RESEARCH ARTICLE

Advanced Platelet-rich Fibrin-mediated Regeneration of Necrotic Immature Permanent Teeth: A Clinico-radiographic Observational Study

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Abstract

Aim: The objective of the study was to assess the regenerative potential of advanced platelet-rich fibrin (APRF) in the regenerative treatment of necrotic immature permanent teeth (NIPT) in the maxillary incisor region.

Study design: After institutional review board clearance, 10 children aged between 8 and 14 years with NIPT in the maxillary incisor region undergoing APRF treatment were enrolled in a prospective clinico-radiographic exploratory observational study. Baseline clinical, radiographic, and vitality testing before the start of treatment were noted. Patients were followed up at 3, 6, and 12 months posttreatment.

Results: After 3, 6, and 12 months of follow-up, all patients (100%) showed complete resolution of clinical signs and symptoms. All patients (100%) showed periradicular healing, and 9 out of 10 patients (90%) showed a clear hard tissue bridge formation at various levels in the root canal on postoperative radiographs. None of the patients (0%) showed a positive response to vitality testing.

Conclusion: APRF is a promising biomaterial in regenerative endodontic treatment (RET). Future randomized trials can be planned to establish superiority or equivalence to conventional PRF.

Keywords: Advanced platelet-rich fibrin, Immature permanent teeth, Necrotic pulp, Open apex, Pediatric dentistry, Regenerative endodontic treatment.

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Introduction

Regenerative endodontic treatment (RET) has been described as a paradigm shift in the current armamentarium of pulp therapy procedures and has attracted enormous attention in recent years.[1,2] It is defined as a biologically based procedure designed to replace damaged tooth structures, including dentin and root structures, as well as the cells of the pulp dentin complex.[3] Based on Cvek's classification of root development, RET is recommended in NIPT in stages 1, 2, and 3 with less than half, half, and two-third root formation, respectively.[1] Endodontic management of NIPT is clinically challenging as they possess thin root walls and wide open apex and are traditionally managed by apexification.[1,4] However, it is important to emphasize that apexification has no potential for root maturation and return of neurogenesis, and hence RET is considered a better treatment option in such teeth.[1,5] Amongst the natural scaffolds utilized in RET, PRF is considered ideal as it binds and localizes specific cells, contains a multitude of growth factors, and undergoes biodegradation over time leading to tissue regeneration and wound healing.[2] Based on the low-speed centrifugation concept (LSCC), Choukroun and Ghanatti[6] described two new advancements, namely APRF and injectable PRF, which contain more cells than conventional PRF.[6] The present study aims to assess the regenerative potential of APRF in NIPT using clinical examination, radiographic method, and vitality testing.

Materials and Methods

This prospective clinico-radiographic exploratory observational study was approved by the Institutional Ethics Committee, and written informed consent was obtained from the parents/guardians of the patients for participation in the study. Around 16 patients between the age group of 8–14 years were initially considered for recruitment in the study, and finally, 10 patients were analyzed after exclusions and dropouts (Fig. 1).

Inclusion Criteria

Were patients in the age group of 8–14 years undergoing APRF treatment in immature permanent teeth for various indications such as necrotic pulp due to trauma or dental caries with open apex (blunderbuss or nonblunderbuss) associated with or without periapical lesion and with or without associated intraoral sinus tract.

Exclusion Criteria

Patients with significant medical comorbidity, presence of internal or external resorption in the tooth, presence of root fracture, and those patients who were lost to follow-up.

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Baseline clinical and radiographic examination was recorded of all the enrolled patients undergoing APRF treatment after taking appropriate consent for participation in the study. As per the existing treatment protocol of our department, in these patients, access was prepared under local anesthesia and rubber dam isolation using round bur and Endo Z bur (Dentsply Maillefer, Tulsa, OK), and working length was confirmed radiographically. The root canals were minimally instrumented and copiously irrigated with 20 mL of 1.5% sodium hypochlorite (NaOCl) for 5 minutes followed by 20 mL of saline for 5 minutes, using a side vented needle. Triple antibiotic paste (TAP) comprising metronidazole (400 mg, J.B. Chemicals and Pharmaceuticals, India), ciprofloxacin (500 mg, Ranbaxy Lab, India), and minocycline (50 mg, Ranbaxy Lab, India) was mixed in equal amounts with distilled water to a get a final concentration not more than 1 mg/mL. The canals were dried with paper points, and prepared TAP was packed into the canal till the cementoenamel junction. The tooth was restored with a 4 mm cavity (3M ESPE, USA), and the patient was recalled after 2 weeks.

In the second appointment, after confirming the absence of clinical signs and symptoms, the triple antibiotic dressing was flushed out with saline. The canals were finally irrigated with 20 mL of 17% ethylene diamine tetra acetic acid (EDTA) and dried using paper points. APRF membrane was then sectioned into pieces using sterile scissors and condensed into the canals, using a finger plugger (Dentsply Maillefer), 1 mm beyond the confines of the working length, followed by placement of a thick layer of biodentine (Septodont, France) and the tooth was restored with 4 mm cavity (3M, ESPE, USA). The patient was recalled after 1 week and the temporary restoration was replaced with a composite restoration (3M ESPE, USA).

Preparation of APRF

Under aseptic precautions, 10 mL of blood was withdrawn from the medial cubital vein of the patient and collected into test tubes without anticoagulants. The blood was centrifuged instantaneously using Choukroun's method of APRF preparation at 1300 rpm for 8 minutes[6] with a centrifugation machine (Duo QUATRO, France). Usually, when blood is centrifuged, it gets divided into three distinct layers namely; straw-colored acellular platelet-poor plasma at the top level, PRF liquid at the intermediate level, and a red fraction of red blood cells at the base level. However, in the case of APRF, since the centrifugation is done at low settings for a lesser period of time, a complete demarcation of all three layers is not visible. Therefore, red blood cells being heavier, settle at the bottom, and the entire straw-colored liquid rich in platelets, and white blood cells (the top layer) is collected and used as the APRF clot (Fig. 2) or can be compressed to form an APRF membrane (Fig. 3). In the current study, APRF membrane was used as a biomaterial/bioscaffold.

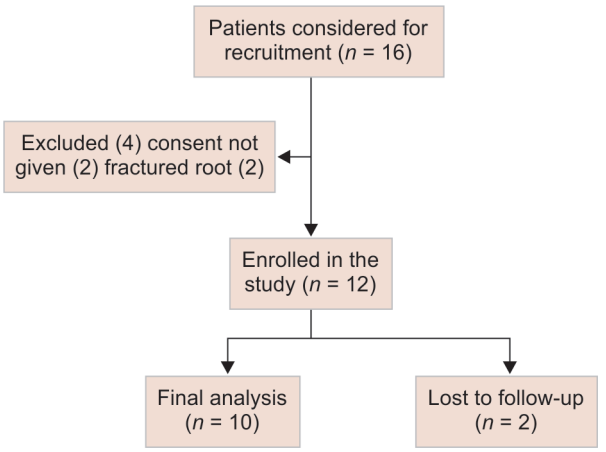


Fig. 1: Patient recruitment algorithm (STROBE)

Follow-Up

Recall visits were scheduled at 3, 6, and 12 months postoperatively. Clinical, radiographic examination and vitality testing were done at each recall visit. The treatment was considered successful clinically when symptoms such as pain, swelling, intraoral sinus tract, tenderness to apical palpation, or percussion were absent. Radiographic success was evaluated by resolution of the periapical lesion, changes in the open apex of the root canal in the form of apical closure, and further root thickening on intraoral periapical radiographs. A positive response to electric pulp testing (EPT) indicated a return of tooth vitality.

Results

All patients presented with a history of trauma in the maxillary incisor region and the minimum, and maximum time elapsed between trauma and the first dental appointment was 2 and 5 years, respectively. Demographic data with presenting clinical and radiological features are described in Table 1.

All the patients tolerated the procedure well and did not complain of any immediate postprocedure discomfort. At 3-, 6-, and 12-month clinical examinations, all patients (100%) were free of pain, associated intraoral swelling, and tenderness to percussion and palpation with complete healing of intraoral sinus

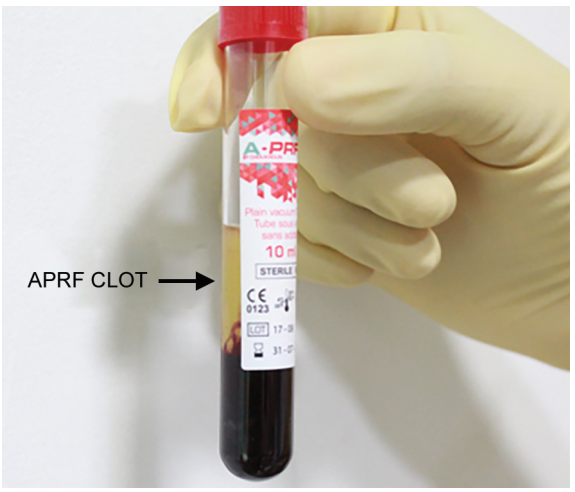


Fig. 2: Formation of advanced PRF after low-speed centrifugation of peripheral blood with clot (top layer) and red blood cells (bottom layer)

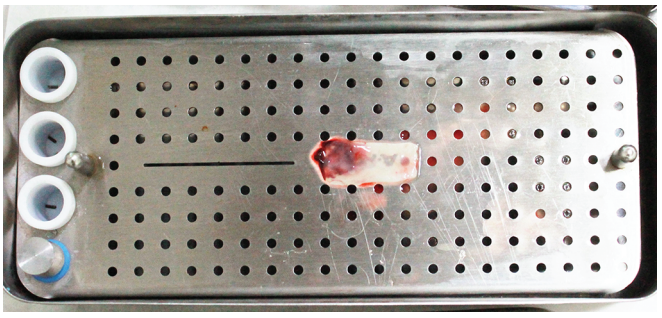


Fig. 3: Advanced PRF membrane

tracts (Table 2). All patients (100%) showed periradicular healing, and 9 out of 10 patients (90%) showed the formation of hard tissue barriers at various levels in the root canal (Table 2). Preoperative and postoperative radiographs at 3 months showed a hard tissue barrier in the middle third of the root canals [Figs 4A and B (#11 & #21)], whereas it was seen in the apical third of the root canal between the coronal bio-dentine and the open root apex [Figs 5A and B (#21)]. There were no further radiographic changes observed at 6 months and 12 months follow-up. However, in one patient (10%), although there was complete resolution of clinical signs and symptoms and the tooth was in function, the radiographic follow-up showed no change in the open apex. None of the patients (0%) responded to EPT at 3, 6, and 12 months follow-up (Table 2).

Discussion

Kim et al. first used the term "revasularization" in endodontics, but later, Huang and Lin proposed the term "revitalization" as the regenerated tissues in the canal space were not just the blood vessels but also hard and soft tissues.[1] However, based on the concept of tissue engineering, American Association of Endodontics (AAE) 2007 adopted the term "Regenerative Endodontics."[1] According to AAE (2018) clinical considerations for RET; the three desired treatment outcomes are as follows: (1) Resolution of clinical signs and symptoms and bone healing which is essential (primary goal), (2) Continued root maturation in the form of increased root wall thickness and length which is desirable (secondary goal), and (3) Return of neurogenesis or positive response to vitality testing (tertiary goal).[7]

The success of regeneration also depends upon the disinfection of the canals and the use of a proper scaffold which gives apical cells a chance to regenerate. NaOCl in concentrations of 1–6% has been used in several studies.[1] In the present study, based on AAE clinical considerations,[7] disinfection of the canal space was obtained by using 1.5% NaOCl and 1 mg/mL concentration of TAP to decrease the cytotoxic damage which may be caused to the stem cells of the apical papilla.

Subsequent to disinfection, an appropriate scaffold is needed for regeneration. Amongst the bioscaffolds, platelet-rich plasma (PRP) and PRF have shown clinical success in RET. However, the preparation of PRP is difficult and time-consuming. In addition, bovine thrombin used in the activation of PRP has been reported to cause the development of antibodies to thrombin, factor V, factor XI, and adverse reactions like hemorrhage, thrombosis,

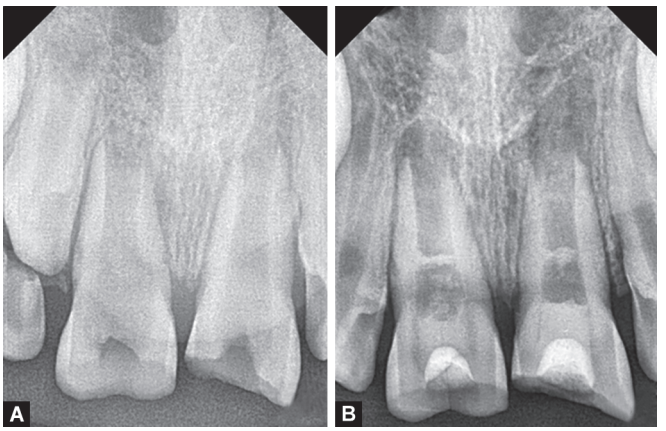
Table 1: Descriptive data of the patients

Patient no.	*Gender*	*Age (years)*	*Age at the time of trauma (years)*	*Involved tooth*	*Clinical features at the time of presentation*	*Radiological features at the time of presentation*
1.	M	9	7.5	11,21	Discolored fractured teeth, intraoral sinus tract, and swelling	Periapical radiolucency, open apex
2.	M	13	9	21	Discolored fractured tooth, associated intraoral sinus tract, and swelling	Periapical radiolucency, open apex
3.	M	9	7.5	21	Discolored tooth	Open apex
4.	F	11	8.5	11	Discolored fractured tooth, intraoral sinus	Periapical radiolucency, open apex
5.	F	10	8	11	Discolored fractured tooth, intraoral sinus	Periapical radiolucency, open apex
6.	M	14	9	21	Discolored fractured tooth, intraoral swelling	Periapical radiolucency, open apex
7.	M	9	7	11	Discolored fractured tooth, associated intraoral sinus tract, and swelling	Periapical radiolucency, open apex
8.	M	9	7	21	Discolored fractured tooth, associated intraoral sinus tract, and swelling	Periapical radiolucency, open apex
9.	F	10	8	11	Discolored tooth	Open apex
10.	F	12	7	11	Discolored fractured tooth	Open apex

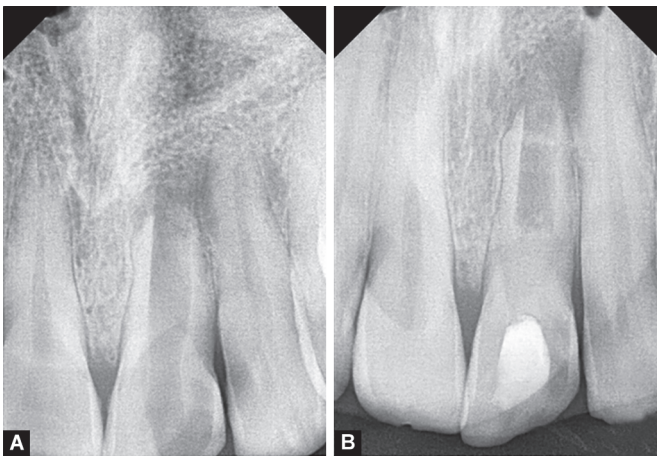
Table 2: Summary of the clinical, radiographic, vitality results at sequential follow ups

Outcome parameter	*3 months (n = 10)*	*6 months (n = 10)*	*12 months (n = 10)*
Resolution of clinical signs and symptoms	10 (100%)	10 (100%)	10 (100%)
Radiological examination			
Periapical healing	10 (100%)	10 (100%)	10 (100%)
Apical barrier formation	9 (90%)	9 (90%)	9 (90%)
EPT (vitality testing)	0 (0%)	0 (0%)	0 (0%)

EPT: Electric pulp testing, data presented as number (percentage)



Figs 4A and B: (A) Preop radiograph; (B) Postop radiograph at 3 months showing the formation of hard tissue barrier in the middle third of the root canals (#11, #21)



Figs 5A and B: (A) Preop radiograph; (B) Postop radiograph at 3 months showing the formation of hard tissue barrier in the apical third of the root canal (#21)

and systemic lupus erythematosus.[8] PRF is a second-generation platelet concentrate with a dense fibrin architecture supporting cellular migration and cytokine enmeshment due to its trimolecular or equilateral fibrin branch junction; while preventing the early invagination of undesired cells.[2] It enhances cellular proliferation, differentiation, and angiogenesis and has a significant and sustained release of key growth factors for upto 28 days which accelerates wound closure and mucosal healing.[2,3,8,9] It requires a single centrifugation cycle, is easy to prepare, requires less placement time, and is strictly autologous as there is no biochemical processing of blood.[9] It has been reported that the PRF causes the proliferation of human dental pulp cells and increases the protein expression of osteoprotegerin and alkaline phosphatase (ALP) activity, both of which are markers of odontoblastic differentiation and reparative dentin formation.[2]

However, in the present study, we have analyzed APRF as a bioscaffold which is an advanced version of PRF. It is based on LSCC, wherein the centrifugation is done at a low speed to separate red blood cells from the blood, and the remaining concentrate is used for regeneration.[10] The different centrifugation speeds and times prevent cell loss from the PRF matrix. APRF, thus formed has a higher concentration of platelets, leukocytes, and growth factors, namely vascular endothelial growth factor (VEGF) and transforming growth factor ß-1, essential for neovascularization and angiogenesis in comparison to PRF. Furthermore, APRF contains monocytes which play an essential role in bone growth, vascularization, and production of VEGF.[10,11] It has an improved collagen matrix synthesis which leads to enhanced recruitment of progenitor cells and hence greater regenerative ability.[12] Although both PRF clot and membrane have reported the same clinical success in RET, the membrane is easier to place and less time-consuming than the clot.[5] In the present study, all cases were treated using APRF membrane, and on follow-up visits, revealed periradicular healing, which is attributed to the multitude of growth factors and tissue healing properties of APRF.

Biodentine, which is an efficient alternative to mineral trioxide aggregate (MTA) considering the enhanced physical, biological, and handling properties[13] was packed directly over the APRF membrane to create a hermetic coronal seal in the current study. It is useful in regenerative endodontics because of its short setting time, high strength, and good marginal adaptability when compared to MTA.[13]

Root maturation after RET of NIPT is attributed to the presence of Hertwig's epithelial root sheath or cell rests of Malassez at the apex, which is resistant to periapical infection and remains vital.[1,14] These stimulate stem cells present in the apical papilla, periodontal ligament, and multipotent stem cells which further differentiate into bone or dentine forming cells and help in root maturation.[14] However, several studies report that root maturation is unpredictable[1], and depends upon the trauma and severity of the periapical lesion both of which may disturb the biological function of Hertwigs epithelial root sheath and its interaction with mesenchymal stem cells in the dental follicle.[1] Furthermore, the outcome of regenerative therapy depends upon the presenting clinical situation. Teeth exhibiting partial necrosis with some vital tissue in the apical portion of the canal have a good prognosis as the residual pulp can regenerate after disinfection. On the other hand, teeth exhibiting full necrosis where the pulp has been completely lost have a poor prognosis as it requires denovo synthesis of pulp.[15] In the current study, the recruited patients had full necrosis of pulp with chronic periapical infection of long duration. The changes observed in the open apex in the postperative radiographs coincided with type 5 of Chen's response,[16] which is the formation of a hard tissue barrier at various levels in the root canal between the coronal biodentine and the root apex. These results are in accordance with the previous studies.[17,18] In addition, the hard tissue barrier was visible radiographically after 3 months, in our study in contrast to the study conducted by Shanthakumar M, which reports no radiographic change at 6 months follow-up examination.[5]

The newly formed tissue in the canal space after RET has been reported to be periodontal like tissue instead of pulp tissue.[1] However, the type of tissue formed can be determined only by histological examination and not *via* conventional periapical radiography or cone beam computed tomography.[1] Histologically, an animal study describes bridge formation at the apical third of the canal by ingrowth of intracanal cementum in revascularized immature necrotic teeth due to the osteoinductive activity of the MTA.[19] In the present study, biodentine packed over APRF has been reported to stimulate odontoblastic differentiation and nodule formation during mineralization.[13]

The tertiary goal of positive response to vitality testing after RET has been reported in 50–60% of cases.[1] Recovery of tooth sensibility indicates the presence of innervated tissue in the root canal space.

This, however, does not necessarily indicate the presence of vital pulp tissue in the canals. Histological and immunohistochemical findings following RET revealed vital tissues like cementum, bone, neuron, and nerve fibers in the canal space, which even though were not pulped tissue, responded to pulp testing.[20,21] In the present study, none of the teeth responded to EPT on 12-month follow-up. However, no conclusion can be drawn from this finding as a recovery of tooth sensibility may take more than 1 year. It has been suggested that the presence of thick layers of materials like MTA and glass ionomer cement; and the fact that these materials limit the growth of new tissue ahead of it might lead to a negative response on vitality testing.[22]

Limitation

The current study was an exploratory observational study to assess the effect of APRF in RET of NIPT in maxillary incisors of pediatric patients. Randomized trials can be planned to further ascertain its efficacy compared to conventional and existing biomaterials in use.

Conclusion

- Advanced PRF is a successful therapeutic biomaterial in RET of NIPT.
- Resolution of clinical signs and symptoms, periradicular healing, and formation of hard tissue bridge in the root canal were visible earliest at 3 months follow-up.
- There was no response to vitality testing at 12 months of follow-up.

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