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Case Report

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Regenerative endodontic of necrotic Immature Permanent Tooth using platelet-rich fibrin: A case report

Ameni Chadlia Belghuith, Amira Kikly, Wided Glii, Fouad Brigui, Lobna Faour Hodroj, Nabiha Douki

*University of Monastir, Dental faculty of Monastir, Department of Dentistry University Hospital Sahloul, Tunisia

Correspondence: Nabiha Douki Zbidi, University of Monastir, Dental Faculty of Monastir, Research Laboratory LR12ES11, Department of Dentistry, University hospital, Sahloul, Tunisia, Email nabiha.douki@gmail.com

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Abstract

The regenerative endodontics is increasingly evolving nowadays. As a matter of fact, the use of Platelet-rich plasma (PRF) as a tissue growth matrix leads to the stimulation of root development for immature teeth. PRF has many qualities related to the presence of abundant growth factors such as pulp revascularization, promoting cell proliferation and enhancing angiogenesis. The aim of this article is to discuss, through a clinical case, the parameters of the protocol for endodontic regeneration of an immature permanent tooth with a necrotic pulp using PRF. A 9 years old patient, victim of a trauma, was referred to our department of dental medicine in Sahloul, Sousse. On clinical examination, the 21 presents a palatoversion and an extrusion of 1mm. After two weeks of retention, the tooth presented a coronal dyschromia with a negative response to the pulp vitality test. Retro alveolar radiographs showed desmodontal enlargement with thin dentinal walls and an open apex. The course of action was to adopt a regenerative endodontic technique with PRF.

At least, the goal of regenerative endodontic therapy is to createa new pulp and dentinal tissue having the same structure and function as the original tissue.

Keywords: Necrotic tooth, trauma, regeneration procedures, platelet concentrates, PRF.

Introduction

The treatment of immature necrotic permanent teeth has been a challenge in endodontics because it is difficult to achieve a tight apical seal when the apices are open using traditional endodontic methods. More recently, the treatment of infected immature teeth has evolved into so-called regenerative procedures, which help complete root construction and root canal wall thickening. In vitro and clinical studies have investigated the possibility of dental pulp regeneration through tissue engineering and improved blood clot revascularization techniques with platelet or fibrin-rich concentrates to increase the concentration of growth factors and the number of stem cells that regenerate dental cells in the canal to ensure continuity of root formation and apical closure.[1]

The aim of this article is to discuss, through a clinical case, the parameters of the protocol for endodontic regeneration of an immature permanent tooth with a necrotic pulp using PRF.

Case Report

Case presentation

The patient was 9 years old and in good general condition. He had suffered a trauma the day before the consultation which caused a palatoversion and an extrusion on the 21 and 22. At exobuccal examination, the upper lip has a sutured wound and oedema and at endobuccal examination, no associated alveolar fracture. Teeth 23,13,14,15 were in the process of (Figure 1a & 1b) eruption. Tooth 21 had a palatal dislocation and a 1mm extrusion as well as tooth 22. The course of action was to reduce the teeth and retain them for 2 weeks and to recommend good disinfection and soft food. After 2 weeks, the removal of dental retention was done (Figure 2a) and at the 1-month check-up, clinically, 21 presented a dyschromia (slight grey coloration especially at the palatal level). At the retroalveolar radiography, 21 showed a desmodontal enlargement and an open apex.

Clinically the crown exhibited discoloration depicting non- vitality and necrosis of the tooth (Figure 2b). The tooth was tender to percussion test and did not respond to cold test and periodontal probing depth was within normal limit. On intra oral peri-apical radiographic examination, the tooth showed anincompletely formed root, thin dentinal walls with open apex. Based on clinical and radiographic findings, the case was diagnosed as pulp necrosis and we decided to perform a regenerative endodontic treatment using PRF as a scaffold material.

Figure 1

A: Pre-operative clinical view.

B: Clinical view after the completion of the restraint.

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Under rubber dam isolation, root canal treatment was initiated (Figure 3, Figure 4). After thorough chemo-mechanical preparation, inter-appointment medication of CaOH2 was given for 2 weeks. On the second visit, the patient was asymptomatic, and the tooth showed no tenderness to percussion and palpation. Under rubber dam isolation, the temporary restoration was removed. The root canal was irrigated with 20 ml of 2.5% NaOCl solution, 5 ml of 17% ethylenediaminetetraacetic acid (EDTA), and 10 ml of normal saline (Figure 5). PRF was prepared by centrifugating patients own blood at 3000 rpm for 12 min (Figure 6) .PRF was prepared as per standard preparation protocol; squeezed in gauze to remove excess fluid, and cut into small linear strips for easy placement (Figure 7) Autologous PRF was introduced into the pulp chamber and carried to the apical portion with a Machtou Hand Plugger (Plugger DrMachtou DENTSPLY SIRONA - 1/2) (Figure 8). The canal orifice was sealed with mineral trioxide aggregate (MTA); a wet cotton pellet was placed over it and sealed with zinc oxide eugenol temporary cement. After 24 h, the patient was recalled, the pellet was removed, the composite filling was done, and post-operative instructions were given. The child was recalled in the sixth (Figure 9), and twelfth months and was evaluated clinically and radiographically. At 12 month follow up, case was asymptomatic interestingly, apical closure, root lengthening and dentinal wall thickening was optimal radiographically. (Figure 10) The protocol thus followed was in accordance with the guidelines proposed by the AmericanAssociation of Endodontists (AAE).

Figure 2

A: Clinical view after removal of the restraint.

B: Crown discoloration.

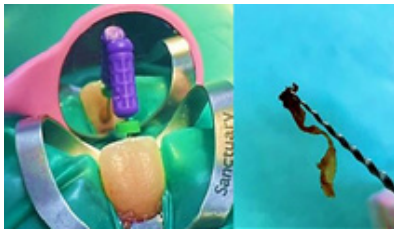

Figure 3 Endodontic treatment (Minimal).

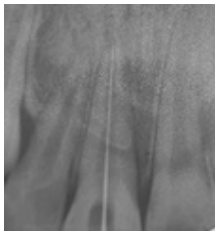

Figure 4 Retro alveolar radiograph file in intracanal.

Figure 5 Per operative Clinical view.

Figure 6 Blood collection and Centrifugation 2700 to 3000 rpm for 12min.

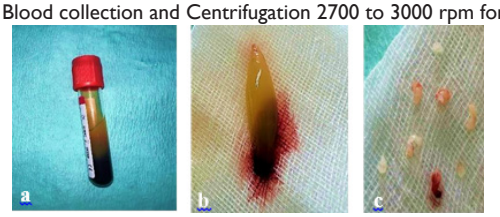

Figure 7

A: Contents of blood after centrifugation.

B: Platelet-rich fibrin membrane.

C: Fragmentation of the PRF into small pieces.

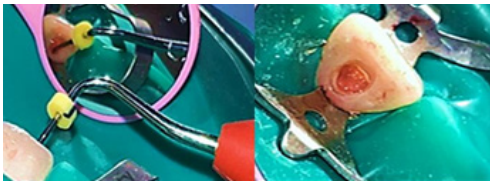

Figure 8 Placement of the FRP fragments using a Plugger Dr Machtou DENTSPLY SIRONA-1/2.

Figure 9 Follow-up radiograph taken 6 months after completion of treatment.

Figure 10 Follow-up radiograph of 12 months after carrying out revascularization treatment. The dentinal walls have thickened, and the apical foramen has almost closed.

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Ethical clearance and consent

Parents were informed of the risks, complications, and possible outcomes of PRF revascularization and gave written informed consent: According to one study, understanding theconcept of PRF (its autologous nature and benefits) is an important point in the acceptance of the procedure, and a booklet or video demonstration is a means of improving parental knowledge about the technique. Legal guardians or parents have the power to assess the child's level of cooperation according to his or her age and cognitive development. The parent's profile is a factor to be taken into consideration; supporting their child without being too reassuring or too silent is of great value.[2]

Discussion

Due to its multiple qualities, PRF-type platelet concentrate gives high potential in pulp revascularization procedures. In fact, it is rich in growth factors, it promotes cell proliferation and differentiation. In addition, this concentrate enhances angiogenesis, acts as a matrix, regulates inflammatory reactions, and has anti-infective properties.[3]

In the endodontic regeneration procedure of immature permanent teeth, only DPSCs and SCAPs have the potential todifferentiate into odontoblasts.[4,5] (Figure 11)

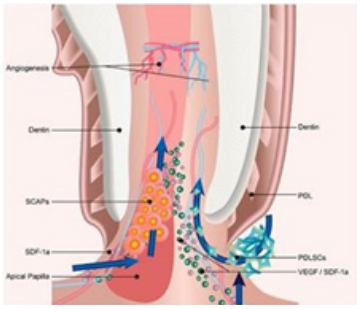

Figure 11 Dental pulp regeneration.[4]

In our case, endodontic regeneration was indicated since it is an immature permanent tooth with pulp necrosis and it is a conservable tooth with no ankylosis or any pathologic signs ofinternal or external root resorption, absence of a periodontal pocket > 3 mm, absence of pathologic tooth mobility and the possibility of placement of a tight surgical field .Relative indications are also noted, including: Patient in good general condition/patient cooperation/parental motivation to ensure regular follow-ups for young patients.

Operative protocol

Successful management of immature permanent teeth using platelet-rich fibrin is a technique-sensitive procedure (Figure 12).

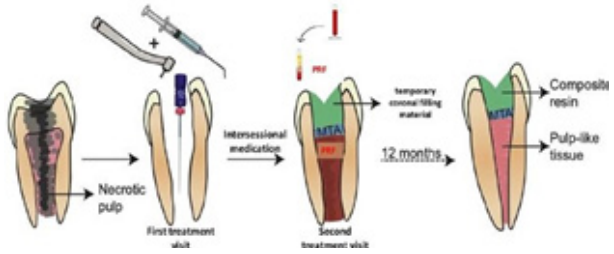

Figure 12 Operating procedure for PRF placement.

Root Canal Disinfection

The first step of the regenerative technique is to create adisinfected root canal mainly by using irrigants andintersessional medication. Minimal instrumentation using K-files is tolerated without the use of rotary instruments in order to avoid the potential fracture of incompletely formed fine roots. Also, to avoid the formation of a smear layer that could obstruct the dentinal walls and tubules and to prevent the attachment of stem cells to the root canal walls.[6,7] Mechanical disinfection must be complemented by chemicalaction: Root canal irrigation and intersessional medication.

Root Canal Irrigation

There are several controversies regarding root canal disinfection solutions (Table 1). Also, final irrigation has always been a highly debated topic.[8-11] Inour case, we used EDTA 17%. In addition to the products usedand their concentrations during this stage of chemical disinfection, two points should be mentioned: The volume andduration of the irrigation: According to the studies, a volume of10 to 20 ml is sufficient to properly irrigate the canal. The operation is repeated every 5 minutes for a duration varying from 15 to 30 minutes/ The irrigation technique: Few authors detail the irrigation technique used, but most agree that it mustbe abundant but at the same time slow and prudent to avoid anyrisk of overflow of the product into the periapical tissues.[12]

Table 1 Root canal irrigants

Ethylenediamide tetraacetic acid solution17% (EDTA)	*Sodium hypochlorite (NaOCl)*	*Chlorhexidine*
*In final irrigation to minimize cytotoxicity to stem cells in apical tissues. *Stimulates the release of growth factors embedded in the dentin matrix: Recruitment of cells involved in regeneration.[8]	*Gentle irrigation with 20mL/root canal, 5 min using a specialized irrigation system that minimizes the possibility of extrusion of irrigants into the periapex (example: closed-tip, side-vented needle). *A low concentration is recommended (1.5% to 2.5%) due to its proteolytic effect.[9,10]	*Chlorhexidine is used because of its antimicrobial activity with a concentration of 2%. Its bacterial remanence gives it a prolonged action. *Nevertheless, it should not be used as the only irrigation solution since it does not have the capacity to dissolve tissues, so irrigation with chlorhexidine could have cytotoxic effects on progenitor stem cells and interfere with the adhesion of DPSC to the root canal walls.[11]

Intersessional medication

- CaOH2

Although considered a "Gold Standard" in endodontics for its osteogenic and antibacterial effects, the use of Ca(OH)2as an intra-root canal medication in pulp regeneration procedures has not been systematic, according to the literature review. From a regenerative point of view, it counteracts the acidosis of inflamed tissues by a buffering phenomenon and thus establishes an environment favorable to tissue regeneration. Its alkaline pH stimulates the underlying vital tissue by increasing the proliferation of SCAPs so that they can initiate a repair response.

According to some authors, calcium hydroxide can be used at any concentration in the regeneration procedure (if the ready-to-use creamy paste is used).[13]

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- Triantibiotic paste:

Scientific research has shown that the tri-antibacterial paste composed of ciprofloxacin, metronidazole and minocycline is effective in disinfecting the canal. Reports have shown that thepaste is able to kill bacteria present in the deep dentin layers ofthe root canal and is also effective against several types of pathogens that can cause endodontic failure. Its greatest disadvantages are the corneal dyschromia caused by minocycline and the risk of allergy and development of resistant bacterial strains. To alleviate these drawbacks, the paste can be kept one millimeter below the ECJ of the tooth to minimize staining, as suggested by various authors.[14,15] In addition, some researchers have stated that sealing of dentinal tubules within the chamber can be performed to decrease the intensity of discoloration.[14]

- Double antibiotic paste:

The study by Iwaya et al. noted the use of a bi-antibiotic paste (without minocycline), or substitution of minocycline with another antibiotic (example, clindamycin; amoxicillin; cefaclor) but this may increase the risk of developing resistant bacterial strains.[12]

According to the articles, there is no consensus on the product used for the inter-sequence medication but in our case the advantage/ disadvantage ratio was balanced for the CAOH2.

PRF

Numerous platelet-rich concentrates, including platelet-rich plasma (PRP) and PRF, have been used for tissue regeneration in several studies. Comparing these two, we find that PRF has several positive points compared to PRP such as the lack of use of anticoagulant or bovine thrombin which reduces the biochemical alterations related to its use, a lower cost and easier handling. Also, PRF has good mechanical properties dueto its complex fibrin matrix architecture which makes it slowlyremodeled, stimulates more efficiently migration, proliferation and differentiation of stem cells as well as presents progressive and slow release of growth factors over a period of 1 to 4 weeks: better effect on bone and dentin regeneration.[15]

Initially, regeneration was based on the blood clot procedure, which has its drawbacks since it is difficult to control bleeding and it is not obvious to obtain periapical hemorrhage in some patients due to anatomical obstacles such as the proximity of the nerve, with the possibility of nerve damage, especially in mandibular premolars.[16,17] Studies have shown that the combination of the reliability of the blood clot (BC) and the action of platelet concentrates (PRF) is betterthan PRF or BC alone.[18]

It has been shown that there are significant differences in the macroscopic morphology and size of PRF membranes after centrifugation between patients. Based on the results of one study, in general, older patients and women produce larger PRF membranes than younger patients and men. In addition, a previous report by Yajamanya et al. showed that the fibrin network of PRF membranes was less dense with aging.[19] This factor may also have played a role in layer separation. Another important parameter examined was the time fromblood collection to the start of centrifugation. The authorsreport that an optimal blood draw of 90 seconds yields PRF.[20]

There are many new forms of PRF that has been developed by changing the centrifugation parameters, mainly its force. In fact, injectable platelet-rich fibrin (I-PRF) was firstly suggested by adjusting spin centrifugation forces in 2014. It is, actually, obtained by a blood centrifugation in non-glass tubes at a lower speed. The I-PRF is enriched with leukocytes that enhances soft and hard tissue regeneration. Besides, this form of PRF is considered as more practical for use because it can remain liquid for about 15 minutes.[21]

In addition to that, reducing the centrifugation g-force resulted in another type of PRF named A-PRF +.[19] Fujioka and al. showed in their study that it contains the greatest number of leukocytes and that it can release higher levels of growth factors compared to L-PRF and A-PRF.[22]

Coronary sealing

Successful revascularization depends on meticulous disinfection, the use of an appropriate scaffold, and proper coronary sealing. In the case presented, an MTA seal was used.MTA remains the most widely used material in endodontic regeneration therapy. It is the most clinically studied material. It is characterized by its marginal adaptation to the tooth walls and provides an effective interface between the permanent restorations and the newly formed tissue. MTA placed directlyon the PRF clot has two advantages: the moisture of the PRF facilitates the uptake of the MTA and the MTA provides signaling molecules for stem cell growth.[23]

Follow-up

The follow-up period is variable. Generally, the follow-up appointment is made after 3 to 6 months from the end of the treatment, and then every 3 months until 1 or 2 years. The success of the treatment is based on the observation of the clinical symptoms on the one hand, and the responses to the pulp vitality tests on the other hand, as well as the periapical healing (resolution of apical radiolucency), the increase in the thickness of the root wall (often observed 1 to 2 years after the treatment) and/or the length of the root and the apical closure, which are radiologically assessed during the follow-up.[12]

The successful outcome of regeneration procedures can be measured as; the ability to accomplish apical closure of the tooth root, a periapical lesion healing response, root lengthening, and dentinal wall thickening, because these indicate the regeneration of tissues (Jadhav et al., 2013; Alagl et al., 2017; Shivashankar et al., 2017).

Some factors may participate in treatment success such as initial apical diameter. Estefan et al (2016) proved that teeth with larger foramen diameters had a greater increase in root thickness and length, as well as partial or complete apical closure.[24] According to the same study age is a key factor inroot development, the youngest age group, 9 to 13 years, showed a significant increase in root length compared to the 14- to 18-year-old group. The authors explain this phenomenon by the greater regenerative potential of stem cells in young people. The etiology of the pulp necrosis also influences the regenerative treatment. Thus, cases treated following dental anomaly or caries have better success rates than cases of severe trauma treated by pulp regeneration.[25] Zeng et al. (2022) found that postoperative failure of the regenerative procedure on immature permanent teeth with trauma could be because of impaired blood supply to the peri apical area and reducedresistance to infection.[26]

Conclusion

Tissue engineering in endodontics is based on the use of a tetrad composed of stem cells, growth factors and a biological matrix that serves as a “scaffold”.[27] The goal of regenerativeendodontic therapy is to create a new pulp and dentinal tissue having the same structure and function as the original tissue, but it is impossible to guarantee that this tissue is a dental pulpcapable of restoring the vitality and the capacity for defense, repair and nutrition of the tooth.

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Conflicts of interest

The authors declare no conflict of interest.

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