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The Effect of Dental Pulp Stem Cell Transplantation on the Regeneration of Mature Pulp Tissue in Rabbits

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ABSTRACT

Background and Objective: Tooth decay is the main cause of pulp and periapical diseases, and when the pulp involves the tooth, there is no other choice but to drain the pulp to save the tooth. The emergence of new methods of regenerative treatments has made it possible to restore dental pulp and dentin. Since regenerative treatments have been limited to immature teeth, this study was conducted to regenerate mature pulp tissue by dental pulp stem cell transplantation along with Platelet Rich Plasma (PRP) Matrix in rabbits.

Methods: This experimental pilot study was conducted on a maxillary central incisor in a 1-year-old 2kg adult male albino rabbit. After completely cleaning the pulp tissue from the root and crown and washing the root canal, File #30 was passed through the apex to cause bleeding. Then dental pulp stem cells along with PRP were injected into the canal and the crown was sealed with glass ionomer cement. After 14 days, a radiograph was taken from the tooth, the extracted tooth was stained by hematoxylin-eosin and examined by light microscope.

Findings: In the histological examination, in the inner wall of the root canal of odontoblasts, small thickness was observed in tubular dentin (about 1 mm) and pseudovascular structures. Furthermore, the entire space of the root canal was filled with a small and scattered number of inflammatory cells and pulp-like living tissue.

Conclusion: Based on the results of this study, the observation of pseudovascular structures indicates the regenerative potential of dental pulp stem cells and opens a window for studies with more samples to effectively regenerate the dental pulp.

Keywords: *Regeneration, Transplantation, Stem Cells, Dental Pulp, Rabbits*.

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Introduction

Tooth decay is the main cause of pulp and periapical diseases, and when the pulp involves the tooth, there is no other choice but to drain the pulp to save the tooth (1). In addition, the progress of secondary caries in the teeth is much faster due to the absence of pain and causes brittleness and early tooth loss (2). The use of dental implants has been popular for a long time, but in addition to being expensive, it has limitations such as the systemic conditions of the patient, the quality of the bone, the location of the implant, and the age of the patient, and non-observance of each of them can lead to the failure of the implant treatment plan (3, 4). The emergence of new methods of regenerative treatments in clinical research has made possible the restoration of dental pulp and dentin (5, 6).

The relatively small space of the dental pulp has a relatively simple cellular structure that can be easily regenerated in terms of tissue engineering (7) and pulp regeneration (Regenerative Endodontics) means the physiological replacement of damaged tooth structures including dentin, root structures and dentin-pulp complex (8). So far, regenerative treatments through various substances such as calcium hydroxide or ciprofloxacin have been very successful. These methods include apexogenesis and revascularization. In most cases, immature teeth with pulp necrosis have been treated in these treatments, and during the 2-year follow-up, acceptable clinical results have been obtained (8, 9). However, in recent studies, the potential of Dental Pulp Stem Cells (DPSCs) has been used for the complete regeneration of the pulp and these cells have been introduced as safe and effective cells for new therapeutic methods of pulp regeneration (capabilities such as angiogenesis, neurogenesis and odontogenic), production of odontoblast-like cells and dental structures (10-14).

Studies in the field of wound healing process have shown that platelets that participate in clot formation release growth factors that play a role in cell division and differentiation and as a result, tissue repair and regeneration. With the recognition of Platelet Rich Plasma (PRP) as a source of growth factors such as PDGF, TGF-β and IGF-I, it was used as a substance to accelerate the regeneration process (15-18). Regenerative treatments and tissue engineering have been limited to immature teeth, but by making changes in the main method, this treatment can also be performed for mature teeth (19). The problems raised for these methods include the contraindications of anesthetics containing vasoconstrictor substances to establish proper blood supply, and the possibility of tooth discoloration, especially with the use of Minocycline. On the other hand, the patient may refuse to perform regenerative treatments due to the need for multiple follow-ups (20). Furthermore, the use of modern methods has shown different results in mature teeth, and the effectiveness of these methods in mature teeth has not been well proven yet (9). Therefore, due to the importance of preserving life in mature permanent teeth, we decided to investigate the regeneration of mature pulp tissue using DPSCs grafting with PRP matrix in rabbits, and if a positive result is obtained, we can investigate this important issue in humans in future studies.

Methods

After obtaining ethics code number IR.SEMUMS.REC.1396.156 from the Ethics Committee of Semnan University of Medical Sciences, this experimental pilot study was conducted on a maxillary central incisor in a 1-year-old 2kg adult male albino rabbit prepared from Razi Vaccine and Serum Research Institute.

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Cell preparation steps: Human dental pulp stem cells (DPSCs) from DPS-13 cell line and IBRC cell code C10896 were purchased from Iran's National Genetic and Biological Reserves Center. Standard procedures of cell culture were performed in the comprehensive laboratory of Semnan University of Medical Sciences. In this study, the fourth and fifth passages of DPSCs were used.

Animal experiment steps: To prepare platelet rich plasma matrix, 2 ml of venous blood was taken from the earlobe of the animal by a 26 catheter and transferred into a tube containing Ethylenediaminetetraacetic acid (EDTA). After transferring the blood sample to the comprehensive laboratory of Semnan University of Medical Sciences, the sample was transferred into a 2 ml microtube with the help of a sampler and then centrifuged at 3000 rpm for 10 minutes to obtain PRP without erythrocytes and leukocytes. Then, the obtained PRP sample was kept at -80°C until use for testing. After general anesthesia, which was done with 60 mg/kg ketamine (ROTEXMEDIA company) intraperitoneally (IP), local anesthesia was also injected with 2% lidocaine solution and epinephrine 1:80000 as infiltration. Then, using a 2# carbide cutter and using a high-speed handpiece on the palatal surface of the left upper incisor tooth, the access cavity was prepared and the pulp was identified. After complete cleaning of the pulp tissue from the root and crown by Barbed broaches and K-file #6 to #30 (Mani company) and washing with normal saline solution and sodium hypochlorite, file #30 (Mani company) was passed through the apex to cause bleeding. K-file #6 (Mani Company) was used for initial access to the apex of the tooth. Then the cleaning of the tooth canal was continued in order to widen the apical foramen to a diameter of 0.5 mm and continued with file #30 (Mani company). During the preparation steps, the tooth canal was washed with 5.25% sodium hypochlorite and finally the canal was dried using sterile paper points.

The PRP obtained by centrifuging the rabbit blood sample exited the temperature of -80°C and reached room temperature. 1×10^6 pulp stem cells along with 0.5 ml of PRP, which was used as a scaffold for placing DPSCs, were injected into the canal using an insulin syringe, and then the tooth was filled with self-healing glass ionomer (GC company). After 14 days, after radiography of the tooth, the animal was sacrificed and the tooth was placed in 10% formalin solution and sent to the Pathology Center of Kowsar Hospital in Semnan for hematoxylin-eosin (H&E) staining. The sent sample was examined for the presence of living pulp tissue, inflammation, necrosis, thickness and type of dentin and the presence of odontoblasts. Since this research was conducted as a pilot study on a number of animal samples, there was no need for statistical comparisons and the results were obtained from the investigated slides.

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 DSPC (Figure 1).

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Figure 1. Histological view: In the microscopic examination, a row of odontoblast cells and a small and scattered number of inflammatory cells, small thickness of tubular dentin and living pulp-like tissue were observed, which emphasizes the ability and potential of DPSC and its induction by PRP for better and more effective endothelial differentiation, cell growth and migration.

Discussion

In near future, dental pulp regeneration can be used as an effective method to restore vitality to necrotic teeth. Since pulp regeneration in necrotic teeth along the root is not possible using DPSCs alone, therefore, several studies have been conducted based on strengthening and inducing the ability of DPSCs for endothelial differentiation and angiogenesis (18, 21). In addition, it has been shown that newly grown tissues in the root canal space have little resemblance to normal pulp tissue and are more similar to cementum, periodontal ligament and bone. The cause of this result is probably related to lack of dental pulp stem cells and apical papilla, which are destroyed by severe root infection. Stem cells responsible for regeneration of new tissues may be taken from systemic blood, local tissue such as bone and periodontal ligament (18, 22). In addition, whether the newly formed tissues can act like natural pulp and stabilize the tooth without causing further infection or loss of the canal is still unclear and more research is needed before performing regenerative procedures so that the long-term prognosis of the treatment is predictable (23).

With the aim of regenerating dental pulp, Angelopoulos et al. investigated the potential effect of mesenchymal cells with growth factor as a support for DPSC in dental pulp regeneration. Their results were associated with the expression of DPSC and angiogenesis, which indicates a successful effort in the field of dental pulp regeneration, which is the main goal of regenerative endodontic treatments. Katata et al. also reached a similar result in their study and emphasized the role of DPSC in pulp regeneration, and these two studies were consistent with the results of our study (18, 21).

The three key components of stem cells, growth factors and scaffolds are necessary for proper tissue regeneration. It has been hypothesized that after debridement, stem cells from the remaining living pulp or apical papilla may regenerate the lost structure of the pulp/dentin complex. However, DPSCs may fail to survive severe root infection (24). In the present study, we tried to evaluate the regeneration of living pulp using DPSCs and PRP scaffold. Since PRP contains growth factors such as transforming growth factor beta 1, platelet-derived growth factor, fibroblast growth factor, vascular endothelial growth factor and epidermal growth factor, it can support cell growth, differentiation and migration of DPSCs (25, 26).

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PRP also has the ability to form a 3D fibrin matrix and acts as a scaffold. Therefore, PRP has been used as a potential scaffold for the endodontic regeneration process as well as in the treatment of bone defects in periodontitis. Some clinical and animal studies have shown that PRP does not appear to increase bone regeneration, and there is limited data on the use of PRP for dental pulp regeneration (27-29). In the study conducted by Katata et al. on the effective regeneration of the pulp by inducing the endothelial differentiation ability of DPSCs, they concluded that vascular markers such as CD31 are positive in the tissue made by DPSCs, and this issue emphasizes the ability of DPSCs for effective dental pulp regeneration (21). In the present study, the observation of pseudovascular structures indicates the potential of DPSC and opens a window for studies with more samples to effectively regenerate the dental pulp. Finally, due to the wide range of cell resources, scaffolds and new growth factors for the formation of tissues and regeneration, it is suggested that more studies be carried out to achieve more effective results and new means and factors effective in restoring vitality to necrotic teeth until reaching an ideal combination.

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