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A REVIEW OF REGENERATIVE ENDODONTICS

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Abstract

An alternative approach for immature necrotic permanent teeth is regenerative endodontic treatment. In this method, which is based on disinfection of the root canal system and periapical region without damaging the existing cells, the aim is to assist in the formation of new tissue within the canal after the stem cells are transported to the root canal and to induce further root development. Studies on stem cells, scaffold and signalling molecules which is triad of regenerative endodontic treatments concept are increasing nowadays thanks to intensive progress of regenerative medicine and tissue engineering. This review outlines the clinical protocols currently used and studies in regenerative endodontic procedures.

Keywords: *Regenerative endodontic treatment, stem cells, calcium hydroxide, immature teeth, pulp necrosis.*

Özet

İmmatür nekrotik daimi dişler için sunulan alternatif bir yaklaşım şekli de rejeneratif endodontik tedavidir. Kök kanalı sisteminde ve periapikal bölgede mevcut hücrelere zarar vermeden dezenfeksiyon hedeflenen bu yöntemde amaç, kök hücrelerin kök kanalına taşınmasıdır. Bu sayede kanal içinde yeni doku oluşumu indüklenerek kök gelişiminin devamlılığı hedeflenmektedir. Rejeneratif tıp ve doku mühendisliği alanındaki gelişmeler sayesinde rejeneratif endodontinin temel aldığı kök hücre, doku iskelesi ve büyüme sinyalleri triadı üzerine çalışmalar son dönemde ağırlık kazanmıştır. Bu derlemede rejeneratif endodontik tedavinin güncel protokol ve çalışmalarına yer verilmiştir.

Anahtar Kelimeler: *Rejeneratif endodontik tedavi, kök hücreler, kalsiyum hidroksit, kök gelişimi tamamlanmamış dişler, pulpa nekrozu.*

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OVERVIEW / GENEL BAKIŞ

Immature teeth with open apex are treated with apexification, which is a method to induce a calcified apical barrier at the root apex. The different materials can be successfully used for the formation of an apical barrier for apexification. Calcium hydroxide is a widely accepted conventional material in multiple visit apexification treatments (1, 2). Due to a need to repeatedly change medication and the lengthy nature of treatment, mineral trioxide aggregate (MTA) have been suggested in the treatment of immature teeth with pulp necrosis (3). Despite all its advantages, it has been shown that apexification with MTA does not strengthen the root structure and induce further root growth compared to calcium hydroxide apexification (4). The development of immature teeth treated with apexification procedures is proven to stop continued root development, while normal pulpal nociception and immune defense are not expected as an outcome (5).

Unlike forming of a hard tissue barrier with apexification, regenerative endodontic treatment is an alternative treatment regime which leads to continued root formation or thickening of the root canal wall (6). Regenerative endodontic treatment is defined as "biologically based processes designed to replace damaged structures such as dentin, root structures and pulp-dentin complex cells" (7).

Terminology

Many terms have been used in the literature for this treatment, such as regenerative endodontics, revascularization and revitalization (8). The term of revascularization is widely used in the literature and relates to the restoration of vascular support in the pulp cavity after traumatic injuries that reduce blood supply to the pulp of immature teeth (9). Revitalization has been proposed because it describes non-specific vital tissue development rather than just blood vessels, as the term of revascularization implies (10). In 2008, "Induced or guided tissue generation and regeneration" has been suggested to describe the technical aspect of the treatment by Huang and Lin (11). The term of regenerative endodontic procedures has been widely accepted and refers to all procedures aimed at providing the regular repair of the dental pulp, including future treatments in the field of regenerative endodontics (12).

In a previous study conducted on 61 immature permanent teeth with pulp necrosis, when the treatment results obtained with revascularization and apexification techniques with MTA or calcium hydroxide were compared, the increase in root thickness and length was found to be significantly higher. In the same study, the survival rate was found to be at the highest rate of 100% in teeth treated by revascularization technique compared with apexification (13). There are three results intended to be obtained with regenerative endodontic treatment (13), demonstrated at Table 1:

Eliminating symptoms and seeing bone healing
Increased root wall thickness and / or root length,
Positive response to vitality test

Table 1. The results intended to be obtained with regenerative endodontic treatment

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The study of Nygaard-Ostby (1961) who assessed the effects of evoked bleeding by over-instrumentation of human or dog root canal systems has pioneered fields of regenerative endodontics (14). Iwaya et al. (2001) showed that increasing in radiographic root development for both root length and root width after the treatment brought about revascularization by them in an immature permanent tooth with a necrotic pulp and sinus pathway (15). Many studies in the literature based on the case report by Banchs and Trope (2004) are considered as the beginning of the modern regenerative endodontic period. This case report describes the treatment of lower right second premolar with incomplete root development with sinus tract. They emphasized that regeneration of pulp of necrotic infected teeth with apical periodontitis may be possible (16).

Both of these case reports have shown important principles of regenerative endodontic procedures: elimination of bacteria in the root canal, creation of a scaffold for the growth of the new tissue, and preventing reinfection by forming a bacterial impermeable layer (17). Although these two case reports showed clinical evidence of continued root growth, they were unable to demonstrate intracanal histology.

Histological Findings in Regenerative Endodontic Treatment

Animal models have shed light on research on this subject due to the difficulty of collecting human samples. Wang et al. (2010) examined the tissue types produced in root canal histologically after disinfecting canines had periapical lesions with triple antibiotic paste. Based on histological examination, it was found that three main types of tissue were formed in the canal cavity. These are cementum-like tissue known as "intracanal cementum" that leads to thickening of dentinal walls in root canal, bone or bone-like tissue, and connective tissue similar to periodontal ligament in the canal space. In addition, only one of the healed cases showed partial survival of the pulp tissue (18). In the study of Yamauchi et al. (2011), it was reported that the newly formed hard tissue in the dentine walls of root canal cavity was distinctly different from dentine or bone-like tissues but resembled cement. However, the collagen matrix organization and maturation were significantly different (19).

In an immature human tooth with irreversible pulpitis had normal periapical tissue, as Shimizu et al. (2012) showed the first histological findings (20). Histological examination revealed a well-vascularized pulp-like tissue consisting of numerous spindle-shaped fibroblasts and mesenchymal cells in the root canal. Although presence of an odontoblast-like cell layer on the dentin wall was showed, nerve-like fibers running alongside the blood vessels were not observed.

In the previous study performed by Martin et al. (2013), histological examination of the lower first molar tooth extracted for crown-root fracture two years after revascularization treatment showed complete dissolution of inflammation and the presence of mineralized and fibrous connective tissue in the root canal; however, the presence of odontoblast-like cells in dentine-like mineralized tissue could not be demonstrated (21).

Case Selection in Regenerative Endodontic Treatment

The majority of cases performed in young patients with immature infected teeth have ceased root development (7). On the other hand, the use of regenerative endodontic treatment in mature teeth has been reported in these cases (22). The American Endodontic Association (AAE) has recommended the use of regenerative endodontic treatment in the teeth of cooperative patients with necrotic pulp, open apex, and pulp

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cavity, none of which requires post-core restoration (22). Shimizu et al. (2012) stated that ideal cases for pulp regeneration should be immature permanent teeth with irreversible pulpitis, necrotic pulp teeth without radiographic evidence of apical periodontitis, or traumatized immature permanent teeth (20). George et al. (2010) argued that the presence of radiolucency in the periradicular region or the pulp vitality test result should no longer be used as a determining factor. In both cases, it is thought that vital pulp tissue or apical papilla cells may still be present in the root canal or apical region of root (23).

Irrigation in Regenerative Endodontic Treatment

Similar to conventional endodontic treatment, microbial control is also important for regenerative endodontic procedures. Clinicians face difficulties in proper cleaning of root canals with funnel shaped apex, wider than coronal aspect. Teeth with open apices often tend to have thin dentinal walls that are susceptible to fracture during or after endodontic treatment. Therefore, mechanical instrumentation is contraindicated in regenerative endodontic therapy and cleaning with chemical agents has been shown to be the main disinfection method (24). In a histological and histobacteriological study of an unsuccessful regenerative endodontic treatment, most bacteria have been shown to form a biofilm on the canal walls and enter the dental tubules. It was also reported that the bacterial majority was concentrated in the apical region, not in the coronal part of the canal (25).

Sodium hypochlorite (NaOCl) is the most widely used agent in all endodontic procedures, including regenerative endodontic treatments (12). It has important advantages, such as particularly strong bactericidal effect, tissue dissolving capacity, and effective lubrication for endodontic instruments (26). According to the current regenerative endodontic treatment protocol recommended by AAE, 17% ethylenediaminetetraacetic acid (EDTA) solution was recommended for irrigation in the second appointment (22). EDTA is an irrigation solution that shows irreversible binding to calcium ions which provides the release of growth factors from the root dentin (27). However, amount of the growth factor release in dentin exposed to EDTA for 5 minutes was found to be higher than after intracanal medicaments using calcium hydroxide, triple antibiotic paste, and antibiotic paste with corticosteroid (28). According to the 2016 protocol of AAE, the recommended procedure is as follows (Table 2):

First Appointment	**Second Appointment (1-4 weeks after 1st visit)**
► Local anesthesia, dental dam isolation and access. ►Copious, gentle irrigation with 20ml NaOCl Lower concentrations of NaOCl [1.5% NaOCl (20mL/canal, 5 min) and then irrigated with saline or EDTA (20 mL/canal, 5 min), with irrigating needle positioned about 1 mm from root end, to minimize cytotoxicity to stem cells in the apical tissues. ►Dry canals with paper points. ►Place calcium hydroxide or low concentration of triple antibiotic paste (mix 1:1:1 ciprofloxacin: metronidazole: minocycline to a final concentration of 0.1-1.0 mg/ml) ►Seal with 3-4mm of a temporary restorative material such as Cavit™, IRM™, glassionomer or	►Anesthesia with 3% mepivacaine without vasoconstrictor, dental dam isolation. ►Copious, gentle irrigation with 20ml of 17% EDTA. ►Dry with paper points. ► Create bleeding into canal system by over-instrumenting (endo file, endo explorer) (K-file at 2 mm past the apical foramen). An alternative to creating of a blood clot is the use of platelet-rich plasma (PRP), platelet rich fibrin (PRF) or autologous fibrin matrix (AFM). ►Stop bleeding at a level that allows for 3-4 mm of restorative material. ►Place a resorbable matrix over the blood clot if necessary and white MTA as capping material. ►A 3–4 mm layer of glass ionomer is flowed gently over the capping material and light-cured for 40 s.

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another temporary material. Dismiss patient for 1-4 weeks.	Bioceramics or tricalcium silicate cements (Biodentine [Septodont, Saint-Maur-des-Foss es, France]) could be used as alternatives to MTA.

Table 2. According to the 2016 protocol of AAE, the first and second appointments procedure of regenerative endodontic treatment.

Besides the importance of bactericidal and bacteriostatic properties of the agents used in chemical disinfection of the root canal system, the survival and proliferative capacity of the stem cells of the patient should not be impaired. Martin et al. (2012) examined the effect of 1.5%, 3%, and 6% NaOCl concentrations on stem cell survival and differentiation in standardized shaped root canals. After irrigation with NaOCl, half of the samples carried out a second irrigation with EDTA. It has been shown that NaOCl decreases the vitality and differentiation of apical papilla stem cells depending on the concentration in the samples examined by real-time polimeraze chain reaction (PCR) and 1.5% NaOCl cause the least damage. In addition, a recent irrigation with 17% EDTA has been shown to reverse the harmful effects of NaOCl (29).

A previous study by Zeng et al. (2016) on the effect of irrigation solutions on growth factor release showed that 1.5% NaOCl + 17% EDTA or 2.5% NaOCl + 17% EDTA significantly increases TGF-ß1 release compared to irrigation group performed with only 17% EDTA (30). It has been proposed that NaOCl is able to remove organic components of the smear layer and thus opening of dentine tubules may be a possible cause of higher release of TGF- ß1. Therefore, it is suggested that NaOCl may be added to the second session of regenerative endodontic treatment protocol (31). In another study, the effect of the use of NaOCl and chlorhexidine before irrigation with EDTA on Transforming Growth Factor (TGF)-ß1 release was investigated (28). It has been concluded that the use of chlorhexidine (CHX) for 5 minutes increases the amount of TGF-ß1 release, whereas the use of NaOCl significantly reduces it.

As an alternative to the EDTA solution, 1% phytic acid (IP6), known for its effective chelating function and biocompatibility, has been proposed in removing the smear layer (32). In the study of Sungur et al. (2019), the effect of phytic acid (IP6) and etidronic acid (HEDP) on the release of TGF-β from the dentine matrix and the behavior of dental pulp stem cells was compared with EDTA (33). The results of the study showed that all chelating solutions increased TGF-β release from the dentin matrix and there was no significant difference between the solutions. In addition, dental pulp stem cell migration was observed in all groups and the highest cell migration was determined after HEDP application. EDTA, distilled water, and IP6 treatment significantly induced cell proliferation. As a result, etidronic acid and phytic acid were found as effective as EDTA in terms of TGF-β release and cell migration (33).

In a study conducted by Trevino et al. (2011), the effect of irrigation solutions on apical papilla stem cells was investigated. In four different groups: 1. EDTA, 2. NaOCl-EDTA, 3. EDTA- CHX, 4. NaOCl-EDTA-Isopropyl alcohol (IPA) -CHX were used and study showed that CHX caused the most damage, while EDTA was the most successful solution for maintaining stem cell vitality (34). Therefore, during disinfection protocol of the root canal for the purpose of regeneration, it is important to maintain the vitality of apical tissues that are the source of new tissue and to ensure the preservation of the dentine that is crucial for important growth factors (35). In a previous study, Soares et al. (2013) proposed the new treatment for revascularization therapy of necrotic immature root canals. In addition to intracanal medication with calcium hydroxide and 2% chlorhexidine gel for 21 days, the treatment consisted of revascularization therapy with manual

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instrumentation of the medium and cervical thirds of the root canal in contrast to many treatment protocols in the literature (36).

Intracanal Medicament Use in Regenerative Endodontic Treatment

The concentrations of both irrigation agents and intracanal medicaments were important for the disinfection of the root canal, the release of growth factors from the dentin matrix, and the balance between survival and proliferation of apical papilla stem cells (8). Calcium hydroxide is the most widely used intracanal medicaments that has the ability to eliminate bacteria due to its high alkalinity (37). The high pH of calcium hydroxide changes the biological properties of the lipopolysaccharide component in the cell wall of gram-negative species and inactivates membrane transport mechanisms leading to bacterial cell death (38). However, due to its high pH as a disadvantage of calcium hydroxide, it has been reported that it causes sudden tissue necrosis at contact and thus destroys differentiation in new pulp tissue (16). Because of their antimicrobial effects in endodontic regenerative procedures, the use of triple antibiotic paste (TAP) containing metronidazole, ciprofloxacin, and minocycline as intracanal medicaments has been proposed (39). Sato et al. (1996) evaluated the potential of triple antibiotic paste to eliminate bacteria in the deep layers of root canal dentin and concluded that TAP has been shown to reliably perform antimicrobial activity against pathogens within 24 hours of the placement of the antibiotic combination, except for a small amount of bacteria (40). Disadvantages of TAP usage have also been reported, such as discoloration, bacterial resistance, and allergic reaction (41).

In a study comparing TAP with calcium hydroxide, TAP has been shown to have better results in reducing colony forming units (37). In a study conducted by Shokraneh et al. (2014), no significant difference was found between TAP and calcium hydroxide in deep dentin in terms of antibacterial activity, whereas triple antibiotic paste in surface dentin proved to have significantly higher antibacterial activity (42). In a study by Ruparel et al. (2012), the effects of TAP, double antibiotic paste (DAP), and calcium hydroxide on human apical papilla stem cells were evaluated. It was found that medicaments used in regenerative procedures negatively affected the survival of apical papilla stem cells except calcium hydroxide paste (43). In order to prevent damage of stem cells from the apical papilla, it is recommended to use TAP at a concentration no greater than 1mg/m L (0.1 -1mg/mL) in RET (22). Chuensombat et al. (2013) reported that the amount of 0.39 mg / mL is a safe dose for TAP although it does not have the ability to destroy all bacteria (44). It is important to use these drugs in a concentration sufficient to have antibacterial activity, although they do not induce toxicity in host stem cells.

Yassen et al. (2015) evaluated the effects of TAP, methylcellulose-based triple antibiotic paste (DTAP), and calcium hydroxide on the microhardness of dentin. Dentin treated with TAP, DTAP, and calcium hydroxide showed a significant reduction in mean microhardness values compared to untreated dentin. However, no significant difference was found in terms of mean microhardness values between the group irrigate with NaOCl and EDTA without medicaments treat, and the dentin groups treated with DTAP and calcium (45).

Coronal Seal in Regenerative Endodontic Treatment

In regenerative endodontic treatment, the most important objective is to provide a healing environment for regeneration. One of the contributing steps of the treatment is an adequate coronal seal on the blood clot. The coronal barrier material in contact with the blood clot is expected to have properties such as preventing bacteria or leakage, demonstrating biocompatibility, and promoting cell proliferation and differentiation (46).

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MTA, bioceramics and tricalcium silicate-based materials such as Biodentine (Septodont, Saint-Maur-des-Foss es, France) and RetroMTA (BioMTA, Seoul, Korea) are recommended as coating material (22). Although MTA is suggested to be placed on the blood clot as coronal barrier material, there are several important disadvantages such as difficulty in use, long setting time (47), need for moisture to set, and color change in crown (48).

In a study which preferred to use Biodentine as a sealing material instead of MTA, revascularization technique was applied in a single visit and it was shown that root development continued and apical closure was provided as a result of follow-up (49). In a study comparing the properties of Biodentine and MTA, Biodentine was found to be advantageous with its high mechanical properties and short setting time; in terms of surface roughness, cytotoxicity, and cell binding has been shown to show similar properties with MTA (50).

Tissue Engineering Approaches in Regenerative Endodontic Treatment

There are two distinctive approaches for regenerative strategies for infected or traumatized teeth: cell transplantation and cell homing (51). Cell transplantation is a cell-based approach in which exogenous stem cells loaded onto the scaffold and signaling molecules are transported to the host root canal system to allow regeneration. The transplanted cells are collected from the host (autologous) or from other individuals (allogeneic) and they can be processed to increase in number or grow in cultures (52). The purpose of cell homing is to provide tissue repair/regeneration through chemotaxis of host endogenous cells to the injured tissue through biological signaling molecules (53). This approach is based on the use of endogenous stem cells in the apical pulp or periapical area, together with tissue scaffolds and signal molecules that stimulate cell migration, proliferation, and differentiation (54).

The attractiveness of the cell homing strategy in clinical endodontics compared to a cell-based treatment is that it is not necessary to isolate or supply exogenous stem cells (52). Cell homing has a number of problems such as regulatory approval issues, stem cell isolation and processing, biological risk of potential immune rejection, risk of pathogen transmission, high costs associated with storage, packaging, and shipping (53).

Regeneration of dental-pulp-like tissue by cell homing was studied by Kim et al. (2010). In this pioneer study, after delivery of collagen scaffold and various growth factors to the root canal, single-rooted human teeth were removed from pulp and inserted into mouse subcutaneous tissue. Histologic examinations revealed that vascularized and innervated tissue with dentin-like hard tissue was obtained in the root canal cavity (55). In a recent study, regenerative enododontic treatment with cell-based approach was performed on 30 patients of traumatic necrotic incisors and 40 patients with Human Dental Pulp Stem Cells (hDPSC) implantation. In the absence of a scaffold, 10 patients have received traditional apexification treatment. Results of study show that regeneration of three-dimensional pulp tissue equipped with sensory nerves and blood vessels obtained hDPSC implantation, but not apexification treatment. Furthermore, histological examination revealed that regenerated three dimensional pulp tissue contained normal structures such as an odontoblast layer, connective tissue, blood vasculature, and neuronal tissue (56).

SUMMARY / SONUÇ

Although apexification with calcium hydroxide and MTA are treatment options with high success rates, they have a negative effect on the prognosis of immature permanent tooth with disadvantages such as ceased

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root development and thin dentinal walls. With the development of tissue engineering techniques, regenerative endodontic treatment is considered to be an important treatment option for immature teeth with pulp necrosis, based on the success of many cases published in the literature. The clinical literature shows that regenerative endodontic treatments with various methods and materials lead to a significant increase in root length and dentin wall thickness.

Even if studies show that pulp tissue has the potential to regenerate the tooth, it should be kept in mind that it may also lead to the development of advanced pulp diseases and the need for retreatment. Further methods are needed for new tissue formation in tissue regeneration with increased anti-microbiological control.

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