

## Regenerative Endodontic Procedure using Platelet-Rich Fibrin to Treat Traumatized Immature Permanent Tooth: a Case Report

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**Abstract:** Regenerative endodontic procedures for the management of traumatized immature permanent teeth have shown promising results in the restoration of functional pulp-dentin complexes. This case report describes the management of a 15-year-old male patient with a traumatized maxillary canine with an immature apex and a necrotic pulp. A regenerative endodontic procedure was performed using calcium hydroxide as intracanal medicament and platelet-rich fibrin (PRF) as scaffold. Recall visits after 4, 8 and 12 months showed complete resolution of periapical lesion with increased canal wall thickness. Cone beam computed tomography (CBCT) scan showed complete closure of the root apex. The patient was asymptomatic and the tooth was not responsive to sensibility testing. The regenerative procedure used induced root development by increasing root wall thickness and closure of apex. The use of bioactive scaffold platelet-rich fibrin enhanced the outcome since it provided a concentrated reservoir of growth factors that are known to induce stem cell proliferation and differentiation.

**Keywords:** Cone Beam Computed Tomography, Immature Tooth, Platelet-Rich Fibrin, Pulp Regeneration

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### I. Introduction

Trauma to immature permanent teeth can lead to various outcomes. Traditional management of such cases include apexification procedures using calcium hydroxide or establishment of apical barriers using materials such as mineral trioxide aggregate (MTA).

Apexification procedures using calcium hydroxide have various drawbacks, such as multiple visits in a short period of time, thin dentinal walls, a compromised crown-root ratio, and an open apex. There is also an increased risk of fracture in teeth treated with apexification (1-4). An alternative to this procedure is establishment of a hard tissue barrier at the apex using materials like MTA, which has excellent sealing properties (5). Although fewer visits are required, this procedure is also associated with similar disadvantages, such as the thinning of dentinal walls and a failure to induce further root development. In this case report, Regenerative endodontic procedure was able to induce root development by increasing root wall thickness and closure of root apex. introduction of the paper should explain the nature of the problem, previous work, purpose, and the contribution of the paper. The contents of each section may be provided to understand easily about the paper.

### II. Case Report

A 15-year-old healthy male patient reported to the department of Conservative Dentistry & Endodontics, Government Dental College & Hospital, Ahmedabad, India, with a complaint of pain in relation to upper front teeth. Patient's parents informed about a history of traumatic injury to the upper front teeth, but could not recall the duration. Pain was dull, aching and continuous, and also felt in the vestibule of maxillary right canine. Medical history was non-contributory.

On intraoral examination, tooth #13 was tender on percussion without loss of any crown structure. Tooth #13 showed no response to sensibility testing. Tenderness to palpation was felt in the facial vestibule near tooth #13. Probing depths were normal and the tooth showed normal physiological mobility. Access opening was attempted by another dentist, but was not completed. Radiographic examination showed periapical radiolucency in relation to the tooth #13 with widening of lamina dura and an immature apex. A diagnosis of previously initiated and symptomatic apical periodontitis with Ellis class IV fracture for tooth #13 was made. Regenerative endodontic treatment was planned for the tooth (Fig. 1).

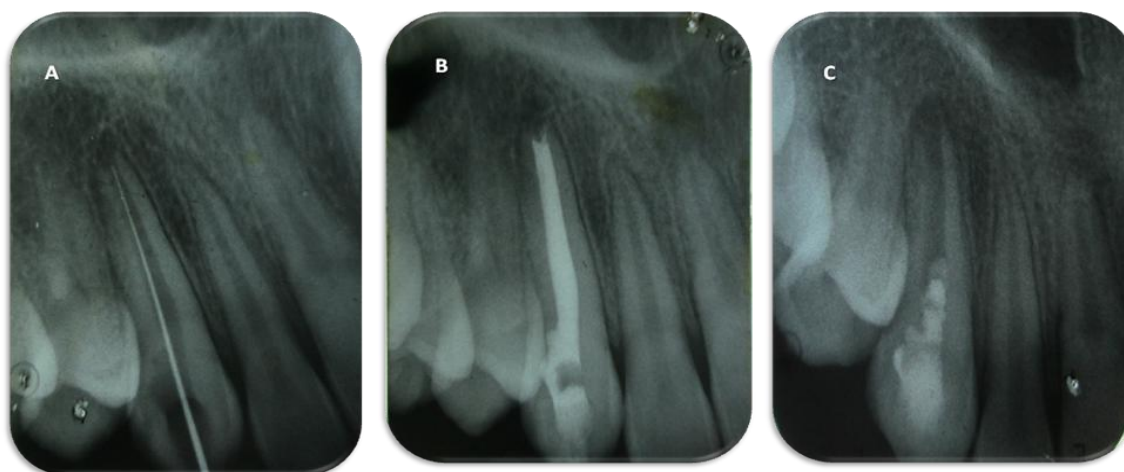


**Figure 1.** Preoperative radiograph showing tooth with an immature apex and periapical radiolucency.

After obtaining informed consent from the parents, the tooth was anaesthetized using 2% lignocaine with adrenaline at a ratio of 1:1,00,000 (Ligno-Aid, Vishal Dentocare Pvt. Ltd., Ahmedabad, India) followed by rubber dam isolation. After removal of temporary restoration, an access cavity extending too distally was seen. Proper access was made and the canal was irrigated with 20 mL of 1% sodium hypochlorite (Vishal Dentocare Pvt Ltd, Ahmedabad, India) for 5 min followed by normal saline. The working length was determined radiographically and apical gauging, showing an apical diameter of 1.2 mm, was performed, which is an ideal requirement for the success of regenerative endodontic procedure. After performing minimal filing, the canal was irrigated with 20 mL of 17% EDTA (PREVEST DenPro) for 5 min. The canal was dried with paper points; and intracanal calcium hydroxide dressing by using Metapex (Meta Biomed Co.,Ltd.) was given for one week (Fig. 2 a & b).

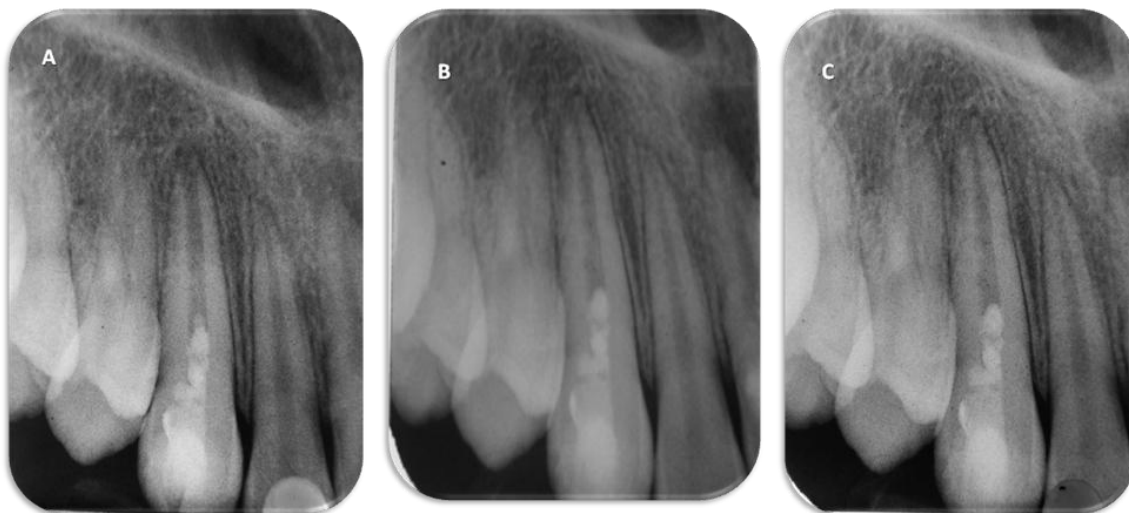
In the next visit, the patient was asymptomatic. There was no tenderness to palpation or percussion. The intracanal dressing was removed by irrigation with normal saline and the canal was flushed again with 20 mL of 17% EDTA for 10 min and dried with paper points. PRF was prepared by drawing 10 mL of blood in a test tube without anticoagulant, and centrifuging immediately using a table top centrifuge (REMI Lab. Inst., India) for 8 min at 3000 rpm. The resultant product consisted of three layers, viz., acellular platelet poor plasma, PRF in the middle, and red blood corpuscles at the bottom.

The acellular plasma was easily removed from the test tube and the PRF was removed with the help of tweezers. The fluid present in the fibrin clot was squeezed out and PRF was placed in the root canal up to middle third, followed by condensing using pluggers. MTA (ProRoot) (2 mm) was then placed on the top and the access cavity was sealed with a wet cotton pellet and IRM (DENTSPLY). On the next day, the cotton pellet was removed and GIC restoration (GC 2) followed with composite was performed (Fig. 2c).

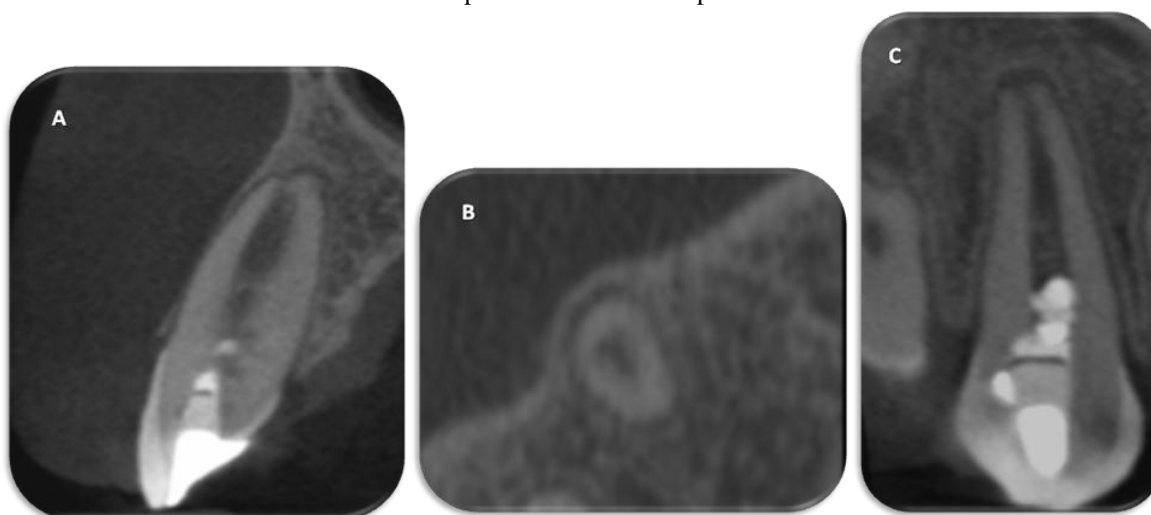


**Figure 2.** A: Radiograph showing working length determination; B: Intracanal dressing of calcium hydroxide; C: Post operative radiograph

The patient was recalled after 4, 8 and 12 months (Fig. 3 a, b, c). A clinical and radiographic evaluation was made considering the following criteria: periapical lesion, root resorption, apical closure, root length and root thickness. A CBCT scan (CareStream CS9300, SNAAP Imaging Centre, Ahmedabad, India) was taken at 13<sup>th</sup> month recall to evaluate the outcome. (Fig. 4)



**Figure 3.** A: Radiograph after four months showing reduction in size of periapical lesion; B: Complete resolution of periapical region with bone regeneration after eight months; C: Radiograph after one year, showing complete closure of root apex.



**Figure 4.** CBCT scan taken one year after to determine apical closure. A: Sagittal Section; B: Axial Section; C: Coronal Section

### III. Discussion

Regeneration of the pulp-dentin complex rather than the conventional apexification procedure is developing as a possible treatment alternative for traumatized permanent immature necrotic teeth. Regenerative endodontics involves biologically based procedures designed to replace damaged tooth structures such as dentine, root, and, even, cells of pulp-dentine complex (6). These procedures are based on three essential components of tissue engineering: stem cells, growth factors, and scaffolds. Addressing these three components of tissue engineering can lead to exciting possibilities for regeneration of dental tissues (7).

An ideal scaffold material should exhibit all the properties required to provide support to the delivery of stem cells and growth factors (8). Recently, a second generation platelet concentrate, PRF has been developed (9). A study found that PRF increases proliferation and differentiation of dental pulp stem cells (10), and it also serves as a continuous reservoir for the slow release of growth factors as it incorporates leukocytes, platelets and a wide range of key healing proteins in a dense fibrin matrix. Smith and Lesot reported that growth factors influence and direct the process of reparative dentinogenesis (11, 12).

In the present case, calcium hydroxide was used as an intracanal medicament in place of double or triple antibiotic paste. It has been found that formulations of various antibiotic combinations in concentrations of 1000mg/mL are highly toxic to allow the survival of stem cells. In contrast, a commercial preparation of calcium hydroxide showed no detrimental effects on survival of Stem Cells of Apical Papilla (SCAP) (13). Moreover, in this study, the choice of irrigant used in the procedure was based on findings from a recent study (14), which states that 6% NaOCl has detrimental effects on SCAP survival by preventing their differentiation along with denaturing dentin derived growth factors, whereas 17% EDTA increases SCAP survival and solubilizes dentin growth factor, and also, thereby increases their bioavailability (15).

The outcome of regenerative endodontic therapy in the present case report might have different possible explanations. In the first hypothesis, the continued development of root can be attributed to Hertwig's epithelial root sheath, which may remain viable after trauma even in non-vital teeth and this may help in continuing the development of root (16). In a subsequent analysis of root length (17), it was shown that continued root development occurs significantly more often with pulp revascularization; however, there were several cases where normal root development occurred even when the pulp was necrotic. On the basis of earlier case reports where new root formation was evident even when immature teeth were lost completely, it can be concluded that Hertwig's epithelial root sheath (HERS) may function separately from the pulp (16).

The second hypothesis is that the increase in thickness of dentinal walls at the apical end of the root might have been formed by dentin and cementum deposited by newly differentiated odontoblasts and cementoblasts. This differentiation may be caused by the various stem cells such as stem cells of apical papilla (SCAP), the dental pulp stem cells (DPSCs), the periodontal ligament stem cells (PDLSCs), dental follicular stem cells (DFSCs) and various other pulp progenitor cells e.g., undifferentiated mesenchymal cells, fibroblasts and subodontoblastic Hoehl's cells. The PRF scaffold acts as an excellent biomatrix incorporating proteins responsible for healing (18). On resorption of the fibrin matrix, various cytokines such as platelet-derived growth factor, TGF- $\beta$ 1, and vascular endothelial growth factor are released, achieving the peak level on day 14, coinciding with cell ingrowth (19). Lovelace (20) has shown that the periapical tissue contains a higher concentration of stem cells compared with the blood from the systemic circulation. Thus it is likely that PRP induces the migration of the stem cells from the periapical region.

At the recall visit after four months, there were no signs of infection, sinus or pain, however the tooth did not respond to vitality tests. Radiographic evaluation showed reduction in size of periapical lesion with bone regeneration. A recall after eight months showed complete healing of the periapical lesion and increased thickening of the dentinal walls. After one year, the radiograph showed complete formation of root apex. A CBCT scan was advised to confirm this, which showed closure of root apex by formation of hard tissue increasing the dentin wall thickness visualized in all three planes.

A better clinical outcome as documented in this case can be ascribed to the use of biogenic materials like PRF scaffold and better disinfection of the canal space without affecting viability of the stem cells.

#### **IV. Conclusion**

Clinical literature indicates that regenerative endodontic treatments using various methods and materials result in a significant increase in root length and dentinal wall thickness. The current status of regenerative endodontics is mainly an empirical approach, while at the same time basic research in dental stem cells is well documented. Efforts are being made to bridge this gap with translational research as well as to discover exciting and meaningful approaches for endodontics in the near future.

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#### **References**

- [1]. Webber RT. Apexogenesis versus apexification. *Dental Clinics of North America*, 28, 1984, 669–697.
- [2]. Kerekes K, Heide S, Jacobsen I. Follow-up examination of endodontic treatment in traumatized juvenile incisors. *Journal of Endodontics*, 6, 1980, 744–748.
- [3]. Rafter M. Apexification: a review. *Dental Traumatology*, 21, 2005, 1–8.
- [4]. Andreasen JO, Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. *Dental Traumatology*, 18, 2002, 134–137.
- [5]. Shabahang S, Torabinejad M. Treatment of teeth with open apices using mineral trioxide aggregate. *Practical Periodontics and Aesthetic Dentistry*, 12, 2000, 315–320.
- [6]. Murray PE, Garcia-Godoy Franklin, Hargreaves K. Regenerative endodontics: A review of current status and a call for action. *Journal of Endodontics*, 33, 2007, 337–390.
- [7]. Diogenes A, Henry MA, Teixeira F et al. An update on clinical regenerative endodontics. *Endodontic Topics*, 28, 2013, 2–23
- [8]. Horst OV, Chavez MG, Jheon AH. Stem cell and biomaterial research in dental tissue engineering and regeneration. *Dental Clinics of North America*, 56, 2012, 495-520.
- [9]. Choukroun J, Adda F, Schoeffler C, et al. Une opportunit  en paro-implantologie. Le PRF (Platelet-Rich Fibrin). *Implantodontie*, 42, 2001, 55–62.

- [10]. Huang FM, Yang SF, Zhao JH, et al. Platelet-rich fibrin increases proliferation and differentiation of human dental pulp cells. *Journal of Endodontics*, 36, 2010, 1628–1632.
- [11]. Del Carso M, Toffler M, Dohan Ehrenfest DM. Use of an autologous leukocyte and platelet-rich fibrin (L-PRF) membrane in post avulsion sites; an overview of Choukroun's PRF. *The Journal of Implant and Advanced Clinical Dentistry*, 1, 2010, 27–35
- [12]. Smith AJ, Lesot H. Induction and regulation of crown dentinogenesis: embryonic events as a template for dental tissue repair? *Critical Reviews in Oral Biology and Medicine*, 2, 2001, 425–437.
- [13]. Ruparel NB, Teixeira FB, Ferraz CC, et al. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. *Journal of Endodontics*, 38, 2012, 1372–1375.
- [15]. Trevino EG, Patwardhan AN, Henry MA, et al. Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. *Journal of Endodontics*, 37, 2011, 1109–1115.
- [16]. Lin P, Lin Y, Lennon DP, et al. Efficient lentiviral transduction of human mesenchymal stem cells that preserves proliferation and differentiation capabilities. *STEM CELLS Translational Medicine*, 1, 2012, 886–897.
- [17]. J M Greer, A J Moule, P J Greer. Resumed tooth development following avulsion of a permanent central incisor. *International Endodontic Journal*, 29, 1996, 266-270
- [18]. Andreasen JO, Borum MK, Andreasen FM. Replantation of 400 avulsed permanent incisors. Factors related to root growth. *Endodontics and Dental Traumatology*, 11, 1995, 69–75.
- [19]. Dohan DM, Choukroun J, Diss A, et al. Platelet-rich fibrin (PRF): a second generation platelet concentrate. Part II: platelet-related biologic features. *Oral Surgery Oral Medicine Oral Pathology Oral Radiology Endodontics*, 101, 2006, 45–50.
- [20]. Lucarelli E, Beretta R, Dozza B, et al. A recently developed bifacial platelet-rich fibrin matrix. *European Cells and Materials*, 20, 2010, 13–23
- [21]. Lovelace TW, Henry MA, Hargreaves KM, Diogenes A. Evaluation of the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after clinical regenerative endodontic procedure. *Journal of Endodontics*, 37, 2011, 133–138.