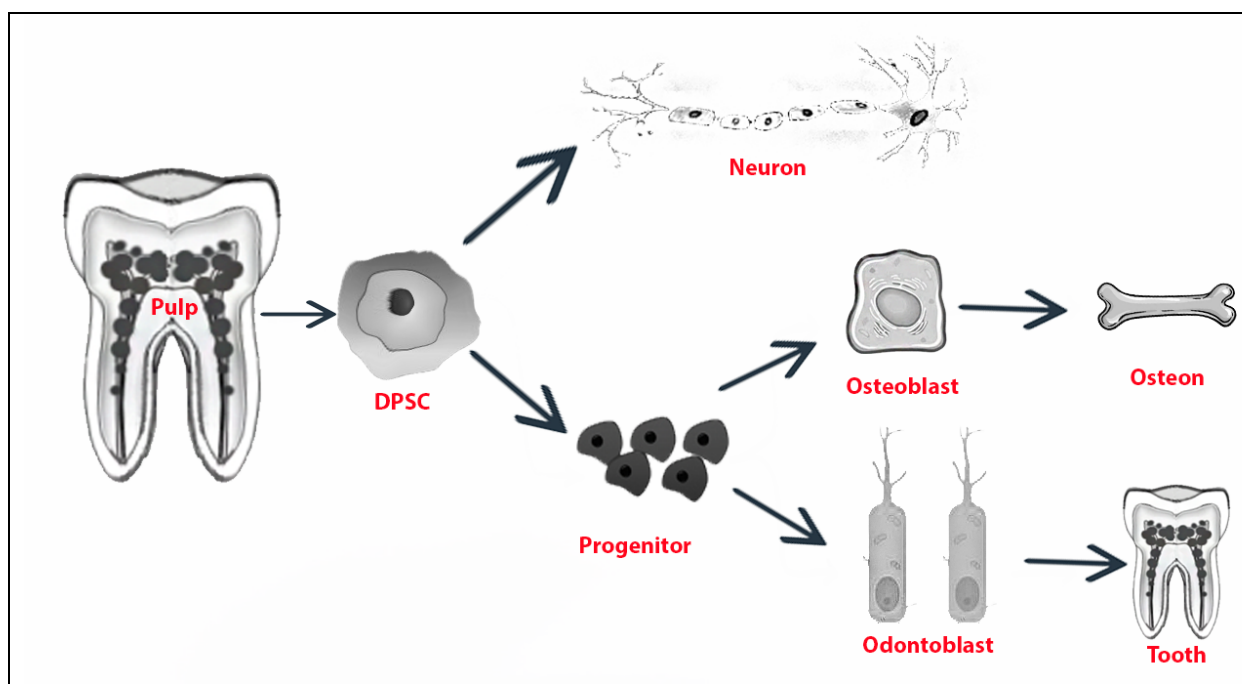


A Review of Dental Pulp Stem Cells in Permanent Teeth.

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Abstract:

Dental pulp stem cells (DPSCs) are considered as a very suitable source of cells for tissue engineering and regenerative medicine.

They can be easily available, accessible and have a close relationship with dental tissues. It has been proved that DPSCs are capable of differentiating into various cell types, which include odontoblasts, osteoblasts and cementoblasts. This makes them potential candidates for the regeneration of damaged or lost teeth and other oral and craniofacial tissues.

The authors of this article made a research and review the current knowledge on DPSCs, including their separation, characterization, and differentiation potential. They also have

discuss the potential applications of DPSCs in tissue engineering and regenerative medicine.

The authors conclude that DPSCs have a very potential for the treatment of many oral and craniofacial diseases. But still further research is needed to optimize the separation, culture, and differentiation of DPSCs and evaluation of their safety and efficacy in clinical trials.

Introduction:

Due to the fact that the oral environment provides a suitable habitat for the growth of microbes, almost all people around the world are at risk of dental caries, periodontal diseases, and tooth loss. In some congenital syndromes and diseases, some teeth (hypodontia) or all teeth are missing (anodontia). Available treatments include artificial tooth replacement with various types of prosthetics and implants, each having its own advantages and disadvantages, and not providing complete and ideal dental functionality and aesthetic [1, 2].

The use of stem cells in treating various diseases has ushered in a new era in tissue engineering. In the field of identification, isolation, proliferation, and application of dental stem cells, extensive research has been done and many successful results have been achieved in this field. With the emergence and development of stem cell science, dental researchers have been thinking about generating tooth buds and implanting them in the site of lost teeth. Stem cells are multipotent cells capable of differentiating into various cell types and have the ability to replace damaged and lost tissues in different parts of the body [3]

All human tissues contain stem cells and (or) precursor cells, which become active in response to growth, development, or tissue repair following disease or trauma. Recently, dental tissues such as periodontal ligament (PDL), dental pulp, and dental follicle have been recognized as available sources of undifferentiated cells [4].

Stem cells extracted from teeth are able to generate various types of tissues, such as bone, cartilage, and nerves. Therefore, these dental stem cells can be used for the regeneration of the entire body. For example, these cells can be used to regenerate knee cartilage in someone who has lost it due to sports-related injuries [5].

Dental stem cells have gained attention for new treatment approaches for diseases such as periodontitis and dental caries, as well as for encouraging dental pulp repair, craniofacial bone regeneration and dental structure construction. These cells are easily accessible and, unlike mesenchymal stem cells in bone marrow, have a much closer relationship with dental tissues, and it is better to use these cells in tissue engineering for the production of dental tissues [6].

Unlike dental epithelial stem cells, undifferentiated ectomesenchymal cells of tooth, do not destroy completely after tooth growth in human. Currently, even at the age of 90, it is possible to isolate precursor stem cells from the permanent teeth pulp. The most prominent feature of DPSC cells is their ability to regenerate the complex pseudo-pulp-dentin, which is composed of a mineralized matrix and tubules lined with odontoblasts and fibrous tissue containing blood vessels. This structure is similar to the natural dentin-pulp complex found in humans [7].

A study in 2008 demonstrated the positive effect of enamel matrix proteins on the differentiation of bone marrow mesenchymal stem cells (BMSCs) into Cementoblast [4].

However, a study which had been done by Minguchuan Yu et al. in Dec 2022, reports:

Bone marrow mesenchymal stem cells (BMSCs)-derived exosomes and microvesicles can effectively improve knee osteoarthritis. We found that microvesicles performed a superior effect on improving mitochondrial function in chondrocytes than exosomes, which may be related to the ability of microvesicles carrying active mitochondria to replace damaged ones in chondrocytes[8].

Another study which had been done in 2021 Says that for bone tissue engineering, DPSCs have shown a higher proliferation capacity compared to BMSCs. Other sources of mesenchymal stem cells are used for hard tissue regeneration; however, the side effects of there isolation methods have limited their practical application. As

mentioned, DPSCs are an easily accessible source of mesenchymal stem cells that possess similar characteristics to bone marrow stem cells for the regeneration of hard tissues. Due to their accessibility and significant differentiation potential, the use of these cells in tissue engineering has gained significant attention in recent years. These cells have shown remarkable results in vivo studies for the treatment of muscular dystrophy, corneal injury, limb ischemia, alzheimer and parkinson disease. They have been extensively studied for bone tissue engineering in animal models and have shown positive outcomes. Considering their high differentiation capacity, safe transport to target tissues, and the creation of a stable but effective environment for their differentiation is important [9].

Therefore, all tissues originate from stem cells. In this way, stem cells can play a very important role in the regeneration and replacement of body lost tissues in medicine and dentistry science [10].

Despite the successful achievements in various tissue regeneration, such as bone defects [11], blood vessel [12], and cartilage in medical science field [12] [Chen, 2006 #33; Rafii, 2003 #28], and despite the considerable efforts and work done in the past, the possibility of successful complete regeneration of tooth buds in dentistry has not been accompanied and tissue engineering has opened a new door for researchers in this field by utilizing dental stem cells [13].

With the emergence and development of stem cell science, dental researchers have been considering the production and transplantation of tooth buds in the region of lost teeth. Despite the positive steps that have been taken in this field, no one has yet succeeded in achieving this important goal in human.

Jalili M. et al in iran regenerated a tooth bud in Rabbits jaw in 2022 [14].

Before it, researchers have been able to produce bone tissue and dentin-pulp complex in a laboratory setting using stem cells from teeth. However, the regeneration of enamel, cementum, and periodontal fibers has not been achieved.

Therefore, in this study, we intend to review the activities that has been done so far in the field of application of dental stem cells in tissue engineering, as well as the repair and reconstruction of dental tissues.

Definition:

Tissue engineering:

Today, researchers are paying a great deal of attention to stem cells. The use of these cells for tissue repair and regeneration is made possible through a scientific field called tissue engineering.

Tissue engineering is a scientific field in which the design and construction of new tissues are carried out to replace lost parts of the body (due to cancer, trauma, disease, etc.). In this science, three key components are used for tissue replacement, which include: 1- Scaffold or extracellular matrix, 2- Signals for morphogenesis (differentiation factor), and 3- Stem cells.

Scaffold:

Scaffold is a structure that provides a three-dimensional biological and physicochemical microenvironment for cell growth, differentiation, adhesion, and migration. Scaffold is used as a carrier for morphogenetic factors in protein therapy and as a carrier for cells in cell therapy.

Growth factors, counting bone morphogenetic protein 2 (BMP 2), are utilized to improve the scaffold properties. A consider in 2018 utilized mixing and freeze drying strategies to create a BMP 2 nHAP COL scaffold [15].

Growth factors:

Growth factors are proteins that bind to their receptors on the cell and induce cell proliferation or differentiation. Many growth factors are pleiotropic, meaning they stimulate cell division or differentiation in multiple cell types, while some others are specific to certain cells.

Mohammed A Mansour et al. in 2021 reported that: Growth factor receptors (GFRs) and receptor tyrosine kinases (RTK) are groups of proteins mediating a plethora of physiological processes, including cell growth, proliferation, survival, differentiation and migration [16].

Some of the bioactive molecules that have been effective in forming dentinal bridge, production of reparative dentin and precursor stem cells stimulation include the following cases:

- Dentin Matrix protein, Bone sialoprotein, Bone Morphogenetic protein BMP2[17]
- Amelogenin[218]

Stem cells:

Stem cells are multipotent cells that have the ability to differentiate into various types of cells and have the capability to replace damaged and lost tissues in different parts of the body.

Progenitor stem cells of matured cells are present in various tissues such as bone marrow, peripheral blood circulation, brain, spinal cord, dental pulp, blood vessels, skeletal muscle, skin, cornea, retina, pancreas, liver, digestive system, umbilical cord blood, umbilical cord, adipose tissue, and other tissues in the body [19,20].

A stem cell is the cell that has the ability to continuously divide and produce precursor cells for differentiation into various types of cells or tissues. A cell that has the power of self-replication (producing similar cells) and minimal differentiation into two separate cell types (multi-lineage differentiation) is considered as a stem cell and these two features are essential for stem cells.

Progenitor/precursor cell:

The property of replication means that a stem cell can convert into an undifferentiated daughter cell similar to their own self, after division to preserve their own generation without any change in their initial characteristics to preserve their own generation without any change in their initial characteristics[21].

Therefore, a cell like a preosteoblast that can only differentiate into an osteoblast and not other mesenchymal lineages like chondrocytes, adipocytes, and it cannot even undergo reverse differentiation and transform back into a mesenchymal stem cell, is not considered a stem cell and is called a progenitor cell or precursor cell.

Types of Stem Cells:

Stem cells are divided into three categories based on their origin:

- 1- Embryonic stem cells derived from embryonic gonads.
- 2- Extra-embryonic stem cells derived from the umbilical cord blood.
- 3- Stem cells of adults (after birth)

Due to ethical and legal issues, the use of embryonic stem cells, despite their high potential for differentiation, has been restricted and there is a greater willingness to use postnatal stem cells of adults in tissue engineering. [2]

History:

In a study conducted by Grontowicz et al. in 2002, the isolation of human embryonic germ cells from fetal gonadal tissue was reported, and they were named primordial germ cells. Then, they obtained pluripotent stem cell lines from embryonic cells.

The medical treatment application of postnatal stem cells began in 1968, when the first successful allogeneic bone marrow transplant was used to treat severe combined immunodeficiency. Since 1970, bone marrow transplants have been successfully used to treat leukemia, lymphoma, various anemias, and genetic disorders.

All human tissues contain stem cells and/or precursor cells after birth, which become active in response to growth, development, or during tissue repair following disease or trauma. Recently, dental tissues such as the periodontal

ligament (PDL), dental papilla, and dental follicle have been identified as available sources of undifferentiated cells [22].

Treatment of lesions and tooth diseases by cell therapy can be performed by a combination of non-dental mesenchymal stem cells and dental stem cells. A study in 2015 says that the dental caries is one of the most widely prevalent infectious diseases in the world. It affects more than half of the world's population. The current treatment of pulp necrosis caused by dental caries is root canal treatment. This treatment can cause weakness and loss of sensation of the tooth, as well as making it more susceptible to secondary infection. [23].

A recent study has shown that the use of mesenchymal stem cells and platelet-rich plasma in periodontal pockets leads to a 4mm reduction in probe depth and the attainment of 4mm clinical attachment and elimination of tooth mobility and gingival bleeding.

Jordan M Iliopoulos et al. found that : Mesenchymal stem cells (MSCs) have been shown to play a role in causing disease or regeneration in the periodontium and play an important role in homeostasis of periodontal tissue [24].

In addition, in another study, it was shown that cells derived from bone marrow rich in c-kit⁺ can be converted into ameloblast-like cells. These studies are progressing day by day, but it seems that for tissue regeneration of dental tissues, dental stem cells may be more desirable than bone marrow stem cells, as they have a closer resemblance to mature dental tissues cells[25].

When researchers transplanted periodontal ligament stem cells (PDLSCs) into the neutral mouse body, they were able to create structures similar to cementum and PDL. This structure resembled natural PDL with a thin layer of cementum and tightly woven surface collagen fibers similar to Sharpey's fibers. Therefore, PDL stem cells have the potential to produce periodontal structures such as cementum and PDL. [21]

Autoradiographic studies with the use of triad thymidine have confirmed that after severe pulp injury due to trauma or accidental mechanical exposure or decay exposure, the adjacent odontoblasts near the wound site as well as the odontoblastoid cells in the cell-rich zone of the pulp layer often experience irreversible damage.

It has also been shown that there is no replicative activity in odontoblasts. Therefore, the theory of the existence of precursor mesenchymal stem cells of odontoblast in the form of undifferentiated mesenchymal stem cells has attracted attention [20].

Past studies have shown that the origin of dental pulp stem cells is from dental papilla cells, which migrate from the neural crest [20].

Dental Stem Cells:

Dental stem cells comprise various types of cells that are classified based on their potential to differentiate into different tissues and their origin. The following is their classification:

1. Odonto ectomesenchymal stem cells
 - a) Group related to dental pulp:
 - A) Dental pulp stem cells
 - B) Stem cells from exfoliated deciduous teeth (SHED)
 - C) Apical papilla stem cells
 - b) Periodontium-associated group
 - A) Dental follicle precursor cells
 - B) PDL stem cells

2- Dental epithelial stem cells

Dental Pulp Stem Cells: DPSCs

Undifferentiated mesenchymal cells (stem cells) are abundant in the primary pulp, but they also exist in the adult pulp.

These cells have been larger than fibroblasts, have a polygonal shape with protrusions around them, and have a large oval nucleus.

These cells are located along the blood vessels of the pulp, but they are also scattered in the center of the pulp. These cells are seen in a spindle-like shape. It is believed that these cells are totipotent, meaning they have the potential to differentiate into odontoblasts, fibroblasts, and macrophages when necessary. However, their numbers decrease with advancing age of the tooth.

The distinction of these cells is induced by potential regulators of bone formation, including members of the TGF- β family and various cytokines. Recently, a similarity in gene expression has been reported between DPSCs and osteoblast precursor cells and bone marrow stromal stem cells (BMSSCs) [26].

DPSCs, like BMSSCs, consist of subpopulations and subgroups that have different proliferation rates and evolutionary potential. For example, DPSCs can be divided into two subgroups: multicolony and single colony. The multi-colony group has the ability to divide continuously up to 20 to 30 times, this number is reported with the criterion (Population Doubling, PD). But the majority (80%) of single-colony cells have PD below 20 times and only 20% of them multiply above 20 times [27].

Applications of dental pulp stem cells:

The ability of old and young teeth to respond to injuries with the formation of reparative dentin indicates that a small population of precursor stem cells remain alive in the pulp of the tooth throughout life [20].

Contrary to the epithelial stem cells, dental undifferentiated ectomesenchymal cells do not completely disappear after tooth eruption in humans. In fact, it is currently possible to isolate precursor stem cells from dental pulp even at the age of 90.

The most prominent feature of DPSCs is their ability to regenerate the dentin-pulp complex, which is composed of a mineralized matrix and tubules covered by odontoblasts and a fibrous tissue containing blood vessels. This structure is similar to the normal dentin-pulp complex found in humans [7].

Below, a recent study about DPSCs with its result is mentioned, which had been done by Xiwen Dong et al. 2022:

To understand the biological effects of dental pulp stem cells (DPSCs) on nucleus pulposus (NP) cells, they carried out RNA sequencing, bioinformatic analysis which unveiled gene expression differences, and pathway variation in primarily isolated patients' NP cells after treatment with DPSCs supernatant. Western blot and immunofluorescence were used to verify these molecular alterations. Besides, to evaluate the therapeutic effect of DPSCs in IVD degeneration treatment, DPSCs were injected into a degeneration rat model in situ, with treatment outcome measured by micro-CT and histological analysis. RNA sequencing and in vitro experiments demonstrated that DPSCs supernatant could downregulate NP cells' inflammation-related NF- κ B and JAK-STAT pathways, reduce IL-6 production, increase collagen II expression, and mitigate apoptosis. In vivo results showed that DPSCs treatment protected the integrity of the disc structure, alleviated extracellular matrix degradation, and increased collagen fiber expression. In this study, we verified the therapeutic effect of DPSCs in an IVD degeneration rat model and elucidated the underlying molecular mechanism of DPSCs treatment, which provides a foundation for the application of DPSCs in IVD degeneration treatment [32].²⁴

Cellular and molecular changes that occur during the formation of primary dentin are repeatedly damaged during the pulp response. Various bioactive molecules, especially the family of beta-transforming growth factor (β TGF-) inducing factors, which are stored in the matrix during the formation of primary dentin, can also be released during processes leading to tissue dissolution, such as dentin resorption. Therefore, the dentin matrix is a potential

reservoir of a mixture of bioactive molecules (especially growth factors) that are ready to be released under suitable conditions [45].^{۲۹}

When DPSC cells were transplanted into an immune-deficient mouse, a pulp-dentine like structure was created, surrounded by human odontoblast-like cells in the vicinity of the pulp tissue [46].^{۳۰}

But BMSSCs cells were able to construct lamellar bone-like structures in the presence of osteocytes and superficial osteoblasts, surrounding a fibrous vascular and hematopoietic tissue and active adipocytes.[16]^{۲۶}

In addition, backscattered electron microscopy results showed that DPSCs cells are capable of producing a mineralized matrix with a globular shape and calcospheritic mineralization pattern similar to primary dentin, without the presence of hematopoietic and adipose tissue [47].^{۳۱}

It has been shown that DPSCs have a greater capacity for differentiation. While it may seem that these cells only differentiate into the types of cells present in the pulp, and since fat cells are not typically part of the pulp, DPSCs do not have the ability to differentiate into these cells. However, it has been found that in cultured environments and under specific differentiation conditions (specific signals), these cells can differentiate into adipocytes, but this does not typically occur in vivo conditions [7].

Properties of human dental pulp stem cells:

- 1) Self-renewal capacity: the ability to produce similar cells (homogeneous differentiation).
- 2) Multilineage differentiation capacity: the ability to differentiate into different cell lineages.
- 3) Colonogenic efficacy: the ability to proliferate and produce colonies.
- 4) Formation of ectopic dentin and associated pulp tissue in vivo: the ability to create ectopic dentin and related pulp tissue in vital organisms.
- 5) Differentiation into adipocytes and neural-like cells: the ability to differentiate into fat cells and neuron-like cells.
- 6) High proliferation potential: the potential for rapid and extensive cell proliferation. [7]

To evaluate the potential of differentiating DPSCs into adipocytes and neurons (similar to the differentiation observed in BMSSCs), DPSCs cells were cultured in a medium containing fat inducers, and after 5 weeks, oil red o-positive fat deposits were visible. RT-PCR method was used to detect the new cells and 2 specific adipocyte transcriptase enzymes, namely PPAR gamma-2 and lipoprotein lipase, were identified.

In addition, the presence of nestin (a marker for neural precursor cells) and GFAP (Glial Fibrillary Acid Protein, a marker for glial cells) has been reported in other culture environments, both at the mRNA and protein level, indicating the neural differentiation of these cells [32].

Gronthos S et al, showed that when DPSCs derived from single-colony were transferred to immunocompromised mouse, 2/3 of the colonies had the ability to produce numerous ectopic dentin on HA/TCP carrier (similar to multicolony strain), but 1/3 of them only produced low to moderate dentin. Analysis of the dentin produced showed structural similarities among all groups [7].

Recently, a specific population of stem cells has been isolated from dental pulp. These cells have the potential to differentiate into smooth muscle cells, skeletal muscle cells, neurons, cartilage, and bone under specific biochemical conditions in a culture environment [11].

Recently, dental pulp stem cells have received attention in the field of reparative endodontics, which refers to the revival of root canal treatment.

Kuttler presented the tertiary dentin formation theory, which suggests that tertiary dentin is composed of a various spectrum of responses, ranging from regular tubular dentin secretion to highly irregular dentin without tubules. These responses are the result of various cellular and molecular processes and manifest as reactions to mild or severe stimuli. Hence the tertiary dentin is categorized into reactive dentin and regenerative dentin [33].

In response to mild stimuli, reactive dentin is secreted by post-mitotic residual odontoblasts, while reparative dentin is secreted by a new generation of pseudo-odontoblastic cells that have differentiated from post-mitotic odontoblasts after their death. Repairative dentin generation involves a complex process of biological events, as well as use and differentiation of stem cells prior to secretion of matrix at the site of Injury. In cases of pulp exposure (visible or affected pulp), reparative dentin can lead to the formation of dentin bridge and the regeneration of the dentin matrix continuity around the pulp [34].

Dentin is composed of a large number of proteins that are capable of stimulating tissue response. Demineralization of dental tissues caused by carious, cavity etching agents and the use of restorative materials can lead to release of growth factors from the inside of dentinal matrix [20].

-Dentonin (as part of the Matrix extracellular phosphoglycoprotein protein from the SIBLING dentinal protein family)[17]

-GDF11 (Growth/differentiation Factor)[235]

-BMP2 Recombinant human[36]

-Emdogain[37]

-TGF β Family[20]

1-Recombinant human insulin-like growth factor.[20]

Ahangari et al, in 2012 researched about the effect of propolis, a natural substance obtained from honeycomb, on stimulating stem cells in the dental pulp of guinea pig teeth for dentin regeneration at the School of Dentistry, Shahid Beheshti University of Medical Sciences. They successfully achieved high-quality tubular dentin regeneration close to natural dentin. [38]

Ahangari et al, examined the effect of propolis on the differentiation and proliferation of pulp stem cells in a culture environment in 2016. They announced that after 21 days, mineralization process were observed in the propolis culture medium [39]

The method of isolation, culturing, identifying, and classifying stem cells has been previously discussed in the general doctoral thesis of Dr. Maryam Jalili. [40]

Three more studies are highly desirable to be added in this article, which are:

1-The Effect of Dentin Matrix Proteins on Differentiation of Autologous Guinea Pig Dental Pulp Stem Cells.

Results

In the case group, the amount of reconstituted dentin, the presence of stem cells and odontoblastic differentiation were higher than in the control group ($P < 0.05$) and the type of regenerated dentin potentially was of higher quality, although it was not statistically significant ($P = 0.924$). However, the amount of regenerated dentin ($P = 0.001$), the presence of pulp stem cells ($P = 0.001$) and the increased quality of regenerated dentin ($P = 0.001$) in both groups on the 1st and 3rd days were significantly different [41].

2-The Effect of Bone and Dentin Matrix Derivatives on the Differentiation of Human Dental Pulp Stem Cells for Osteogenesis and Dentinogenesis in a Scaffold-Free Culture.

Results:

In the first group, hDPSCs were differentiated into odontoblast and in the second group into osteoblast. The mean expression percentage of the dentin sialophosphoprotein (DSPP) marker in differentiated cells in the first group was significant at days 3, 7, and 10 ($p \leq 0.0001$). Also, the mean expression of the bone sialoprotein (BSP) marker was significant in the second group on days 3, 7, and 10 ($p \leq 0.0001$) [42].

3-The effect of dental pulp stem cell transplantation on the regeneration of mature pulp tissue in rabbits.

Results:

In the microscopic examination of the sliced tooth after 14 days by an oral and maxillofacial pathologist, the pathology report showed that a row of living odontoblast cells was observed in the inner wall of the root canal where the pulp tissue was completely drained. Furthermore, the entire space of the root canal was filled with a small and scattered number of inflammatory cells (below 30%) as well as pulp-like living tissue. Although this tissue was not completely similar to the normal dental pulp, it was a sign of pulp formation. In addition, observation of formation of small pseudovascular structures emphasizes the ability and potential of DPSC and its induction by PRP for better and more effective endothelial differentiation, as well as cell growth and migration. In histological examination, small thickness of tubular dentin (about 1 mm) was shown, which is an emphasis on the regeneration of tooth dentin tissue induced by DPSC [43].

In 2018, Gomes A et al. researched about the various types of tissue sources of stem cells and their applications in the oro-facial region in a review article and have found a hopeful result of possibilities for regenerating orofacial soft and hard tissues in addition to tooth germs and suggested the establishment of a dental stem cells bank [44].

Park B. et al. observed in 2018 that papillary and tooth follicular stem cells exhibit high differentiation potential into bone tissues in an extramatrix environment, and pulp and periodontal stem cells exhibit high differentiation potential into neuronal tissues in an intratissular environment [45].

Zhai Q et al. announced in 2019 in a review study from China that theoretically, dental stem cells are able to regenerate various tooth structure tissues, but this has not been implemented in practice yet [46].

Dr M Jalili et al conducted an experimental study on an albino rabbit maxillary central incisor and found a result that the entire space of the root canal was filled with a small and scattered number of inflammatory cells and pulp-like living tissue [47].

Conclusion:

Researchers have examined the differentiation of dental pulp stem cells in vivo and in vitro conditions, and in both cases, they obtained positive results. However, despite the numerous efforts and studies conducted in the past, the complete regeneration of dental bud tissues has yet to be successfully achieved.

Hence it has been done in animal trial phase but not yet in human.

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