CASE REPORT

# A Comparative Evaluation of Revascularization Done in Traumatized Immature, Necrotic Anterior Teeth with and without Platelet-rich Fibrin: A Case Report

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## Abstract

The aim of this paper is to present two methods of revascularization done in traumatized immature, nonvital anterior teeth using platelet-rich fibrin (PRF) and natural blood clot as a scaffold material. This was performed after disinfection of the root canal space using tri-antibacterial paste. In one tooth, PRF prepared from autologous blood was placed in the canal and in the other tooth, natural bleeding was induced to obtain a fresh blood clot. The patient was recalled regularly at 1, 3, 6, 9, and 12 months' interval for evaluation. After 12 months, on clinical examination, both teeth showed negative response to percussion and palpation tests but positive response to cold and electric pulp tests. On radiographic examination, the tooth treated with PRF exhibited comparatively faster root lengthening, complete closure of the root apex, more thickening of the root dentinal walls, and narrowing of root canal space compared to conventionally revascularized tooth.

**Keywords:** Nonvital teeth, Open apex, Platelet-rich fibrin, Revascularization, Traumatized necrotic teeth.

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## Introduction

The occurrence of trauma to the anterior teeth in children is the most commonly seen clinical entity. The endodontic management of such traumatized anterior teeth with an immature open apex and nonvital pulp tissue is a challenging procedure for a clinician. As these teeth pose a great problem due to thin dentinal walls, open apices, and wide root canal space, the oldest method of calcium hydroxide apexification was modified to mineral trioxide aggregate (MTA) single-visit apexification.[1,2] However, this technique also resulted in various drawbacks, which finally lead to the development of a biological approach called pulp revascularization or regenerative endodontic procedure.[3]

"Regenerative endodontics" is the emerging and exciting field of the contemporary endodontic science, which is defined as biologically based procedures designed to replace damaged structures, including dentine and root structure as well as cells of the pulp–dentine complex.[3] For the success of this treatment, three critical components have been researched like stem cells, signaling molecules, and a three-dimensional physical scaffold. All three components are important for the in-growth of new tissues from the periapical area in an empty canal. Natural blood clot, collagen, and platelet-rich plasma (PRP) have been investigated as potential scaffold materials for revascularization.[4–6] Numerous studies have shown successful results in both conventional method using blood clot and PRP-assisted revascularization procedures. However, there is still questionable long-term predictability with moderate anticipated benefits noticed with these techniques and should be balanced with an informed knowledge of relative advantages and potential risks.[4–7]

Choukroun et al.[8] from France introduced a second-generation platelet concentrate known as PRF, which revolutionized the various specialties of dentistry. In the arena of contemporary nonsurgical endodontics, using PRF for the revascularization procedure is a new vista, and evidence-based Medline search revealed few case reports pertaining to the use of PRF in regenerative endodontic procedure.[9–13] The aim of this paper is to discuss the clinical and radiological outcome of the patient where both blood clot and PRF were used as a scaffold materials in the revascularization procedure in two (bilateral) necrotic immature permanent teeth.

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## Case Description

An 11-year-old boy reported to the Department of Pedodontics and Preventive Dentistry complaining of pain in the upper front teeth since 15 days. Patient reported a 6-month history of trauma of the front teeth due to fall. Following trauma, the patient did not seek any treatment of the traumatized teeth. On intraoral examination, both the maxillary right and left lateral incisors were discolored with Ellis class IV fracture and were tender to percussion test. The

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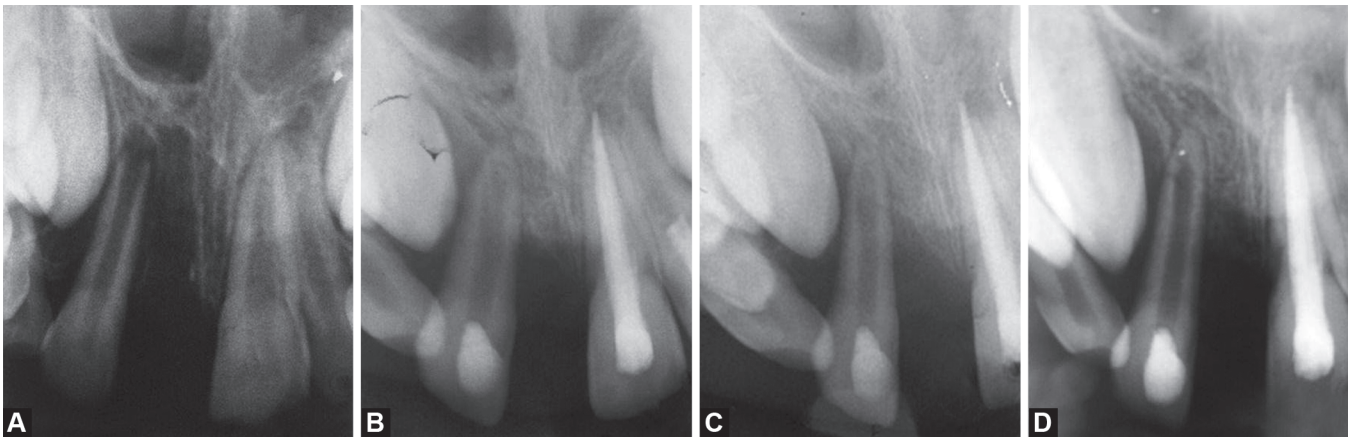
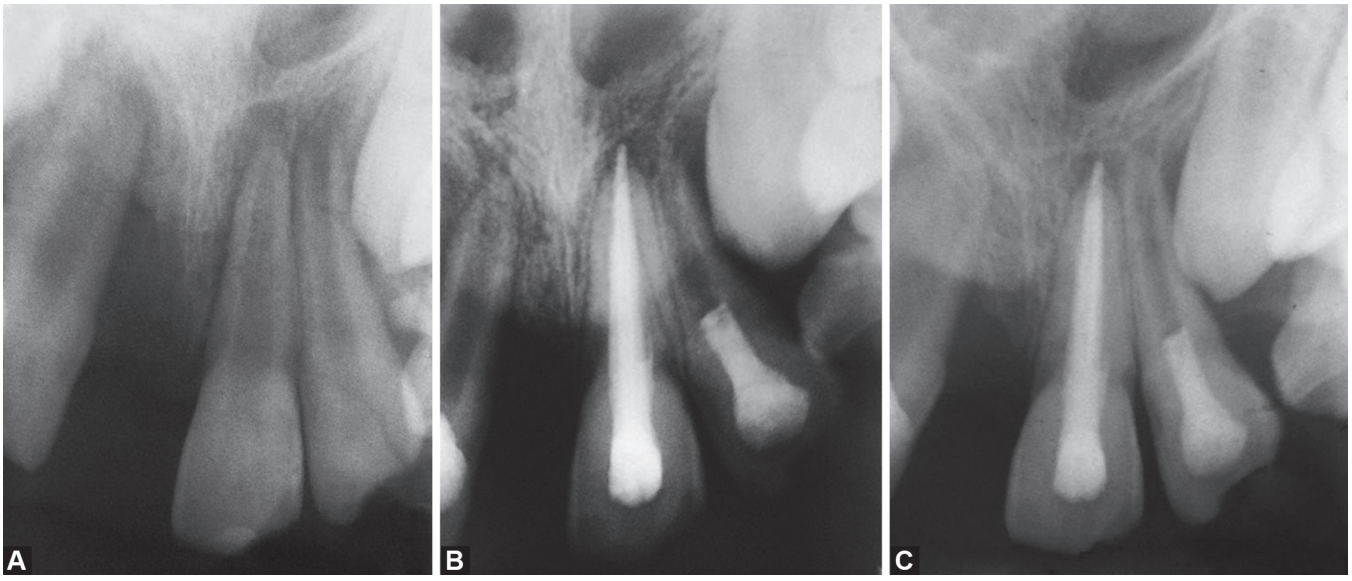
teeth did not respond to cold and electric pulp test and periodontal probing depth was within the normal limits. The maxillary right central incisor was completely avulsed. On periapical radiographic examination, both teeth exhibited an incompletely developed root with thin dentinal walls and wide open apex (Figs 1A and 2A). Based on clinical and radiographic presentations, the diagnosis of class IV fracture with apical periodontitis was made and decision was made to perform a regenerative endodontic treatment using blood clot in the left lateral incisor and PRF in right lateral incisor. Detailed treatment protocol was narrated to the parents and written informed consent was taken. The protocol and consent form were approved by the Institutional Ethics Committee (College of Dental Sciences, Davangere, India) to conduct the procedure.

Following rubber dam application, local anesthesia was obtained using 2% lidocaine with 1:100,000 epinephrine. Access cavity was prepared in both teeth using a round endo access bur (Dentsply Maillefer, Switzerland). Upon access opening, bleeding from the canal was not evident in two teeth. Minimal instrumentation was done and the canals were copiously irrigated using 10 mL of 5.25% of sodium hypochlorite solution (Rasayan Laboratory, Mumbai, India). Working length was determined by placing a file of 70 k size (Mani, Japan). The two canals were then dried with paper points. A mixture of ciprofloxacin (Cifran 500 µg; Ranbaxy Laboratories Ltd., Mumbai, India), metronidazole (Metrogyl 400 µg; J.B. Chemicals, Mumbai, India), and minocycline (Minoz 50 µg; Ranbaxy Ltd., Mumbai, India) with equal proportions was prepared, mixed with distilled water, and placed into the canal to a depth of 2 mm short of the apex using endodontic plugger (Mani, Japan). A cotton pellet was placed and the access opening was temporarily restored with Cavit (3M ESPE, Germany). The patient was recalled after 7 days and the tooth




**Figs 1A to D:** Radiographic outcome in PRF-treated permanent maxillary right central incisor: (A) Tooth showing incompletely developed root with wide and open apex, thin dentinal walls, and wide root canal space with periapical radiolucency; (B) At third month follow-up, tooth showing slight elongation in the root with accelerated closure of the apex and resolution in periapical radiolucency; (C) At the sixth month follow-up, tooth showed marked increase in the root length, obliteration of the root canal space, and excellent closure of the root apex with normal periapical anatomy; (D) At the 12th month follow-up, PRF-treated tooth exhibited excellent root lengthening, complete closure of the apex with normal periradicular architecture, thickened dentinal walls, and narrowing of the root canal space




**Figs 2A to C:** Radiographic outcome in blood clot-mediated revascularization done in permanent maxillary left central incisor: (A) Tooth showing incompletely formed root with wide, open apex, and thin root dentinal walls, wide root canal space, and widened periodontal ligament space; (B) At the sixth month follow-up, apex still found open; (C) At 12th month follow-up, blood clot-treated tooth exhibited continued root elongation and favorable closure of the apex with periradicular architecture and slightly thickened root dentinal walls

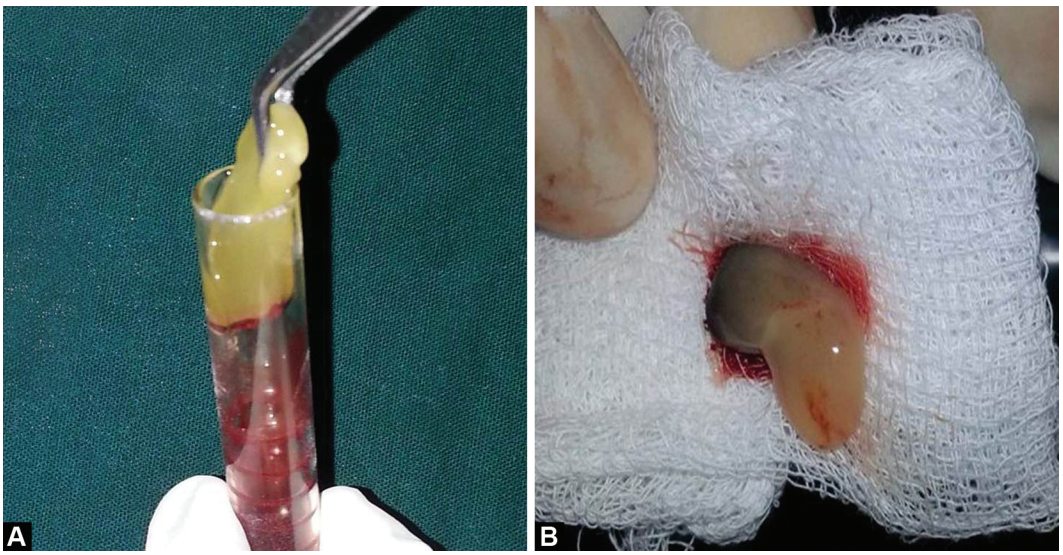
was found asymptomatic. Under rubber dam isolation, the access cavity in both the teeth reopened and the canals were thoroughly irrigated using saline solution to flush out antibiotic paste and finally the canals were dried with paper points. In the left lateral incisor, conventional method was followed by inducing natural blood clot within the canal. The periapical tissue was intentionally punctured using a sterile 23 G needle. When frank bleeding was noticed at the cervical part of the root canal, a dry cotton pellet was inserted 2 mm into the canal and held for 8–10 minutes to allow the blood to clot. In the right lateral incisor, the PRF used was prepared from the autologous blood by centrifugation method 10 minutes prior to the procedure (Fig. 3). The PRF membrane obtained was placed inside the canal and pushed 1 mm beyond the working length and coronally up to the cementoenamel junction (CEJ) level using an endodontic hand plugger (Dentsply Maillefer, Switzerland). A thin layer of white MTA (Pro Root MTA; Dentsply, Switzerland) was placed directly over the PRF membrane and blood clot in two treated teeth followed by placement of a wet cotton pellet and the teeth were temporarily restored using Cavit. The patient was recalled after 1 day for removal of cotton pellet. The access cavities were restored using glass ionomer cement [GIC (Universal Restorative, Tokyo, Japan)]. The patient was recalled every 1, 3, 6, 9, and 12 months for clinical and radiological assessments. Follow-up examination revealed normal responses to percussion, palpation, and normal pocket probing depths, and a positive response to cold and electric pulp test were found in both treated teeth similar to the adjacent teeth. At 6 months, on radiographic examination, the PRF-treated tooth showed accelerated root growth with complete closure of the apex (Figs 1B and C), whereas in tooth treated with blood clot the apex was found open (Fig. 2B). At 12-month examination, the PRF-treated tooth revealed accelerated root end growth, enhanced thick dentinal walls with narrowing of the canal space, faster closure of the apex, and normal periradicular architecture (Fig. 1D) as compared to the tooth treated with blood clot Fig. 2C).

## Discussion

Regenerative endodontic procedure for traumatized necrotic teeth as described by Banchs and Trope (14) leads to apexogenesis and maturogenesis in nonvital immature permanent teeth. The thickened dentinal walls and increased root length with closed apex increase the fracture resistance, thereby enhancing the long-term prognosis of the tooth. Different scaffold materials such as blood clot, collagen, and PRP are used for this purpose.[4–6] Therefore, in the present case, this novel endodontic procedure was opted to determine the efficacy of two different scaffolds, the blood clot and PRP in revascularization therapy as it is evident from previous data that PRF satisfies many criteria of an ideal scaffold material.[9–13]

In traditional method, revascularization is performed by inducing bleeding into the pulp canal by mechanically irritating the periapical tissues.[7] It has been hypothesized that even in the presence of apical periodontitis and periapical infection, pulp tissues may still be remaining along with Hertwigs epithelial root sheath in the apical region; and these tissues can proliferate and form odontoblast-like cells, once the inflammatory condition is treated and the canal becomes totally disinfected.[3,14,15] The apical papilla consists of a very specific stem cell tissue formation that is seen apically to the differentiated pulp tissue of the developing tooth, and these are called stem cells from the apical papilla.[7] The apical papilla has the potential of remaining undamaged because it is loosely connected to the dental pulp and has therefore a greater potential to regenerate the pulp tissue and continue the root development.[3,7,15] Therefore, the induced blood clot acts as a scaffold for the growth of new tissues into the empty canal space. However, this procedure is traumatic and causes discomfort for the patients especially in young children, while mechanically irritating the periapical region.[6] Moreover, the induction and maintenance of a blood clot is extremely difficult and this fact is evident in Petrino et al.[16] and Ding et al.[17] case series. They experienced difficulty in inducing bleeding into the canal in some of their patients which finally led to unfavorable treatment outcomes. In addition, it has been claimed that the precise placement of MTA to a desired and required level over a blood clot is a technically sensitive procedure.[16] All these factors tempted us to use the newer biologic matrix such as PRF in comparison with the natural blood clot, for the revascularization procedure in this patient.

To overcome the disadvantages associated with blood clot or PRP-induced revascularization, a novel biologic scaffold like PRF has been tested.[9–13,18] When compared to blood clots, platelet




**Figs 3A and B:** (A) Picture showing retrieval of platelet-rich fibrin gel formed after centrifugation; (B) Platelet-rich fibrin clot

concentrates increased the cell proliferation over time as they contain increased concentration of growth factors. Moreover, many *in vitro* studies showed the direct dose–response influence of many growth factors like platelet-derived growth factor (PDGF) on cell migration, proliferation, and matrix synthesis.[19] It has the characteristic of polymerizing naturally and slowly during centrifugation. The high-density fibrin clot acts as a biological matrix by supporting cell migration and cytokine release. When compared to other synthetic polymers and collagen scaffolds, it is more advantageous in terms of cost, immune response, toxicity levels, and inflammation.[18–20] Lucarelli et al.[21] reported that PRF generates a 210-fold higher concentration of platelets and fibrin when compared to the initial input whole blood volume. In addition, leukocytes in the fibrin clot act as anti-inflammatory agent and immune response regulator and provide a vasculoendothelial growth factor to promote angiogenesis.[18–20] Few studies have demonstrated that the PRF has a very significant slow sustained release of many key growth factors like PDGF and transforming growth factor-β for at least 1 week and up to 28 days, which means that PRF could release growth factors with its own biological scaffold for wound-healing process as compared to the natural blood clot.[18–22]

Pertaining to procurement, this technique involves simplified processing and lack of biochemical reagents for its activation.[8] However, it needs a speedy blood collection and immediate centrifugation, before the clotting cascade is initiated. Because no anticoagulants are added, the blood coagulates as soon as it comes in contact with the glass surface. The PRF is associated with other disadvantages such as the invasive procedure of drawing blood in children and the requirement of specialized equipment for its processing. Another drawback reported is difficulty in handling and placing PRF membrane inside the root canal as it is jelly-like consistency and sticks to the instrument.[11–13]

Based on the American Association of Endodontists policy on regenerative procedure, currently we do not have evidence-based standardized treatment protocols for endodontic regeneration procedures.[23] In many case reports,[9–13] the PRF-treated tooth showed excellent results, with continued radiographic evidence of the development of the pulp–dentin complex with an absence of clinical symptoms. Studies[3,4,7] done with blood clot have also shown similar results. In the present case, the clinical follow-ups of both revascularized teeth showed the absence of clinical symptoms such as negative response to palpation and percussion tests and positive responses to cold and electric pulp tests. On radiographic examination, the PRF-treated tooth showed accelerated growth of the root with thick dentinal walls and faster periapical closure within a span of 6 months as compared to blood clot-treated tooth. This could be explained based on the fact that the sustained release of growth factors from PRF over a period of 7–14 days enhanced the recruitment, retention, and proliferation of undifferentiated mesenchymal and endothelial cells from the periapical tissues, resulting in the formation of new hard and soft tissues within the empty canal.[7,23] Nagaveni et al. in 2015[9] used similar technique using a PRF in a 10-year-old male patient with immature permanent central incisor with apical periodontitis. At 12-month follow-up, interestingly the tooth responded positively to cold and an electric pulp test. Radiographically, continued thickening of the root canal walls, root lengthening, and closure of the apex was evident. The promising results with PRF were also shown by publications of Shivashankar et al.[13], Keswani and Pandey[11], Mishra et al.[12], Johns et al.[10], and Geeta et al.[24]

The successful clinical outcome and radiographic presentation of accelerated root growth, with length, enhanced dentinal thickness, and closure of the apex obtained in the PRF-treated tooth as compared to blood clot explains that PRF has ideal properties to be used for regeneration of vital tissues in traumatized necrotic immature teeth. Based on the significant results obtained from the present report, we can say that PRF may be a boon to regenerative endodontic therapy. However, the current case report serves as a rationale for conducting future research, and long-term prospective trials are absolutely essential for comparing conventional blood-induced regenerative techniques with PRF-assisted regenerative treatment outcomes in cases of immature necrotic permanent teeth before concluding PRF as a boon to pulp revascularization procedure.

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