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Application of Stem Cells in Endodontics

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Abstract

The discovery of stem cells in dental pulp has expanded our understanding concerning the various mechanisms that play a substantial role in the maintenance of dental pulp homeostasis to improve health and also regarding the pulp response to injury. Stems cells play a pivotal role in the physiology of the dental pulp tissue throughout the entire lifespan of the tooth. The ideology that these cells are intimately associated with regulating pulp angiogenesis as a response to cariogenic challenges is also inferred. In recent times, we are witnessing an increasing number of passionate individuals showing enthusiasm in the field of regenerative endodontics as stem cells exhibit potential benefits in dental pulp tissue engineering. The article here focuses predominantly on stem cells—the dental stem cells and non-dental stem cells which play an active part in benefitting pulpal necrosis tooth, the cardinal objective of these cells in the field of endodontic therapy, and the current, as well as the future role, stem cells could play in the clinical practice of endodontics eventually.

Keywords: *Dental stem cells, Pulpal necrosis, Stem cells, Regenerative.*

Introduction

Stem cells, also known as "progenitor or precursor" cells, can be defined as clonogenic cells that have the potential for multi-lineage differentiation as well as self-renewal capability [1]. It's possible to classify stem cells based on their origin.

1. Embryonic/fetal and,
2. Postnatal/adult stem cells.
3. As the name implies, embryonic stem cells are those cells that are taken from the inner cell mass of the early embryo, popularly known as 'blastocyst,' and can self-divide and self-renew for a long period without differentiating. On the contrary, adult stem cells are fetched from the mature tissue, which is property lineage-restricted and named depending on their tissue of origin. These cells are principally involved in the repair and regeneration of local tissue [2,3]. Stem cells can also be classified according to their mobility (Table 1) [4].

Table 1. Classification of Stem Cells

Totipotent stem cells	These are differentiated into all embryonic and extraembryonic cells
Pluripotent stem cells	These are differentiated into the type of cells except cells of the embryonic membrane
Multipotent stem cells	These are differentiated into more than one mature cell

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Adult Stem Cells

It is also well-known as somatic or postnatal stem cells; adult stem cells are multipotent and exhibit traits of differentiating into a limited number of cell lines. Adult stem cells play a functional role in the repair and regeneration of local tissues. The type of adult stem cells are described in Table 2.

Table 2. Types of Adult Stem Cells

Hematopoietic Stem Cells	Mesenchymal Stem Cells
These are multipotent primitive cells that have the potential to grow into various types of blood cells, such as the myeloid-lineage and lymphoid-lineage cells. Hematopoietic stem cells are present in different organs of the body, including the bone marrow, peripheral blood, and umbilical cord blood [5].	A type of adult stem cells, these are also called mesenchymal stromal cells. Being multipotent, these stem cells are found in the bone marrow, oral and maxillofacial structures, skin, and adipose tissue [5, 6].

There are many general mesenchymal stem cells, such as Adipose-derived stem cells, Umbilical cord-derived stem cells, (Table 3), Amniotic fluid-derived stem cells, Bone marrow-derived mesenchymal stem cells and Dental tissue-derived stem cells.

Table 3. Mesenchymal Stem Cells

Adipose-derived stem cells	Such cells are generally isolated from human fat using liposuction in most cases [7].
Umbilical cord-derived stem cells	As the name suggests, these cells are derived from the blood of the umbilical cord [5].
Amniotic fluid-derived stem cells	These cells have the potential for isolation from the aspirates of amniocentesis during genetic screening or collection at the time of delivery [5].
Bone marrow-derived mesenchymal stem cells	These stem cells have the pride of being the first successfully accomplished stem cell therapies. Also known as derived mesenchymal stem cells, peripheral blood stem cell collection is used more frequently than bone marrow aspiration in recent times [5].
Dental tissue-derived stem cells	Having the potential to be isolated from both, developing and permanent teeth, extracted or exfoliated teeth, tooth follicles, pulp and apical papilla, gingiva, and periodontal ligament, these stem cells could be termed as one of the most easily obtainable types of stem cells [4].

Induced Pluripotent Stem Cells

The ability of these cells to divide makes them comparable to embryonic stem cells, except for the fact that any ethical restraints do not bind them. Transfecting genes available in the embryonic stem cells to a donor cell using vectors produces induced pluripotent stem cells [8].

Dental Stem Cells

These are impressive with their accessibility potential as they surpass dental tissue-derived stem cells to be termed the most accessible stem cells.

The dental pulp of healthy teeth including both primary and permanent teeth, the periodontal ligament that’s inclusive of the apical region of the developing tooth, and other tooth structures are the most commonly used regions for isolating dental stem cells [9]. Dental hard tissue formation is possible with the involvement of two major cell types: epithelium-derived ameloblasts that are responsible for enamel formation and

mesenchymal-originated odontoblasts that oversee dentin production [10]. Various types of dental stem cells are listed in Table 4.

Table 4. Types of Dental Stem Cells

Dental pulp-derived stem cells (DPSC)	Secured from the pulp of permanent teeth, these are the commonest sources of dental tissue-derived stem cells. These are well-equipped to produce pulp dentin complex in-vivo and can also differentiate into odontogenic, osteogenic, adipogenic, myogenic, and neurogenic components both in-vivo and in-vitro [11]
Stem cells from human exfoliated deciduous teeth (SHED)	These cells are obtained from exfoliated teeth and exhibit better proliferation compared to DPSC. SHED can differentiate into adipogenic, neurogenic, and odontogenic components and prove beneficial in the field of tissue regeneration that involves orofacial bony structures [11]
Periodontal ligament stem cells (PDLSC)	Fetched from the separated periodontal ligaments of the third molars, there are progenitors present in PDLSC, which contribute toward self-renewal of oral structures, including cementum and bone [11]
Dental follicle stem cells (DFSC)	The third molars of the human teeth are surrounded by follicles that contribute toward these stem cells. Comprising of pluripotent characteristics, DFSCs are capable enough to differentiate into osteoblasts, adipocytes, and neuroblasts in-vitro and periodontal ligament in-vivo [12]
Stem cells from apical part of papilla (SCAP)	Every tooth in the developing stage consists of these cells that are present in their apical part. High proliferation, regeneration, and migration capabilities are some of the unique traits possessed by SCAP.
Human dental epithelial stem cells (hDESC)	These cells can be obtained in two ways—either from the third molars or the epithelial sheaths that disintegrate into the rest of Malasez [11]
Periosteum-derived stem cells	These cells are derived from the human periosteum and are multipotent with chondrogenic, odontogenic, myogenic, and adipogenic capabilities both in-vitro and in-vivo.
Salivary-gland derived stem cells	Capable of forming duct cells and acinar cells in-vitro, these stem cells are obtained from the salivary gland and possess high potential to produce mucin and amylase.

The regenerative property of human dental stem cells can be extremely helpful from the practical standpoint and in retrieving autologous dental stem cells. However, deriving a sub-population of stem cells can be quite challenging. Without a doubt, there are stem cells available in every single tooth, but the main barricade for obtaining them lies in the eligibility criteria—there are only some teeth that meet the required standards for extracting these cells. Extracting SHED from canines and deciduous incisors having no pathology but staying intact with at least one-third of the root remaining is possible. But, in the real-time world, it doesn't stop with one carious tooth but extends to many more in most clinical cases. Also, the longer the time taken for exfoliation of the tooth, the higher the resorption needed

for the root having no pulp, which in turn means zero availability of stem cells. Unlike the bone marrow that is replenished post-extraction, the DPSCs in adults is restricted only to the availability of the third molars [13].

According to recent publications, even the human inflamed pulps containing the mesenchymal stem/progenitor cells comprise regenerative potentials [14], and there are treasures of information yet to be discovered in connection to the inflamed periapical tissue. RS *et al*., when exploring various means to utilize somatic mesenchymal stem cells (MSCs) fetched from other sources with the help of a bio-mimetic dental pulp extracellular matrix (ECM)-incorporated scaffold, the researchers found the presence of odontogenic differentiation of PDLSCs and HMSCs independent of the exogenous addition of differentiation and growth factors which were a result of the stimulation of the dental pulp stem derived ECM scaffold. Epithelial-mesenchymal transition is also possible in the case of epithelial rests of Malassez (ERMs) [15].

Cai*et al*. researched about utilizing non-dental stem cells for dental treatments and came up with a unique methodology for growing teeth using stem cells derived from human urine [16]. In the laboratory, the research team made use of pluripotent stem cells (iPSCs) obtained from human urine in a group of mice to promote the growth of tooth-like structures and the team reported a success rate of around 30%. The generated teeth behaved just like the human teeth when comparing their physical properties but there was a huge difference witnessed in terms of the tooth's hardness—the resulting tooth exhibited only one-third of the hardness levels of a regular human tooth. Clinical application of dental stem [17] cells can be summarized in Table 5.

Table 5. Clinical Applications of Dental Stem Cells

Study design	Type of stem cells	Purpose of utilization	Procedure of obtainment	Outcome	Method of assessment	Clinical applications	Authors observation
Invitro	Mesencymal stem cells [MSCs]	Is to evoke bleeding from periapical tissue and influx MSCs into root canal system for mature teeth with apical lesions.	Canal has been opened debridement is done till the intra canal bleeding from apical region achieved and blood sample is collected in cartilage during LA.	MSCs found compartmentalized mainly within vasculature structures which is presented in lesion according immunohistochemical analysis.	Intracanal bleeding from the periapical tissues was achieved, and intracanal blood samples were collected. Total RNA was isolated and used as a template in quantitative reverse transcription polymerase chain reactions using MSC-specific arrays.	According to hypothesis that intracanal bleeding evoked by the over instrumentation of periapical tissues elicits the influx of cells with MSC markers into the root canal system of mature, fully formed teeth with apical lesions.	This study bleeding technique delivers undifferentiated MSCs into the root canal systems of adult patients with mature teeth. [18]
Invivo	Apical papilla and Dental pulp	To regenerate lost pulp and dentinal wall structure in the root canal region and regenerate vascularization and produce new dentin	Stem cells of apical papilla and dental pulp is isolated, characterized, seeded onto synthetic scaffolds consisting of poly-D, L-lactide/glycolide, inserted into the tooth root canal and injected into mice	The root canal space was filled with pulp-like tissue with vascularity and layer of dentin-like tissue was deposited onto the canal dentinal wall. There is formation odontoblast.	Histological analysis has been done to evaluate pulp/dentin regeneration using SCAP. As been examined after 3 months.	extracted human teeth has been horizontally sectioned. The root canal space was enlarged and sealed with MTA Cement. Root fragments were soaked at room temperature. Fragments were then rinsed in sterile PBS, soaked in PBS, and then incubated at 37°C for 3–7 days to remove residual sterilization agents and to ensure that there is no microbial contamination	The purpose of this study is to show pulp/dentin regeneration can be accomplished with progenitor cell–mediated tissue engineering approach.[19]

Invivo	dental pulp stem cell (DPSC)-derived ECM (DPM)	ECM-based cultured DPSCs has to be evaluated for dental pulp regeneration. DPM was used to imitate the original dental pulp niche, which was compared to a conventional polystyrene culture surface	Collection of extracted tooth, cell culture and identify, dish coated with DPM and immunoflurosence staining has been done and observed under electric microscope	DPM-based cultivation and could serve as a cell niche and modulate DPSC behaviour, also provided an alternative to harvest tissue-specific ECM and provided a strategy for ECM-cell interaction.	Histological analysis has been done DSP and DMP1 were used to identify whether the newly formed tissues followed the process of mineralization	The ECM has potential bioactive agents for inducing DPSC reaction, which affects repair and regeneration in dental pulp tissue. Involvement of DPM and DPSCs were further investigated by histological staining in vivo. The results demonstrated that the DPM influenced the structural integrity of the regenerated dental pulp ECM	DPM provides an advantageous environment for the DPSCs to preserve themselves before the implantation, and ECM plays an essential part in reconstructing the dental pulp tissue.[20]
Invitro	Periodontal ligament stem cells comparing with calcium silicate-based biomaterials	Biological interactions, that is cytocompatibility, cell differentiation and mineralization potential, between calcium silicate-based biomaterials and periodontal ligament stem cells (PDLSCs) which is clinical used in endodontic procedures involving direct contact with periodontal tissues.	Criteria involved periodontal ligament stem cells intervention, culture media treated with calcium silicate-based sealers or cement, comparisons/control, untreated culture media outcome. PDLSCs viability, proliferation, migration, differentiation and mineralization and study selection process	Calcium silicate-based sealers and Calcium sealers cements which is group of endodontic materials exhibit adequate cytocompatibility and favour the osteo/ cementog-enic differentiation and mineralization potential of PDLSCs, has been evidenced	Assess the biological interactions of PDLSCs with calcium silicate-based sealers (CSSs) and cements (CSCs) as inclusion. Evaluation of the ‘biological interaction’ of the cytotoxicity, cytocompatibility, cell plasticity or differentiation potential, and bioactive properties of PDLSCs cultured in CSC or CSS-conditioned media.	Calcium silicate sealer are used as root canal filling materials and calcium silicate cement referred to as ‘putty’ biomaterials are reserved for defect repair. These both biomaterial subsets will be placed in direct contact with biological tissues i.e one of cell population of periodontal tissue stem cells during endodontic procedures	Calcium silicate-based cement and sealers, as a group of endodontic materials, exhibit adequate cytocompatibility, osteogenic, cementogenic differentiation, and mineralization potential of human periodontal ligament stem cells, as evidenced in this study. Within the limitations of the in vitro nature of the included studies, this

							work supports their potential use in stem cell therapy and biologically based regenerative endodontic procedures.[21]
Invitro	collagen I gel containing isolated rat dental pulp cells (DPC)	Evaluate the ability of vascularized tissue to develop within a pulpless tooth using tissue engineering with stem cells	Non vital tooth with pulpless chamber, filled with collagen I gel containing isolated rat dental pulp cells (DPC) and angiogenic growth factors, was placed into a hole created in the femoral cortex or into its own tooth socket, respectively. Histological and biochemical characteristics of the tissue were evaluated for 4- and 8-weeks post-transplantation	Tooth revascularization and tissue generation was observed in the femur group, and role of vascular supply in tissue regeneration. The addition of cells and growth factors significantly promoted connective tissue production in the tooth chamber	Rats has been collected, dental pulp cells preparation, isolation, culturing is done, scaffolded growth factor preparation, two groups have been done as experimental group. Histological assessment has been done	As vascular and tissue regeneration can be induced in an empty root canal space as long as a blood clot does not impede tissue ingrowth but Direct access to a vascular supply together with addition of cell and growth factor will participate in regenerative situation.	As hypothesize that a direct vascular supply plays a pivotal role in tissue regeneration in an empty root canal. Study aims to evaluate the ability of vascularized tissue to develop within a pulpless rat incisor, to promote dental pulp cell (DPC) survival and to ultimately regenerate dental pulp tissue.[22]

Stem Cells Therapy in Endodontics

Apexogenesis or Apexification

Any irreversible pulpal injury can result in pulp necrosis which in turn leads to the infection of the root. If the patient is younger and has immature roots, there exists the slightest ray of hope that it's still possible to preserve at least some of the vital pulp tissue, which in turn goes ahead with the root formation but to do all this we need a much more conservative approach. But, if the entire root canal is fully infected, we have no choice but to go ahead with the endodontic treatment. In such cases, it becomes mandatory to remove the rest of the pulpal tissue up to the developing root apex, thereby cutting off any contact with the apical papilla. If the permanent tooth is an immature one, it's possible to regenerate the tissue into the apex using stem cells that are available in the SCAP, vital pulp tissues, PDL, or alveolar bone. In-vitro or in-vivo tissue regeneration also becomes possible when the stem cells and growth factors are seeded on scaffolds. The primary objective of using stem cells in the field of pulpal regeneration is mainly to take a conservative approach while treating immature permanent teeth. There are greater possibilities to increase the root length and thickness, thereby leading to total root formation when we make use of short-term regenerative endodontic procedures [23].

Advantages

1. It has a low risk of immune injection.
2. Not much risk of pathogenic infection.

Disadvantages

1. Very less case report has be published.
2. If tissue becomes infection there is changes of necrosis.

Pulp Revascularization

1. Any immature tooth affected by pulpal necrosis has the potential to obstruct root development due to caries or trauma, thereby leading to thinner root canal walls and blunderbuss apices.
2. Getting hold of a seal with conventional obturation techniques is quite problematic, which in turn makes root canal treatment a difficult affair primarily due to the unavailability of an apical stop. Such thin root canal walls increase the risk of fracture multifold times.
3. In the absence of intra-pulpal infection and scaffold and when the environment is favorable to tissue growth, there are higher chances for pulpal tissue regeneration of immature teeth that are infected.
4. Such circumstances pave the way for the repopulation of mesenchymal cells that erupt from the dental papilla or apical periodontium [24, 25].

Whole Tooth Regeneration

By seeding various cell types on biodegradable scaffolds, we've had successful regeneration of tooth-like tissues. In-vitro harvesting, growing, and differentiation of cells and seeding cells onto scaffolds for their in-vivo implantation has been a common technique followed for regeneration. There have also been times when the scaffolds have been once again re-implanted onto the extracted tooth socket or the jaw [26].

Stem Cell Therapy

In stem cells of dental pulp which has multipotent stem cells in the dental pulp and have their phenotype in peri vascularity. Studies has shown that DPSCs and SHED develop from the same origin, that is, dental pulp**.** SHED and DPSC cells are capable of regenerating dentin and pulp-like tissues. The stem cells present as autologous or allogenic stem cells, and these are delivered to the tooth via injection with the help of matrix [27]. Major advantages and disadvantages of this method are.

1. It is a quick method.
2. Very easy delivery into tooth or root canal.

3. It has less pain.
4. The cells can be easily harvest.
5. Disadvantage.
6. Cell survival rate is low.
7. The cells don't produce new functional pulp.
8. Risk of complication.

Objectives of Stem Cells Therapy in Endodontics [28]

A majority of the research focusing on stem cell therapy primarily aims for the below-given targets:[29]

1. Regeneration of pulp dentin complex.
2. Regeneration of damaged coronal dentin.
3. Regeneration of resorbed root, cervical or apical dentin, and repair perforations.
4. Whole tooth regeneration.

Banking of Dental Stem Cells

The fundamental goal of stem cell therapy includes harvesting and storing these cells safely until and unless their need arises due to an accident or disease. Research data obtained until now have clear indications pointing toward the use of primary teeth for stem cell therapy in the field of regenerative medicine when compared to the use of orthodontic, wisdom, or extracted teeth [30].

Collection, Isolation, and Preservation

Step 1. Tooth Collection

As mentioned above, primary teeth are better choices for banking as they are composed of healthy pulp in comparison to permanent teeth after orthodontic extraction, wisdom tooth, or bicuspid tooth.

The first step involves keeping the selected tooth in some liquid, either fresh milk, a sterile solution, or a frozen gel pack to transfer it safely to the laboratory. Blood flow to the tooth until the time of extraction is mandatory, and this is evident when the exfoliated tooth comprises of red coloration of the pulp, indicating decent blood flow. The Gray coloration of the pulp is a clear indication that the blood flow to the tooth has been compromised. Such teeth can no longer be recovered as they are necrotic. Once the tooth recovery is successfully made, it can be transferred to a hypotonic phosphate-buffered saline solution that stops any drying up of the tissues during transportation. It is possible to transfer only up to four teeth in one vial. Once the transfer is done, the vial is tightly sealed with utmost care and placed into a thermette (a temperature phase change carrier) which, in turn, is placed into an insulated metal transport vessel. All these steps must be followed diligently to maintain the sample in a hypothermic state during transportation, and the whole procedure is referred to as 'sustentation' [5, 29].

Stem Cell Isolation

Once the kit or vial safely reaches the tooth bank, every cell inside it is patiently isolated, and the tooth surface is thoroughly cleaned using different disinfectants and by maintaining a strict protocol. The pulp tissue is extracted from the pulp chamber, and an MSM medium is used to culture the cells by maintaining suitable corrections [29]. It's possible to extract various cell lines, including adipogenic, odontogenic, and neural ones, by bringing variations to the MSC medium. In case contaminations are higher than recommended, procedural changes using STRO 1 or CD 146 can be done [29]. The total time taken from the moment the cells are harvested to the time until they reach the processing facility must not be greater than 40 hours.

Stem Cell Storage

Either approach given below can be used for storing the stem cells.

Cryopreservation

This technique involves preserving cells in liquid nitrogen vapor at a temperature of −150° to preserve their latency and potency. When done this way, there are higher chances of storing the cells for longer and keeping them viable for use [29].

Magnetic Freezing

The object is chilled below the freezing point with the help of a Cell Alive System (CAS) that makes use of a magnetic field. This way, there is even distribution of low temperatures causing no damage to the cell wall. As per Hiroshima University, following such a procedure helps in elevating the cell survival rate of the teeth to up to 83% [12, 29].

Technique of Stem Cell Identification

The stem cells are stained with specific antibody markers using a flow cytometer it's also called as fluorescent antibody cell sorting (FACs) [30].

Advantages of Flow Cytometry

1. It has the ability to escape dead cells and identify the cell size and structure.
2. The dentin thickness is checked in the root canal after cleaning and shaping.

Immunohistochemical staining: It uses the antibody-based method to detect a specific protein in the sample.

Physiological and histological criteria: These includes phenotyping, chemotaxis, proliferation, differentiation, and mineralizing.

Current Trends and Future Perspectives of Stem Cells Regeneration

Figuring out a well-suited autologous stem cell source in humans is the foremost requirement when we want to make use of tooth regeneration technology, and this is already discussed above. Concerning this, pulpal stem cells owing to their ability to differentiate into dental epithelial and mesenchymal cells might be a suitable cell source for regeneration. These cells can be obtained from the patient's very own somatic cells. It's possible to bank exfoliated deciduous teeth, third molars, teeth extracted for orthodontic reasons, and supernumerary teeth as the dental stem cells remain in the viable state in the frozen tissues. Individuals can avoid any ethical or immunological issues arising from the use of allogeneic cells by relying upon their cell tissues. The high proliferative nature of the SHED or the stem cells from the immature third molar teeth and the increased occurrence of traumatic dental injuries in the early permanent dentition makes these cells a preferred choice for banking them.

The future is full of surprises, and in the times to come, it's even possible that gene therapy could be the key to regenerating dental tissues using genetically altered cells to deliver physiological-specific growth factors. Research for identifying exact novel genes and coming up with a suitable vehicle to deliver the specific cells safely is in the infancy stage. But incorporating all the ethical constraints involved in using gene therapy and coming up with in-vivo clinical applications to regenerate dental tissues remain a distant dream [31].

Conclusion

The ability of the dental pulp cells to differentiate into odontoblasts and the possibility of regenerating the dental pulp have paved the way for a plethora of strategies that help in the revitalization of necrotic teeth. Somatic cells that can be extracted from one's a deciduous tooth or permanent tooth must be preserved carefully as these could help in endodontic treatment. To achieve this, the clinician or the endodontist involved must possess knowledge about the current concept of stem cells and impart the same to their patients.

Conflict of Interest

There is no conflict of interest.

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References

[1] Fischbach, G. D., Fischbach, R., L., 2004, Stem cells: science, policy, and ethics. *The Journal of clinical investigation*, 114(10):1364-1370.

[2] Ashri, N. Y., Ajlan, S. A., & Aldahmash, A. M., 2015, Dental pulp stem cells. Biology and use for periodontal tissue engineering. Saudi medical journal, 36(12), 1391–1399.

[3] Almeida, P. N., Cunha, K. S., 2016, Dental stem cells and their application in Dentistry: a literature review. Revista Brasileira de Odontologia, 73(4):331.

[4] Rao M. S., 2004, Stem sense: a proposal for the classification of stem cells. Stem cells and development, 13(5), 452–455.

[5] Rai, S., Kaur, M., & Kaur, S., 2013, Applications of stem cells in interdisciplinary dentistry and beyond: an overview. *Annals of medical and health sciences research*, 3(2), 245–254.

[6] Lin, L. M., & Kahler, B., 2017, A review of regenerative endodontics: current protocols and future directions. *Journal of Istanbul University Faculty of Dentistry*, 51(3 Suppl 1), S41–S51.

[7] De Ugarte, D. A., Morizono, K., Elbarbary, A., Alfonso, Z., Zuk, P. A., Zhu, M., Dragoo, J. L., Ashjian, P., Thomas, B., Benhaim, P., Chen, I., Fraser, J., & Hedrick, M. H., 2003, Comparison of multi-lineage cells from human adipose tissue and bone marrow. Cells, tissues, organs, 174(3), 101–109.

[8] Takahashi, K., & Yamanaka, S., 2006, Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. Cell, 126(4), 663–676.

[9] Caplan A. I., 1991, Mesenchymal stem cells. *Journal of Orthopaedic Research*: official publication of the Orthopaedic Research Society, 9(5), 641–650.

[10] Bluteau, G., Luder, H. U., De Bari, C., & Mitsiadis, T. A., 2008, Stem cells for tooth engineering. *European cells & materials*, 16, 1–9.

[11] Mitsiadis, T. A., Orsini, G., & Jimenez-Rojo, L., 2015, Stem cell-based approaches in dentistry. *European cells & materials*, 30, 248–257.

[12] Huang, Y. H., Yang, J. C., Wang, C. W., Lee, S. Y., 2010, Dental stem cells and tooth banking for regenerative medicine. *J ExpClin Med*, 2(3):111-117.

[13] Ravindran, S., Huang, C. C., & George, A., 2014. Extracellular matrix of dental pulp stem cells: applications in pulp tissue engineering using somatic MSCs. *Frontiers in physiology*, 4, 395.

[14] Alongi, D. J., Yamaza, T., Song, Y., Fouad, A. F., Romberg, E. E., Shi, S., Tuan, R. S., & Huang, G. T., 2010, Stem/progenitor cells from inflamed human dental pulp retain tissue regeneration potential. Regenerative medicine, 5(4), 617–631.

[15] Xiong, J., Gronthos, S., & Bartold, P. M., 2013, Role of the epithelial cell rests of Malassez in the development, maintenance, and regeneration of periodontal ligament tissues. Periodontology 2000, 63(1), 217–233.

[16] Cai, J., Zhang, Y., Liu, P., Chen, S., Wu, X., Sun, Y., Li, A., Huang, K., Luo, R., Wang, L., Liu, Y., Zhou, T., Wei, S., Pan, G., & Pei, D., 2013, Generation of tooth-like structures from integration-free human urine induced pluripotent stem cells. Cell regeneration (London, England), 2(1), 6.

[17] Laird, D. J., De Tomaso, A. W., & Weissman, I. L.,2005, Stem cells are units of natural selection in a colonial ascidian. Cell, 123(7), 1351–1360.

[18] Chrepa, V., Henry, M. A., Daniel, B. J., & Diogenes, A., 2015, Delivery of Apical Mesenchymal Stem Cells into Root Canals of Mature Teeth. Journal of dental research, 94(12), 1653–1659.

[19] Huang, G. T., Yamaza, T., Shea, L. D., Djouad, F., Kuhn, N. Z., Tuan, R. S., & Shi, S., 2010, Stem/progenitor cell-mediated de novo regeneration of dental pulp with newly deposited continuous layer of dentin in an in vivo model. Tissue engineering. Part A, 16(2), 605–615.

[20] Zhang, X., Li, H., Sun, J., Luo, X., Yang, H., Xie, L., Yang, B., Guo, W., & Tian, W., 2017, Cell-derived micro-environment helps dental pulp stem cells promote dental pulp regeneration. Cell proliferation, 50(5), e12361.

[21] Sanz, J. L., Guerrero-Gironés, J., Pecci-Lloret, M. P., Pecci-Lloret, M. R., & Melo, M., 2021, Biological interactions between calcium silicate-based endodontic biomaterials and periodontal ligament stem cells: A systematic review of in vitro

studies. *International Endodontic Journal*, 54(11), 2025–2043.

[22] Srisuwan, T., Tilkorn, D. J., Al-Benna, S., Abberton, K., Messer, H. H., & Thompson, E. W., 2013, Revascularization and tissue regeneration of an empty root canal space is enhanced by a direct blood supply and stem cells. Dental traumatology: official publication of International Association for Dental Traumatology, 29(2), 84–91.

[23] Friedlander, L. T., Cullinan, M. P., & Love, R. M., 2009, Dental stem cells and their potential role in apexogenesis and apexification. *International Endodontic Journal*, 42(11), 955–962.

[24] Kottoor., J, 2013, Biomimetic endodontics: barriers and strategies. Health Sciences, 2(1):JS007.

[25] Ding, R. Y., Cheung, G. S., Chen, J., Yin, X. Z., Wang, Q. Q., & Zhang, C. F., 2009, Pulp revascularization of immature teeth with apical periodontitis: a clinical study. *Journal of endodontics*, 35(5), 745–749.

[26] Gandhi, A., Gandhi, T., 2011, Madan N. Dental pulp stem cells in endodontic research: a promising tool for tooth tissue engineering. RSBO. 8(3):335-40.

[27] Harikumar., Kavitha, A., Jayaprada, J., Shetty, S.R.,2010, Regenerative Endodontics. *Ind J Dent Adv*, 2(2):203-209.

[28] Shi, S., Bartold, P. M., Miura, M., Seo, B. M., Robey, P. G., & Gronthos, S., 2005, The efficacy of mesenchymal stem cells to regenerate and repair dental structures. Orthodontics & craniofacial research, 8(3), 191–199.

[29] Arora, V., Arora, P., & Munshi, A. K., 2009, Banking stem cells from human exfoliated deciduous teeth (SHED): saving for the future. *The Journal of clinical pediatric dentistry*, 33(4), 289–294.

[30] Goldstein, G., Slizys, I. S., & Chase, M. W., 1961, Studies on fluorescent antibody staining. I. Non-specific fluorescence with fluorescein-coupled sheep anti-rabbit globulins. *The Journal of experimental medicine*, 114(1), 89–110.

[31] Baranwal, K., A, 2015, Regenerative Stem Cells for Endodontic Tissue Engineering: Past, Present and Future. Ann ProsthodontRestor Dent, 1(1):20-5.