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Case Report

Management of dens evaginatus with an immature permanent tooth with apical periodontitis using platelet-rich fibrin in regenerative endodontics: case reports and literature review

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Abstract: The regenerative endodontic treatment (RET) of human immature permanent tooth with apical periodontitis using platelet-rich fibrin (PRF) has shown successful results in incisor. The purpose of this case series is to report four cases of the management of dens evaginatus in immature mandibular second premolars with apical periodontitis using PRF in regenerative endodontics. Four dens evaginatus with immature teeth with apical periodontitis were treated via RET using PRF as a scaffold. During the follow-up period, all teeth showed a resolution of periapical radiolucencies, and three teeth showed continuous root development and apical closure. Two teeth displayed a positive response to pulp sensibility tests. On the basis of the short-term results of the present case series, we can conclude that RET is an ideal treatment method for dens evaginatus with an immature permanent tooth with apical periodontitis. In addition, PRF is a potentially ideal scaffold material for RET, and the operation is easier with less risk. Therefore, PRF is recommended for use in DE or other cases in which it is difficult to induce bleeding.

Keywords: Immature permanent tooth, platelet-rich fibrin, apical periodontitis, dens evaginatus, regenerative endodontic treatment

Dens evaginatus (DE) is a rare dental anomaly that is caused by abnormal proliferation during development [1]. The prevalence of DE with varying estimates reported is 0.5% to 4.3%. The DE predominantly occurs in the mandibular premolars and usually occurs bilaterally [2]. Studies have suggested that 82.5% of the occlusal tubercles of DE are eventually fractured or worn, and in 26.3% of these, the result was pulpal necrosis [2]. If pulpal necrosis or periapical periodontitis occurs before the completion of root formation, treatment can be very challenging due to incomplete root formation with an open apex. These teeth often are ultimately extracted.

For many decades, clinicians have resorted to traditional apexification techniques or the use of an artificial apical barrier method with mineral trioxide aggregate (MTA) to treat these teeth [3]. However, these teeth are prone to root fracture after such procedures because neither of these methods can increase the root thickness or length.

In recent years, many case reports of the regenerative endodontic treatment (RET) of human traumatized immature permanent anterior teeth with necrotic pulps or apical periodontitis have been published [4-7]. The conventional method of RET using a blood clot as a scaffolding material has been shown to have successful results. However, it is very difficult to induce bleeding and place the MTA over a blood clot [8, 9].

Platelet-rich fibrin (PRF), a second-generation platelet concentrate, was first introduced by Choukroun *et al* [10]. PRF is a matrix of autologous fibrin containing a large quantity of plate-

Table 1. Current published case reports of the use of platelet-rich fibrin in regenerative endodontics

Authors	Age	Tooth	Etiology	Diagnosis	Scaffold	Clinical outcomes	Post-treatment vitality responses	Follow-up period (months)
Shivashankar et al [8], 2012	9	Incisor	Trauma	SAP	PRF membrane	Continued thickening of the dentinal walls, root lengthening, regression of the periapical lesion and apical closure.	Yes	12
Mishra, et al [9], 2013	11	Incisor	Trauma	SAP	PRF membrane	Resolution of periapical rarefaction, further root development and apical closure.	Yes	12
Keswani et al [10], 2013	7	Incisor	Trauma	SAP	PRF fragments	Continued thickening of root canal walls, root lengthening and apical closure.	Yes	15
Johns, et al [11], 2014	9	Incisor	Trauma	Pulp necrosis	PRF membrane	Continued thickening of the dentinal walls, root lengthening, regression of the periapical lesion and apical closure.	No	10
Jadhav, et al [12], 2015	16	Incisor	Trauma	SAP	PRF membrane	Periapical healing, apical closure, root lengthening and dentinal wall thickening was optimal radiographically.	N/A	18
Nagaveni et al [13], 2015	10	Incisor	Trauma	SAP	PRF membrane	Continued root development, thick dentinal walls with narrowing of the canal space, continued periapical closure, and normal periradicular architecture.	Yes	12
Ray et al [14], 2015	11	Incisor	Trauma	Pulp necrosis, AAP	PRF membrane	Continued root development, increase in root length.	Yes	36

SAP, symptomatic apical periodontitis; AAP, asymptomatic apical periodontitis; N/A, information not available.

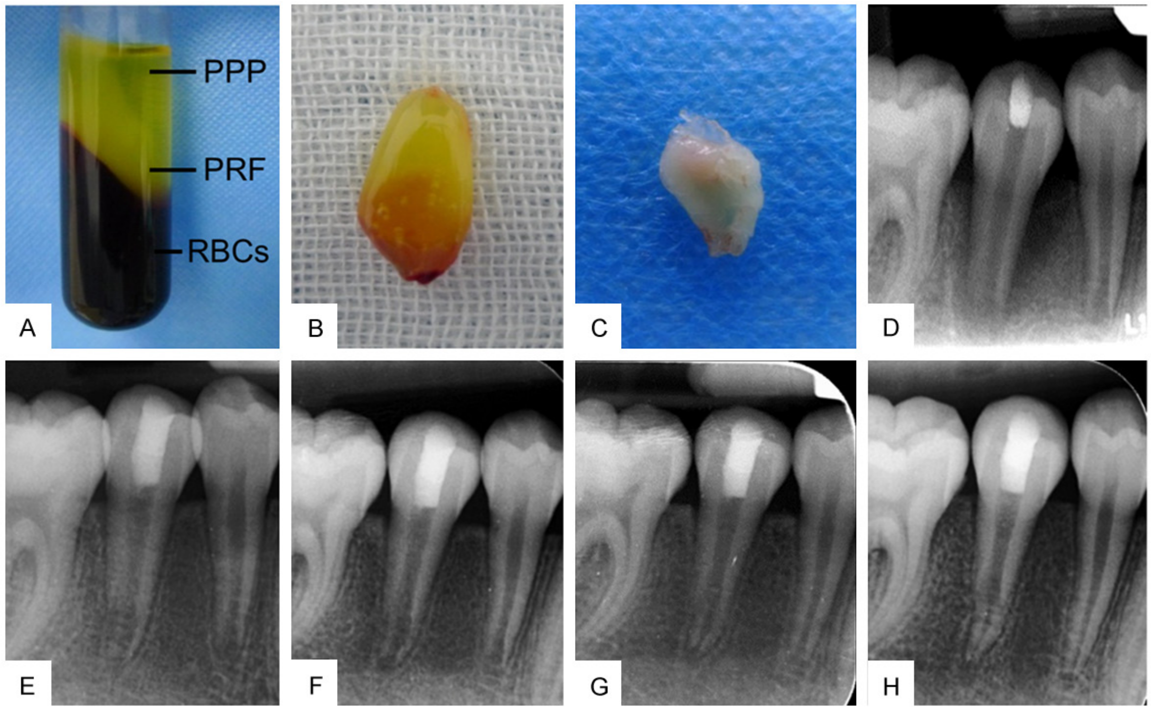

Figure 1. PRF preparation and periapical radiographs in case 1. A. 3 layers obtained after centrifugation: platelet-poor plasma at the top, platelet-rich fibrin in the middle, and red blood cells at the bottom. B. Platelet-rich fibrin clot on sterile dry gauze. C. Platelet-rich fibrin membrane. D. Preoperative intraoral periapical radiograph of tooth #29 showing an incomplete apex with a periradicular radiolucency. E. A 3-month follow-up radiograph showing that the periapical radiolucency healed completely, and the root elongated. F. The 6-month follow-up radiograph showing continued root development. G. The 9-month follow-up radiograph showing continued root development and the narrowing of the apical foramen. H. The 12-month follow-up radiograph showing complete root development and closure of the root apex.

lets, growth factors and leukocytes [11]. PRF collected via Choukroun's technique is very simple and inexpensive to use and does not require any chemical agents.

Rowe suggested that the inferior alveolar nerve can be directly damaged during root canal preparation due to over-instrumentation [12]. Considering the location of mandibular premolar teeth, it seems that using a K-file introduced into the apical tissues beyond the apical foramen to form a blood clot has a potential risk.

As shown in **Table 1**, we know that the teeth were anterior teeth with a history of trauma, and none were cases of premolars with DE [13-19]. Therefore, using PRF as a scaffold in RET is a novel method to treat DE with apical periodontitis. The purpose of this case series is to describe four cases of the management of dens evaginatus with an immature permanent tooth with apical periodontitis using PRF in regenerative endodontics.

Case report

Four patients (11-16 years of age) were referred to the Department of Endodontics and Operative Dentistry for the treatment of immature permanent premolars. The teeth included four mandibular second premolar teeth. The teeth had the developmental anomaly dens evaginatus, which resulted in apical periodontitis. On clinical examination, the teeth were sensitive to percussion and palpation. None of the teeth responded to pulp sensibility tests. Radiographic examination of the teeth showed immature root with open apices and periapical radiolucencies. After clinical and radiographic examination, a diagnosis of pulpal necrosis with symptomatic apical periodontitis was made. After explaining the risks, benefits, and treatment options to the patient's parents, a written informed consent was obtained for performing RET using PRF. The protocol and consent forms were approved by the Institutional Ethics Committee of the School and Hospital of Stomatology, Fujian Medical University.

Table 2. Summary of clinical and radiographic findings

Case no.	Gender	Age	Tooth no.	Etiology	Diagnosis	Periapical radiolucency resolution (months)	Increase in root length	Increase in dentinal wall thickness	Apical closure	Post-treatment vitality responses	Follow-up period (months)
1	Female	12	29	DE	SAP	3	Yes	Yes	Yes	Yes	12
2	Male	11	20	DE	SAP	3	Yes	Yes	Yes	No	18
3	Male	12	20	DE	SAP	3	Yes	Yes	Yes	Yes	18
4	Female	16	29	DE	SAP	9	No	No	No	No	12

SAP, symptomatic apical periodontitis; DE, dens evaginatus.

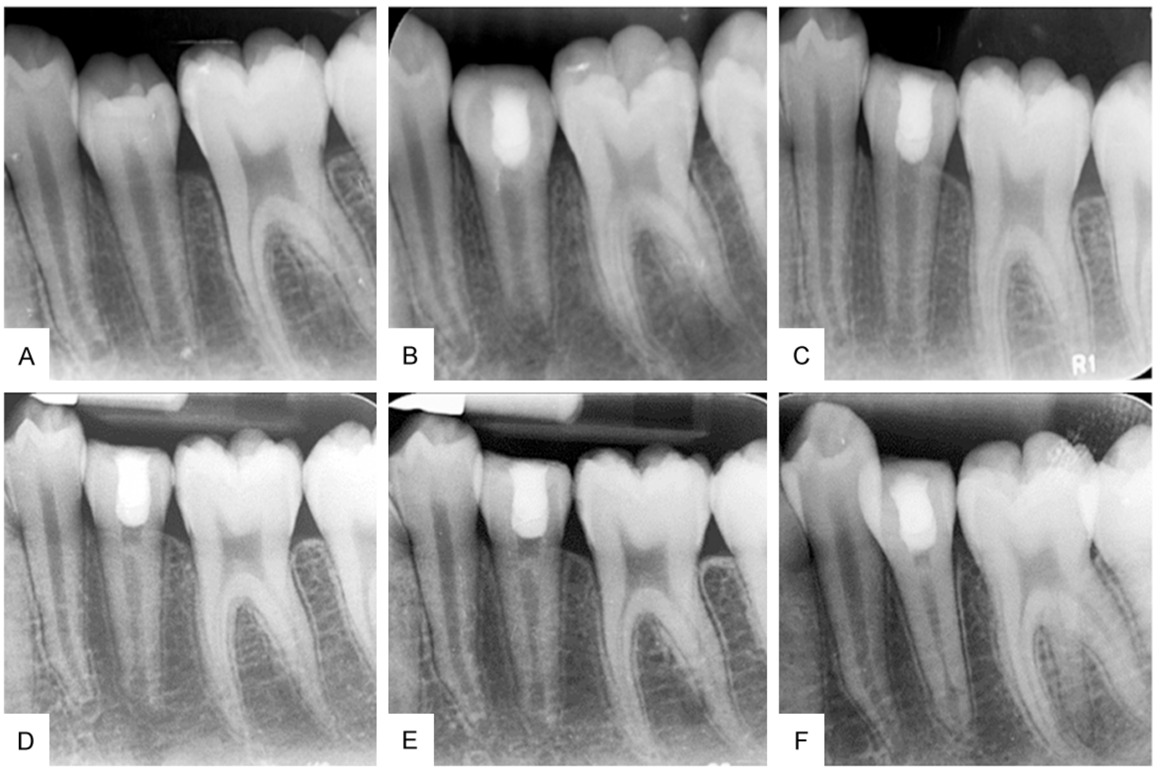

Figure 2. Periapical radiographs in case 2. A. A preoperative periapical radiograph of tooth #20 showing an immature root and an open apex. B. A 3-month follow-up radiograph showing tooth #20 have a periapical radiolucency around the root apex. C. A 6-month follow-up radiograph showing that the periapical radiolucency healed completely and the root canal walls slightly thickened. D, E. Radiographs taken after 9 and 12 months showing continued root development. F. The 18-month follow-up radiograph showing complete root development and the closure of the root apex.

On the initial visit, an access cavity was made after local anesthesia and rubber dam isolation. Without mechanical instrumentation, the canal was irrigated with 1% sodium hypochlorite before being dried with paper points. Subsequently, a triple antibiotic paste consisting of a powder of 100 mg each of ciprofloxacin, metronidazole, and cefaclor mixed with 1 ml of sterile water was placed into the canal and filled to a level just below the cementoenamel junction using a syringe under a microscope. The access cavity was then temporized with Cavit (3M ESPE, Seefeld, Germany).

The patients were recalled after 4-6 weeks. Local anesthesia was administered with 2% lidocaine with 1:100 000 epinephrine. The tooth was isolated with a rubber dam, and the access cavity was reopened. Then, the antibiotic paste was gently washed out of the canal with sterile saline solution. Next, the canal was irrigated with 10 ml of 17% EDTA solution and dried with paper points. Concurrently, the PRF preparation was accomplished as described by Choukroun *et al* [10]. Briefly, a 5 ml sample of whole blood was drawn intravenously from the patient's forearm (antecubital vein). The blood

Figure 3. Periapical radiographs in case 3. A. Preoperative intraoral periapical radiograph showing an incomplete apex with a periradicular radiolucency of tooth #20. B. The 3-month follow-up radiograph showing that the periapical radiolucency healed completely. C. The 6-month follow-up radiograph showing the elongation of the root. D, E. Radiographs taken after 9 and 12 months showing continued root development. F. The 18-month follow-up radiograph showing complete root development and a lateral opening in the mesial wall of the root.

sample was transferred to a 10-ml test tube without an anticoagulant and centrifuged immediately using a tabletop centrifuge at 400 g for 10 min. After centrifugation, three distinct layers were formed in the tube: red blood cells (RBCs) at the bottom, a platelet-rich fibrin clot (PRF) in the middle and platelet-poor plasma (PPP) at the top (**Figure 1A**). A syringe was used to draw out the PPP at the top, which was discarded. Sterile tweezers were inserted into the tube to gently grab the PRF clot, and the RBCs were removed using scissors. The PRF clot was compressed between the sterile dry gauze to squeeze out the fluids (**Figure 1B**), which resulted in a membrane (**Figure 1C**). The prepared PRF membrane was cut into segments of approximately 3 mm. Under a surgical microscope, the fragments were placed into the canal space with Buchanan Hand Plugger Size #2 (Sybron Endo, Orange, CA) up to the level of the cementoenamel junction (CEJ). A 3-mm-thick layer of white mineral trioxide aggregate (Dentsply Tulsa Dental Specialty, Tulsa, OK, USA) was placed directly over the PRF membrane followed by a moist cotton pellet and Cavit. One week later, the patients were recalled, and Cavit and cotton pellet was replaced with a bonded resin restoration (Filtek Z350 XT: 3M ESPE Dental Products, St Paul, MN).

All the patients returned to the clinic for follow-up appointments at 3, 6, 9, 12 and 18 months. Assessments at each of these appointments included an evaluation of clinical signs and symptoms, periapical status, the development of the root, and pulp sensibility tests.

In all follow-up visits, all teeth were asymptomatic (**Table 2**). The radiographic examination (in cases 1, 2, and 3) revealed that complete resolution of the radiolucency, continued root development and closure of the root apex (**Figures 1-3**). However, there was still a lateral opening in the mesial wall of the root (**Figure 3**). Although the radiographs in case 4 revealed that the periapical radiolucent lesion was eliminated

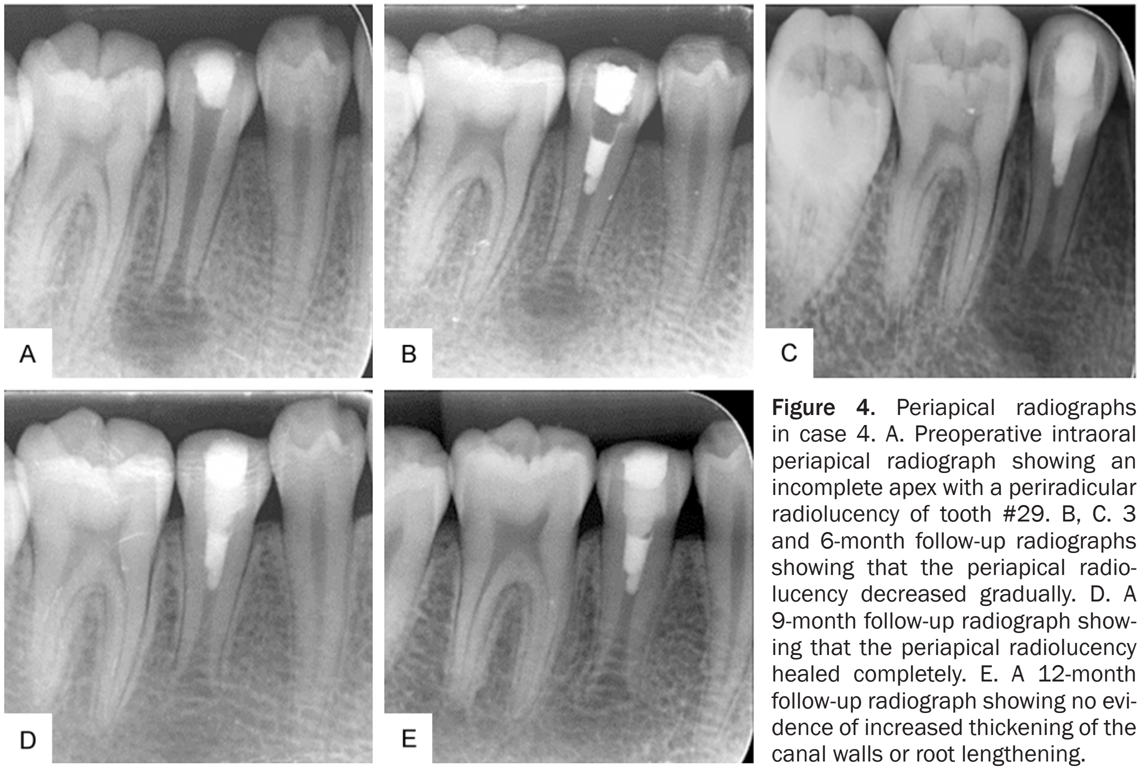

Figure 4. Periapical radiographs in case 4. A. Preoperative intraoral periapical radiograph showing an incomplete apex with a periradicular radiolucency of tooth #29. B, C. 3 and 6-month follow-up radiographs showing that the periapical radiolucency decreased gradually. D. A 9-month follow-up radiograph showing that the periapical radiolucency healed completely. E. A 12-month follow-up radiograph showing no evidence of increased thickening of the canal walls or root lengthening.

completely, they did not show evidence of the increased thickening of the canal walls or the lengthening of the root (**Figure 4**). In addition, the teeth presented in cases 1 and 2 had a positive response to pulp sensibility tests, including a cold test and EPT. All the patients are scheduled for further follow-up.

Discussion

One of the most serious potential complications of dens evaginatus is pulpal necrosis. Once the tubercle of DE is fractured or worn, the exposure of dentinal tubules provide a pathway for bacterial invasion. Subsequently, pulpal inflammation or necrosis can occur. If the pulp becomes inflamed or necrotic before the completion of root formation, the root formation will be interrupted. Although the ideal treatment method for DE is prophylactic restoration and the preservation of the vital pulp to promote root development, for various reasons, pulpal necrosis before the completion of root formation can still be commonly observed in clinical cases, especially in Asian populations.

Once the pulp of a tooth with an immature root becomes necrotic, with or without a periapical lesion, the affected tooth should be subjected to an apexification procedure with calcium hydroxide or mineral trioxide aggregate. However, these teeth are prone to root fracture after these procedures because neither of these methods can increase the root thickness or length. In recent years, more and more case reports of RET have been published. The conventional method of RET using a K-file introduced into the apical tissues beyond the apical foramen to form a blood clot as a scaffold material has had success, including continued root development and apical closure. The new method of RET using PRF to replace the blood clot as a scaffold material has also been successful in incisors (**Table 1**).

The mandibular mental foramen is variably positioned, with 67% under the second premolar, 24% between the first and second premolars, 6% under the first premolar, and the remaining 3% behind the second premolar [20]. The presence of periapical infection weakens the bone barrier between the apex and the neurovascular bundle. Moreover, a mandibular canal surrounded by dense cortical bone does not always exist. In most cases, the neurovascular bundle follows a path through cancellous bone, which makes the mandibular canal more vulnerable to various factors from the root canal [21]. Therefore, the location of these four DE cases was considered. For the mandibular second premolar teeth, a K-file was introduced into the apical tissues beyond the apical foramen to initiate bleeding into the root canal, where it is easier to damage the mental nerve and cause sensory disturbances. In addition, it is very difficult to induce bleeding and place the MTA over a blood clot to maintain it [8, 9]. Moreover, it is known that the concentration of growth factors in the blood clot is limited. Therefore, to avoid these disadvantages in RET, PRF was used as a scaffold in these four cases.

The use of PRF as a scaffold in RET has several advantages: First, PRF has a tri-molecular or an equilateral fibrin branch junction that makes its architecture flexible [10]. This architecture not only supports cytokine enmeshment and cell migration, it also allows the easy placement of the MTA over PRF. Second, PRF has a very significant slow-sustained release of various growth factors, like platelet-derived growth factor, vascular endothelial growth factors and transforming growth factor beta over a period of 7-14 days [22]. These growth factors can facilitate cellular proliferation, differentiation, angiogenesis, and the healing potential of soft and hard tissues. Lastly, leukocytes in PRF act as anti-inflammatory agents and play a significant role in the self-regulation of inflammatory and infectious phenomena in RET [22].

In case 1 and 2, the radiographic examination of the treated teeth revealed continued root development and apical closure at 12-18 months after treatment. The most plausible explanation is that PRF acted as a scaffold to the disinfected root canal because there was no bleeding in the root canal before placing the PRF. It has been shown that some vital pulp tissue and Hertwig's epithelial root sheath (HERS) may still survive even in the presence of a periradicular lesion [23, 24]. A study has shown that PRF can increase dental pulp cell proliferation and differentiation [25]. After the disinfection of the root canal and under the influence of PRF and HERS, the dental pulp cells might have differentiated into odontoblasts-like cells, leading to continued root development and apical closure.

In case 3, the radiographic examination of the treated teeth revealed continued root development and apical closure at 18 months after treatment. However, there was still a lateral opening in the mesial wall of the root. We speculate that the mesial wall of HERS may be destroyed by periapical infection, such that the mesial wall of the root cannot continue development. In case 4, the radiograph did not show evidence of increased thickening of the canal walls and continued root development at 12 months after treatment. This could be attributed to long-standing periapical infection. The vitality of the HERS is reduced as the duration of the infection period increases. In case 4, the tooth has a larger periapical radiolucency and an immature root with an open apex, suggesting that root development was halted when the patient was approximately 12 years old. It is highly likely that necrosis and infection were present for over 4 years. The HERS may have been destroyed completely, causing root development to cease.

Based on the short-term results of the present case series, we can conclude that RET is an ideal treatment method for DE with an immature permanent tooth with apical periodontitis. In addition, PRF is a potentially ideal scaffold material for RET, making the procedure easier and less risky. Therefore, PRF is recommended for use in DE or other cases in which it is difficult to induce bleeding.

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Disclosure of conflict of interest

None.

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