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CASE REPORT

Maturogenesis of an immature necrotic tooth with an extensive perirapical lesion using platelet rich fibrin

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Introduction: Earlier, treatment of immature necrotic tooth focussed only on surgical endodontic management and apexification procedures. Regenerative endodontic therapies have called for a paradigm shift in management of such cases. Prognosis becomes challenging though at times due to chronicity of infection, inadequate disinfection of canal space and cytotoxic nature of irrigants used hindering the survival of the apical stem cells. **Case presentation**: This report highlights the management of a long-standing case of an immature necrotic tooth with an extensive periapical lesion in a 12 year old male patient. The protocol of regenerative endodontic procedure using platelet rich fibrin (PRF) was followed. Follow up visits at 1week, 3months and 12 months revealed a successful clinical and radiological outcome leading to progressive maturogenesis of the tooth. **Conclusion**: The case report favours the possibility of stem cells of apical papilla being viable even in chronic periapical lesions. Hence conservative approach involving regenerative endodontic therapies should always be sought for as first line of treatment in such cases.

Keywords: apexogenesis, regenerative endodontics, platelet rich fibrin, periapical abscess

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Introduction

Impediment to root development and pulpal necrosis often succeeds traumatic injury of immature permanent teeth. This leads to failure of apex closure, thin dentinal walls of roots which ensues an augmented risk of root fracture [1].

Conventionally, the choice of treatment for necrotic immature teeth was apexification which comprised of repeated calcium hydroxide dressing in multiple visits for establishing a calcified barrier at the tooth apex prior to root canal obturation [2]. In addition to the unpredictable treatment timings, numerous visits, hazards of reinfection due to the impediment in achieving long-standing seals with interim restorations, calcium hydroxide leads to reduction of microhardness in dentine as it disrupts the bonds between collagen fibers and hydroxyapatite owing to its proteolytic nature. On the other hand, artificial barrier techniques are associated with considerable success, but the roots remain thin and prone to fracture due to absence of continued development of root [3].

Regenerative endodontic procedures may be deemed as a more effective treatment modality in this regard since immature teeth comprise of periapical tissues which are rich in vascular supply and stem cells, which under appropriate conditions possess the ability for tissue regeneration. The triad of factors that govern the regenerative endodontic treatment procedure are the stem cells, a biomimetic scaffold, presence of growth factors in root canal space for stimulating cellular growth and differentiation. Prognosis of such treatment becomes challenging in the presence of extensive preoperative periapical lesion [4], the chronicity of periapical disease, the cytotoxic nature of irrigants used and the inadequate disinfection of the root canal system [5].

The intent of this case report is to depict the management of a necrotic immature tooth with an extensive periapical lesion and its successful clinical and radiological outcome by using Platelet rich fibrin (PRF), a second-generation platelet concentrate as a scaffold in the regenerative endodontic procedure.

Case description

A 12-year-old male patient reported to the Department of Paedodontics and Preventive Dentistry with chief complaint of pain in the upper front tooth region since 2 days. The patient was prescribed antibiotics and analgesics by a medical practitioner and was advised for dental treatment a day before. The patient's preceding medical and dental history was non-contributory. There was a history of trauma to the maxillary incisors due to a fall 4 years back. Clinical examination (Fig. 1A) revealed Ellis class II fracture of 21. Small, fluctuant labiogingival swelling present apical to 21 was suggestive of abscess. Tooth 21 and 22 exhibited Grade I mobility, were tender on percussion and did not respond to Cold test and Electronic pulp test. Following radiographic evaluation using intraoral periapical radiograph (Fig. 1B), incomplete root formation and wide open apex of 21 associated with periodontal ligament space widening and an extensive periapical radioluscent lesion was evident. Tooth 22 had a closed apex and widening of periodontal ligament space was evident.

A diagnosis of pulp necrosis with acute exacerbation of chronic periapical abscess was reached at for tooth 21. Regenerative endodontic procedure was planned for 21 using PRF. Tooth 22 was diagnosed with pulpal necrosis with

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Fig.1. (A) Preoperative clinical picture showing Ellis class II fracture of 21. Small, fluctuant labiogingival swelling present apical to 21 suggestive of abscess. (B) Preoperative radiograph showing 21 with immature root apex associated with periodontal ligament space widening and an extensive periapical radioluscent lesion. Tooth 22 has a closed apex and widening of periodontal ligament space is noted.

symptomatic apical periodontitis. Conventional root canal treatment was planned and carried out for this tooth which is not a part of this report and shall not be discussed further.

Treatment modalities were discussed with the parent and the patient and consent was obtained. The tooth was anaesthetised by administration of local anaesthesia in the form of Lignocaine hydrochloride (2%) containing epinephrine (1:2,00,000) (Dentocare Pvt. Ltd) following which an access cavity preparation was done using a safe end bur (MANI, INC.) in tooth 21. From the canal, a voluminous amount of pale-yellowish fluid blended with blood drained out (Fig. 2A). Copious irrigation of the canal space was then carried out with 20 mL of 1% sodium hypochlorite (NaOCl) (Parcan; Septodont, Saint-Maur, France) and saline using a 27-gauge side-venting needle (Fig. 2B). Working length was determined with the aid of intraoral periapical radiograph (Fig. 2C). Canal was dried using sterile absorbent paper points. One coat of a single component total etch adhesive (Adaper ™ Single Bond 2, 3M ESPE, and USA) was applied with a nylon-bristled brush and in the access cavity till the CEJ and light cured. Following which a triple antibiotic paste was applied (Fig. 2D). The enteric coating of the drugs, Ciprofloxacin (Ciplox Tab 250mg, Cipla, India), Metronidazole (Flagyl Tab 400mg, Nicholas Piramal India Ltd.), Minocycline (Divaine tab 100mg, Cipla, India) were removed and then powdered. They were mixed in a ratio of 1:3:3 as mentioned by Takushige et al [6]. To achieve a paste like texture, propylene glycol was added to the drug mixture. A reamer was used to place the triple antibiotic paste in the canal. Following which a closed dressing was given using Type II Glass ionomer cement (GC Corporation Tokyo, Japan). At the next visit after 1 week, the patient was asymptomatic. The treated tooth had undergone discolouration (Fig. 2E) and was non-tender on percussion. Under rubber dam isolation, the access cavity was reopened and the triple antibiotic paste was removed by irrigating the canal with saline. Irrigation of the canal was carried out using 20 mL of saline and 20 mL of 1% NaOCl. Normal saline was used for final irrigation of the canal. The canal was then dried using sterile paper points. For the preparation of PRF, the procedure illustrated by Choukroun et al. was used [7]. Five milliliters of venous blood was drawn from patient's forearm and collected in a 10-mL test tube which did not have anticoagulant. Immediately, the test tube containing the blood sample was placed on a centrifugal machine (R-8C, REMI Laboratories, Mumbai, India) at 3000 revolutions per minute for 10 minutes. Formation of three distinct layers were seen in the tube (Fig. 2F.i): acellular plasma at the top, platelet-rich fibrin clot in the middle and red blood cells at the bottom. The straw-coloured acellular plasma at the top was removed. A sterile tweezer was inserted into the test tube to gently hold the fibrin clot and then take it out of the tube (Fig. 2F. ii). It was squeezed to remove excess fluid and then cut into pieces (Fig. 2G. i) and placed in the access cavity using a tweezer (Fig. 2G. ii). A finger plugger was used to place the PRF matrices more apically till 3 – 4mm short of the cementoenamel junction (Fig. 2H). Mineral trioxide aggregate (MTA-Angelus) was placed over the PRF till the cementoenamel junction (Fig. 2I). An intraoral periapical radiograph was taken to ensure the proper placement of MTA (Fig. 2J). Following which a moist cotton pellet was placed over the MTA, and glass ionomer cement (GC Corporation Tokyo, Japan) restoration was done. At the recall visit after 2 days, the cotton pellet was removed and a layer of resin-modified glass ionomer cement (Vitrebond; 3M ESPE) of around 2 - 3 mm was placed on top of the MTA and light-cured. Restoration of the access cavity was done with composite resin (Ivoclar Vivadent, Liechtenstein). Composite build up of the tooth was done followed by veneering of the labial surface of the tooth since it had undergone discoloration (Fig. 3).

Patient was recalled for follow up visits at 1 week, 3 months and 12 months. At the follow-up visit after 1

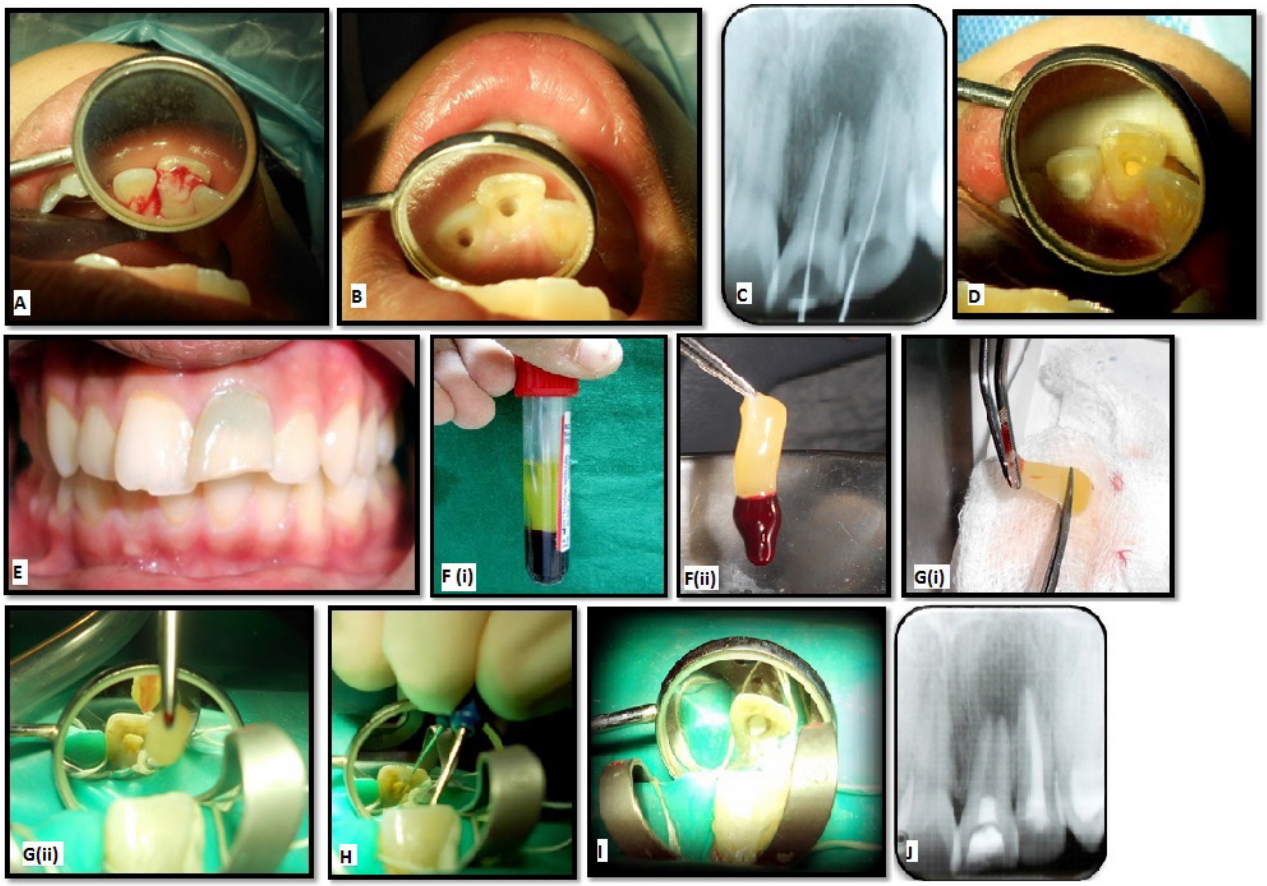

Fig.2. (A) Profuse pus discharge following access opening of 21, 22. (B) Following abscess drainage and irrigation of root canals of 21, 22. (C) Working length determination. (D) Application of triple antibiotic paste in root canal space of 21. (E) Crown discolouration of 21. (F) Preparation of PRF from patient's blood sample: i) Formation of 3 distinct layers, ii) A sterile tweezer used to gently hold the fibrin clot. (G) i) Sectioning of PRF, ii) placement of PRF in the access cavity with the help of a tweezer. (H) Use of finger plugger for placing the PRF more apically. (I) MTA plug placement following placement of PRF. (J) IOPAR showing the MTA plug.

week, the patient was completely asymptomatic. The tooth did not exhibit any tenderness to percussion. Radiographically there was slight decrease in radioluscency periapically (Fig. 4A). At 3 months follow up visit, the patient continued to be asymptomatic. The radiograph exhibited further reduction of radiolucency at the periapical region(Fig. 4B). At 12 months, the patient was asymptomatic. Radiographic examination (Fig. 4C) revealed complete resolution of the extensive periapical radioluscency. The periapical area showed normal trabeculation. The radiograph showed continued root growth, enhanced thickness of root wall and closure of the root apex. The tooth was not responsive to pulp testing. The evolution of radiographic findings has been summarised in Table I.

Discussion

Pulp necrosis often follows traumatic injury in immature permanent tooth with open apex. This leads to occurrence of a microbial biofilm and a shift in the microenvironment in the periapical region, prompting the death of the undifferentiated cells from the apical papilla and Hertwig's epithelial root sheath (HERS) [8]. Traumatic injury also

Fig.3. Postoperative Clinical pictures following composite build up and veneering.

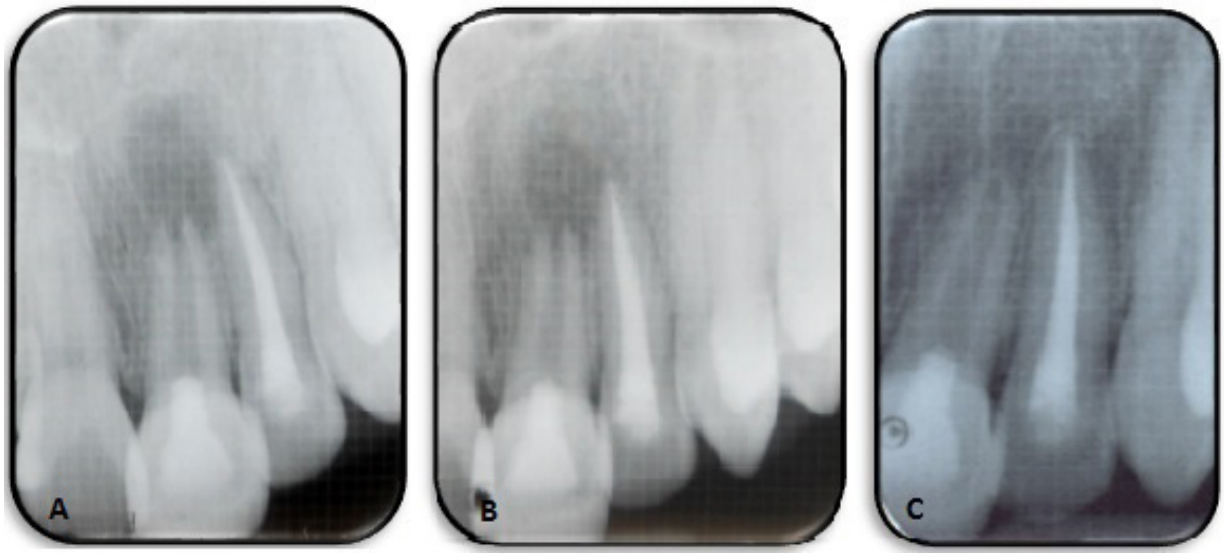

Fig. 4. Follow up radiographs showing gradual regression of periapical radioluscency at (A) 1 week, (B) 3months, (C) 12 months. Complete resolution of periapical radioluscency, root elongation and apex closure are evident.

Table I. Evolution of radiographic changes

Pre operative radiographic findings	Incomplete root formation and wide open apex of 21. Periodontal ligament space widening and presence of an extensive periapical radiolucent lesion.	
Post operative radiographic findings at follow up visits	At 1 week	Slight decrease in periapical radioluscency. Presence of open apex.
	At 3 months	Further significant reduction of periapical radiolucency. Presence of open apex. Absence of significant changes in root growth.
	At 12 months	Complete resolution of the extensive periapical radioluscency. Normal trabeculation at the periapical region. Root elongation suggestive of continued root growth, enhanced thickness of root wall and closure of the root apex suggestive of progressive tooth maturogenesis.

affects tooth maturogenesis of immature permanent teeth adversely most probably due to damage to HERS. Regardless of the development of a chronic periapical lesion and loss of tooth vitality in this case, an effective outcome was accomplished. There was absolute remission of the symptoms and tooth maturogenesis was achieved. The stem cells of the apical papilla (SCAP), which play significant role in physiologic root development may also promote progressive development of root during regenerative endodontic procedures. The development of a normal dentin–pulp complex implies not only the persistence of SCAP but also the presence of epithelial cells, originating from HERS or its disintegration as epithelial rests of Malassez (ERM), that permit them to reiterate the initial events of tooth development [9].

A research study [10] revealed that there was histological resolution of periapical lesions in 79% of necrotic immature teeth along with an increase in root thickness and length (74%). Complete apical closure was appreciated in 42% cases whereas partial closure was seen in 32% cases. These outcomes are indicative of the fact that even after infection, the viability of SCAP and cells of HERS is not lost and they persist in continuity or in proximity to the root. The case presented here supports these data with the clinical observation that even in extensive periapical lesions, SCAP, HERS, and ERM are more resilient than anticipated, which help them to endure untoward conditions and their development progresses. It seems that SCAP cells have the intrinsic ability to endure and thrive in the hypoxic environment which can be attributed to its niche typically being poor in vascular supply, its nourishment depending on passive diffusion from the adjacent tissues [11,12].

The treatment procedure that was carried out in this case was similar to that of any other regenerative endodontic procedure. The vital initial step in regenerative endodontic procedures is infection control of root canal space. The antibiotic mixture comprising of Ciprofloxacin, Metronidazole, Minocycline aided in root canal disinfection, as it is quite effective against the polymicrobial nature of endodontic and periapical lesions [13]. Crown discolouration was observed in this case even after sealing the dentinal tubules with dentine bonding agent. In a study by Bakhtiar et al. discoloration was reported in all revascularized teeth after application of dentine bonding agent prior to application of triple antibiotic paste. However, Biodentine was used rather than MTA as the sealing material. Authors accredited the staining to the interaction between Biodentine and PRF [14]. An in vitro study observed that the use of the adhesive agent reduced the severity of staining but did not prevent it [15]. However, in a study by Bezgin et al [16], there was no discolouration reported in the revascularized teeth where adhesive application was done prior to intracanal medication with triple antibiotic paste. Furthermore, regenerative endodontic procedures itself can promote discoloration to some extent [17].

Ensuing proper disinfection, the presence of biomimemetic scaffold becomes elemental in promoting the in growth of new tissue [18]. PRF, a second generation platelet concentrate was used as a scaffold in this case. Its dense fibrin matrix comprises of a huge number of cytokines, leucocytes, platelets and key healing proteins [19]. The elastic and resistant membrane of fibrin aids in the migration of fibroblasts and endothelial cells and harbours growth factors that help in revascularization. Studies reveal that that PRF exhibits a considerable slow, sustained release of essential growth factors like platelet derived growth factor (PDGF) , transforming growth factor beta 1 (TGFb1) and vascular endothelial growth factor (VEGF) for over a period of at least 1 week to 28 days, achieving a peak level at 14th day. PRF triggers cellular differentiation leading to generation of numerous cell types and augments angiogenesis. Furthermore, the granulocytes comprising of lymphocytes and cytokines released by T helper cells and macrophages, though present in small amounts in PRF, serve a crucial part in the self-regulation of inflammatory and infectious episode [20].

Radiographically, the tooth in concern exhibited regression of the extensive periapical lesion and tooth maturogenesis was appreciated as there was progressive thickening of the dentinal walls, lengthening of root, and apical closure was evident in 12 months which can be attributed to the survival of SCAP in a hypoxic environment as discussed earlier and also to the use of PRF. Huang et al. [21] showed that SCAP, osteoblasts, endothelial cells, fibroblasts, epithelial cells exhibit receptors to the growth factors encountered in platelets which can subsequently lead to their proliferation into the canal space from the periapical region. Hence, PRF serving as a reservoir of tissue regenerating and restorative factors, enhances the development of root.

The tooth continued to be unresponsive to the pulp sensibility tests. The probable cause according to a study by Palma PJ et al. [9], can be due to the distance between the crown and pulp tissue which was possibly restricted to the apical region of the tooth. To achieve neuronal function after regenerative endodontic procedures, a longer follow up may be required. A few studies after a follow up period of 2 to 3 years revealed a positive response to pulp sensibility tests [22,23]. However, concerning pulp sensibility testing in two recent studies with a follow up period of 2 to 5 years, tooth remained unresponsive [9,24].

Conclusion

Conservative approach for treating immature teeth with long standing endodontic infection entitles our attention. The clinical observations of this case signify massive healing potential of immature necrotic teeth with extensive chronic periapical lesion favouring the possibility that SCAP survive in hypoxic environment and are important in the healing process. Application of endodontic therapies that do not hinder the viability of the stem cells of apical papilla are a vital factor leading to successful maturogenesis of immature necrotic teeth.

Author's contribution

BC (Investigation, Methodology, Writing – original draft, Writing – review & editing)
AR (Conceptualization, Supervision, Writing – review & editing)

Conflicts of interest

the authors of this article declare no conflict of interest.

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