Role of Stem Cells in Dentistry: A Review

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Abstract

The current strategy of dental treatment is based on 3 elements- restore, retain and revitalize. Replacing the lost or diseased tissue with the same natural tissue is a challenge in the field of dentistry. Keeping this in mind, the use of stem cells has emerged as a potential way for its ideal replacement. Stem cells are undifferentiated cells, which can differentiate or multiply into particular types of cells and tissues. Besides stem cells, scaffolds and growth factor abandoned their role in the field of regeneration. Stem cells can be easily isolated from the orofacial region and have the property to differentiate into osteogenic, odontogenic, adipogenic, neurogenic components. This review article will discuss both embryonic and adult stem cells, sources, and their role in the process of regeneration along with tissue engineering strategy.

Keyword: *Stem Cells, Sources of Stem Cells, Tissue Engineering.*

Introductions

Stems cells are special and multitalented cell types that can divide indefinitely and have the capacity of self-replication to give rise to a peculiar cell type.[1] Research of Stem cell is a very challenging topic in the era of modern biomedical investigations and stands steadily at the core of "Regenerative medicine". Substantial use of stem cells in various fields for the process of tissue regeneration and/or repair has gained tremendous interest.It can radically change the treatment of some diseases like Parkinson's disease, Alzheimer's disease,lymphoma, leukemia, brain and spinal cord injuries, teeth missing, blindness and vision impairment, hearing loss, heart failures and even paralysis.[2,3] The investigators isolated a class of pluripotent stem cells from a human embryo in the year 1998 and grow them in culture.[1] The emerging problem faced by medical and dental practitioners is organ failure or tissue loss. Various prostheses or mechanical devices have been used to replace the original tissues but none of them restore the original form and function of the damaged or loosed tissues. Hence, the integrity of the host tissue is not properly maintained. Additionally, these prostheses can induce inflammatory reactions in the host tissue and can undergo wear upon prolonged use as well.[4] Stem cell development and its implementation in these cases dramatically reduced the problems as it restores the normal form and function of the original tissue.

During recent years stem cell research is being continued for the development of novel regenerative therapies in dentistry. This article is focussing on the basics of stem cells and their possible role in dentistry. Unspecialized cells found in all multicellular organisms, which under appropriate signal can generate cells with different and more restricted properties, are called a stem cell stem cell or progenitor cells. All the stem cells, regardless of their source, have three general properties: prolonged self-renewal capacity; they are unspecialized; asymmetric replication to become one of the many different types of cells on receiving the appropriate signals from their environments. Signals can be in the

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form of chemicals, e.g- growth factors, physical like the contact of cell with the adjacent cells, and contact with molecules in the environment.

Different sources of stem cells:

Sources can be either of the following:

1. Embryonic stem cells
2. Adult stem cells

Apart from these, stem cells can also be harvested from other sources like fetal stem cells, Umbilical cord stem cells, placental derived stem cells.

Embryonic stem cells (ESCs): ESCs- as the name suggests these stem cells are derived from the embryos, 2-11 days old (blastocytes).[5] These blastocytes contain 50-150 cells and differentiate into 3 germ layers:- endoderm, mesoderm, and ectoderm.[6] ESCs can grow in the laboratory and later used in research purposes. However, the inner cell layer of a fertilized embryo has been used for the cell culture. Legal or ethical consideration is required for this type of culture. Another variety of embryonic cells is Embryonic germ cells (EGCs). EGCs are pluripotent stem cells, originates from the primordial germ cells, give rise to gametes, and have the capacity to differentiate into all the three germ layers(4). Nevertheless, major drawbacks of this ESCs are they have the potential to induce tumorigenesis and immune rejection.[7,8]

Adult stem cells: Adult stem cells are otherwise called as postnatal or somatic stem cells. These cells are easier to isolate and no ethical or legal consideration is needed for this. The main goal of this adult stem cell is to maintain and repair the tissue of the concerned area and this cell founds in a particular area of the tissue called "stem cell niche". As mesenchymal tissues are the resident of most of the adult stem cells, they are togetherly called multipotent mesenchymal stromal cells (MSCs) or mesenchymal stem cells. Originally these were found in the bone marrow cells and are of 2 varieties a) hematopoietic stem cells and b) bone marrow stromal cells.[9] Other than bone marrow they can also be derived from the dental tissues, adipose tissues, skin, and other adult tissues.[10]

Sources of stem cell for tooth development: Epithelial-mesenchymal interactions lead to the formation of the tooth. Epithelial stem cells (EpSC) form ameloblasts, whereas Mesenchymal stem cells (MSC) give rise to osteoblasts, odontoblasts, cementoblasts, and fibroblasts of the periodontal ligament. These stem cells are influenced by chemical transmitters called signals.

Mesenchymal Stem Cells: For tissue engineering following are the mesenchymal progenitors that have been assessed.

Stem cells from human exfoliated deciduous teeth (SHED): Mesenchymal progenitors are mainly isolated from the pulpal connective tissue of deciduous incisors of humans.[11] They possess unique characteristics like self-renewal and multi- differentiation. Along with this, it can stimulate the formation of bone matrix in a lamellar pattern by organizing the host tissue.[12]

Adult dental pulp stem cells (DPSC): Authors have been investigated that adult dental pulp carries precursors can form odontoblast under suitable signals like pulp capping materials.[13] Pericytes found in the pulp can differentiate into both osteoblasts and odontoblasts. It also has the properties of self-renewal and self- differentiation. Another unique characteristic of DPSC is that it can differentiate into pro-osteoblasts even after 2 years of refrigeration, because the surface antigen present remained unchanged and give rise to dental tissue.[14]

Stem cells from apical papilla (SCAP): As the name suggest it is derived from the apical part of the human dental papilla. SCAP has higher proliferative capacity as well as higher efficiency than periodontal ligament stem cells. Along with the above it has an added advantage is that it can be easily isolated from human third molars. For immature stem cells developing dental papilla tissue provides greater source than the developed dental papilla tissues. SCAP has better in vitro proliferation capacity and also has superior regeneration capacity as compared to DPSCs.[15]

Stem cells from the dental follicle (DFSC): It has been observed after *in vivo* implantation of dental follicle cells new periodontal ligament, cementum, and alveolar have been formed.[16] Usually DFSCs are obtained from extracted 3rd molar at an early age.

Periodontal ligament stem cells (PDLSC): Progenitors present in the periodontal ligament have the property of self-renewal and can produce other tissues particularly cementum, periodontal ligament, and alveolar bone.[17] PDLSCs can also be obtained from the extracted tooth. The use of nitric oxide along with the proliferating PDLSC could be a challenging approach

for the management of periodontal diseases.[18] Authors have investigated the formation of PDL and cementum when PDLSCs were allowed to culture with apical tooth germ cells upon transplantation in mice.[19]

Bone marrow-derived mesenchymal stem cells (BMSC): It was observed BMSCs have the capability to produce cementum, periodontal ligament, and alveolar bone when these cells had implanted inside the deformed periodontal tissues. BMSCs have less odontogenic potential than DPSCs indicating all embryonic origin is quite different from each other.[20] They are closely related to bone morphology as well as with the pathology so mainly used in the regeneration process. These are mostly used in cases of regeneration as they have a very close relationship with the bone morphology and pathology. On the other hand it acts like microenvironmental modulators as it strives anti-inflammatory actions soon after transplantation.[21] BMSCs have tremendous effect in cases with osteopenia and bone defects using tissue engineering or cryotherapy.[22]

Tooth germ progenitor cells (TGPCs): TGPCs are spindle-shaped in morphology and have a high proliferation rate, usually isolated during the late bell stage from the dental mesenchymal tissue of 3 rd molar.[23] Unlike dental papilla stem cells TGPCs have odontogenic, osteogenic, neurogenic differentiation capacity.[24] A *in vitro* study showed TGPCs were capable of producing tube-like structures, signifying the confirmation of vascularization.[24]

Salivary gland derived stem cells (SGSCs): These stem cells arelined with epithelial cell, contains exocrine secretionand originates from endoderm. Mainly submandibular gland is selected from the isolation of stem cells as they have high proliferative capability and have the ability of expressing the associated markers.[25]

Epithelial Originated Dental Stem Cells: Epithelial derived dental stem cells are believed to be lost soon after the eruption of the tooth into the oral cavity. Enamel regeneration would be difficult as enamel producing ameloblasts are lost. Stem cell is the alternative approach for the creation of enamel surface. These stem cell types of stem cells can be isolated from-

1. **Epithelial stem cells from developing molars:** Epithelial stem cells usually isolated from the 3rd molar of juvenile or newborn animals.[26]
2. **Epithelial stem cell from rodent incisor:** An EpSC niche,found in the labial cervical loop of rodent incisor is accountable for the enamel matrix formation.[27] Since EpSC require implantation of rodent cells in the human mouth, it cannot be used in humans for treatment purpose.

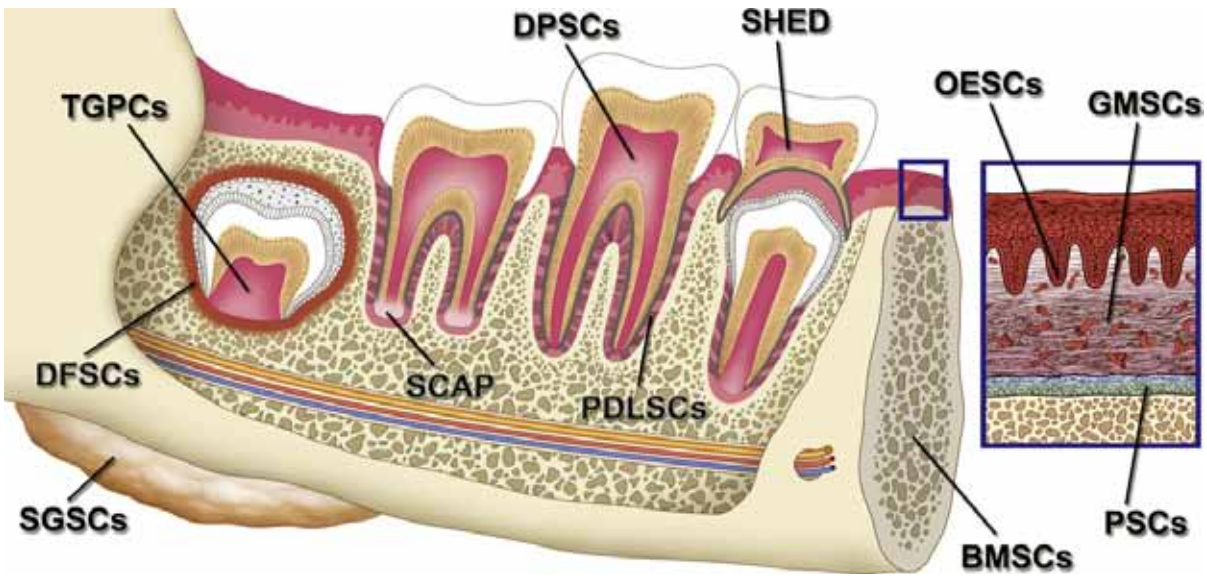

Fig. 1: Sources of adult stem cells:- Tooth germ progenitor cells (TGPCs), Dental follicle stem cells (DFSCs), Salivary gland derived stem cells (SGSCs), Stem cells from apical papilla (SCAP), Dental papilla stem cells (DPSCs), Periodontal ligament stem cells (PDLSCs), Stem cell from human exfoliated deciduous teeth (SHED).

Types of Stem Cell:

Stem cells can be totipotent, pluripotent, or multipotent.

Totipotent (omnipotent) can differentiate into any cell type.

Pluripotent stem cells- it can differentiate into nearly all cells, but not all cell types.

Multipotent stem cells can differentiate only into cells of a closely related family.

Identification of Stem Cells:

4 ways to identify the stem cell:

1. Fluorescent antibody cell sorting:-with the use of flow cytometer stem cells can be identified from clusters of different cells by staining the cell with antibody markers.
2. Immunomagnetic bead selection.
3. Immunohistochemical staining
4. By using physiological and histological criteria- like differentiation, proliferation, mineralizing activity.

Possible Uses of Stem Cell Technology:

Tissue engineering

Repair of defective tissues/organs

Delivery of gene therapies

Delivery of chemotherapeutic agents

Regenerative dentistry: Both mesenchymal and epithelial cells are required for the regeneration process or to laid down odontogenic cells. Mostly Pre natal germ cells are preferred over postnatal for regeneration because they possess high propensity regarding the genesis of normal tooth structure.[28]An injectable self hydrogel (autogenic stem cells) along with inorganic bone is used for temporomandibular joint regeneration.[28,29] Bioengineered TMJ can be constructed by using a biomimetic capsule that contains adult mesenchymal stem cells.[29] For regeneration of young permanent tooth apexification and apexogenesis are the ideal candidates, as they permit root development in both the lateral and vertical directions. Thus, improving the prognosis. A study has been suggested that DPSCs and BMSCs have the potential to induce dentinogenesis and osteogenesis respectively, as a result of the formation of dentin like tissues (30). SCAPs can also produce odontoblasts and the later is responsible for root completion in immature permanent tooth and deposit dentin like structures. When SHED is implanted inside the scaffold, it was able to produce odontoblast like cells.[31] It was observed by *Gotlieb EL et al,* that when SHED was incorporated into the polylactic acid scaffolds containing bone morphogenic protein 2 (BMP-2) and transforming growth factor (TGF-b1), produced structures that are similar to pulp tissue components.[32]

To date, tooth prosthesis has significantly lower characteristics in terms of esthetics and function as compared to natural teeth. Therefore the field of tooth engineering is an upcoming research project which uses stem cells to create a natural tooth by which replacing a missing tooth or missing part of a tooth and its attachment apparatus is facilitated. Proper isolation and culture of stem cells for both in vivo and in vitro conditions play an important role in tooth engineering.

Protocols for tooth engineering: Tooth regeneration is completely dependent on the close association of epithelial and mesenchymal cells along with the interaction of stem cells with the extracellular matrix molecule and scaffold to facilitate tooth engineering (fig-2). Usually scaffold is a crucial supporting structure that forms the framework for cellular function and behavior. Materialused in scaffold should not be toxic, less side effect, highly biocompatible, and should be easy to prepare. Undoubtedly, it might be said that scaffold material could indicate the direction for tissue engineering.[33]

The generation of periodontal tissues is dependent on four basic components. The appropriate signals, cells, body signals, and scaffolds (Fig-3). Collagen matrix type I, hydroxyapatite and tricalcium phosphate has been widely used in scaffold to increase the osteoinductive property.[34] Overall, tissue engineering is dependent on 3 essential elements: cells, scaffold, and growth factors (Fig-4).

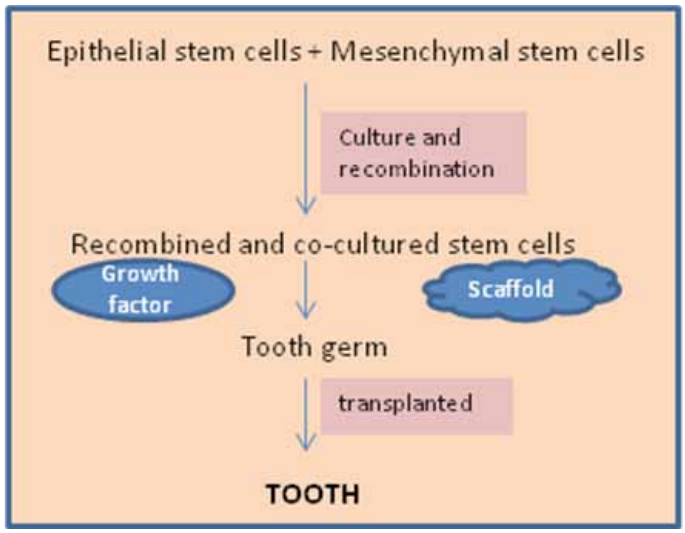

Fig 2: Formations of fully functional tooth after introduction of tooth germ into the alveolar bone.

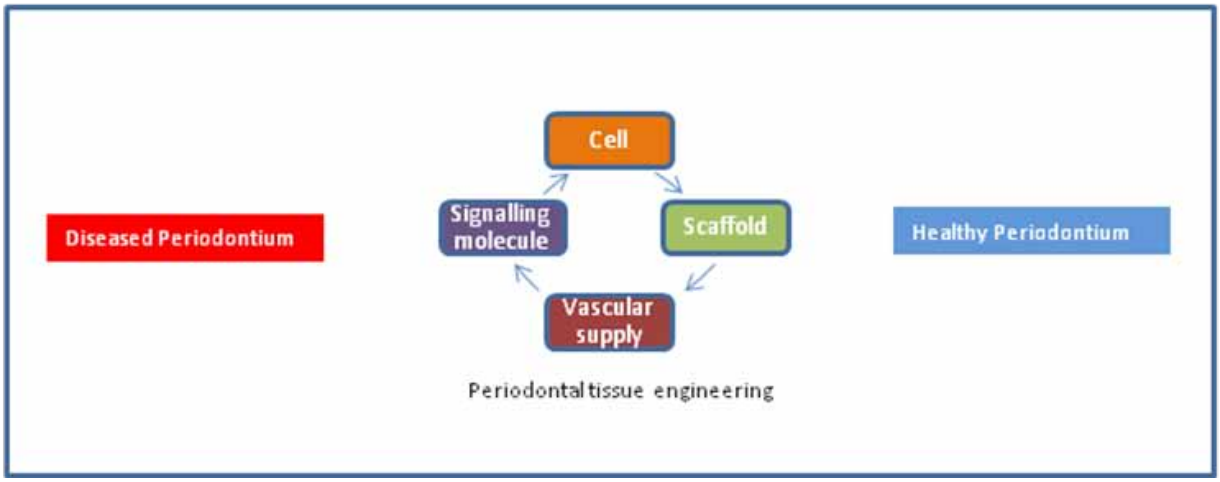

Fig 3: Necessary elements for periodontal tissue engineering

Tissue Engineering Strategies:

1. **Cell injection technique:** This approach involves the injection of talented cells, mainly stem cells inside the defects and it indicates regeneration of tissues over there. Some of the disadvantages of this technique are improper localization of injected cells and poor engraftment. To improve the localization and also to avoid communication with the immune system, the use of a delivery vehicle has been tested. The introduction of a delivery vehicle has been a successful approach as cells found inside the vehicle have the capacity of proliferation and differentiation.[35]

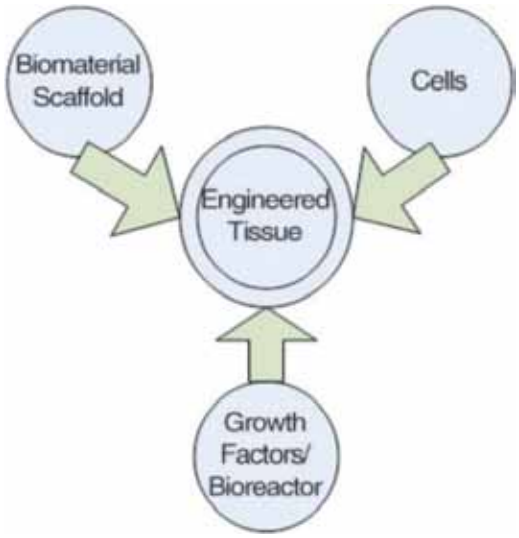

Fig 4: Triad of tissue engineering

2. **Cell induction therapy:** To get the better off from the cell injection technique, this technique was planned to induce osteoinductive property at the site of the requirement by enrolling the circulatory body cells. Any exogenous biological constituents should not be used for the process of osteogenesis.

3. **Cell seeded scaffolds:** This approach involves the isolation of a particular group of cells from a biopsy specimen, which was taken from the donor or recipient. Mostly, mesenchymal cells are preferred for this strategy. Oral mucosal or gingival derived mesenchymal cells are the best choice for this approach due to their immunomodulatory and anti-inflammatory properties. These MSCs can treat various immune-related diseases and help in the repair of wounds. Isolated cells are enlarged in culture medium and then seeded into the synthetic or artificial scaffold (Fig- 5), which holds the mesenchymal cells in position during their growth period and determines the shape of the tissue as well.[36] Here, cells differentiate, proliferate, and finally form the desired tissue. The new tissues formed are then implanted into the subjects.

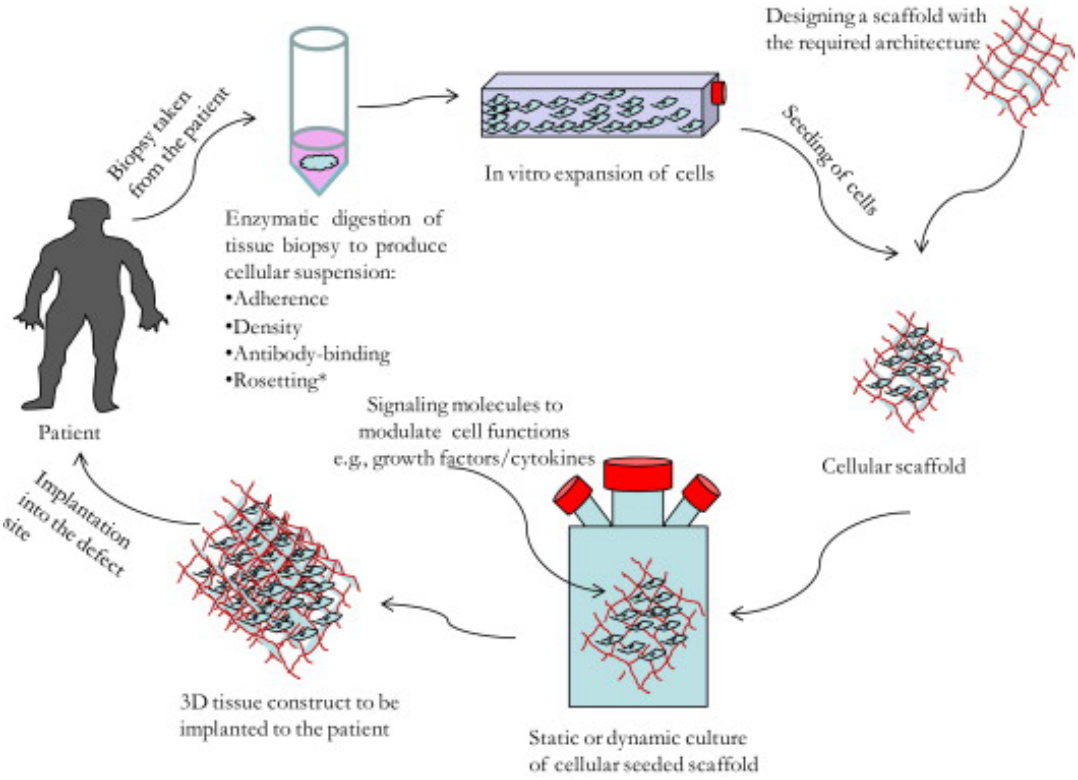

Fig. 5: Schematic presentation of cell-matrix tissue engineering strategy by Tomlinson et al (37).

Conclusion and Challenges

The use of stem cells in regenerative dentistry is capable of bringing quantum advances in treatment for our patients. Apart from some practical obstacles still to be overcome, the stem cell knowledge can be implemented in regenerative dentistry. More advancement in the field of stem cell research should be done upon which we can build future strategies for the tissue engineering of teeth. Stem cells have plenty of application in the field of dentistry, dental professionals must recognize the outcome of the emerging zone of regenerative dentistry and should implement those during conventional dental treatment. To make the nonvital tooth fully functional, researchers need to grow blood and nerve supply to that particular tooth. Stem cell brought many promising hope among the researchers. Shortly, stem cell is the future of dentistry.

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Ethical Permission: Approved

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References

1. Kumar S, Singh NP. Review Article Stem cells : A new paradigm. Indian J. Hum. Genet. 2006;12(27):4–10.
2. Yang B, Qiu Y, Zhou N, Ouyang H, Ding J, Cheng B. Application of Stem Cells in Oral Disease Therapy : Progress and Perspectives. Front Physiol. 2017;8:197.
3. Narang S, Sehgal N, Pradesh M. Stem cells : A potential regenerative future in dentistry Sources of Stem Cells Growth of Stem Cells in the Laboratory. Indian J Hum Genet. 2012; 18(2): 150–154
4. Chopra H, Hans MK, Shetty S. Stem cells-the hidden treasure : A strategic review. Dent Res J (Isfahan) 2013; 10(4): 421–427.
5. Nadig RR, Hills SM, Layout K. Stem cell therapy – Hype or hope ? A review. J Conserv Dent 2009;12:131-8.
6. Rai S, Kaur M, Kaur S. Applications of Stem Cells in Interdisciplinary Dentistry and Beyond : An Overview. Ann Med Health Sci Res. 2013;3(2):245-54.
7. Vyas S, Vyas K, Satish M, Shende V, Srivastav R. Stem Cells—The Future of Dentistry : A Review. J Indian Acad Oral Med Radiol. 2011; 23 (5): 370-72.
8. Mouli C, Kumar M, Senthil B, Pathiban S, Priya R, Subha R. J. Pharm. Sci. & Res. 2012; Vol.4(7): 1872 - 1876 Stem Cells in Dentistry- A Review . 2012;4(7):1872–6.
9. Locatelli F, Stefano PD. New insights into haematopoietic stem cell transplantation for patients with haemoglobinopathies. Br J Haematol. 2004;125(1):3-11
10. Ding DC, Shyu WC, Lin SZ. Mesenchymal Stem Cells. Cell Transplant. 2011;20:5–14.
11. 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; 13;100(10):5807-12.
12. BM Seo, W Sonoyama, T Yamaza, C Coppe, T Kikuiri, K Akiyama, et al. SHED repair critical-size calvarial defects in mice. Oral Dis. 2008; 14(5): 428–434.
13. About I, Bottero MJ, de Denato P, Camps J, Franquin JC, Mitsiadis TA. Human dentin production in vitro. Exp Cell Res. 2000; 258(1):33-41.
14. Papaccio G, Graziano A, d'Aquino R, et al. 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):319-325.
15. Egusa H, Sonoyama W, Nishimura M, Atsuta I, Akiyama K. Stem cells in dentistry--part I: stem cell sources. J Prosthodont Res. 2012;56(3):151-165.
16. Götz W, Schierholz J, Zeilhofer F, Kühn U, Möhl C, Sippel C, et al. Isolation of precursor cells (PCs) from human dental follicle of wisdom teeth. Matrix Biol. 2005; 24(2):155-65.
17. Bm S, Miura M, Gronthos S, Pm B, Batouli S, Brahim J, et al. Investigation of multipotent postnatal stem cells from human periodontal ligament.Lancet. 2004; 364 (9429): 149-155.
18. Orciani M, Trubiani O, Vignini A, Mattioli-Belmonte M, Di Primio R, Salvolini E. Nitric oxide production during the osteogenic differentiation of human periodontal ligament mesenchymal stem cells. Acta Histochem. 2009; 111(1):15-24..
19. Zh Y, Xj Z, Nn D, Zf M, Xu L, Jj W, et al. Apical tooth germ cell-conditioned medium enhances the differentiation of periodontal ligament stem cells into cementum/periodontal ligament-like tissues. J Periodontal Res. 2009;44(2):199-210.
20. Yu J, Wang Y, Deng Z, Tang L, Li Y, Shi J, et al. Odontogenic capability : bone marrow stromal stem cells versus dental pulp stem cells. Biol Cell. 2007; Aug;99(8):465-74,
21. Apoptosis LFTC, Akiyama K, Chen C, Wang D, Xu X, Qu C, et al. NIH Public Access. 2013;10(5):544–55.
22. Sui B, Chen J, Zhang X, He T, Zhao P, Zheng C, et al. Gender-independent efficacy of mesenchymal stem cell therapy in sex hormone-deficient bone loss via immunosuppression and resident stem cell recovery. Exp Mol Med. 2018; 50: 1-14.
23. Ikeda E, Yagi K, Kojima M, Yagyuu T, Ohshima A, Sobajima S, et al. Multipotent cells from the human third molar: feasibility of cell-based therapy for liverdisease. Differentiation. 2008; 76(5): 495-505.
24. Yalvac ME, Ramazanoglu M. Isolation and characterization of stem cells derived from human third molar tooth germs of young adults : implications in neo-vascularization, osteo-, adipo-

and neurogenesis. Pharmacogenomics J. 2010; 10 (2):105–13.

25. Kishi T, Takao T, Fujita K, Taniguchi H. Clonal proliferation of multipotent stem/progenitor cells in the neonatal and adult salivary glands. Biochem Biophys Res Commun 2006;340:544-52.
26. Honda MJ, Sumita Y, Kagami H, Ueda M (2005) Histological and immunohistochemical studies of tissue-engineered odontogenesis. Arch Histol Cytol 68: 89-101.
27. Harada H, Kettunen P, Jung HS, Mustonen T, Wang YA, Thesleff I (1999) Localization of putative stem cells in dental epithelium and their association with Notch and FGF signaling. J Cell Biol 147: 105-120.
28. 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.
29. 29.Shete M, Byakodi R, Kshar A, Paranjpe A. stem cell therapy in dentistry: An overview. IJSS case reports and reviews 2015; 1(8):50-54.
30. Batouli S, Miura M, Brahim J, Tsutsui TW, Fisher LW, Gronthos S, et al. Comparison of stem-cellmediated osteogenesis and dentinogenesis. J Dent Res. 2003;82:976–81.
31. Cordeiro MM, Dong Z, Kaneko T, Zhang Z, Miyazawa M, Shi S, et al. Dental pulp tissue engineering with stem cells from exfoliated deciduous teeth. J Endod 2008;34:962-9.
32. Gotlieb EL, Murray PE, Namerow KN, Kuttler S, Garcia-Godoy F. An ultrastructural investigation of tissue-engineered pulp constructs implanted within endodontically treated teeth. J Am Dent Assoc 2008;139:457-65.
33. Wang X, Luo Y, Yang Y, Zheng B, Yan F, Wei F, Friis T, Crawford RW, Xiao Y. Alteration of blood clot structure by bone substitute biomaterials (beta-tricalcium phosphate) significantly delays the bone healing process. J Mater Chem B Mater Biol Med. 2018. https://pubs.rsc.org/en/content/articlelanding/2018/tb/c8tb01747f#!divAbstract.
34. Park H, Choi B, Hu J, Lee M. Injectable chitosan hyaluronic acid hydrogels for cartilage tissue engineering. Acta Biomaterialia 2013;9:4779–86.
35. Vacanti JP, Langer R, Upton J, Marler JJ. Transplantation of cells in matrices for tissue regeneration. Advanced Drug Delivery Reviews 1998;33:165–82.
36. Tomlinson MJ, Tomlinson S, Yang XB, Kirkham J. Cell separation: terminology and practical considerations. Journal of Tissue Engineering 2012; 4:1–14.