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Regenerative endodontic procedure using PRF (Platelet –rich fibrin) as a scaffold in an immature necrotic mandibular premolar teeth with symptomatic apical periodontitis : a case report.

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ABSTRACT

Aim and objective: This case study's objective is to illustrate how regenerative endodontic procedure (REP) can be used to treat an immature necrotic tooth with symptomatic apical periodontitis.

Background: Endodontics is transitioning into a new era of tissue engineering and regeneration. We may need to adopt a more biological strategy to encourage the body's innate wound healing process to revitalize the pulp tissue within the canal space rather than routine root canal treatment for immature necrotic teeth. Compared to apexification procedure, outcome studies using REP on immature permanent teeth showed larger improvements in both root width and root length.

Case description: In this case report, a 14-year-old female patient came in with a chief complaint of pain in her lower right premolar tooth. On clinical examination deep dentinal caries was revealed, radiographically; periapical radiolucency was seen with an immature root and wide open apex. A finding of immature permanent tooth with symptomatic apical periodontitis was confirmed. Regenerative endodontic procedure (REP) was chosen as the course of treatment. Local anesthesia was administered, access opening of the tooth#45 was carried out, radiographic measurement of the working length was done and the tooth was biomechanically preparated upto 30k file. Calcium hydroxide dressing was given for 1 week. Following week TAP (triple antibiotic paste) was placed for about 2 weeks. After complete disinfection of the canal , in the 3rd week blood clot was induced within the canal using a sterile 15k file 2mm beyond the apex. PRF fragment membrane using an endodontic plugger was placed into the pulp chamber. Biodentine was placed over the PRF membrane and the tooth was restored with GIC. The patient underwent evaluations after 3, 6, 9 and 12 months.

Conclusion: Immature necrotic permanent teeth with symptomatic apical periodontitis may respond well to REP using PRF as a scaffold. In this case report pulp regeneration outcome was characterised by prolonged root growth and a positive feedback to pulp vitality tests.

Clinical significance: The treatment for an immature necrotic tooth with symptomatic apical periodontitis reported in this case report is a biologically based procedure. It can thereby be used as an alternative approach to apexification procedure.

Key words: Regenerative endodontic procedure(REP)**,** immature teeth**,** open apex**,** scaffolds**,** Platelet-rich fibrin(PRF), biodentine,

BACKGROUND :

The goal of the fresh and incredibly interesting branch of study known as tissue engineering is to restore damaged tissue. As a result, it has an incredible impact on dental practise recently. The interplay of material sciences, biocompatibility, integrated cells, natural or synthetic scaffolds, and specific signals is required for the development of newly formed tissue. The American Association of Endodontists approved the name "regenerative endodontics" based on the tissue engineering approach in 2007.

The three most frequent causes of pulp and periradicular disorders are caries, pulpitis, and apical periodontitis. The complete eradication of the diseased pulp tissue from the root canal space is the viable treatment option in a conventional root canal therapy. Nevertheless, the viewpoint has changed nowadays towards biologically based treatment approaches, such as Regeneration Endodontic Procedure(REP) and vital pulp therapy utilising pulp stem/progenitor cells. [1]

Regeneration endodontic therapy, as opposed to apexification procedure for young permanent teeth, takes a more biological approach to promote the body's innate wound healing mechanism to repair the essential tissue within the root canal space. It has the potential to revive immunological and sensory function in an adult necrotic teeth, these newer treatment approach supports the regeneration or repair of the pulp-dentin complex with or without the removal of the whole pulp. The primary Stem cells mainly from Apical papilla (SCAPs), together with growth factors and morphogens, and a scaffold that can support cell development and differentiation are the fundamental elements of RET. [2] On the basis of the classification of a Cvek (Endodontics and Dental Traumatology 8, 1992,45)[3] regenerative endodontics should be considered as a treatment option in young permanent tooth with incomplete root formation .

Nosrat et al. have compiled a list of the desirable qualities of a scaffold for effective regeneration which includes revascularization via blood clot, scaffold implantation using platelet rich plasma (PRP) or platelet rich fibrin (PRF).[4-6] The treatment outcome has 3 goals: first is disappearnce of the clinical signs and symptoms, second is the development and maturation of root apex, third is revascularization of pulp or return to neurogenesis.[7]

Endodontics is transitioning into a new era of tissue engineering and regeneration. However additional research into this biologically based procedure is warranted. The purpose of this case report is to demonstrate how regenerative endodontic therapy (REP) may be used to treat a developing permanent tooth with symptomatic apical periodontitis.

CASE DESCRIPTION:

A 14 year old female who had a primary complaint of pain in her lower right posterior tooth region reported to the department of Conservative Dentistry and Endodontics. History revealed that the pain started two weeks previously which was continuous in nature, moderate in intensity, aggrevating and relieved on its own. The patient lacked any pertinent medical history. Clinical examination showed deep dentinal caries in the lower right second premolar (tooth#45) and marked tenderness to both percussion and palpation tests. The tooth responded negatively to cold and EPT tests. (Endo Ice Coltene Whaledent and EPT) The depth of periodontal probing was normal and there was no mobility of the tooth. Radiographically; radiolucency involving enamel, dentin and pulp with immature root and wide open apex, thin dentinal walls and an evident periapical radiolucency around the apex of the tooth #45 was seen. **(figure 1)** A diagnosis of an immature permanent tooth with symptomatic apical periodontitis was confirmed based on the radiological and clinical results.

Since the patient was young and the root was incompletely formed thereby Regenerative endodontic procedure (REP) was determined as the therapy option. The treatment plan was explained to the patient, and the patient's parent signed a written consent form.

TREATMENT PROTOCOL:

Local anesthesia (2%lidocaine with 1:100,000 epinephrine) was administered. After Rubber dam isolation access opening of the tooth#45 was carried out. Radiographic measurement of the working length was done. The canals were biomechanically prepared upto 30k file. 10ml of 1.5% sodium hypochlorite and 17% EDTA was used to irrigate the canal and final irriagation was done using saline. The canals were then dried using paper points. Calcium hydroxide dressing was given for 1 week. The cavity was temporarily sealed placing a cotton pellet inside the pulp chamber and a temporary sealing material.

The patient reported back after 7 days. The access cavity was reopened, and the calcium hydroxide paste was thoroughly flushed from the canal using saline irrigation. Paper points were then used to dry the canal. Equivalent amounts of ciprofloxacin, metronidazole, and minocycline in the ratio (1:1:1) were combined with distilled water to form a thick paste that was then placed into the canal using a lentulo spiral. A cotton pellet and a temporary sealing substance were placed within the pulp chamber to temporarily seal the cavity.

After 14 days, the patient reported back with no symptoms. 10ml blood was withdrawn from patients right arm and was transferred into a test tube without an anticoagulant and was centrifuged (3000rpm) for 10 min. Using sterile tweezers, PRF was separated from the test tube following centrifugation. After being transformed into membrane form, this fibrin membrane was cut into small pieces using surgical scissors. **(figure2A and 2B)**

Local anesthesia was administered to the patient, after isolating the tooth with rubber dam the access cavity was reopened, thorough saline irrigation was flushed within the canal to remove the anitibiotic mixture. Canal was prepared for the placement of the PRF by drying it with paper points.

A sterile 15k file was introduced 2 mm beyond the apex to induce bleeding within the canal, intracanal bleeding was thereby induced upto 3mm below the CEJ.**(figure3A)** Following that with the use of an endodontic plugger, PRF fragment membrane was inserted into the pulp chamber.**(figure 3B)** PRF membrane covered the roof of the entire pulp chamber. **(figure3C).** Over the PRF membrane, biodentine was applied, and GIC was used as a temporary filling material. **(figure3Dand 3E)** During the 3 month follow up the patient reported back, she was asymptomatic and radiographically the periapical lesion was healing. A composite restoration was done after partial removal of the GIC material.

FOLLOW UP :

The patient underwent evaluations after 3, 6, 9 and 12 months. The tooth #45 was asymotomatic and was not sensitive to percussion on the 3rdmonth review. On pulp sensibility test it showed negative response. On the 6th month follow up the pulp sensibility test with cold and EPT elicited a delayed positive response. On the 9th and 12th month follow up radiographically, the periapical lesion resolved and mild thickening of the dentinal walls was appreciated, pulp sensability test showed positive response to both cold test and EPT.**(figure 4A-D)**

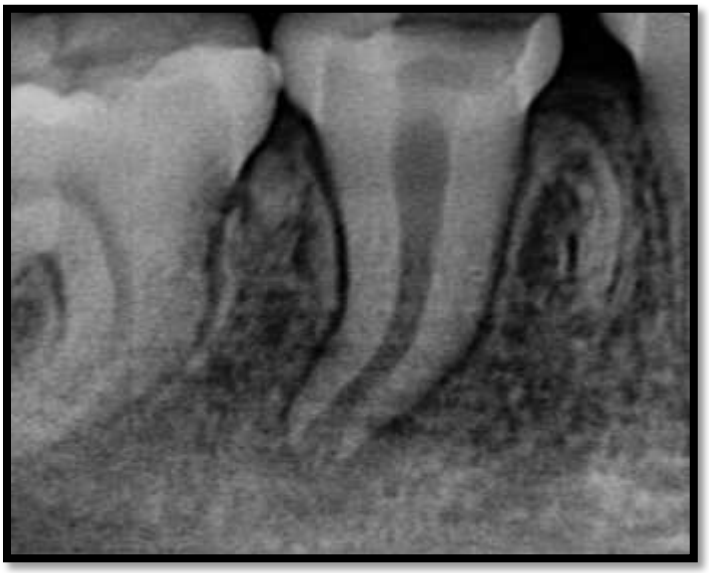


Figure1: pre-operative radiograph showing deep dentinal caries with periapical radiolucency in an immature root apex.

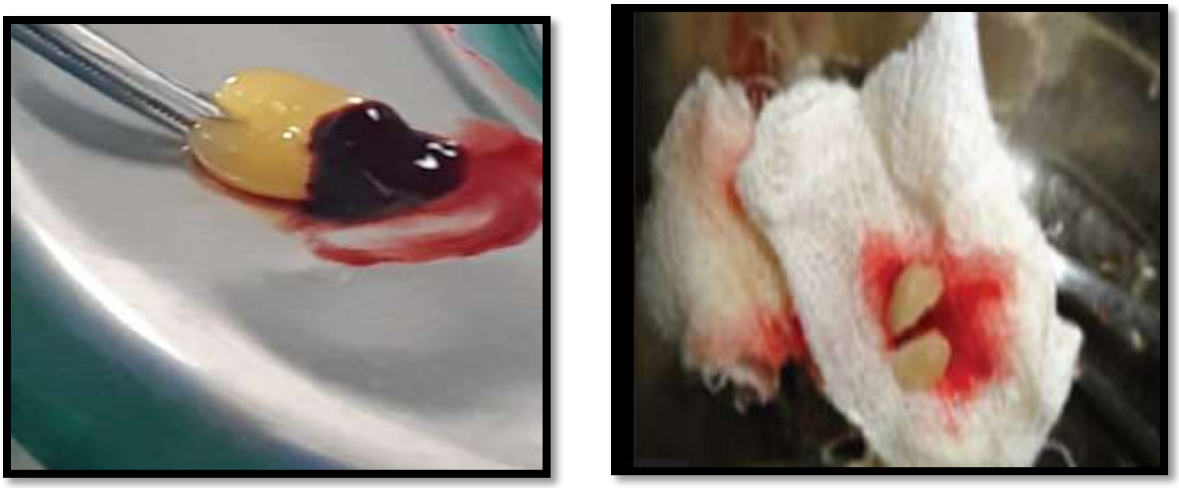
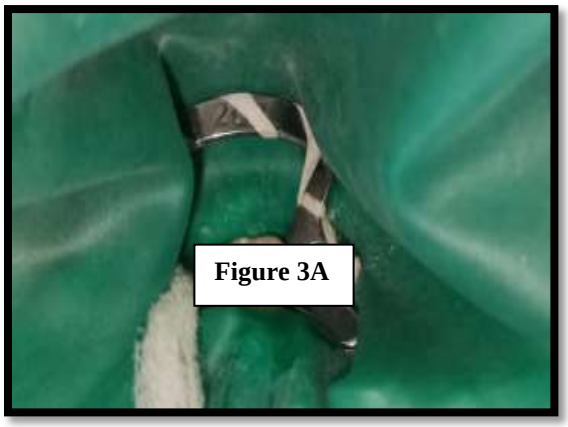


Figure 2A and 2B : (A) PRF mrembrane (B) fibrin membrane fragmented into small pieces



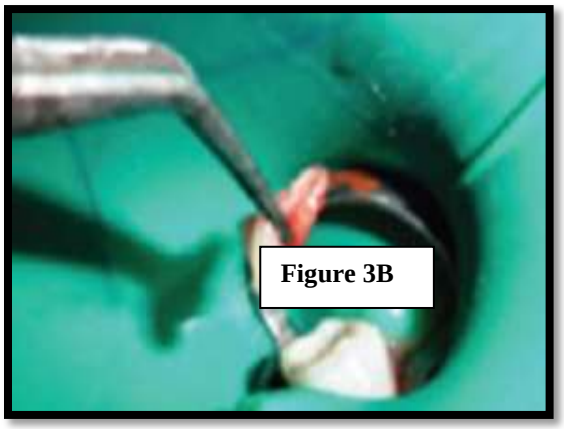


Figure 3C

Figure 3D

Figure 3E

Figure 3A to 3E: (A) induced bloot clot (B and C) PRF fragment membrane placement (D) biodentin placement (E) GIC restoration

Figure 4A

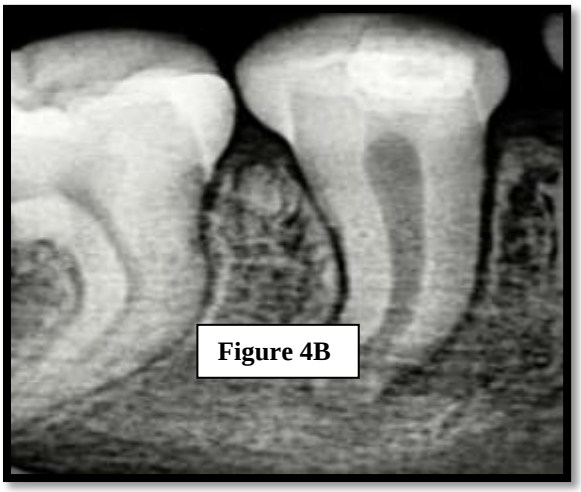


Figure 4B

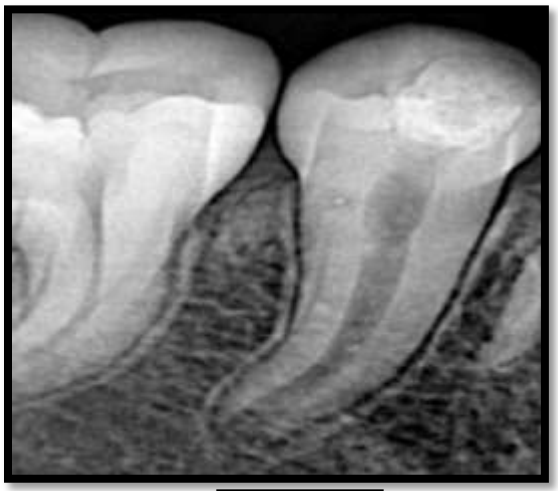


Figure 4C

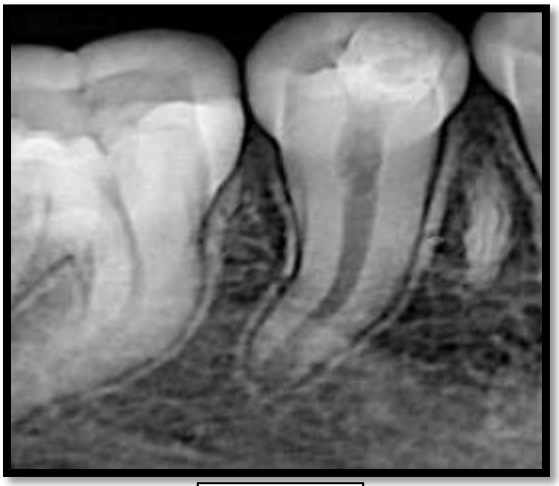


Figure 4D

Figure 4A-D: (A) 3 month follow up (B) 6 month follow up (C) 9 month follow up (D) 12 month follow up

DISCUSSION :

Apexification and regenerative endodontic procedure (REP) are the two therapeutic choices for immature permanent non-vital teeth with apical periodontitis. While a strong tissue barrier has been created at the apex of the canals using apexification with calcium hydroxide, biodentine, and mineral trioxide aggregate, there is no additional root development or thickening of the root canal walls, which is a significant disadvantage. The underveloped, short, and weak roots cannot support large masticatory load and is frequently brittle.[8,9] Root end closure, thickening of the root canal walls, and revascularization of the pulp are some advantages that regenerative endodontic procedures offer over the conventional apexification method.[10] Hence, we have opted to utilise REP as the treatment option.

Only if the canals are sufficiently cleaned, which is a prerequisite for regeneration/repair, can the endogenous bioactive chemicals be targeted to promote regeneration. The fundamental aspect for the success of this procedure is the complete elimination of the bacteria and decontaminate the root canal space. The efficacy of triple antibiotic paste in an immature teeth with apical periodontitis was investigated by William Windley et al who reported that the colony forming unit (CFU) counts decreased in the samples where triple antibiotic paste was used as a dressing for two weeks.[11] Hoshino et al. conducted a study to investigate the antibacterial efficiency of this paste and later concluded that this drug combination can adequately remove bacteria from the diseased dentine of root canals.[12]

The effect of triple antibiotic paste on the in vitro survival of stem cells from the apical papilla was investigated (Ruparel et al. 2012, Althunairy et al. 2014)[13,14] It was concluded that to prevent harming stem cells from the apical papilla, it is advised to use triple antibiotic paste in RET at a dosage of no more than 1 mg mL-1 (0.1–1 mg mL-1) (AAE 2016)[7]. In the current study triple antibiotic paste was used as a disinfection protocol for 2 weeks.

NaOCl (1.5%) was chosen as an irrigant because of its slightest toxic reaction to SCAP and its potential to release more TGF-1 from the canal walls when paired with 17% EDTA.[7,15] Dental pulp stem cells are more likely to adhere to EDTA-treated dentin because it has improved surface wettability and increased synthesis of the odontoblast-like cell marker dentin sialophosphoprotein (DSPP), in addition to removing the smear layer.[16- 18]

By releasing growth factors locally or by initiating a signalling cascade when stem cells adhere to the extracellular matrix and to one another in a three-dimensional environment, a scaffold is crucial for controlling stem cell development.[19]

The usage of blood clot as a scaffold in REP has been promoted. Blood induced from the root apex allows endogenous hemostatic components to flow into the canal space and form a fibrin clot that supports procedures necessary for SCAP survival and development.[20,21] The advantages of an intracanal blood clot include the availability of a homologized scaffold composed of cross-linked fibrin and containing the growth factors necessary to promote SCAP migration, differentiation, vascularization, and tissue regeneration, as well as the absence of a foreign body response (Jadhav et al.2012; Chrepa et al., 2017)[22,23] Several studies, however have discovered that in clinical practise, it wasn't always feasible to produce enough blood to act as a scaffold,

thereby raising the risk of sealing material failure.[24] Also, a number of studies have indicated that platelet concentrates play a more beneficial function in regeneration than only using blood clots as a scaffold. Thereby in this case report blood clot as a scaffold was used along with PRF.

The use of a physical scaffold is crucial in tissue engineering. When utilised as scaffolds in regenerative endodontic procedure, platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) two autologous platelet concentrates, have shown encouraging clinical and radiographic results (REP).[25-27] First-generation autologous plasma known as PRP has higher concentrations and quantities of growth factors that can promote tissue regeneration in both hard and soft tissues. In contrast, PRF contains a three-dimensional structure and bioactive molecules that encourage the growth and division of stem cells. Since it exclusively includes endogenous components, it is a better fibrin network for cytokine and growth factor storage as well as cell migration. PRF was utilised as a framework in this case study. Young permanent teeth with necrotic pulps can be revascularized with PRF because it provides a scaffold rich in growth factors and encourages cellular proliferation and differentiation. After the administration of PRF to a tooth with pulpal necrosis and an open apex, Shivashankar et al. found signs of root lengthening, periapical lesion regression, thickening of the dentinal walls, and apical closure.[28] In this present case report pulp regeneration was seen along with persistent root development and a positive reaction to pulp vitality tests.

Biodentine was employed as a covering material over the PRF scaffold because it has the ability to stimulate TGF-1 secreted from the radicular dentin, a quicker setting time than mineral trioxide aggregate, and less discolouration. Its a bioactive substance that promotes the survival and development of apical papilla stem cells which creates a layer of hydroxyapatite crystal at their interface with the biological environment making it more biocompatible and also helping in hard tissue induction potential.[29]

In the current study all the aforementioned goals were achieved. The periapical lesion healed and the affected tooth showed no clinical indications or symptoms, pulp regeneration was seen along with increased thickening of canal walls and continuous root maturation was observed. The tooth also responsed positive for the pulp vitality tests.

CONCLUSION :

The current case report shows that REP using PRF as a scaffold may be a good substitute for treating immature necrotic permanent teeth with symptomatic apical periodontitis. This case report also gives the subject of regenerative endodontics a fresh viewpoint. To ascertain the effectiveness and survival rate of teeth receiving regenerative endodontic therapy, more clinical trials should be done on immature necrotic permanent tooth.

CLINICAL SIGNIFICANCE: The procedure described in this case report is a biologically based procedure and a more conservative approach in treating an immature necrotic teeth with symptomatic apical periodontitis. Thereby instead of apexification, it can be employed as a therapy modality.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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