case report

Revitalization of a Geminated Tooth with a Necrotic Pulp and an Open Apex Using Platelet–rich Plasma (PRP) and Mineral Trioxide Aggregate (MTA): A Case Report

Abstract

Aim: To successfully revascularize a geminated maxillary lateral incisor with an open apex using platelet-rich plasma (PRP) and mineral trioxide aggregate (MTA). Method: A 21-year-old male was referred for endodontic treatment of a geminated nonvital left maxillary lateral incisor (tooth #10) with asymptomatic apical periodontitis. After preparing an access cavity, its necrotic pulp was removed. The canal was irrigated with 5.25% NaOCl solution and dried with sterile paper points. A triple antibiotic mixed with distilled water was packed in the canal and left for 40 days. Ten ml of whole blood was drawn by venipuncture of the antecubital vein for preparation of PRP. After removal of the antibiotic mixture, the PRP was injected into the canal space up to the cementoenamel junction level. Three millimeters of white MTA was placed directly over the PRP clot. Two days later, the tooth was double-sealed with permanent filling materials. Results: Patient was recalled for 3, 6, 12 and 18 months clinical/radiographic follow up and is scheduled for a 24 months re-evaluation. Clinical examination, 12 months later revealed no sensitivity to percussion or palpation tests. Radiographic examination of this tooth showed appreciable regeneration of the root substance and resolution of the associated lesion. The tooth was not responsive to cold tests; however sensitivity test with an electric pulp tester (EPT) now elicited a delayed positive response. Conclusions: On the basis of short-term results of the present case, it appears that PRP has the potential of an ideal scaffold for regeneration of vital tissues in teeth with necrotic pulp and a periapical lesion.
Key Words
Fusion; Gemination; Mineral Trioxide Aggregate (MTA); Platelet-rich Plasma (PRP);
Regenerative endodontic procedures; Revitalization

Introduction
Although millions of teeth are saved each year by root canal therapy, an ideal form of therapy
might consist of a regenerative approach in which a diseased or necrotic pulp tissue is
removed and replaced with a healthy pulp tissue to revitalize teeth. Regenerative endodontic
procedures may be defined as biologically based procedures designed to replace damaged
structures, including dentin and root structures, as well as cells of the pulp-dentin
complex (1). The objectives of regenerative endodontic procedures are to regenerate pulp-like
tissue, ideally, the pulp-dentin complex, regenerate damaged coronal dentin, such as
following a carious exposure and regenerate resorbed root, cervical or apical dentin (1).
Endodontic therapy in permanent teeth diagnosed with pulpal necrosis and immature
root development is fraught with challenges. It is difficult to get an appropriate apical seal in
teeth with open apices by using the conventional endodontic treatment methods. The
discontinued development of dentinal walls after the pulp necrosis can also lead to a weak
root structure with thin dentinal walls and poor crown-to-root ratio which makes the tooth
susceptible to future fractures (2). Traditionally, multiple-visit apexification with calcium
hydroxide was the treatment of choice in necrotic immature teeth, which would induce
formation of an apical hard tissue barrier (3). Although this approach was predictable and
successful (4), long-term use of calcium hydroxide has several disadvantages such as
multiple treatment appointments, probable recontamination of the root canal system during
treatment period and increased brittleness of root dentin, which increases the risk of future cervical root fractures (4, 5) and ankylosis. Recently, an alternative, biologically based, regenerative approach has been advocated on the basis of prior revascularization studies from the trauma literature (6, 7). The publication of several case series has lead to growing recognition of the potential for successful outcomes by using regenerative procedures in treating the necrotic immature permanent tooth (8).

Hargreaves et al (9) have identified three components contributing to the success of regenerative procedures. These include stem cells that are capable of hard tissue formation, signaling molecules for cellular stimulation, proliferation and differentiation and finally, a 3-dimensional physical scaffold that can support cell growth and differentiation. Platelet-rich plasma (PRP) has been mentioned as a potentially ideal scaffold for regenerative endodontic treatment regimens (9) in dentistry while platelet enriched plasma and fibrin glue is being used for conjunctival autograft in post pterygium surgery in ophthalmology (10).

This article describes successful revitalization of a geminated maxillary lateral incisor by using PRP as a 3-dimensional physical scaffold and MTA to create a hard tissue barrier and a probable source of signalling molecules for the growth of stem cells (11).

Method

A 21-year-old male patient was referred to the Department of Conservative Dentistry and Endodontics, Himachal Dental College, Sundernagar (H.P.), India for endodontic treatment of tooth #10 (maxillary left lateral incisor). His medical history was non-contributory. The patient had experienced intermittent spontaneous pain in that area for previous months.
Patient gave a history of trauma approximately 10 years earlier, wherein there was a direct impact on upper anteriors.

Clinical examination revealed an irregular unilateral morphology of tooth #10 (Fig. 1A), with an increase in the mesiodistal width suggestive of a possible union of two clinical crowns. The tooth was distolingually rotated. Distinct developmental occlusogingival grooves between the two clinical crowns were noticed (Fig. 1A, 1B). The rest maxillary and mandibular permanent teeth were evaluated by OPG (Fig. 1C) for any other anomaly and were found to be normal in shape and structure.

Fig. 1A, 1B, 1C
The tooth was tender on percussion. A draining sinus tract was also observed in relation to the involved tooth. No periodontal pockets were present and thermal and electrical pulp testing elicited a negative response.

A periapical radiograph revealed that tooth #10 had two partially separated crowns with a single root and a single root canal (Fig. 2A). There was widening of the periodontal ligament space with periapical radiolucency in relation to the involved tooth. The sinus tract when traced by using a gutta-percha cone of size 20 (Dentsply Maillefer, Ballaigues, Switzerland) from the buccal aspect into the lesion pointed to tooth #10. On the basis of the clinical and radiographic findings (Fig. 2A), diagnosis of a “geminated nonvital tooth with symptomatic apical periodontitis” was made.

Considering various treatment options for tooth #10, a decision to perform a regenerative endodontic procedure with the aid of PRP was made. The patient was informed that this treatment was an attempt to initiate further root development and that the proposed treatment may not have a successful outcome. A written informed consent was obtained from the patient.

After administration of local anaesthesia, that is, 2% lidocaine (Sepotodont, St Maur Des Fosses Cedex, France) with 1:80000 epinephrine, the tooth was isolated with rubber dam (Hygenic, Coltene/Whaledent, France) and cotton rolls. Endodontic access cavity (Fig. 2B) was prepared on the involved tooth using a diamond coated fissure bur (Diatech, Heerbrugg, Switzerland) in a high speed handpiece with copious water spray. No haemorrhage was observed from the root canal. The necrotic pulp was removed with the help of a barbed broach (Mani, Inc, Tochigi, Japan) and H-files (Mani, Inc, Tochigi, Japan) under copious irrigation with sterile saline 0.9% w/v (Alkem Laboratories Ltd., India). Coronal flaring was carried out with Gates Glidden drills (Mani, Inc, Tochigi, Japan) and working length was
determined with the help of apex locator (Root ZX; Morita, Tokyo, Japan), which was later confirmed with a radiograph (Fig. 2C).

The root canal was irrigated with 5.25% sodium hypochlorite (Sigma-Aldrich Chemicals, Bangalore, India) copiously for 20 minutes without any instrumentation, followed by sterile saline 0.9% w/v (Alkem Laboratories Ltd., India). The root canal was then dried with sterile paper points (Dentsply, Maillefer, Baillaigues, Switzerland). Equal proportions of ciprofloxacin (Cifran, Ranbaxy, India), metronidazole (Metron, Ulticare-Alkem Laboratories, India) and minocycline (Divaine, Cipla, India) were ground and mixed with distilled water to a thick paste consistency. This antibiotic mixture was placed in the canal using a lentulo spiral (Mani, Inc, Tochigi, Japan). The access cavity was sealed with Cavit (3M ESPE AG, Seefeld, Germany) (Fig. 2D).
The patient returned to the endodontic clinic after 30 days and was without any symptoms. Tooth #10 was asymptomatic to both percussion and palpation tests. Local anesthesia was obtained by using 2% lidocaine with 1:80,000 epinephrine (Septodont, St Maur Des Fosses Cedex, France). After rubber dam (Hygenic, Coltene/Whaledent, France) isolation, the temporary restoration was removed and the antibiotic mixture was washed out.
by using sterile saline 0.9% w/v (Alkem Laboratories Ltd., India) irrigation. The canal was
dried with sterile paper points (Dentsply, Maillefer, Baillaigues, Switzerland).

PRP was prepared by using the procedure as described by Okuda et al (12) and
Kawase et al (13). A 10 ml sample of whole blood was drawn by venipuncture of the
antecubital vein of the patient’s right arm. Blood was collected in a 15-ml sterile glass tube
coated with an anticoagulant (acid-citrate-dextrose, Health Line Pharmaceuticals Pvt. Ltd.,
India). Whole blood was initially centrifuged at 2,400 rpm for 10 minutes in a centrifuge
(H.L. Scientific Industries, India) to separate PRP and platelet-poor plasma (PPP) portions
from the red blood cell fraction. PRP and PPP portions were again centrifuged in a centrifuge
(H.L. Scientific Industries, India) at 3,600 rpm for 15 minutes to separate the PRP from the
PPP. PRP was then injected into the canal space up to the level of the cementoenamel
junction (CEJ). Three millimeters of white MTA (Pro-Root MTA; Dentsply Tulsa Dental
Specialties, Tulsa, OK, USA) was placed directly over the PRP clot. A moist cotton pellet
was placed over the MTA and provisionally restored with Cavit (3M ESPE AG, Seefeld,
Germany).

The patient was recalled after 2 days and was asymptomatic. After rubber dam
(Hygenic, Coltene/Whaledent, France) application, the provisional restoration and cotton
pellet was removed and setting of the MTA (Pro-Root MTA; Dentsply Tulsa Dental
Specialties, Tulsa, OK, USA) was confirmed. The tooth was then double-sealed with 2 mm of
glass ionomer cement (GC Corporation, Tokyo, Japan) and universal composite resin
restorative material (3M ESPE Dental Products, St Paul, MN). The patient was recalled for 3,
6, 12 (Fig. 3A) and 18 months (Fig. 3B), clinical/radiographic follow up and is scheduled for
a 24 months re-evaluation.
Clinical evaluation after 12 months revealed that the involved tooth was asymptomatic and was not sensitive to percussion or palpation tests. The tooth was not responsive to cold tests; however, sensitivity test with an electric pulp tester (Parkell, Edgewood, NY, USA) now elicited a delayed positive response compared to the contralateral tooth. Radiographic examination showed resolution of the periapical lesion, further root development and continued apical closure of the root apex in tooth #10. The patient is now scheduled for a 24 months re-evaluation.

Fig. 3A, 3B

Fusion and gemination are developmental anomalies of dental hard tissues with complex and unusual anatomy. Gemination is considered as a result of a failed attempt of a tooth bud to
split into two. The structure most often presents two crowns, either totally or partially separated, with a single root and one root canal. It is characterized by a normal teeth number. An extra crown is usually present. The etiology is unknown, but trauma has been suggested as a possible cause, though a familial tendency has also been suggested.[14] Clinically, it is often difficult to differentiate between fusion and gemination and it is common to refer to these anomalies as “double teeth” (15). The differential diagnosis between gemination and fusion of a normal and a supernumerary tooth is difficult. Traditional terminology such as fusion and gemination should be used as a potential embryologic cause of the anomaly and not as an exact diagnosis (16). Concerning treatment, an exact differentiation between fusion and gemination may not be critically important (17).

Treatment of necrotic immature teeth has always been a challenge in endodontics. The primary objective of endodontic therapy in infected root canal systems is to disinfect the root canal system through chemical and mechanical means without injuring and irritating the periapical tissue. However, in teeth with immature root development, the removal of microorganisms by mechanical means is limited as a result of the thin, fragile dentinal walls of the roots. Disinfection of the root canal system in these cases often relies on irrigation and intracanal medicaments. All of this invariably injures the periapical tissue. After completion of traditional endodontic therapy, these teeth often have a poor crown-to-root ratio and are susceptible to fracture (18) or ankylosis.

Traditionally, the treatment of immature permanent teeth with necrotic pulp involves long-term application of calcium hydroxide to induce apexification at the root apex (3). However, the remaining thin fragile dentinal walls predispose these teeth to fracture and some studies have shown that long-term use of calcium hydroxide can weaken dentin (5). An alternative technique for apexification with calcium hydroxide is an artificial apical barrier
technique, which is done by placing barrier material in apical portion of the canal. The material of choice for this technique is mineral trioxide aggregate (MTA) (19), which has been shown to have high success rates (20) and reduce the number of required clinical sessions. Both of the mentioned methods (i.e. apexification and artificial apical barrier techniques) share the same disadvantage of not allowing the continuation of root development, which leads to a fragile root structure.

Revascularization is a regenerative treatment and a biologically based alternative approach to treat necrotic immature teeth that, unlike apexification and artificial apical barrier techniques, allows continuation of root development (21). Regeneration of the pulp dentin complex had once been thought to be impossible. However, if a suitable environment could be achieved, i.e. absence of intracanal infection and presence of a scaffold conducive to tissue ingrowth, then regeneration of pulp may take place (and the pulpal space might become repopulated with mesenchymal tissue resembling the dental pulp (22)). Recently, the use of platelet-rich plasma (PRP) has been proposed as a method for increasing growth factor delivery. Compared to whole blood, PRP contains increased concentrations of platelets and, therefore, platelet-derived growth factors such as transforming growth factor β (TGFβ1), platelet-derived growth factors (PDGFs), and vascular endothelial growth factor (VEGF), which have been shown, in vitro, to promote cell migration, differentiation, and matrix synthesis. Although histologic studies are still lacking, the clinical outcome of these endodontic regeneration studies is promising. The publication of several case series has lead to growing recognition of the potential for successful outcomes by using regenerative procedures in treating the necrotic immature permanent tooth.

The radiographic appearance of wide root canal systems and the presence of blunted roots strongly suggest a history of incomplete root development. Endodontic infection or
physical trauma of a developing tooth might lead to an unusual situation for root development (23). 5.25% sodium hypochlorite was used as an intracanal irrigant as reported previously (24). Triple antibiotics (a mixture of ciprofloxacin, metronidazole and minocycline paste) were used as an intracanal medicament to disinfect the root canal of this tooth as suggested by other investigators (24) and increase the chances of revascularization (25,26). Tetracycline has been shown to enhance the growth of host cells on dentin via exposure of embedded collagen fibers or growth factors and not by an antimicrobial action (27). Like many recent cases (24, 28) MTA was placed over PRP to isolate the root canal from the external surface of the tooth and create a hard tissue barrier at its contact point with PRP. It might have also provided signaling molecules for the growth of stem cells (11).

**Conclusion**

In the presented case, resolution of the periapical lesion, further root development and continued apical closure of the root apex occurred within 6 months, which suggests the potential ability of PRP in regenerative endodontics. Histological backup regarding the nature of formed tissue(s) after this procedure in humans needs to be evaluated. However, pulp tissue, periodontal ligament, dentin, cementum and bone have been found within the root canals of teeth in experimental animals undergoing regenerative endodontic procedure (29).

On the basis of promising results of the present case, it appears that PRP is potentially an ideal scaffold for regeneration of vital tissues in a tooth with a necrotic pulp and periapical lesion. Further studies with long-term clinical follow-up and histological evaluation are recommended.

**REFERENCES**


