

Harnessing the Potential Role of PRF and Blood Clot Combination in Revitalization of Tooth with Necrotic Pulp and Open Apex through Bioroot Tissue Engineering -A Case Report

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ABSTRACT

This case report explores the utilization of platelet-rich fibrin (PRF) in conjunction with a blood clot for the revitalization of a tooth with necrotic pulp and an open apex through bioroot tissue engineering. Traditional treatment modalities for such cases often fall short in achieving optimal outcomes, necessitating novel approaches to promote pulp revascularization and root maturation. In this report, we present a case wherein a combination of PRF and a blood clot was employed to stimulate tissue regeneration and facilitate the closure of the apex. The procedure involved meticulous disinfection of the root canal followed by the placement of PRF and a blood clot, creating an environment conducive to pulp revascularization and root development. Subsequent follow-up assessments revealed encouraging signs of pulp revascularization, root canal narrowing, and apex closure, indicating successful revitalization of the tooth. This case highlights the potential of PRF and blood clot combination in bioroot tissue engineering as a promising alternative for the management of teeth with necrotic pulp and open apex. Further research and clinical trials are warranted to validate the efficacy of this approach and establish standardized protocols for its implementation in routine endodontic practice.

Key words: PRF, Open apex, blood clot, revascularisation

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I. Introduction

The clinical entity most frequently observed in youngsters is trauma to the anterior teeth. It is difficult for a clinician to manage such traumatised anterior teeth with an immature open apex and nonvital pulp tissue using endodontic techniques¹. Due to the difficulty in apexifying teeth with thin dentinal walls, open apices, and large root canal spaces, the traditional calcium hydroxide method was changed to mineral trioxide aggregate (MTA) single-visit apexification². The creation of a biological strategy known as pulp revascularization or regenerative endodontic process was ultimately prompted by the numerous problems that this technology also produced³.

Mesenchymal stem cells and dental pulp stem cells can be preserved throughout operations to promote intracanal revascularization and sustained root growth⁴. The ability of the apical papilla cells to survive after pulp necrosis, which can multiply into canal space, forms the basis for the revascularization of the teeth⁵. The positioning of growth factors and stem cells in the canal space requires an adequate scaffold for stem cell differentiation and proliferation. In revascularization operations, scaffolding materials such as blood clot, collagen, platelet-rich plasma (PRP), and platelet-rich fibrin (PRF) can be employed⁶.

The ability to achieve apical closure of the tooth root, a healing response to periapical lesions, root lengthening, and thickening of the dentinal wall can all be used to measure the success of the REP⁷.

There are numerous studies on blood clot-based and PRF revascularization and a few studies on combination of blood clot and PRF. The aim of this paper is to discuss the clinical and radiological outcome of the patient where both blood clot and PRF combination as a scaffold materials in the revascularization procedure in necrotic immature permanent teeth.

II. Case Description

A 15-year-old female patient came to the department with a chief complaint of discoloured and fracture tooth in upper front region of jaw (fig 1). Patient reported a 2-year 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, the maxillary left central incisor were discolored with Ellis class IV fracture and were tender to percussion test. The teeth did not respond to cold and electric pulp test and periodontal probing depth was within the normal limits. On periapical radiographic examination, both teeth exhibited an incompletely developed root with thin dentinal walls and wide open apex. From the periapical radiographic the mesio-distal 2-D width of root apex (apical third) and diameter of apical opening was measured (table 1). On the basis of clinical and radiographic findings, a pulpal diagnosis of necrotic pulp and a periapical diagnosis of *asymptomatic apical periodontitis* were made for tooth 21. Since the case was in accordance with AAE Clinical Considerations for a Regenerative Procedure Revised 5/18/2021, decision was made to perform a regenerative endodontic treatment using blood clot and PRF in the left central incisor.

TREATMENT PLAN

PHASE 1

ROOT CANAL PREPARATION AND IRRIGATION PROTOCOL

Treatment Methods

General Steps: The tooth was isolated under rubber dam and access cavity was prepared with a round diamond bur and long straight fissure bur. The canal was minimally instrumented and was copiously irrigated with 5.25% sodium hypochlorite and dried with sterile paper points. The triple antibiotic paste (containing metronidazole-400 mg, ciprofloxacin-200 mg, and minocycline- 100 mg in the ratio 1:1:1 by weight) was prepared in a creamy consistency using propylene glycol and macrogol ointment taken in 1:1 ratio by weight [13] and was placed inside the canal up to a maximum of 2 mg. The access cavity was then closed with cotton pellets and intermediate restorative material. The patient was recalled after three weeks. In the second sitting, the tooth was checked for any discoloration, abscess, mobility, or pain on percussion and after disinfecting the mouth with betadine solution, the tooth was re-accessed under rubber dam isolation. The antibiotic paste was removed using sterile saline solution. No instrumentation of the canal space was performed and the canal space was dried using sterile paper points. Following the general step, the patients were randomly categorised into three groups with 20 patients in each group as mentioned earlier.

The tooth was anesthetised using 2% lidocaine with 1:100,000 epinephrine and access cavity was prepared with a round diamond bur and long straight fissure bur, working length was determined as 20mm by placing a file of 70 k size (Mani, Japan) using ingles radiographic method. The canal was minimally instrumented and was irrigated with 20ml of **1.5%** sodium hypochlorite for 5 minutes followed by 20ml of **saline for 5min** using a needle with closed end and side-vent that minimizes the possibility of extrusion of irrigants into the periapical space and dried with sterile paper points. The modified triple antibiotic paste (containing metronidazole-400 mg, ciprofloxacin-200 mg and clindamycin 100 mg in the ratio 1:1:1 by weight final concentration of 0.1-1.0 mg/ml) was prepared in a creamy consistency using propylene glycol taken in 1:1 ratio by weight and was placed inside the canal using lentulospirals up to a maximum of 2 mg. The access cavity was then closed with cotton pellets and intermediate restorative material ie **zinc poly carboxylate**. The patient was recalled after three weeks (fig 2).

PHASE 2

ROOT CANAL PREPARATION AND IRRIGATION PROTOCOL

In the second sitting, the tooth was checked for any discoloration, abscess, mobility, or pain on percussion. The tooth was anesthetised using 2% lidocaine without vasoconstrictor and the tooth was re-accessed. The antibiotic paste was removed using 30ml of **17% EDTA** for 5 min followed 5ml **Saline for 5 min** (5 mL, 1 min). No instrumentation of the canal space was performed and to remove the medicament on the canal space addition to irrigation it was activated by ultrasonic irrigation activation and dried using sterile paper points.

PRF PREPARATION :

5 ml of whole venous blood is collected in sterile vacutainer tubes of 6 ml capacity without anticoagulant. The vacutainer tubes are then placed in a centrifugal machine(Remi Cm 8 PLUS) at 3000 revolutions per minute (rpm) for 10 minutes. This results in the separation of blood samples into three layers: a red cell base at the bottom with acellular plasma on the topmost layer, and a clot of PRF in the middle. The resultant PRF clot is then pressed in between gauze pieces to attain a firm membrane.

PRF PLACEMENT

Before placing PRF in to the root canal , bleeding was induced into the canal system by over-instrumenting by rotating a pre-curved K-file at 2 mm past the apical foramen with the goal of having the canal filled with blood . The PRF membrane obtained was placed inside hand plugger . (Dentsply Maillefer, Switzerland). A thin layer of white MTA (Pro Root MTA; Dentsply, Switzerland) was placed directly over the PRF membrane 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 and GIC was placed.

The patient was recalled every 1, 6, 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.(fig3, fig 4)



Fig 1 :Pre operative radiography



Fig 2 : PHASE 1

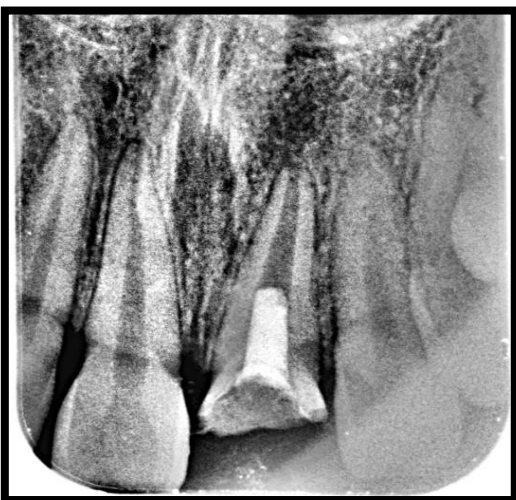


Fig 3: 6 month follow up

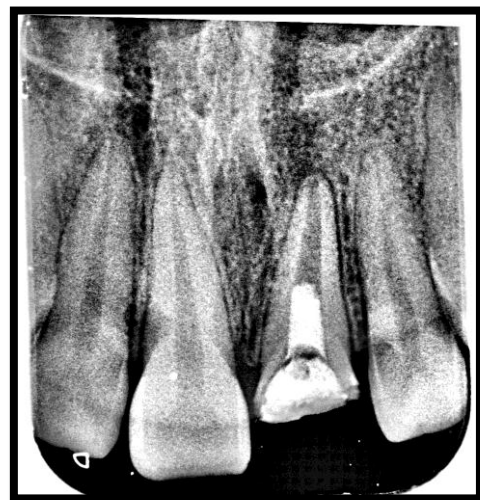


Fig 4: 12 month follow up

Root surface	Mesio-distal 2-D	Mesio-distal 2-D width	Mesio-distal 2-D width
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	width (Apical third)	(Apical third) After 3 months radiographic review	(Apical third) After 1 year radiographic review
mesial	0.6mm	0.8mm	0.9mm
distal	0.8mm	0.9mm	1mm
Apical opening	1.2ml	0.5mm	0.1mm

III. RESULTS

The mean success rate for root lengthening in mesial root after 6 month was 62% and after 1 year was 68%. The mean success rate for root lengthening in distal root after 6 month was 68% in distal root and after 1 year was 72 %. The mean success rate for apical reduction after 6 month was 0.5mm and after 1 year was 98%.. Apical closure occurred with PRF and blood clot combination.

IV. DISCUSSION

Overall, the case report suggests that PRF is highly effective in promoting various aspects of tissue regeneration and healing in regenerative endodontic procedures. These outcomes include improved root lengthening, significant apical closure or reduction, complete healing of periapical lesions, dentinal wall thickening, and positive vitality tests. These findings underscore the potential of PRF as a valuable adjunctive therapy in regenerative endodontics for enhancing clinical outcomes and promoting long-term tooth vitality⁸.

PRF is a second-generation platelet concentrate derived from the patient's own blood. It contains a high concentration of platelets, leukocytes, and growth factors, all of which play crucial roles in tissue healing and regeneration. Unlike other platelet concentrates, PRF is prepared without anticoagulants or additives, making it a biocompatible and bioactive material for clinical applications.

PRF acts as a reservoir for various growth factors, including platelet-derived growth factor, transforming growth factor-beta, vascular endothelial growth factor and insulin-like growth factor⁷. Upon activation, these growth factors are gradually released from the fibrin matrix, stimulating cell proliferation, differentiation, and angiogenesis. VEGF and PDGF, among other growth factors present in PRF, play pivotal roles in angiogenesis – the formation of new blood vessels. Angiogenesis is crucial for supplying nutrients and oxygen to the regenerating tissues within the root canal space, facilitating the proliferation and differentiation of stem cells.

PRF contains leukocytes that secrete chemotactic factors, such as stromal cell-derived factor-1, which attract mesenchymal stem cells to the site of injury. Within the root canal space, these MSCs undergo differentiation into odontoblast-like cells, promoting dentinogenesis and pulp tissue regeneration⁸.

Since infection inhibits stem cell activity, regeneration, and repair, it is believed that disinfecting the root canal system is essential. Chemical disinfection of the root canal is not solely dependent on bacteriocidal/bacteriostatic properties of the agents as these irrigants should not damage the survival and proliferative capacity of the patient's stem cells. Adequate irrigation with 20 ml of NaOCl while utilising an irrigation device such as needle with a closed end and side vents that reduces the chance of irrigants being extruded into the periapical area. In order to reduce cytotoxicity to stem cells in the apical tissues, lower concentrations of NaOCl-1.5% was done. These concentrations was followed by irrigation with saline or EDTA (20 mL/canal, 5 min), with the irrigating needle positioned approximately 1 mm from the root end⁹.

The incorporation of PRF And Blood clot into regenerative apical opening procedures offers a synergistic approach to promoting tissue regeneration within the root canal space. Through its diverse mechanisms of action, combination of PRF and a blood clot enhances angiogenesis, stem cell recruitment, and tissue differentiation, leading to improved clinical outcomes in endodontic therapy. Further research is warranted to optimize the application techniques and validate the long-term efficacy of this promising treatment modality.

REFERENCE

- [1]. Jadhav G, Shah N, Logani A. Revascularization with and without platelet-rich plasma in nonvital, immature, anterior teeth: a pilot clinical study. *J Endod.* 2012;38(12):1581–1587
- [2]. Gawthaman, M., Vinodh, S., Mathian, V.M., Vijayaraghavan, R. and Karunakaran, R., 2013. Apexification with calcium hydroxide and mineral trioxide aggregate: Report of two cases. *Journal of Pharmacy and Bioallied Sciences*, 5(Suppl 2), pp.S131-S134.
- [3]. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: a review of current status and a call for action. *J Endod* 2007; 33: 377–390
- [4]. Kwack, K.H. and Lee, H.W., 2022. Clinical potential of dental pulp stem cells in pulp regeneration: current endodontic progress and future perspectives. *Frontiers in Cell and Developmental Biology*, 10, p.857066.
- [5]. Torabinejad M., Corr R., Buhrlay M., Wright K., Shabahang S. An animal model to study regenerative endodontics. *Journal of Endodontics*. 2011;37(2):197–202.
- [6]. Kwack, K.H. and Lee, H.W., 2022. Clinical potential of dental pulp stem cells in pulp regeneration: current endodontic progress and future perspectives. *Frontiers in Cell and Developmental Biology*, 10, p.857066.

- [7]. Alagl A., Bedi S., Hassan K., AlHumaid J. (2017). Use of platelet-rich plasma for regeneration in non-vital immature permanent teeth: clinical and cone-beam computed tomography evaluation. *J. Int. Med. Res.* 45, 583–593.
- [8]. Pavlovic, V., Ciric, M., Jovanovic, V., Trandafilovic, M. and Stojanovic, P., 2021. Platelet-rich fibrin: Basics of biological actions and protocol modifications. *Open Medicine*, 16(1), pp.446-454.
- [9]. Lin LM, Shimizu E, Gibbs JL, Loghin S, Ricucci D. Histologic and histobacteriologic observations of failed revascularization/revitalization therapy: a case report. *J Endod.* 2014. February;40(2):291–5.