purpose have lesser therapeutic effect than the cells extracted from the primary teeth. (17)

The pulpal cells have a graeter potential in differentiating into moyocytes,neurocytic cells,.adipocytes ,chondrocytes and osteocytes.(17)

Advantage of banking SHED cells

- it saves cells from natural damage
- it is painless
- simple procedure
- cost effective-it is just as less as one third of umbilical cord blood storage cost(18,19)

Collection and processing of stem cells:- (18,19.20)

1.To collect the teeth required for SHED banking:-

This procedure of collection of stem cells banking fronm SHED is mostly decision of the parent /care giver of child.The exfoliated tooth is immediately placed in asterile normal saline solution then it is to be informed to the bank.

Indication for the tooth which is to be used for banking:-

- The pulp must be red in colour-this ensures the viability of teeth i.e. until exfoliation there was proper blood supply received to the pulpal tissues.
- Periodontally sound and vital endodontically.(18)
- Not more than 1/3rd of the must be resorbed in case of deciduous teeth.(21)
- The primary teeth are extracted due to abnormal eruption of succeduous teeth to avoid crowding .

Contraindication:- (18,19)

- If there is gray colour pulp then it is compromised due to necrosis and which has no viable cells for the future stem cell use.
- The traumatized teeth-reduced cell viability
- Teeth affected by abscess,tumors and cysts.
- Teeth sectioned during surgery
- Root fractured teeth
- Discoloured teeth-pulpal degeneration.

After collecting –transportation

It is transported in a" hypotonic phosphate buffer salt solution".This solution provides the required nutrients and prevents drying of the tissues during transportation.(19)

Sustentation

The temperature changes during transportation may cause hypothermia.Hence the cells are placed in thermette –A carrier which helps in maintaining temperature and then into insulated metal transport vessel called sustentation.

Save –A-tooth can also be used during transportation.stem cells viability is born sensitive for time and temperature.Hence the total time for harvesting and processing must be within 40 hours.(19)

2.Isolating the stem cells

- The tooth is washed thrice with Dulbecco's phosphate buffered saline (PBSA).
- Disinection reagent –povidine iodine .
- Again washed using PBSA
- Pulp tissue isolated with small forceps or dental extractor.
- Pulp washed with salt water.
- Tissue digestion done using collagenase type I and dispase for 1 hour at 37℃ or Tripsin EDTA may be used.
- To obtain a single cell suspension the cells isolated are passed through a 70 µm filter(18,20)

3.Culturing of stem cells:- (18,19,20)

The stem cells are cultured in mesenchymal stem cell medium constitute of

- Alpha modified medium with 2mm glutamine
- 15% fetal bovine serum(FBS)
- 0.1Mm L-ascorbic acid phosphate
- 100U/ml penicillin and 100ug/ml streptomycin at 37ºC and 5% CO2 in air.
- Colonies are usually seen by 24 hours.

4. Storage of the stem cell

a. **Cryopreservation**

Cryo preservation means cold preservation at sub zero temperature which is effective and also helps in maintenance of the cells to be viable during transportation .it is technique wherein slow cooling process at 1 to 2℃/min done along with the Dimethyl SulphOxide (DMSO)- a cryoprotectant,which avoids intracellular damage of cells due to ice damage.(18)

b. Magnetic freezing

Here the freezing point is lowered to about 6-7 ° C by appling a weak magnetic field around the water and tissue.It ensures a damageless distribution of low temperature in the cell wall .Also the ice expansion and drainage of nutrition because of capillary action by using regular freezing technique is reduced .This method of magnetic freezing is considered to be relatively cheaper and more reliable when it is compared to the cryopreservation(16,17,22,23)

Dental Pulp Stem Cells (DPSC)

The dentine-pulp complex formed in the odontogenesis process of teeth during formation.These are generally formed by spontaneous proliferation and thickening of the ectomesenchymal cells on the future maxilla and mandible of primordial mouth.These spontaneous thickening in the specialized region leads to development of tooth germd which may have undergone a sequential changes from initial bud stage to the bell stage of hystogenesis.The odontoblastic cells also helps in organic matrix production which leads to mineralization.

The main objective behind the scientific research is that ,to find a better way on stimulating those existing odontoblastic cells and improvising their defence action against noxious stimuli.

A change of daughter cells remains under preodontoblast which are migrated from neural combs.They don't have a direct interaction with ectodermal cells and remains undifferentiated (24)

While many researches are going on the proper understanding of pulpal tissues growth and its

significantly improved.yet it is unclear for proper pulpal harnessment and regeneration has they involve many specific molecular signaling properties in dental regeneration.These cells can be obtained by direct local application of cell population by harvesting them from autologous SHED or non autologous teeth.(5)

Some studies have proved that DPSCs are capable of producing dental tissues in vivo including dentin, pulp, and crown-like structures. Whereas other investigations have shown that these stem cells can bring about the formation of bone-like tissues. The invitro studies proves that DPSCs helps in bio-teeth formation ,which serves a nice tooth reconstruction in future clinically.(15)

Isolation of Dental Pulp Stem Cells

To produce odontoblast cells are seeded in enzymatic isolates which gives rise to myocytes,osteocytes,neurocytes and chondrocytes(6)

Stem Cells of Apical Papilla (SCAP):

The cells that are located at the apex of root of developing teeth are the SCAP. [25,26,27], and they are distinct from pulp cells [12]. This cells forms the root portion and it's a potential source of mesenchymal cells. Generally when a variety of stem cell isolated in a medium give rise to different cells ,these SCAP cells also produces cells which as osteocytic cells,odontoblastic cells and adipocytes [28]. When these cells transplanted into an immunocompromised mice it has a capacity to produce dentin like structures using scaffolds. [28].

Periodontal Ligament Stem Cells(PDLSC)

The presence of PDLSC progenitor/stem cells in mice was first reported by McCulloch in 1985 (29) . On further research on PDLSC Seo et al and his collegues reported about the multipotent mesenchymal cells in the human periodontal ligament in 2004. Seo illustrated that, in the enzymatically digested PDL there are presence of clonogenic stem cells .(13)

The PDLSCS transported into immunocompromised rodent it produced cementum /PDL like cells that aided in tissue repair of periodontal tissues.(30,6)On further in vitro studies ,they found that the Hertwig's epithelial root sheath cells promoted the differentiation of PDLSCs.(31)

The PDLSC cells can be differentiated into cementoblast line cells ,adipocytes and collagen forming cells under a suitable culture medium.(6,32) The PDLSCs as of Bone morphogenetic mesenchyal stem cells can undergo osteogenic, adipogenic, and chondrogenic differentiation.(33) .These PDLSCs can also differentiate as neuronal precursors (34) .The periodontal ligament progenitor cells which was isolated from third molar was used in a intra bony defect as bone graft .over a follow up 32 to 70 months revealed a marked improvement in those sites(34)

Dental Follicle Precursor Cells (DFPC'S):-

The ectomesenchymal condensation around the tooth germ is dental follicles that gives rise to three tiisue of periodontium : periodontal ligament, cementum and alveolar bone(14,27,35). DFPCs were more proliferative than DPSCs and SCAP(36).

These DFPCs was initially isolated from human third molar teeth .(14,18) The progenitor cells of ectomesenchyme gives rise to dental follicle cells in cap stage .it consist of progenitor cells in the developing tooth germs for for periodontal ligament cells, cementoblasts, and osteoblasts. These DFPCs believed to have a greater plasticity when compared to other dental stem cells as they are isolated from developing tooth germ(18,37).However DFPCs cloned shows a heterogenous differentiation(35).when DFPSCs transplanted in rodent which are immune compromised shows differentiation into cementoblasts(38,39)and osteogenic cells (40). In invitro studies DFPSC were able to differentiate into odontoblast (41,42).The DFSCs and

SHED cells can differentiate into neural cells under a culture medium(43)

Application of Stem Cell In Dentistry

a. Regeneration of bone

Now a days stem cells is proven for its capacity to regenerate bone.(16,18)The stem cells isolated can be used for rebuilding bony defects of cranio facial defects (44).Mesenchymal stem cells (MSC) isolated can effectively differentiate into osteoblasts and can serve for rebuilding of bone defects in the oromaxillofacial region by bone regeneration. (45)

They may be useful for treatment of periodontal diseases, osteoporosis, bone fractures, augmentation of the alveolar ridge of maxilla and mandible, sinus lift, filling of large bone defects after surgical treatment of cancer or after injury and repair of inborn bone.(44)

b. Tissue Regeneration:

The finest goal of tissue regeneration in Stem cells is to replace the lost tissue of the tooth mostly as dentin and pulpal regeneration.according to Bohl et al. in 1998 the cells were cultured in PGA polyglycolic resulted in a tissue of higher density similar to the native pulp.(20,46) .A study conducted on mice where SHED cells were seeded in scaffold led to rise of odontoblast like cells.(20,47)

c. Formation of root

the apical papilla stem cells helps in continued root formation.A study conducted in minipigs ,the apical papillary tissue were removed with intact pulpal tissues which yet showed presence of incomplete root formation.(20)

d. Pulp implantation

the cultured pulpal tissues are transferred to a cleaned and shaped canal system.In in vitro the pulpal tissues are grown in a biodegradable scaffold as sheers with protein such as collagen I or fibronectin. There are special technique needed for proper adherence of cell in root canal wall which is a major limitation.(48,49)

e. Bio root engineering

Stem cells from periapical follicle isolated from apical end of developing root of human third molars evaluated the application of these cells in PDL regeneration/cementum regeneration as well as for bio root engineering.(47)

f.Tooth engineering

A study was conducted on the mouse mandible for growing teeth on it by nakao et al in 2007 .in this study the mesenchymal cells were embedded on the collagen gel and then impalanted into the adult mice .This lead to development of ameloblast,odontoblastic cells,periodontal ligament,root and alveolar structures.**(figure 3)**Thus implantation of these tissues led to stages of eruption and maturation which suggest futher human studies in future.(50,51)

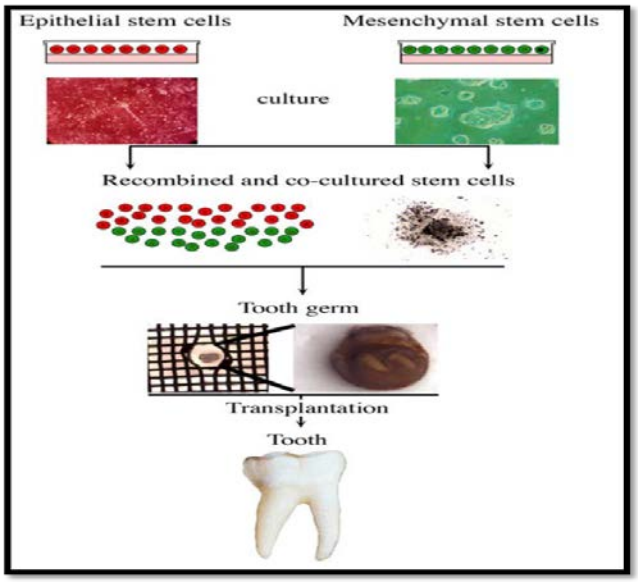

Figure 3-tooth engineering

Stem Cell Banks

There are about 40 blood cord banksin overall world Today, there are over 40 cord blood banks worldwide, both public and private.

Cord blood banks in India

- Reliance Life Sciences, Delhi,
- Life cell, Chennai,
- Cordlife Sciences and Cryobanks in Kolkata and New Delhi, respectively,
- Histostem, cord blood bank in Mumbai.(17,52)

Conclusion

Stem cells plays a major role in both medical and dental field which as been proven in many laboratory level studies .The awareness regarding the stem cell collection storage for the future use is increasing which demands a upcoming human based studies for a better use of these cells in day to day treatment options.

References

1. Mohamed Nihal Buhari, Saravana Kumar. Success Rate of Stem Cells in Dentistry – A Review. J. Pharm. Sci. & Res. Vol. 6(1), 2014, 48 - 51

2.Clive N. Svendsen and Allison D.Ebert.Encyclopedia of stem cell research.vol 1 and 2.

3. Shalini H , Dr. Sankari.Stem Cells in Periodontal Regeneration . JDMS. Volume 12, Issue 2 (Nov.- Dec. 2013), PP 59-63.

4. Marawar PP, Mani A, Sachdev S, Sodhi NK, Anju A. Stem cells in dentistry: an overview. Pravara Med Rev 2012;4(2):11-15.

5. Paloma Dias telles, Maria Aparecida de Andrade Moreira machado, Vivien Thiemy sakai,

Jacques Eduardo Nor.Pulp tissue from primary teeth: new source of stem cells. J Appl Oral Sci. 2011;19(3):189-194.

6. Sura Ali Ahmed Fouad, “Dental Stem Cells: A Perspective Area in Dentistry.” International Journal of Dental Sciences and Research, vol. 3, no. 2A (2015): 15-25.

7.Fischbach GD, Fischbach RL. Stem cells: science, policy, and ethics. J Clin Invest 2004; 114: 1364-1370

8.Watt FM, Driskell RR. The therapeutic potential of stem cells. Philos Trans R Soc Lond B Biol Sci 2010; 365: 155-163

9.He F, Chen X, Pei M. Reconstruction of an in vitro tissue-specific microenvironment to rejuvenate synovium-derived stem cells for cartilage tissue engineering. Tissue Eng Part A 2009; 15: 3809-3821

10. Miura M, Gronthos S, Zhao M, Lu B, Fisher LW, Robey PG, et al. SHED: Stem cells from human exfoliated deciduous teeth. Proc Natl Acad Sci U S A 2003;100:5807-12.

11. Gronthos S, Mankani M, Brahim J, Robey PG, Shi S. Postnatal human dental pulp stem cells (DPSCs) in vitro and in vivo. Proc Natl Acad Sci U S A 2000;97:13625-30.

12. 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.

13. Seo BM, Miura M, Gronthos S, Bartold PM, Batouli S, Brahim J, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament. Lancet 2004;364:149-55.

14. Morsczeck C, Götz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C et al. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol 2005;24:155-65.

15. Xi Wing, Xin-Jia Sha, Guang-Hui Li, Fu-Sheng Yang, Kun Ji, Ling-Ying Wen, Shi-Yu Liu, Lei Chen, Yin Ding. Comparative characterization of stem cells from human exfoliated deciduous teeth and dental pulp stem cells. Archives of Oral Biology 2012:57, 9. 1231-1240.

16. Seo BM, Miura M, Sonoyama W, Coppe C, Stanyon R, Shi S. Recovery of stem cells from cryopreserved periodontal ligament. J Dent Res. 2005; 84(10):907–12.

17.Zhang W, Walboomers XF, Shi S, Fan M, Jansen JA. Multilineage differentiation potential of stem cells derived

from human dental pulp after cryopreservation. Tissue Eng. 2006;12(10):2813–23.

18.Vipin Arora, Pooja Arora, AK Munshi Banking Stem Cells from Human Exfoliated Deciduous Teeth (SHED): Saving for the Future, J Clin Pediatr Dent 33(4): 289–294, 2009

19. Freshney Ian R et al. Culture of human stem cells. Chapter 8: 187–207,2007.

20. Madan KA, Sudhindra BM, Khatri S, Madan P. Stem Cells from Human Exfoliated Deciduous Teeth- A Boon to Dentistry. Int J Oral Health Med Res 2016;3(2):93-95.

21. Nayyar AS, Turakhia H, Chawla S. Dental Stem Cell Banking, The Future is Here: A Case Report. J Dent Oral Biol. 2016; 1(3): 1011.

22. Chueh LH, Huang GT. Immature teeth with periradicular periodontitis or abscess undergoing apexogenesis: A paradigm shift. J Endod. 2006;32:1205–13.

23.paccio G, Graziano A, d'Aquino R, Graziano MF, Pirozzi G, Menditti 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.

24. Peneva M., Vanyo Mitev, Nikolai Ishketiev. Isolation of mesenchymal stem cells

from the pulp of deciduous teeth. Journal of imab .book 2.pg 84-87

25. Sedgley CM, Botero TM. Dental stem cells and their sources. Dent Clin North Am 2012; 56: 549-561.

26.Huang GT, Sonoyama W, Liu Y, et al. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. J Endod 2008;34:645–51.

27 .Miki Taketomi Saito, Karina Gonzales Silvério, Márcio Zaffalon Casati, Enilson Antonio Sallum, Francisco Humberto Nociti Jr. Tooth-derived stem cells: Update and perspectives. World J Stem Cells 2015 March 26; 7(2): 399-407

28.Sonoyama W, Liu Y, Fang D, Yamaza T, Seo BM, Zhang C, Liu H, Gronthos S, Wang CY, Wang S, Shi S. Mesenchymal stem cellmediated functional tooth regeneration in swine. PLoS One 2006; 1: e79

29. McCulloch CA. Progenitor cell populations in the periodontal ligament of mice. Anat Rec 1985;211:258–62. 65.

30.Sonoyama W, Seo BM, Yamaza T, et al. Human Hertwig's epithelial root sheath cells play crucial roles in cementum formation. J Dent Res 2007;86: 594–9. 66.

31. Gronthos S, Mrozik K, Shi S, et al. Ovine periodontal ligament stem cells: isolation, characterization, and differentiation potential. Calcif Tissue Int 2006;79:310–7. 67.

32. Gay IC, Chen S, MacDougall M. Isolation and characterization of multipotent human periodontal ligament stem cells. Orthod Craniofac Res 2007;10: 149–60. 68.

33.Techawattanawisal W, Nakahama K, Komaki M, et al. Isolation of multipotent stem cells from adult rat periodontal ligament by neurosphere-forming culture system. Biochem Biophys Res Commun 2007;357:917–23. 69.

34.Feng F, Akiyama K, Liu Y, et al. Utility of PDL progenitors for in vivo tissue regeneration: a report of 3 cases. Oral Dis 2010;16:20–8.

35. Luan X, Ito Y, Dangaria S, Diekwisch TG. Dental follicle progenitor cell heterogeneity in the developing mouse periodontium. Stem Cells Dev 2006; 15: 595-608.

36. Patil R, Kumar BM, Lee WJ, Jeon RH, Jang SJ, Lee YM, Park BW, Byun JH, Ahn CS, Kim JW, Rho GJ. Multilineage potential and proteomic profiling of human

dental stem cells derived from a single donor. Exp Cell Res 2014; 320: 92-107.

37. Volponi AA, Pang Y, Sharpe PT. Stem cell-based biological tooth repair and regeneration. Trends Cell Biol 2010;20:715–22. 71.

38.Handa K, Saito M, Tsunoda A, et al. Progenitor cells from dental follicle are able to form cementum matrix in vivo. Connect Tissue Res 2002;43:406–8. 73.

39.Handa K, Saito M, Yamauchi M, et al. Cementum matrix formation in vivo by cultured dental follicle cells. Bone 2002;31:606–11. 74.

40.Honda MJ, Imaizumi M, Suzuki H, et al. Stem cells isolated from human dental follicles have osteogenic potential. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;111:700–8. 75.

41. Guo W, Gong K, Shi H, et al. Dental follicle cells and treated dentin matrix scaffold for tissue engineering the tooth root. Biomaterials 2012;33:1291–302.

42.Guo W, He Y, Zhang X, et al. The use of dentin matrix scaffold and dental follicle cells for dentin regeneration. Biomaterials 2009;30:6708–23. 77.

43. Morsczeck C, Vollner F, Saugspier M, et al. Comparison of human dental follicle cells (DFCs) and stem cells from human exfoliated deciduous teeth (SHED) after neural differentiation in vitro. Clin Oral Investig 2010;14:433–40

44. Sameksha arora, ravneet malhi, saru khosla, shreya sabharwal, ankur sabharwal, vaibhav verma. Utilization of Stem Cells in Dentistry: A Review. International Healthcare Research Journal 2017;1(6):3-8.

45. Hass R, Kasper C, Böhm S,Jacobs R. Different populations and sources of human

mesenchymal stem cells (MSC): A comparison of adult and neonatal tissue-derived MSC. Cel Communication and Signaling 2011;9(12):1-14.

46.Bohl KS, Shon J, Rutherford B, Mooney DJ. Role of synthetic extracellular matrix in development of engineered dental pulp. J Biomater Sci Polym Ed, 1998;9:749-64.

47. George T, Sonoyama W, Shi S. The hidden treasure in apical papilla: the potential role in Pulp/ Dentin regeneration and BioRoot engineering. J Endod 2008;34(6):645-51.

48.Murray PE, Godoy FG, Hargreaves KM. Regenerative Endodontics: A Review of Current Status and a Call for Action. J Endod 2007;33(3):377–90.

49. Dewan RG, Kochhar R, Bhandari PP, Tyagi N. Regenerative Endodontics In The Light Of Recent Research. Indian Journal of Dental Sciences 2013;2(5):132-5.

50. Nakao K, Morita R, Saji Y, Ishida K, Tomita Y, Ogawa M, Saitoh M, Tomooka Y, Tsuji T (2007) The development of a bioengineered organ germ method. Nat Methods **4**: 227-230.

51. G. Bluteau, H-U. Luder, C. De Bari, T. A. Mitsiadis .stem cells for tooth engineering . European calls and material .vol 16,2008,pg 1-9.

52. Sangamesh N C, Ashish Aggarwal, Ankkita Chakarvarty, stem cells- an update Journal of Dental Sciences & Oral Rehabilitation : Jan-March 2012,