Revascularization Induced Maturogenesis of Non-Vital Immature Permanent Tooth Using Platelet-Rich-Fibrin: A Case Report

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The aim of this report is to describe a novel method of revascularization therapy done in a non-vital, immature permanent tooth using Platelet-rich fibrin (PRF),in a recently developed scaffold material to overcome limitations associated with the traditional method of revascularization using natural blood clot. PRF prepared from autologous blood was placed in the root canal and patient was followed up regularly at one, three, six, nine and 12 months for detailed clinical and radiographic evaluation. At 12 months, radiographic examination revealed root elongation, root end closure, continued thickening of the root dentinal walls, obliteration of root canal space, and normal periradicular anatomy. However, more long term prospective trials and histological studies are highly needed before to testify PRF a panacea for the regenerative endodontic therapy in children.

Key words: immature teeth, platelet-rich-fibrin, revascularization

INTRODUCTION

The sequels of trauma or carious attack in immature permanent teeth are pulp necrosis, infection and arrested root development. Cessation of root development results in roots with thin dentinal walls, open apex, poor crown root ratio with increased risk of root fracture, difficult instrumentation and sealing of the canal. Literature shows numerous treatment options for management of these teeth like conventional non-surgical endodontic treatment, apical surgery and single visit apexification.[1,2] Although the traditional method of calcium hydroxide apexification was found to be effective in inducing apical barrier formation, the studies exhibited that it has various disadvantages.[3,4] Later single visit apexification using mineral trioxide aggregate (MTA) popularized the endodontic field. However, some authors showed that MTA apexification does not lead to further root growth and risk of root fractures are high due to thin dentinal walls.[2] Because of these reasons there is a quest for an alternative biological approach which includes regenerative endodontic procedures. Revascularization or maturogenesis procedure involves three critical components for the successful outcome like the stem cells, growth factors and 3-dimensional physical scaffold without these an empty canal does not induce in-growth of new tissues from the apical region.[5]

PRF, a second generation platelet concentrate was developed by Choukron *et al* in France.[6] Various studies have been published in different fields depicting that PRF can cause both hard and soft tissue re-growth.[7,8] In the arena of regenerative endodontic therapy, use of PRF as a scaffold material to induce root growth in immature necrotic teeth is a new vista which attracted very few clinicians.[9-11] Therefore, the aim of present paper is to describe both clinical and radiological outcome of the case with immature non-vital tooth, in that PRF was used as scaffold material to induce maturogenesis of the root. This case is another contribution to the realm of existing regenerative endodontic literature about using PRF as a valid scaffold material for revascularization of immature necrotic teeth.

Case report

An 11-year-old boy reported to the Department of Pedodontics and Preventive Dentistry, College of Dental Sciences, Davangere, Karnataka, India seeking treatment for the fractured upper front tooth due to trauma. There was a history of trauma to the upper front region three month back due to fall. The medical status was non-contributory. On intraoral examination, the right maxillary central incisor had Ellis and Davey's class IV fracture (fracture involving enamel, dentine with pulp exposure leading to non-vital tooth). Clinically the crown exhibited discoloration depicting non-vitality and necrosis of the tooth (Figure 1). The tooth was tender to percussion test and did not respond to cold and electric pulp test and periodontal probing depth was within normal limits. On intra oral peri-apical radiographic examination, the tooth showed an incompletely formed root, thin dentinal walls with wide

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open apex. Based on clinical and radiographic findings, the case was diagnosed as pulp necrosis with symptomatic apical periodontitis and we decided to perform a regenerative endodontic treatment using PRF as a scaffold material. The detailed treatment protocol was narrated to the parents and written informed consent was taken. Both treatment protocol and signed consent form were approved by the Institutional Ethics Committee (College of Dental Sciences, Davangere, India) to perform the treatment procedure.

Local anesthesia was given using two percent Lidocaine with 1:100000 Epinephrine and rubber dam application was done for isolation. Proper access cavity was prepared using a round bur (Dentsply Maillefer, Switzerland). After access opening pulp was completely removed and found to be necrotic and there was no bleeding from the canal. The necrotic pulp was completely removed and the canal was thoroughly irrigated using five ml of 5.25 percent of sodium hypochlorite solution (Rasayan Laboratory, Mumbai, India). Next, working length was determined by keeping an 80 size k file (MN, Mani, Tochigi, Japan) 1 mm short of the apex and confirmed on periapical radiograph. After this the canal was completely dried using paper points. A thick mixture of Ciprofloxacin (Cifran 500 mg, Ranbaxy Laboratories Ltd., Mumbai, India), Metronidazole (Metrogyl 400 mg, J.B. Chemicals, Mumbai, India) and Minocycline (Minoz 50 mg, Ranbaxy Ltd., Mumbai, India) with equal proportions was prepared after grinding using mortar and pestle and mixing with distilled water. The prepared paste was placed inside the canal to a depth of two mm short of the apex and one mm below the cemento-enamel junction (CEJ) of the tooth to confine the paste within the root part using an endodontic plugger (Mani, Japan) in order to minimize staining resulting from Minocycline. A cotton pellet was placed over this paste and the access cavity was temporarily filled with Cavit (3M ESPE, Germany). The patient was recalled after one week and there were no signs and symptoms. The access cavity was reopened under rubber dam isolation and the canal was copiously irrigated using saline to remove antibiotic paste. Finally the canal was dried with paper points.

Next, for preparation of PRF, five ml of blood was drawn intravenously from the forearm (antecubital vein) of the patient using an 18 gauge needle and collected in a sterile plastic vaccutube without adding any anticoagulants. Immediately after this the tube was centrifuged (Remi Model, Mumbai, Maharashtra, India) under 3000 revolutions per minute (RPM) for 15 minutes. After centrifugation three layers were formed in the whole blood. They are:

1. Top layer - Platelet Poor Plasma (PPP) - acellular straw colored fluid
2. A middle layer - Platelet-rich fibrin clot
3. A bottom layer - Red Blood Cells (RBCs) (Figure 2, A)

A sterile tweezer was inserted into the test tube to remove the PRF clot (Figure 2, B). The PRF gel was pressed between the sterile dry gauze to squeeze out fluid which resulted in a membrane. The membrane was cut into small fragments using scalpel blade and placed incrementally inside the canal using an endodontic hand plugger (Dentsply Maillefer, Switzerland). Then white MTA (Pro Root MTA; Dentsply, Switzerland) was placed directly over the PRF membrane and a wet cotton pellet was placed over the MTA and restored with Cavit. The patient was recalled after one day to remove cotton and confirm the setting of MTA. Finally the access opening was restored using Glass Ionomer Cement [GIC (Universal Restorative, Tokyo, Japan)]. The patient was kept under observation and recalled every one, three, six, nine and 12 months to evaluate the clinical and radiographical changes. During each follow-up examination the clinical findings exhibited normal findings like no sensitivity to percussion, and palpation tests and with normal pocket probing depths. There was a positive response to cold and electric pulp test fairly similar to adjacent teeth after 3 months onwards. On radiographic examination compared to preoperative radiograph in Figure 3, A, (showing open apex, discontinuity in the lamina dura, and periapical radiolucency) in subsequent follow ups (Figure 3, C and Figure 4, D, E and F) we could appreciate the closure of the

Figure 1. Clinical photograph showing permanent maxillary right central incisor with Ellis and Davey class IV fracture and crown discoloration (fracture involving enamel, dentine with pulp exposure leading to pulp necrosis).

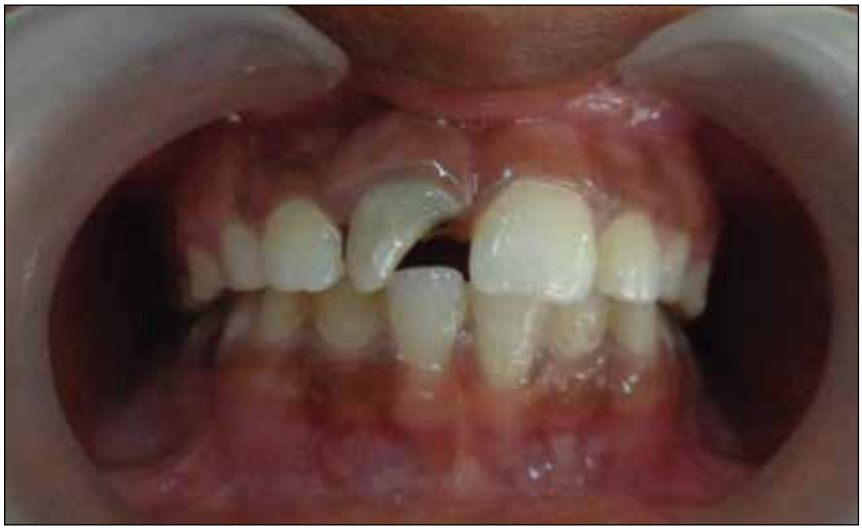


Figure 2. [A] Picture showing 3 layers formed after centrifugation: 1. Platelet-poor-plasma 2. Platelet-rich-fibrin and 3. Red blood cells. [B] Picture showing removal of platelet-rich-fibrin gel.

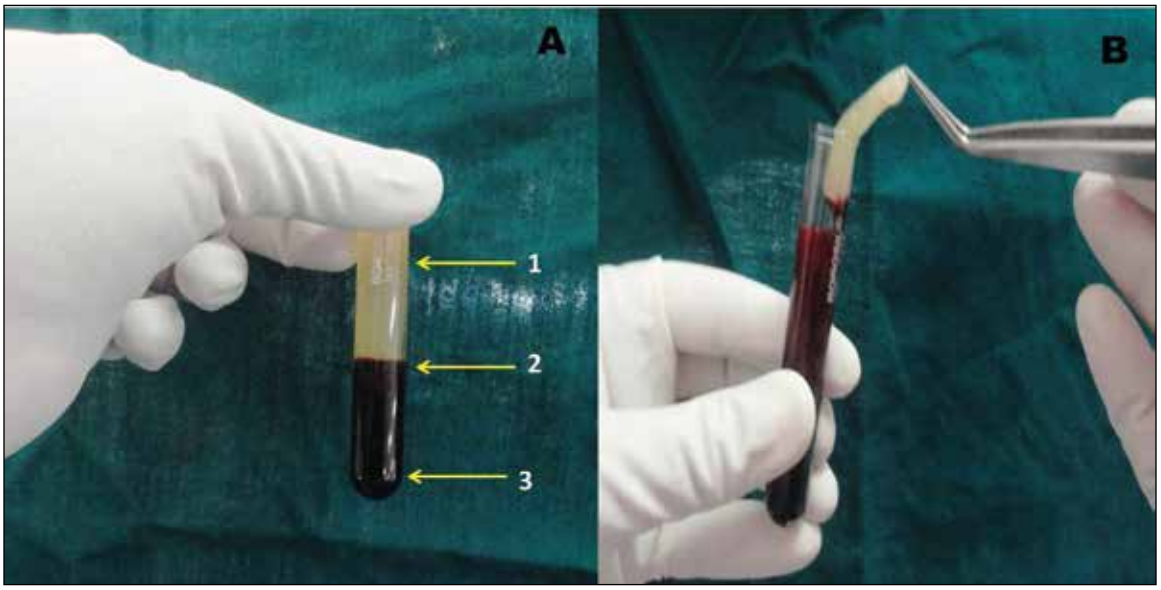


Figure 3. [A] Radiographic picture of permanent maxillary right central incisor showing incompletely formed root with wide, open apex and thin root dentinal walls, wide root canal space and widened periodontal ligament space. [B] At 1 month follow up, tooth showing slight elongation in the root. [C] At 3rd month follow-up, tooth showed continued root elongation and favorable closure of the apex and normal periodontal ligament space.

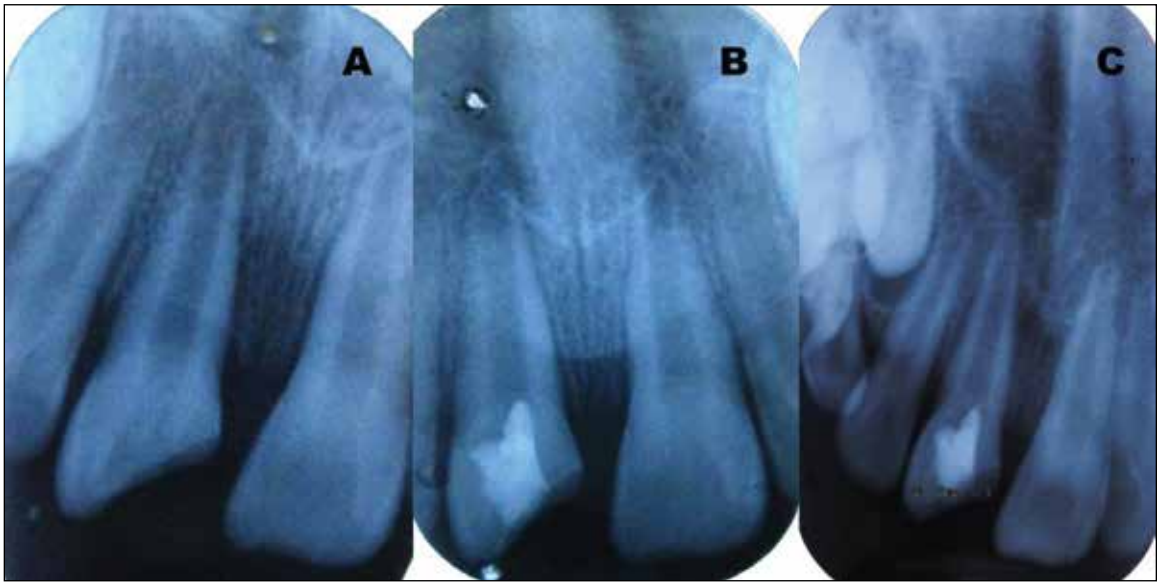
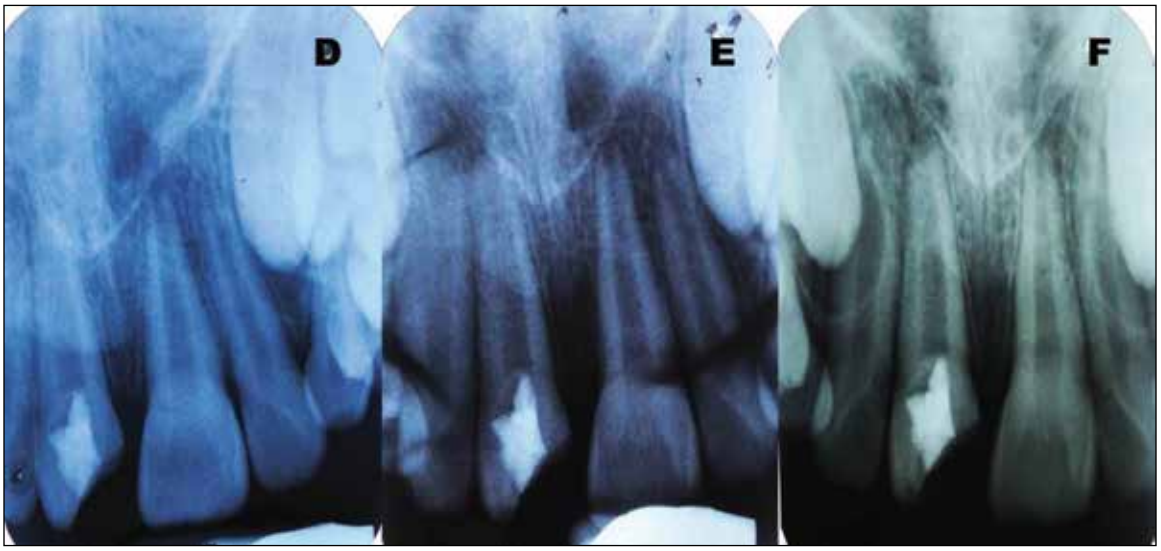


Figure 4. [D] At 6th month follow up, tooth revealed marked increase in the root length compared to adjacent tooth, obliteration of the root canal space and complete closure of the root apex. [E] At 9th month, tooth showed marked root lengthening, thickened root dentinal walls, excellent closure of the root apex with normal peri-apical architecture. [E] At 12 month follow up, PRF treated tooth exhibited excellent root length, complete peri-apical closure with normal peri-radicular architecture, thickened root lateral walls and narrowing of the root canal space.



apex, continuity in lamina dura with normal periapical architecture. At 6, 9 and 12 months follow up (Figure 4, D,E,F) obliteration in the root canal space from middle to apical third of root was evident which was absent in Figure 3, A, and B radiographs showing the radiolucent wide root canal space. In Figure 4, D, E, and F we could appreciate the thickening in the root walls especially at the middle to apical third compared to preoperative radiograph (Figure 3, A).

DISCUSSION

Revascularization or maturogenesis of non-vital immature teeth with a wide, open and blunderbuss apex is a novel treatment modality to re-establish the repair, vitality and regeneration of tissues resulting in further growth of the underdeveloped root and thickening of fragile dentin walls.[5]

The primary step in the revascularization therapy includes creating an aseptic environment so that in-growth of new tissues can take place inside the canal space which further leads to completion of root development and periapical closure. Scientific research has demonstrated that the tri-antibacterial paste consisting of ciprofloxacin, metronidazole and minocycline is very effective in disinfection of the canal.[12,13] In the present case, complete disinfection of the canal was obtained using this paste. Reports have shown that the paste is effective in killing the bacteria present in the deep layers of root canal dentin and also effective against all types of pathogens which cause endodontic failures.[12,13] However, the major disadvantage associated with use of triple antibiotic paste is crown discoloration resulting from minocycline.[14] Therefore, in the present case care was taken to keep the paste one mm below the CEJ of the tooth in order to minimize staining as suggested by various authors.[14,15] Addition to this, some investigators have stated that sealing of dentinal tubules within the chamber can be done to decrease the intensity of discoloration.[15,16]

Research shows that three critical components are highly essential for the successful outcome of this procedure.[17] The first component is the stem cells which help in differentiation and supports the root development. The second factor is growth factors which induce cellular proliferation and differentiation and last component is the physical matrix or scaffold. According to Hargreaves *et al* [18] use of appropriate scaffold is very essential to initiate differentiation and growth of new cells as an empty canal will not lead to in-growth of tissues from the periapical area. Apart from natural blood clot various authors have used different scaffolds like collagen and Platelet-Rich-Plasma for the revascularization process. However, each material is associated with various disadvantages, demerits, and contradictory issues. Therefore, we used PRF in this patient for maturogenesis of immature necrotic permanent tooth which is a new biologically based scaffold matrix developed to overcome the limitations associated with both conventional blood clot and new matrices like PRP, collagen etc.,

Compared to PRF, the traditional method of natural blood clot induced revascularization procedures has various draw backs like inducing bleeding to obtain fresh blood clot is extremely difficult and more painful procedure especially in pediatric patients. Petrino *et al* [15] has stated that several attempts were made to induce bleeding in one of the patient of their case series. Moreover, maintenance of blood clot within the canal and placement of MTA over this blood clot is still a technically difficult procedure. In addition, condensation of MTA results in apical displacement of the material. All these factors lead to the generation of a novel biologic approach like PRF for the maturogenesis treatment.[15] However, PRF has few disadvantages like difficult handling due to its jelly consistency and requirement of a specialized equipment for its processing.

PRF, an autologous healing biomaterial contains platelets, leukocytes, growth factors and key healing proteins (cytokines) in a dense fibrin matrix.[6] On radiographic evaluation the tooth treated with

PRF showed continued root growth, thickening of the root dentinal walls, narrowing of root canal space and closure of the root apex after 12 months. On clinical examination, the tooth exhibited negative response to palpation and percussion tests, positive responses to electric and cold pulp tests similar to adjacent normal teeth. This finding was found similar to previous PRF induced revascularization cases.[8-11] A recently published case report also exhibited excellent results of root maturogeneis following revascularization using PRF. The most plausible explanation for this success could be attributed to Huang et al[19] hypothesis who stated that PRF causes proliferation of human dental pulp cells and increase the protein synthesis and alkaline phosphatase activity. So the human dental pulp cells present in the apical papilla remain vital and may differentiate into odontoblasts like cells under the influence of hertwig´s epithelial root sheath (HERS) thereby enhancing the development of both hard and new tissue within the empty canal space.[17,19] Therefore various in vitro and animal in-vivo studies have investigated that the radiographic evidence of root development and increased root thickness might be due to in-growth of dentine, cementum or bone.[19,20] Moreover, studies done in avulsed[21] and necrotic teeth[22] showed that only 30% of pulp tissue regeneration occurs following revascularization treatment. However, more human histologic studies are highly essential to evaluate whether the revascularization procedure truly replicate the pulp–dentin complex.

CONCLUSION

Based on the 12 months successful clinical and radiographic results obtained from the present case, we can conclude that PRF is potentially an ideal scaffold material for regeneration of pulp-dentin complex in non-vital, immature permanent teeth. However, more long term prospective trials and histological studies are strictly needed before to testify PRF a panacea for the regenerative endodontic therapy in children.

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