

Rejuvenating Tooth Pulp Naturally: Two Intriguing Case Reports on Successful Regeneration using Platelet-rich Fibrin Scaffold in Young Central Incisor and Mandibular Molar

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Abstract

Endodontics is entering a revolutionary phase of tissue engineering and regeneration. A shift toward adopting a more biological approach is necessary to stimulate the body's intrinsic wound healing process; thereby revitalizing pulp tissue in the canal space. Treating necrotic immature permanent teeth with endodontic intervention can be a complex task for clinicians. It is possible to achieve positive results through regenerative treatment, as long as appropriate cases are selected. However, there is currently no consensus on the most effective disinfection protocol or method to ensure consistent clinical outcomes. Research on regenerative treatments for immature permanent teeth has demonstrated remarkable advancements in root width and length, surpassing the outcomes obtained through the apexification procedure. To shed light on this matter, this article presents two distinct case reports. Both involve regenerative treatment in traumatized immature maxillary central incisor teeth, as well as an immature mandibular molar with irreversible pulpitis. In these cases, biodentine was implemented as coronal barrier, resulting in successful clinical outcomes.

Keywords: Biodentine, Immature teeth, Open apex, Platelet-rich fibrin, Regenerative endodontic procedure, Scaffolds.

INTRODUCTION

Immature permanent teeth have traditionally presented challenges in endodontic treatment. There are two commonly employed approaches: Apexogenesis, which aims to maintain pulp tissue vitality and allow natural root formation, and apexification, a procedure that involves the use of specific materials to create a barrier and close the root apex. However, apexification has several disadvantages. The use of calcium hydroxide necessitates repeated placement of the intracanal medication, increasing the risk of reinfection. In addition, extensive instrumentation within the root canal can weaken the canal walls.^[1,2] To address the issue of intracanal medication replacement, newer materials like mineral trioxide aggregate (MTA) or Biodentine have been introduced as alternatives to calcium hydroxide. However, none of these materials promote root completion; they merely serve as mechanical barriers to close the root canal's lumen.^[3]

In contrast, regenerative endodontic therapy leverages biological and engineering principles to restore the pulp-dentine complex that has been compromised due to factors such as caries, traumatic injury, or abnormalities. It has been

proposed as the most suitable treatment option for young permanent teeth with necrotic pulp and apical periodontitis. The desired outcomes of this therapy are similar to those achieved through apexogenesis – a crucial process for preserving the vitality of the pulp.^[4]

A blood clot acts as a biological scaffold within the root canal system and has shown promising results in most regenerative endodontic therapies.^[5,6] Other physical scaffolds have also been found to promote tissue regeneration. The use of platelet-rich fibrin (PRF), a second-generation platelet concentrate, can enhance healing capabilities through the presence of platelets, growth factors, and cytokines. What sets PRF apart is its completely autologous origin.^[7,8] This case report details a regenerative endodontic procedure for a traumatic

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necrotic maxillary central incisor with asymptomatic apical periodontitis and a mandibular second molar with symptomatic irreversible pulpitis employing triple antibiotic paste (TAP), biodentine, and PRF.

CASE DESCRIPTION

Case 1

An 11-year-old male patient along with his guardian reported to the Department of Conservative Dentistry and Endodontics with a chief complaint of a broken tooth in the upper front region. The patient had a traumatic accident that occurred 4 months ago. Medical history was non-contributory. Dental history was non-significant other than the history of trauma to the upper left central incisor. Clinical examination reveals teeth discoloration (Figure 1a). Electric pulp testing along with a cold test was done, and it showed a negative response in the concerned teeth compared to the upper right central incisor and also right and left lateral incisors. On percussion or palpation, no tenderness was present. On clinical examination, gingival tissues were healthy with normal texture and contour, and also periodontal probing depth was within normal range for all teeth. A periapical radiograph (Figure 1b) was taken, and it showed incomplete root development with parallel root walls in the upper left central incisor with no apparent periapical radiolucency. Furthermore, cone-beam computed tomography (CBCT) was advised (Figure 1c-f). The diagnosis was pulp necrosis with asymptomatic apical periodontitis. The treatment plan was to try revascularization of concerned teeth in an attempt to promote root maturation in length and width. The treatment plan was discussed with the patient's guardian. The potential risks, complications, and benefits of the recommended treatment were thoroughly explained and the patient provided consent by signing the necessary documentation.

Local anesthesia was administered, consisting of 2% lidocaine with 1: 100,000 epinephrine. After isolating the upper left central incisor with a rubber dam, an access opening was created (Figure 1g). A radiographic measurement of the working length was performed (Figure 1h). The canals were then mechanically prepared up to an international organization for standardization (ISO) #55 K file (Mani Inc., Japan). A solution of 10 mL of 1.5% sodium hypochlorite (Neelkanth [zodenta], India) and 17% ethylenediaminetetraacetic acid (EDTA) (MEDA BIOMED CO. Ltd, Korea) was used for irrigation, followed by final irrigation with saline (Infutec Healthcare Ltd., India) and paper points were used to dry the canal. Calcium hydroxide dressing was applied and left in place for 1 week. The cavity was temporarily sealed with a cotton pellet and a temporary sealing material.

After 7 days, the patient returned for follow-up. The access cavity was reopened and the calcium hydroxide paste was thoroughly rinsed out using saline irrigation. The canals were

dried using paper points. A thick paste, composed of equivalent amounts of ciprofloxacin, metronidazole, and minocycline (TAP was prepared with a ratio 1:1:1 proportion) mixed with distilled water, was then placed into the canal using a lentulo spiral. A small cotton roll along with temporary sealing material was placed in the pulp chamber to temporarily seal of the prepared cavity (Figure 1i).

After another 14 days, the patient reported no symptoms. A sample of 10 mL of blood was withdrawn from the patient's right arm and transferred into a test tube without an anticoagulant. The sample was centrifuged at 3000 rpm for 10 min, and the PRF was separated from the test tube using sterile tweezers (Figure 1j and 1k). The PRF was then transformed into membrane form and cut into small pieces using surgical scissors. The patient received local anesthesia, and after isolating the tooth with a rubber dam, the access cavity was reopened. Thorough saline irrigation was performed to remove the antibiotic mixture. The canal was then prepared for the placement of the PRF by drying it with paper points.

A sterile file, with a diameter of ISO #15 K, was inserted 2 mm past the apex of the tooth to induce bleeding within the root canal. This resulted in bleeding occurring up to 3 mm below the cemento-enamel junction. Afterward, a piece of PRF membrane was placed in the pulp chamber using an endodontic plugger (Figure 1l). The PRF membrane covered the entire roof of the pulp chamber. Biodentine was then applied (Figure 1m) over the PRF membrane, and a temporary filling material, glass ionomer cement (GIC), was used.

During the 3-month follow-up (Figure 1n), the patient returned and reported no symptoms. X-rays showed healing of the periapical tissues. After partially removing the GIC material, a composite restoration was performed.

Further evaluations were conducted at 6 and 12 months. During the 6-month follow-up (Figure 1o), there was a delayed positive response to cold and electric pulp testing. At the 12th month follow-ups (Figure 1p), radiographs revealed an increase in radicular dentin length and width with more apical closure in the upper left central incisor. Post-CBCT image (Figure 1q) was also obtained to visualize the apical end of the tooth. Pulp sensibility testing showed positive responses to both cold and electric pulp tests.

Case 2

A 16-year-old girl came to the Department of Conservative Dentistry and Endodontics with a main complaint of jaw pain on the lower right side that had been bothering her for the past 18–20 days. She had been fine before this but suddenly experienced moderate-to-severe pain in the back tooth region on the lower right side of her jaw. She described the pain as sharp and localized. No significant medical or dental history was noted. On examination, it was found that the right second permanent molar had deep cavities and was tender when tapped. X-rays revealed incomplete root formation

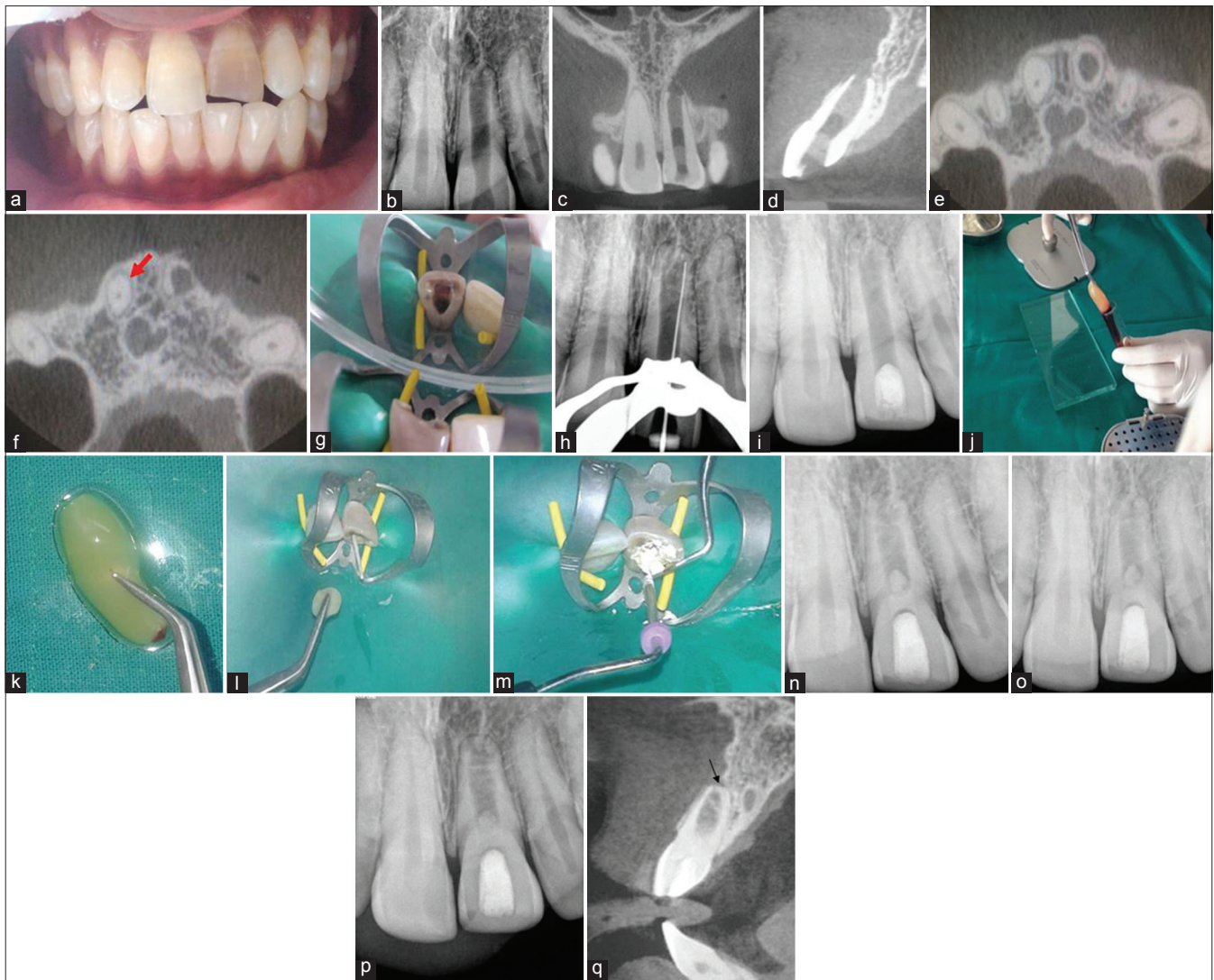


Figure 1: (a) Pre-operative photograph, (b) Pre-operative RVG of upper left central incisor which shows incomplete root development due to traumatic injury, (c and d) Pre-operative cone-beam computed tomography (CBCT) images showed incomplete root development and parallel root walls of an upper left central incisor, (e and f) Pre-operative CBCT images: Axial slicing at different root lengths, (g) Access opening in rubber dam, (h) Working length determination, (i) Triple antibiotic paste, (j and k) PRF obtained from 10 mL of intravenous blood of the patient, (l) PRF placement, (m) Biodentine placement, (n) 3-month follow up, (o) 6-month follow up, (p) 1-year follow-up RVG shows an increase in radicular dentin length and width with more apical closure in the upper left central incisor, (q) 1-year follow-up CBCT

and increased space in the ligament that surrounds the tooth (Figure 2a). The diagnosis was determined to be symptomatic irreversible pulpitis with an open apex. It was decided that root canal treatment was the best course of action given the circumstances. The parents were thoroughly informed about the possible complications and alternative treatment options and gave their consent.

The root canal treatment was performed in two appointments. In the first appointment, the patient was given local anesthesia, and the tooth was isolated using a dental dam. The decayed areas were removed and access opening was done. After locating the canals and removing the pulp tissue, the tooth was irrigated with 10 mL of sodium hypochlorite solution to disinfect it.

To minimize any harm to the stem cells present in the apical tissues, the root end was irrigated with a saline solution using a needle. The working length of the canals was determined (Figure 2b), and they were dried with paper points. A thin layer of TAP, consisting of ciprofloxacin, metronidazole, and minocycline, was applied to the canals. The pulp chamber was sealed with a bonding agent to prevent staining before the antibiotic paste was inserted. The tooth was then temporarily restored with GIC (Figure 2c). The patient was scheduled for a second appointment in 4 weeks.

During the second appointment, the effectiveness of the initial treatment was evaluated and there were no signs of persistent infection. PRF was obtained by drawing 10 mL of blood from the patient's arm and centrifuging it. The PRF membrane was

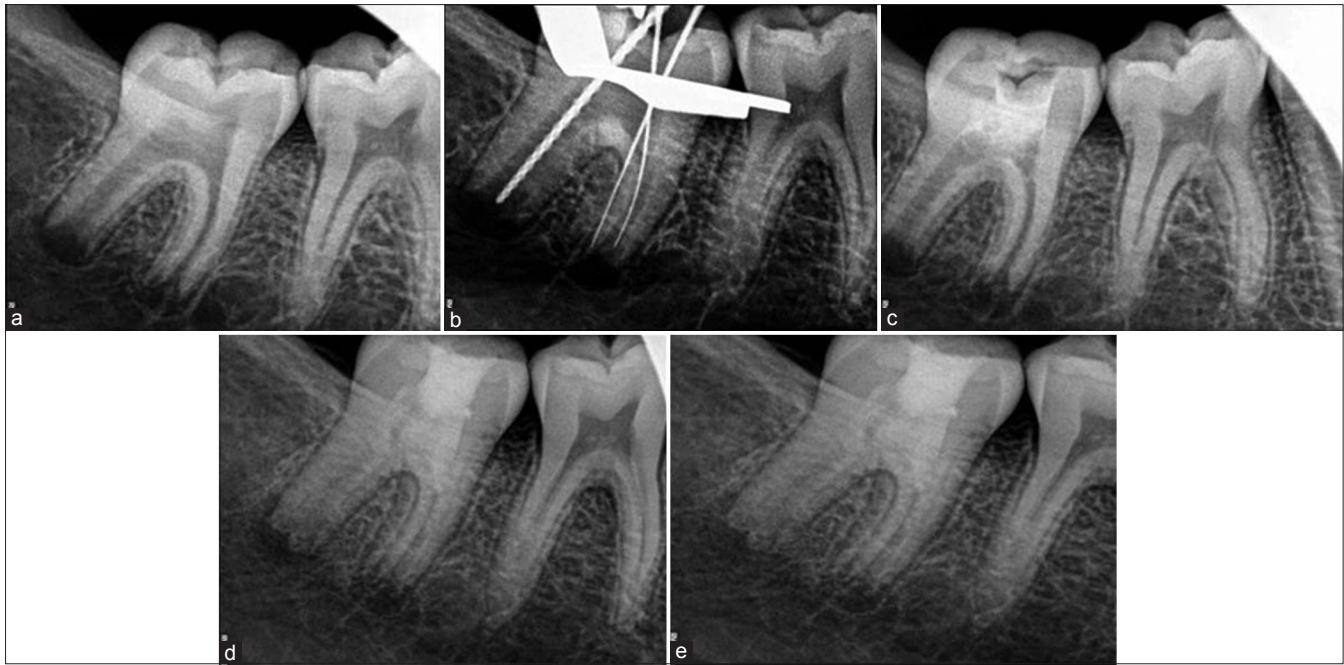


Figure 2: (a) Pre-operative RVG of mandibular right second molar suggestive of irreversible pulpitis and incomplete root development, (b) Working length determination, (c) Placement of triple antibiotic paste and temporarily restored with glass ionomer cement, (d) Placement of platelet-rich fibrin and biodentine, (e) 1-year follow-up RVG shows an increase in root apex formation

placed in each canal after gentle irrigation with EDTA. The bleeding was stopped before restoring the tooth with biodentine and composite material (Figure 2d).

The patient was called back every 3 months for follow-up examinations. There was no pain or discomfort during palpation or when the tooth was tapped, and it did not react to cold or electric pulp tester testing. At the 1-year follow-up (Figure 2e), an increase in root apex formation was observed.

Discussion

Apexification and regenerative endodontic procedure is two viable treatment options for immature permanent non-vital teeth with apical periodontitis. While apexification using substances such as calcium hydroxide, biodentine, and MTA can create a strong tissue barrier at the apex of the canals, it fails to promote additional root development or thickening of the root canal walls. This limitation significantly hampers the ability of the underdeveloped, short, and weak roots to sustain substantial masticatory forces, often resulting in brittleness.^[9] In contrast, regenerative endodontic procedures offer several advantages over the conventional apexification method.^[10] These include the ability to promote root end closure, thicken the root canal walls, and revascularize the pulp. These benefits make regenerative endodontic procedures a more desirable treatment option for this case.

To promote regeneration, the canals must be thoroughly cleaned and prepared, as this is a necessary step before any endogenous bioactive chemicals can be targeted for the

regeneration process. To ensure the success of this procedure, the complete elimination of bacteria and decontamination of the root canal space are essential. A study conducted by Windley *et al.* explored the effectiveness of TAP in treating immature teeth with apical periodontitis. They found that the colony forming unit counts significantly decreased in samples where the paste was used as a dressing for 2 weeks.^[11]

The impact of TAP on the viability of stem cells derived from the apical papilla was studied by Ruparel *et al.*^[12] and Althumairy *et al.*^[13] Their findings indicated that to protect stem cells from the apical papilla (SCAP), it is recommended to use TAP in regenerative endodontic treatment at a concentration of no more than 1 mg/mL (0.1–1 mg/mL), as stated by the American Association of endodontists (2016). For the current cases, a TAP was utilized as a disinfection method for 2 weeks. A 1.5% concentration of NaOCl was chosen as an irrigant due to its minimal toxic effects on SCAP and its ability to enhance the release of transforming growth factor (TGF-1) from the canal walls when combined with 17% EDTA.^[5] The use of EDTA-treated dentin is preferred as dental pulp stem cells have a greater tendency to adhere to it, attributed to improved surface wettability and increased synthesis of the odontoblast-like cell marker dentin sialophosphoprotein, in addition to the removal of the smear layer.^[14]

A scaffold is essential for controlling stem cell development, either through releasing growth factors locally or initiating a signaling cascade when stem cells adhere to the extracellular matrix and each other in a three-dimensional environment.^[15]

One suggested that scaffold for regenerative endodontic procedures is a blood clot. When blood is induced from the apex of the root, hemostatic components are allowed to flow into the canal space, thereby forming a fibrin clot.^[16] This clot acts as a homogenized scaffold composed of cross-linked

fibrin, containing necessary growth factors for promoting the survival, development, migration, differentiation, vascularization, and tissue regeneration of SCAP. Moreover, using an intracanal blood clot as a scaffold avoids any foreign body response.^[17,18]



Figure 3: PRICE 2020 flowchart

However, some clinical studies have revealed difficulties in producing enough blood to serve as a scaffold, which increases the risk of sealing material failure. In addition, other research suggests that platelet concentrates may have a more beneficial role in regeneration compared to solely using blood clots as a scaffold.^[19] Hence, in this particular case report, a combination of blood clots as a scaffold and PRF was utilized.

The use of a physical scaffold plays a crucial role in tissue engineering, particularly in regenerative endodontic procedures. PRF and platelet-rich plasma (PRP) have shown promising clinical and radiographic results as autologous platelet concentrates in this field.^[20,21] PRP, considered first-generation autologous plasma, contains a higher concentration and quantity of growth factors that can effectively promote tissue regeneration in both hard and soft tissues. On the other hand, PRF offers a three-dimensional structure and bioactive molecules that specifically encourage the growth and division of stem cells. Being composed solely of endogenous components, PRF provides a superior fibrin network for the storage of cytokines and growth factors, as well as for cell migration. Thus, in this particular case study, PRF was utilized as a framework. Revascularization of young permanent teeth with necrotic pulps has shown successful outcomes when PRF is used, as it provides a scaffold rich in growth factors that facilitate cellular proliferation and differentiation. In a study conducted by Shivashankar *et al.*, the administration of PRF to a tooth with pulpal necrosis and an open apex resulted in positive outcomes such as root lengthening, regression of periapical lesions, thickening of dentinal walls, and apical closure.^[22]

Biodentine was utilized as a protective substance to cover the PRF scaffold due to several advantages it offers. First, Biodentine has the capacity to stimulate the secretion of TGF-1 from the radicular dentin. In addition, it has a faster setting time compared to MTA, while also causing less noticeable discoloration. Moreover, Biodentine consists of bioactive properties that support the survival and growth of apical papilla stem cells. It creates a layer of hydroxyapatite crystal at the interface with the biological environment, promoting increased biocompatibility. Furthermore, Biodentine aids in the induction of hard tissue, enhancing its potential for creating new and strong tissue.^[23]

In the present two studies, the affected teeth displayed no clinical signs or symptoms, indicating successful treatment. Pulp regeneration was observed, along with the notable thickening of canal walls and continuous root maturation. The teeth also responded positively to pulp vitality tests.

CONCLUSION

Pulp revascularization is considered a viable option instead of apexification when treating developing teeth with pulp necrosis and irreversible pulpitis, whether or not there is an associated periapical disease. This procedure is relatively easy to perform and has shown positive outcomes compared to apexification.

Unlike apexification, pulp revascularization promotes the thickening of dentin walls, helps in closing the apex of the tooth, and prevents any weakening of the tooth structure.

This case report was written in compliance with the Preferred Reporting Items for Case Reports in Endodontics 2020 guidelines (Figure 3).^[24]

DECLARATION OF PATIENT CONSENT

The author certifies that they have obtained all appropriate patient consent forms. In the form, the patients have given their consent for images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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