

The Efficacy of Advanced Platelet-rich Fibrin in Revascularization of Immature Necrotic Teeth

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ABSTRACT

Aim: The present study aims to compare advanced platelet-rich fibrin (A-PRF) and conventional blood clot as scaffolds in the regenerative endodontic procedure (REP) and to evaluate their effectiveness in the development of traumatized nonvital immature teeth roots.

Material and methods: Regenerative endodontic procedure was carried out on 20 traumatized nonvital immature maxillary incisors of 17 patients between the ages of 8 and 12 years. Irrigation with 1.5% sodium hypochlorite (NaOCl) and 17% ethylene diamine tetra-acetic acid was done following minimal mechanical debridement. Canal disinfection was achieved using calcium hydroxide paste. According to the scaffold type, teeth were randomly allocated into A-PRF ($n = 10$) and conventional blood-clot groups ($n = 10$). Apical width and root dimensions (length and thickness) were analyzed radiographically and statistically after 3-, 6-, and 12-month follow-up.

Results: Fifteen patients with 18 teeth (A-PRF $n = 9$, blood clot $n = 9$) completed the follow-up, and 2 patients were excluded. Patients in both groups were asymptomatic. There was a significant increase within each group in respect to root length, root thickness at one- and two-thirds, and root apex width for all timepoints. While percent of change between the two groups was statistically insignificant.

Conclusion: Regenerative endodontic procedure for traumatized immature nonvital teeth with either conventional blood clot or A-PRF as scaffold was comparable, except in cases where adequate bleeding cannot be achieved.

Clinical significance: Advanced platelet-rich fibrin provides a suitable scaffold that can be used in REP of teeth with close proximity to vital structures such as inferior alveolar nerve (IAN) or mental nerve, where using a sharp instrument to induce bleeding can cause damage and also in cases where adequate bleeding cannot be achieved.

Keywords: Open apex, Randomized clinical trial, Revitalization.

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INTRODUCTION

One of the most common diseases affecting humans nowadays is dental caries.¹ Children and adolescents with immature permanent teeth have an incidence rate of ~21%.² Dental-pulp necrosis takes place in nearly 7% of cases with deep caries in immature permanent teeth.³ About 25% of this age group are subjected to dental trauma, which results in pulp necrosis in nearly 27% of them.⁴ Such cases usually have poor prognosis owing to their thin dentinal walls liable to fracture⁵ and open apices that cannot be easily sealed.⁶

The traditional treatment for such cases included a procedure called "apexification," which involved elimination of the intradicular infection through intracanal medication using calcium hydroxide and to stimulate the calcification at the root apices. Unfortunately, it requires multiple visits, and therefore longer duration for the root filling to be completed.⁷

This drawback leads to the development of single-visit apexification technique using mineral trioxide aggregate (MTA), which provides an artificial barrier for the obturating material to be compacted against.⁸ However, continuation of root maturation could not be obtained by either of these "apexification" techniques predisposing to fractures.⁹ All of these reasons raised the need to develop REP.¹⁰

Regenerative endodontic procedures aim to restore the cellular element of the pulp-dentin complex (PDC) and replace the affected dentin and root tissues in order to regain the natural physiology of the PDC.¹¹ They have emerged as an alternative procedure to permit the complete development of the immature

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roots utilizing the basics of tissue engineering to eliminate pathogens, stimulate stem cells, and apply appropriate scaffolds and signaling molecules.¹²

Pulp revascularization is based on the residual pulp, apical and periodontal stem cell's ability to differentiate¹³ and regenerate a highly vascularized and a well-organized living tissue that fills the available pulp space. Subsequently, new odontoblasts will differentiate from these stem cells inducing hard-tissue apposition. The regenerated pulp tissues will restore nerve function regaining the pulp-defensive proprioceptive reflexes, and providing a useful alarm system against tissue injury, and therefore protecting the tooth from the pulp damage or fracture.¹⁴

Pulp revascularization permits continuation of root growth, increase in dentinal wall thickness, and achievement of natural apexification. Stem cell proliferation and differentiation into odontoblasts require specific growth factors and scaffolds to create an organized structure for their distribution and arrangement.¹⁵

The most widely used scaffold in the field of REP is based on using sharp sterile instruments to induce bleeding into the root canal followed by blood clot formation. However, bleeding is not usually easy to achieve, and the formed blood clot does not apply for the ideal scaffold criteria. Sometimes, this procedure will cause discomfort to the patient during the process of mechanical irritation of periapical tissues.¹⁶

Finding new alternative scaffolds other than the usual blood clot became a necessity in REP. So, researchers are currently examining other three-dimensional scaffolds, one of which is the platelet concentrates.¹³ Platelet concentrates have been classified into four main types: pure platelet-rich plasma (P-PRP), leukocyte-rich PRP (L-PRP), pure platelet-rich fibrin (P-PRF), and leukocyte-rich PRF (L-PRF). The leukocyte-rich PRF is subdivided into the Choukroun's PRF, injectable PRF (I-PRF), and advanced PRF (A-PRF).¹⁷

Platelet-rich plasma was the first generation of human-derived platelet-based scaffolds.¹⁷ However, it is not 100% autologous. Then, Choukroun's PRF was developed in REP to avoid the disadvantages of PRP as PRF showed marked increase in the released leukocytes and growth factors.¹⁸ A major advantage of the preparation procedure has led to the development of A-PRF.¹⁹ Advanced PRF is a new generation of platelet concentrate that uses lower G-forces (1500 rpm/14 minutes) to obtain higher growth factor release and neoangiogenic potential compared with PRF (2700 rpm/12 minutes).²⁰ Advanced platelet-rich fibrin releases a significantly higher quantity of growth factors such as vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), transforming growth factor-beta (TGF- β), and chemotactic molecules eotaxin and chemokine (c-c motif) ligand 5 (CCL-5), compared with PRF.²¹

Therefore, this study was designed to evaluate and compare the achieved revascularization using conventional blood clot and A-PRF.

MATERIALS AND METHODS

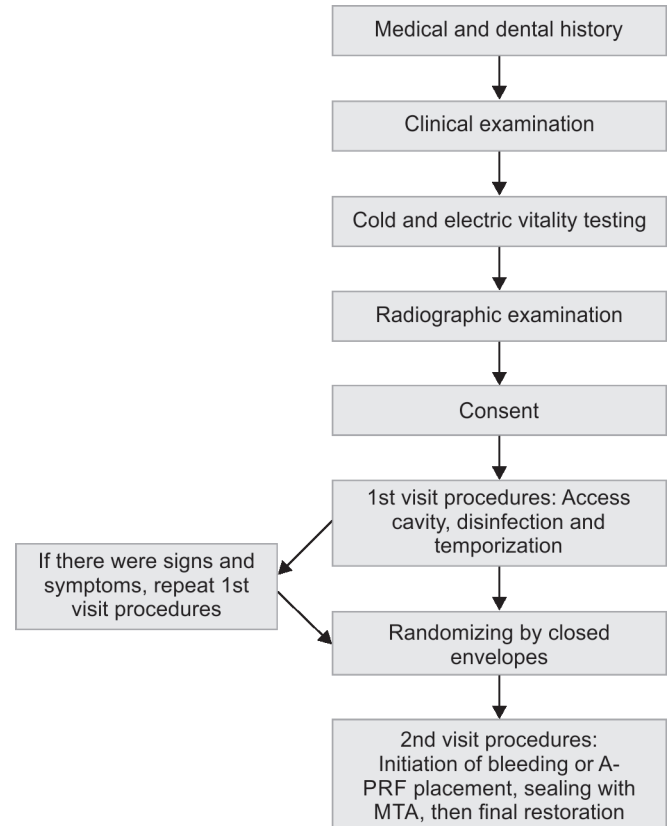
Sample Size Determination

Based on a previous study,²² the sample power was calculated using G* power 3.1 (Heinrich Heine University Dusseldorf, Germany), indicating that the sample size should be a minimum of 8 teeth per group. Therefore, 10 teeth were allocated in each group to cover the possible dropout. Twenty immature maxillary permanent incisors (17 patients) of both genders were included in this study (Flowchart 1).

Study Setting

Patients were selected from the outpatient clinic of the Endodontic Department, Faculty of Dentistry, Mansoura University, Egypt, between 2019 and 2020. This trial was approved by the Ethical Committee of Mansoura University (Ref. no. 03131118). Before starting the treatment, full explanation of the study aim and treatment steps was given to all the patients' guardians. A full medical and dental history was obtained for each case to exclude medically compromised patients. Included patients were asked to sign a printed informed consent in which they conformed to attend the clinical and radiographic follow-up at 3-, 6-, and 12-month intervals. The envelope method was used for randomization.

Flowchart 1: Flowchart showing a summary for study setting and treatment steps



Eligibility Criteria

Inclusion criteria: Patients who belonged to the age group of 8–12 years, medically free, no sex predilection, having permanent maxillary incisors with necrotic pulp (either due to dental caries or trauma that did not require post and core treatment) and immature apex, either tubular or blunderbuss, with or without apical radiolucency, not responding to cold or electric pulp testing (EPT), and not mobile. The number of days before starting treatment did not make a difference since the pulp was already necrotic.

Exclusion criteria: Patients who have or showed an allergic reaction against any materials used in the study, root resorption, root fracture, radiographic, or clinical signs of ankylosis; patients who were uncooperative or whose legal guardians did not sign the study participation consent.

Randomization and Blinding

Each case was represented by a code and a group name, which were then sealed in a sequentially numbered opaque envelope. Upon enrollment of a new case, the in-line envelope was chosen and then opened to start the intervention.

Clinical Diagnostic Procedure and Informed Consent

Personal, medical, and dental history, treatment plan and follow-up notes, and clinical and radiographic examination results were all recorded utilizing a detailed dental diagnostic chart. Before beginning any procedure, each patient's parent was asked to sign an informed consent explaining intended treatment, the possible outcomes, complications, and follow-up period required. The pulp was considered to be necrotic when a preoperative

negative response was obtained to cold test carried out by ethyl chloride spray (Chloroethyl; IGS Aerosols GmbH, Wehr, Germany) and to EPT using the pulp tester DY310 (Denjoy, China).

Treatment Procedure

At the first appointment, local anesthesia was achieved using infiltration technique with 1.8–3.6 mL of lidocaine local anesthetic solution with 1:100,000 epinephrine. The root canal system was accessed, and rubber dam isolation was obtained. Negotiation of the canals was performed using a stainless-steel hand K-files size # 15. The working length was determined using an electronic apex locator (DentaPort ZX, J Morita, Japan) and then confirmed with intraoral periapical radiograph to be 0.5–1 mm shorter than the radiographic apex. The root canal systems were slowly irrigated with 25-gauge, side-vented ENDO-TOP irrigation needles (CERKAMED, ul, POLAND) first using 1.5% NaOCl (20 mL/canal, 5 minutes) followed by saline (20 mL/canal, 5 minutes), while the needle was positioned about 1 mm from the root apex. Paper points were used for drying the canals and then calcium hydroxide (UltraCal XS, Ultradent) was delivered to the canal system to a point just beneath the cemento-enamel junction (CEJ) followed by placement of a cotton pellet and sealing of the cavity with glass ionomer (Fuji IX; GC America, Alsip, IL).

At the second appointment (3 weeks later), the participants were recalled for completion of REP. A clinical examination was carried out to exclude any sensitivity to percussion or palpation. In case of any observed sensitivity, swelling, or sinus tract formation, the first visit treatment steps were repeated. After achieving adequate local anesthesia without vasoconstrictor using 3% Mepivacaine (Scandonest, Septodont, Canada), rubber dam isolation was obtained. Glass ionomer was removed using a high-speed handpiece. Irrigation with 17% ethylenediaminetetraacetic acid (EDTA) (30 mL/canal, 5 minutes) was done to remove intracanal medicament followed by final flushing using saline (5 mL/canal, for 1 minute). Intracanal drying was done with paper points (DiaDent, Korea).

In group I (blood-clot group), bleeding was induced by rotating a pre-curved K-file #25 (Mani Inc., Japan) at 2 mm past the apical foramen with the goal of the whole canal filled with blood to the level of CEJ. Once a blood clot is formed, a collagen membrane is placed followed by MTA (ProRoot MTA; Dentsply Tulsa Dental, Tulsa, OK) application, then a moist cotton pellet was placed to promote MTA setting. The tooth was temporarily filled for 2 days to allow the complete setting of the MTA. After that, glass-ionomer cement base and resin-composite restoration were used to restore the tooth.

In group II (A-PRF group), a standard venipuncture was withdrawn from the median cubital vein. A tabletop centrifuge at 1400 rpm for 14 minutes was used to centrifuge the blood sample in a test tube without any anticoagulant immediately after withdrawal. Three layers were obtained: an uppermost straw-colored layer of acellular PPP, a middle layer of PRF clot, and a lower sedimented red blood cells (RBCs) layer. After discarding the uppermost layer with a pipette, the fibrin clot was transferred using a tweezer to a sterile dappen dish. The RBCs layer was discarded. After fragmenting the freshly prepared A-PRF, the fragments were placed layer-by-layer into the canal with a finger plugger, then the collagen membrane was placed. Mineral trioxide aggregate was applied, and the tooth was restored in the same manner as group I.

In the blood-clot group, one case dropped out because of sustained pain (9/10). Also, in A-PRF group, one case dropped out during follow-up for an unknown reason (9/10). No other adverse effects happened during follow-up.

Radiographic Diagnostic Procedure and Standardization

A film-holding device (Kerr X-ray and phosphor plate holders) was used to standardize the dimensions of periapical radiographic images. A radiographic stent was used to achieve a standard film-tooth-cone relationship throughout the study. DIGORA Optime intraoral digital imaging plate system was used to X-ray images that were scanned and saved in a JPEG format.

Computer-assisted Digital Image Analysis (Digital Morphometric Study)

The resulting images were analyzed by a blind examiner unaware of patient allocation on Intel® Core i7® based computer using VideoTest Morphology® software (Russia) with a specific built-in routine for area and distance measurement. Length was measured using manual line tool with the help of Genius multitouch digital tablet against the instrument calibration measures.

The root length was measured as a straight line from the CEJ to the radiographic apex of the tooth.²³ The root width (dentinal wall thickness) was measured at two levels: 1/3 and 2/3 of root length. The thickness of dentin wall was calculated by subtracting the average pulp space width (mm) from the average root width (mm). This protocol was used to calculate the preoperative and postoperative radiographic measurements. The percentage of change in the radiographic dimensions of preoperative and postoperative stages was calculated using the formula:

$$\text{Percentage of change} = \frac{(\text{Postoperative value} - \text{Preoperative value}) \times 100}{\text{Preoperative value}}$$

The two-visit protocol of revascularization was done to all teeth by a single operator.

Statistical Analysis

Data were tabulated, coded, and then analyzed using the computer software program Graph Pad Prism 9. Descriptive statistics were calculated in the form of mean \pm standard deviation (SD). Shapiro-Wilk and Kolmogorov-Smirnov tests were used to assess normality of data. The significance of difference was tested using Student's *t*-test (unpaired) to compare between means of two different groups of numerical (parametric) data and repeated one-way analysis of variance (ANOVA) to compare between different follow-up intervals within the same group followed by *post-hoc* Bonferroni correction. A *p*-value < 0.05 was considered statistically significant.

RESULTS

A total of 18 teeth (15 patients) completed the 12-month follow-up (blood clot *n* = 9, A-PRF *n* = 9). The dropout in the blood-clot group was because of pain in one case (9/10) with survival rate of 90%. The drop out in A-PRF group was caused by loss of one patient during follow-up for an unknown reason (9/10). So, the survival rate for A-PRF teeth treated was 90%.

Response to Sensitivity Testing

When the patients' responses to vitality testing were compared, a gradual shift in the response percentage from negative to positive during the follow-up period was observed. After 12 months, a total percentage of 22.2% of conventional-group patients and 33.3% of A-PRF-group patients showed a positive response to vitality testing.

Radiographic Results

Radiographic images showed increase in both length and thickness at one- and two-thirds of the root at different time periods with narrowing of root apices in both groups. Also, gradual periapical healing was noticed in cases with periapical lesions throughout the follow-up period (Fig. 1).

Statistical Results

Repeated measures ANOVA followed by *post-hoc* Bonferroni test for comparison between preoperative, 3-, 6-, and 12-months' time periods within the blood-clot group: root length results revealed nonsignificant increase at all time intervals, except when 12-month results were compared with both preoperative and 3-month ones. While the thickness at one-third of the root revealed significant increase in between all intervals. At two-thirds of the root, there was a nonsignificant increase, except when 12-month results were compared with preoperative ones. For root apex width, there was significant increase at all time intervals, except when each of 3-month and 12-month results were compared with preoperative ones (Table 1).

Repeated measures ANOVA followed by *post-hoc* Bonferroni test for comparison between preoperative, 3-, 6-, and 12-months' time periods within the A-PRF group: root length revealed nonsignificant difference between all intervals, except when each of 6- and 12-month results were compared with the preoperative ones. While the thickness at one-third of the root revealed significant increase in between all intervals. At two-thirds of the root, there was significant increase between all intervals, except when 12-month results were compared with 6-month ones. For root apex width, there was significant increase at all time intervals, except when 6-month results were compared with 3-month ones (Table 2).

While the percent of change between the two groups relative to root length, thickness at 1/3 and 2/3, and root apex width was statistically insignificant at all time intervals (Table 3).

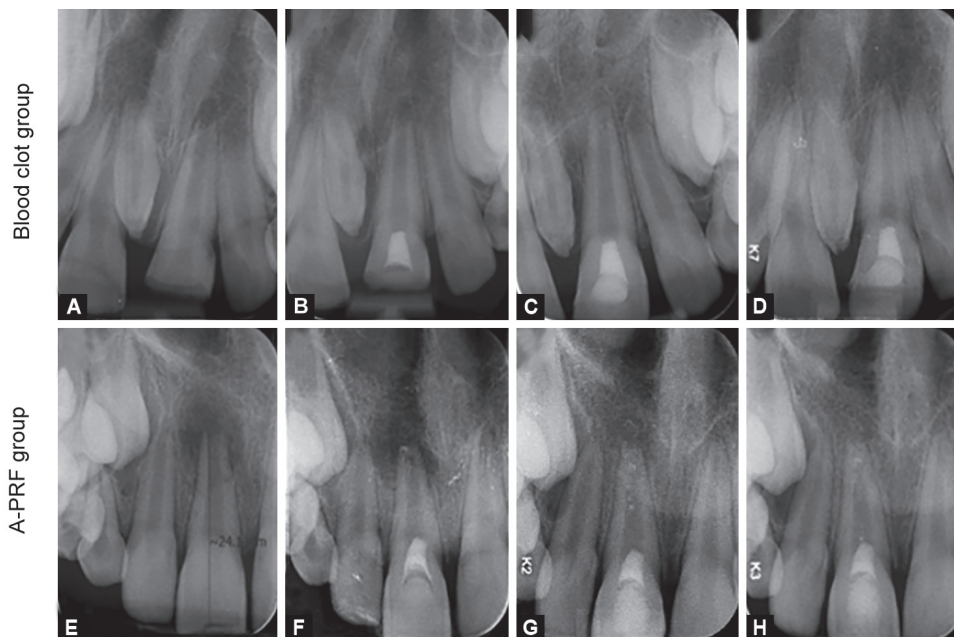
DISCUSSION

Following the guidelines of the American Association of Endodontists (AAE) (8.5.2014), there are three goals of REP. First: eliminate the pain and swelling symptoms that the patient suffered from before scaffold placement procedure, achieve bone healing, and apical radiolucency resolution within 6 months of treatment. Second: to increase root dentin thickness and root length and decrease root apex diameter within 12 months of treatment. Third: to obtain a positive response to pulp vitality testing, indicating the regeneration of a well-organized vital pulp tissue.

There are three main requirements that need to be followed to achieve a successful revascularization; first, complete disinfection of the root canal; second, introduction of the intracanal matrix to allow tissue growth; and third to tightly seal the access filling.²⁴ For a scaffold to be considered ideal, certain criteria must be met. A scaffold should permit binding and localization of cells, be rich in growth factors, and be biodegradable in nature. Even though the blood clot follows most of these criteria, it is poor in growth factors.²⁵

Regarding root-canal disinfection, NaOCl was found to be cytotoxic when used at a concentration more than 3% to the periodontal ligament stem cells and stem cells residing in the periapical papilla (SCAP).²⁶ Due to the minimal cytotoxicity of NaOCl with a concentration of 1.5% to SCAP, especially when used with 17% EDTA,²⁷ and to the release of higher levels of TGF- β 1 from the walls of the root canal,²⁸ it was used for irrigation in the present study.

The use of 17% EDTA removes the smear layer and stimulates dentin sialophosphoprotein (DSPP) release, which increases dentin surface wettability allowing dental pulp stem cells to adhere to dentin wall.²⁹ Martin et al. reported that the use of 17% EDTA for final irrigation corrected the hazardous effects of using highly concentrated NaOCl solutions improving SCAP survival and differentiation.³⁰



Figs 1A to H: Radiographic images for blood clot group (A to D): #21, blood clot-treated 9-years-old male with 12-months follow-up showing increase in root length, thickness, and narrowing of root apex. For A-PRF group (E to H): #11, A-PRF-treated 9-years-old female with 12-months follow-up showing periapical healing with increased root length, thickness, and narrowing of root apex

Table 1: Repeated measures ANOVA followed by *post-hoc* Bonferroni for mean (\pm SD) radiographic measurements at each time point in millimeters

Blood clot group	Preop	3 months	6 months	12 months
Length	14.9 \pm 1.84	15.8 \pm 1.95 <i>P</i> ₁ = 0.3596	16.3 \pm 1.94 <i>P</i> ₁ = 0.1637 <i>P</i> ₂ = 0.1809	16.8 \pm 2.13 <i>P</i> ₁ = 0.0378* <i>P</i> ₂ = 0.0380* <i>P</i> ₃ = 0.1375
1/3 Thickness	4 \pm 0.63	4.2 \pm 0.65 <i>P</i> ₁ = 0.0114*	4.4 \pm 0.7 <i>P</i> ₁ = 0.0007* <i>P</i> ₂ = 0.0041*	4.7 \pm 0.69 <i>P</i> ₁ = 0.0004* <i>P</i> ₂ = 0.0016* <i>P</i> ₃ = 0.0102*
2/3 Thickness	3.1 \pm 0.59	3.3 \pm 0.54 <i>P</i> ₁ = 0.1963	3.4 \pm 0.63 <i>P</i> ₁ = 0.1524 <i>P</i> ₂ = 0.2926	3.7 \pm 0.76 <i>P</i> ₁ = 0.0148* <i>P</i> ₂ = 0.0844 <i>P</i> ₃ = 0.1136
Root apex width	1.8 \pm 0.54	1.5 \pm 0.52 <i>P</i> ₁ = 0.0794	1.4 \pm 0.48 <i>P</i> ₁ = 0.0278* <i>P</i> ₂ = 0.0233*	1 \pm 0.43 <i>P</i> ₁ = 0.0214* <i>P</i> ₂ = 0.0278* <i>P</i> ₃ = 0.0775

Data expressed as mean \pm SD. * Significant <0.05. *P*, probability; *P*₁, significance vs preoperative; *P*₂, significance vs 3M; *P*₃, significance vs 6M

Table 2: Mean (\pm SD) radiographic measurements at each time point in millimeters

APRF group	Preop	3 months	6 months	12 months
Length	14.5 \pm 2.34	15 \pm 2.33 <i>P</i> ₁ = 0.589	15.4 \pm 2.29 <i>P</i> ₁ = 0.0007* <i>P</i> ₂ = 0.0961	16 \pm 2.42 <i>P</i> ₁ = 0.004* <i>P</i> ₂ = 0.0589 <i>P</i> ₃ = 0.0816
1/3 thickness	3.1 \pm 0.73	3.3 \pm 0.74 <i>P</i> ₁ = 0.0045*	3.5 \pm 0.78 <i>P</i> ₁ = 0.0034* <i>P</i> ₂ = 0.0067*	3.8 \pm 0.76 <i>P</i> ₁ = 0.0001* <i>P</i> ₂ = 0.0003* <i>P</i> ₃ = 0.0053*
2/3 thickness	2.5 \pm 0.57	2.7 \pm 0.52 <i>P</i> ₁ = 0.0053*	2.9 \pm 0.54 <i>P</i> ₁ = 0.0001* <i>P</i> ₂ = 0.0114*	3.2 \pm 0.49 <i>P</i> ₁ = 0.0022* <i>P</i> ₂ = 0.0139* <i>P</i> ₃ = 0.1327
Root apex width	2 \pm 0.67	1.8 \pm 0.72 <i>P</i> ₁ = 0.0045*	1.6 \pm 0.63 <i>P</i> ₁ = 0.0053* <i>P</i> ₂ = 0.1704	1.2 \pm 0.51 <i>P</i> ₁ = 0.0011* <i>P</i> ₂ = 0.0114* <i>P</i> ₃ = 0.0042*

Data expressed as mean \pm SD. *Significant < 0.05. *P*, probability; *P*₁, significance vs preoperative; *P*₂, significance vs 3M; *P*₃, significance vs 6M

Calcium hydroxide is the recommended intracanal medicament in REP³¹ to minimize the tooth discoloration risk by minocycline in the triple antibiotic paste (TAP). Triple antibiotic paste demineralizes intertubular dentin through interaction with calcium ions forming an insoluble component via chelation and incorporating into the tooth structure resulting in discoloration.³² The TAP acidity (pH = 2.9) causes more demineralization of the intertubular dentin, decrease in the phosphate/amide ratio, and reduction of the dentin fracture resistance and microhardness.³³

An appropriate intracanal matrix in REP had been achieved using blood clot scaffolds.³⁴ However, few clinical trials reported that inducing sufficient bleeding into root canals to formulate a scaffold was not always possible, which caused increase in collapse possibility of sealing material.³⁵ Another drawback was the possibility of injuring the mental nerve or the IAN during mandibular premolars treatment due to the close relation of premolar apices to the mental and IAN foramina.³⁶ Mechanical instrumentation beyond the root apex or overfilling with Gutta-Percha can cause

Table 3: Student's *t*-test unpaired for comparison of root length, thicknesses at 1/3 and 2/3, and root apex width between blood clot and A-PRF groups within percent of change of 3-, 6-, and 12-months follow-up intervals

		Blood clot	A-PRF	Unpaired <i>t</i> -test
3 months	Length	6.4 ± 6.29	3 ± 2.27	<i>P</i> = 0.1829
	1/3 thickness	4.5 ± 1.71	7.6 ± 3.24	<i>P</i> = 0.0596
	2/3 thickness	6.9 ± 6.59	8.8 ± 4.77	<i>P</i> = 0.5398
	Root apex width	-18.5 ± 10.04	-15.7 ± 7.63	<i>P</i> = 0.56
6 months	Length	11.1 ± 6.62	6.2 ± 1.71	<i>P</i> = 0.0861
	1/3 thickness	9.2 ± 2.67	12.8 ± 4.24	<i>P</i> = 0.1277
	2/3 thickness	9.6 ± 7.86	18.2 ± 5.9	<i>P</i> = 0.0541
	Root apex width	-24 ± 11.06	-27.3 ± 14.87	<i>P</i> = 0.6895
12 months	Length	14.7 ± 6.09	9.4 ± 3.17	<i>P</i> = 0.0965
	1/3 thickness	16.5 ± 4.67	23.8 ± 7.19	<i>P</i> = 0.0846
	2/3 thickness	18.1 ± 6.32	28 ± 12.17	<i>P</i> = 0.1378
	Root apex width	-45.3 ± 16.28	-41.9 ± 11.2	<i>P</i> = 0.6931

Data expressed as mean ± SD. *Significance < 0.05. *P*, probability

iatrogenic paresthesia of mental nerve. Also, radicular lesion and apical periodontitis might erode the bone protecting the IAN.³⁷

Several studies reported accelerated growth and regeneration when platelet concentrates were used as scaffold compared with only blood clot.⁵ This could be explained by increased growth factor concentration in platelet concentrates providing a prolonged and higher release. So, these concentrates were a better scaffold allowing for cellular proliferation and differentiation.³⁸ Among these growth factors was TGF-β1, which plays a major role in cellular proliferation, differentiation, and dentin regeneration. Wang et al.³⁹ reported that the lack of TGF-β in dentin and bone-forming cells caused failure of root growth, reduction in density of root dentin matrix, and delayed tooth eruption.

Advanced PRF has the advantages of being easy to prepare without any added anticoagulants, making it purely autologous, so it is an ideal scaffold for regeneration. In addition, A-PRF also functions as a reservoir releasing various growth factors at the application site.⁴⁰ Kobayashi et al.²¹ reported that A-PRF releases more growth factors for a longer period than that released by PRP and PRF at different time periods 15 minutes, 59 minutes, 8 hours, 1 day, 3 days, and 10 days, making it more suitable for regenerative procedures.

In the current trial, there was no difference in the clinical evaluation results between the A-PRF and blood clot groups relative to resolution of swelling, pain, sinus/fistula, or mobility, which could be attributed to the application of an effective and standardized disinfection protocol. The study also revealed that there was a significant increase within each group in respect to root length, root thickness at one- and two-thirds, and root apex width for all time points. While percent of change between the two groups was statistically insignificant. This was in agreement with Shivashankar et al.,⁴¹ Murray study results.⁴²

Revascularization studies that were based on inducing bleeding into root canals in immature teeth with follow-up periods between 9 and 22 months reported periapical lesions healing in 90–100% of the cases.⁴³ Another experimental study found complete resolution of the periapical lesions in both blood clot and blood clot mixed with PRF groups.³⁴ Another clinical trial comparing the efficacy of blood clot, platelet pellet, PRP, and PRF

as regenerative scaffolding materials reported similar healing for a 28-month follow-up period.³⁸ Studies reported 80–94% success rate regarding root length and thickness relying on the implanted scaffold type.²⁷

In the present study, the A-PRF group showed significant increase in the length of root at 6 and 12 months and in root thickness at one- and two-thirds at all-time intervals. Similarly, Saoud et al.⁴⁴ and Nagata et al.⁴⁵ reported an increase rate of 0–34.8% in root length and 43.5–45% in root thickness after treatment of traumatized immature teeth followed by a 12–19-month follow-up period. On the other hand, Narang et al.⁵ found that using PRF followed by 18 months of follow-up resulted in a success rate of 60% for dentin wall thickness and of 99% for root length.

The difference between A-PRF and blood clot group outcomes might be attributed to the variation in the participants' age. However, there was no statistically significant difference when the participants' mean age was compared in both groups. Estefan et al.⁴⁶ suggested that the participants' age affected the rate of increase in root thickness and length due to alteration in the quality of fibrin clot formed caused by variation in the fibrin network pattern and interaction with platelets with age.⁴⁷

Nazzal et al.⁴³ reported that REP of immature tooth is most likely to cause an increase in root thickness rather than root length due to the damage of the epithelial root sheath of Hertwig's cells needed for root formation. According to Zhou et al.,³⁴ histologic evaluation revealed that the root thickening and elongation were caused by deposition of cementum-like tissue and scattered bone-like tissue at the canals' lateral walls and at the apical region. However, root-bone ankylosis and calcification of pulp canals are some of the complications that could result from REP of permanent immature nonvital teeth.^{48,49}

Restoring tooth sensitivity is essential to regain the defensive proprioceptive reflexes of the pulp providing a useful alarm system protecting against tooth fracture or tissue damage.⁵⁰ Electric pulp test negative results may be due to the fact that coronally placed MTA layer acted as an insulator, or that more than a year is required for nerve fibers' and blood vessels' complete regeneration within the root canal. Another possible reason is that the canal walls'

thickening is due to deposition of cementum-like tissue lacking the characteristic dentin tubular structure.⁵¹

The response of the tooth-to-pulp vitality testing is affected by thickness of placed MTA as it affects the new tissue growth ahead of it.⁵² Even if the tooth showed a negative response to pulp vitality testing, this tooth is considered to be vital since tooth radiographic maturation after the REP is due to deposition of cementum-like hard tissue in the root canal by cementoblast-like cells.⁵³ In a histological study conducted by Lei et al.,⁵⁴ regenerated nerve fibers and neurons were found and confirmed immunohistochemically in mandibular immature premolar with successful REP that was extracted for orthodontic reasons.

Coronal seal was achieved using MTA over the blood clot or A-PRF through the formation of hydrated silicate gel and calcium hydroxide. The latter has numerous physicochemical and biological characteristics; due to its alkaline pH which has an antibacterial and stimulative effect for reparative dentin deposition by activating growth factors within the proximal dentin controlling cellular proliferation and differentiation.⁵⁵

The limitations of the present study included that the cause of trauma could not be specified, the patients' gender and age were not standardized, the two-dimensional radiographic evaluations, the lack of uniformity in groups distribution, and limited follow-up period. Pulse oximeter and laser doppler flowmetry should have been used for pulp vitality testing to evaluate blood supply. The histological evaluation of the newly formed tissue within the root canal was not done in the present study, so the precise nature of tissues formed in response to REP was not determined. As a recommendation, APRF is an excellent scaffold for REP. However, longer follow-up periods could be addressed in future studies for pulp vitality testing to display positive results. Histological changes in the root canal should be evaluated to determine precisely which types of tissues were formed.

CONCLUSION

Regenerative endodontic procedures for traumatized immature nonvital teeth with either conventional blood clot or A-PRF as scaffolds were comparable, except in cases where adequate bleeding cannot be achieved.

REFERENCES

- Selwitz RH, Ismail AI, Pitts NB. Dental caries. *Lancet* 2007;369(9555): 51–59. DOI: 10.1016/S0140-6736(07)60031-2.
- Dye BA, Thornton-Evans G, Li X, et al. Dental caries and sealant prevalence in children and adolescents in the United States, 2011–2012. *NCHS Data Brief* 2015;(191):1–8. PMID: 25932891.
- Hintze H. Approximal caries prevalence in Danish recruits and progression of caries in the late teens: A retrospective radiographic study. *Caries Res* 2001;35(1):27–35. DOI: 10.1159/000047427.
- Hecova H, Tzigkounakis V, Merglova V, et al. A retrospective study of 889 injured permanent teeth. *Dent Traumatol* 2010;26(6):466–475. DOI: 10.1111/j.1600-9657.2010.00924.x.
- Narang I, Mittal N, Mishra N. A comparative evaluation of the blood clot, platelet-rich plasma, and platelet-rich fibrin in regeneration of necrotic immature permanent teeth: A clinical study. *Contemp Clin Dent* 2015;6(1):63–68. DOI: 10.4103/0976-237X.149294.
- Kerezoudis NP, Valavanis D, Proutzos F. A method of adapting gutta-percha master cones for obturation of open apex cases using heat. *Int Endod J* 1999;32(1):53–60. DOI: 10.1046/j.1365-2591.1999.00180.x.
- Thomson A, Kahler B. Regenerative endodontics—biologically-based treatment for immature permanent teeth: A case report and review of the literature. *Aust Dent J* 2010;55(4):446–452. DOI: 10.1111/j.1834-7819.2010.01268.x.
- Rafter MJDT. Apexification: A review. *Dent Traumatol* 2005;21(1):1–8. DOI: 10.1111/j.1600-9657.2004.00284.x.
- Kerekes K, Heide S, Jacobsen I. Follow-up examination of endodontic treatment in traumatized juvenile incisors. *J Endod* 1980;6(9):744–748. DOI: 10.1016/S0099-2399(80)80186-5.
- Lv H, Chen Y, Cai Z, et al. The efficacy of platelet-rich fibrin as a scaffold in regenerative endodontic treatment: A retrospective controlled cohort study. *BMC Oral Health* 2018;18(1):139. DOI: 10.1186/s12903-018-0598-z.
- Huang FM, Yang SF, Zhao JH, et al. Platelet-rich fibrin increases proliferation and differentiation of human dental pulp cells. *J Endod* 2010;36(10):1628–1632. DOI: 10.1016/j.joen.2010.07.004.
- Freed LE, Vunjak-Novakovic G, Biron RJ, et al. Biodegradable polymer scaffolds for tissue engineering. *Biotechnology (NY)* 1994;12(7): 689–693. DOI: 10.1038/nbt0794-689.
- Torabinejad M, Corr R, Buhley M, et al. An animal model to study regenerative endodontics. *J Endod* 2011;37(2):197–202. DOI: 10.1016/j.joen.2010.10.011.
- Torabinejad M, Faras H. A clinical and histological report of a tooth with an open apex treated with regenerative endodontics using platelet-rich plasma. *J Endod* 2012;38(6):864–868. DOI: 10.1016/j.joen.2012.03.006.
- Kim SG, Zhou J, Solomon C, et al. Effects of growth factors on dental stem/progenitor cells. *Dent Clin North Am* 2012;56(3):563–575. DOI: 10.1016/j.cden.2012.05.001.
- Shivashankar VY, Johns DA, Vidyant S, et al. Platelet rich fibrin in the revitalization of tooth with necrotic pulp and open apex. *J Conserv Dent* 2012;15(4):395–398. DOI: 10.4103/0972-0707.101926.
- Ehrenfest DMD, Rasmusson L, Albrektsson T. Classification of platelet concentrates: From pure platelet-rich plasma (P-PRP) to leucocyte- and platelet-rich fibrin (L-PRF). *Trends Biotechnol* 2009;27(3):158–167. DOI: 10.1016/j.tibtech.2008.11.009.
- Kawazoe T, Kim HH. Tissue augmentation by white blood cell-containing platelet-rich plasma. *Cell Transplant* 2012;21(2–3):601–607. DOI: 10.3727/096368911X605538.
- Fujioka-Kobayashi M, Miron RJ, Hernandez M, et al. Optimized platelet-rich fibrin with the low-speed concept: Growth factor release, biocompatibility, and cellular response. *J Periodontol* 2017;88(1): 112–121. DOI: 10.1902/jop.2016.160443.
- Dohan Ehrenfest DM, Pinto NR, Pereda A, et al. The impact of the centrifuge characteristics and centrifugation protocols on the cells, growth factors, and fibrin architecture of a leukocyte- and platelet-rich fibrin (L-PRF) clot and membrane. *Platelets* 2017;29(2):171–184. DOI: 10.1080/09537104.2017.1293812.
- Kobayashi E, Flückiger L, Fujioka-Kobayashi M, et al. Comparative release of growth factors from PRP, PRF, and advanced-PRF. *Clin Oral Investig* 2016;20(9):2353–2360. DOI: 10.1007/s00784-016-1719-1.
- Rizk HM, Salah Al-Deen MS, Emam AA. Pulp revascularization/revitalization of bilateral upper necrotic immature permanent central incisors with blood clot vs platelet-rich fibrin scaffolds – A split-mouth double-blind randomized controlled trial. *Int J Clin Pediatr Dent* 2020;13(4):337–343. DOI: 10.5005/jp-journals-10005-1788.
- Cehreli ZC, Isbitiren B, Sara S, et al. Regenerative endodontic treatment (revascularization) of immature necrotic molars medicated with calcium hydroxide: A case series. *J Endod* 2011;37(9):1327–1330. DOI: 10.1016/j.joen.2011.05.033.
- Trope M. Treatment of the immature tooth with a non-vital pulp and apical periodontitis. *Den Clin North Am* 2010;54(2):313–324. DOI: 10.1016/j.cden.2009.12.006.
- Nosrat A, Seifi A, Asgary S. Regenerative endodontic treatment (revascularization) for necrotic immature permanent molars: A review and report of two cases with a new biomaterial. *J Endod* 2011;37(4): 562–567. DOI: 10.1016/j.joen.2011.01.011.
- Chang YC, Huang FM, Tai KW, et al. The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells.

- Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2001;92(4):446–450. DOI: 10.1067/moe.2001.116812.
27. Kontakiotis EG, Filippatos CG, Tzanetakakis GN, et al. Regenerative endodontic therapy: A data analysis of clinical protocols. *J Endod* 2015;41(2):146–154. DOI: 10.1016/j.joen.2014.08.003.
 28. Zeng Q, Nguyen S, Zhang H, et al. Release of growth factors into root canal by irrigations in regenerative endodontics. *J Endod* 2016;42(12):1760–1766. DOI: 10.1016/j.joen.2016.04.029.
 29. Huang X, Zhang J, Huang C, et al. Effect of intracanal dentine wettability on human dental pulp cell attachment. *Int Endod J* 2012;45(4):346–353. DOI: 10.1111/j.1365-2591.2011.01982.x.
 30. Martin DE, De Almeida JFA, Henry MA, et al. Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. *J Endod* 2014;40(1):51–55. DOI: 10.1016/j.joen.2013.07.026.
 31. Diogenes A, Ruparel NB. Regenerative endodontic procedures: Clinical outcomes. *Dent Clin North Am* 2017;61(1):111–125. DOI: 10.1016/j.cden.2016.08.004.
 32. Tanase S, Tsuchiya H, Yao J, et al. Reversed-phase ion-pair chromatographic analysis of tetracycline antibiotics: Application to discolored teeth. *J Chromatogr B Biomed Sci Appl* 1998;706(2):279–285. DOI: 10.1016/S0378-4347(97)00563-x.
 33. Eymirli A, Nagas E, Uyanik MO, et al. Effect of laser-activated irrigation with ethylene diaminetetraacetic acid and phytic acid on the removal of calcium hydroxide and triple antibiotic paste from root dentin. *Photomed Laser Surg* 2017;35(1):43–48. DOI: 10.1089/pho.2016.4146.
 34. Zhou R, Wang Y, Chen Y, et al. Radiographic, histologic, and biomechanical evaluation of combined application of platelet-rich fibrin with blood clot in regenerative endodontics. *J Endod* 2017;43(12):2034–2040. DOI: 10.1016/j.joen.2017.07.021.
 35. Jiang X, Liu H, Peng C. Clinical and radiographic assessment of the efficacy of a collagen membrane in regenerative endodontics: A randomized, controlled clinical trial. *J Endod* 2017;43(9):1465–1471. DOI: 10.1016/j.joen.2017.04.011.
 36. Denio D, Torabinejad M, Bakland LK. Anatomical relationship of the mandibular canal to its surrounding structures in mature mandibles. *J Endod* 1992;18(4):161–165. DOI: 10.1016/S0099-2399(06)81411-1.
 37. Scarano A, Di Carlo F, Quaranta A, et al. Injury of the inferior alveolar nerve after overfilling of the root canal with endodontic cement: a case report. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007;104(1):e56–e59. DOI: 10.1016/j.tripleo.2007.01.036.
 38. Ulusoy AT, Turedi I, Cimen M, et al. Evaluation of blood clot, platelet-rich plasma, platelet-rich fibrin, and platelet pellet as scaffolds in regenerative endodontic treatment: A prospective randomized trial. *J Endod* 2019;45(5):560–566. DOI: 10.1016/j.joen.2019.02.002.
 39. Wang Y, Cox MK, Coricor G, et al. Inactivation of Tgfb2 in Osterix-Cre expressing dental mesenchyme disrupts molar root formation. *Dev Biol* 2013;382(1):27–37. DOI: 10.1016/j.ydbio.2013.08.003.
 40. Masuki H, Okudera T, Watanebe T, et al. Growth factor and pro-inflammatory cytokine contents in platelet-rich plasma (PRP), plasma rich in growth factors (PRGF), advanced platelet-rich fibrin (A-PRF), and concentrated growth factors (CGF). *Int J Implant Dent* 2016;2(1):19. DOI: 10.1186/s40729-016-0052-4.
 41. Shivashankar VY, Johns DA, Maroli RK, et al. Comparison of the effect of PRP, PRF and induced bleeding in the revascularization of teeth with necrotic pulp and open apex: A triple blind randomized clinical trial. *J Clin Diagn Res* 2017;11(6):ZC34–ZC39. DOI: 10.7860/JCDR/2017/22352.10056.
 42. Murray PE. Minireview of the clinical efficacy of platelet-rich plasma, platelet-rich fibrin and blood-clot revascularization for the regeneration of immature permanent teeth. 2018;6(1):1–5. DOI: 10.5321/wjs.v6.i1.1.
 43. Nazzal H, Kenny K, Altimimi A, et al. A prospective clinical study of regenerative endodontic treatment of traumatized immature teeth with necrotic pulps using bi-antibiotic paste. *Int Endod J* 2018;51(Suppl 3):e204–e215. DOI: 10.1111/iej.12808.
 44. Saoud TMA, Zaazou A, Nabil A, et al. Clinical and radiographic outcomes of traumatized immature permanent necrotic teeth after revascularization/revitalization therapy. *J Endod* 2014;40(12):1946–1952. DOI: 10.1016/j.joen.2014.08.023.
 45. Nagata JY, de Almeida Gomes BPF, Rocha Lima TF, et al. Traumatized immature teeth treated with 2 protocols of pulp revascularization. *J Endod* 2014;40(5):606–612. DOI: 10.1016/j.joen.2014.01.032.
 46. Estefan BS, El Batouty KM, Nagy MM, et al. Influence of age and apical diameter on the success of endodontic regeneration procedures. *J Endod* 2016;42(11):1620–1625. DOI: 10.1016/j.joen.2016.06.020.
 47. Yajamanya SR, Chatterjee A, Babu CN, et al. Fibrin network pattern changes of platelet-rich fibrin in young versus old age group of individuals: A cell block cytology study. *J Indian Soc Periodontol* 2016;20(2):151–156. DOI: 10.4103/0972-124X.176390.
 58. Nazzal H, Ainscough S, Kang J, et al. Revitalisation endodontic treatment of traumatised immature teeth: A prospective long-term clinical study. *Eur Arch Paediatr Dent* 2020;21(5):587–596. DOI: 10.1007/s40368-019-00501-0.
 49. Chen MY, Chen KL, Chen CA, et al. Responses of immature permanent teeth with infected necrotic pulp tissue and apical periodontitis/abscess to revascularization procedures. *Int Endod J* 2012;45(3):294–305. DOI: 10.1111/j.1365-2591.2011.01978.x.
 50. Ou KL, Chang CC, Chang WJ, et al. Effect of damping properties on fracture resistance of root filled premolar teeth: A dynamic finite element analysis. *Int Endod J* 2009;42(8):694–704. DOI: 10.1111/j.1365-2591.2009.01570.x.
 51. Yang J, Yuan G, Chen Z. Pulp regeneration: current approaches and future challenges. *Front Physiol* 2016;7:58. DOI: 10.3389/fphys.2016.00058.
 52. Torabinejad M, Turman M. Revitalization of tooth with necrotic pulp and open apex by using platelet-rich plasma: A case report. *J Endod* 2011;37(2):265–268. DOI: 10.1016/j.joen.2010.11.004.
 53. Wang X, Thibodeau B, Trope M, et al. Histologic characterization of regenerated tissues in canal space after the revitalization/revascularization procedure of immature dog teeth with apical periodontitis. *J Endod* 2010;36(1):56–63. DOI: 10.1016/j.joen.2009.09.039.
 54. Lei L, Chen Y, Zhou R, et al. Histologic and immunohistochemical findings of a human immature permanent tooth with apical periodontitis after regenerative endodontic treatment. *J Endod* 2015;41(7):1172–1179. DOI: 10.1016/j.joen.2015.03.012.
 55. Tomson PL, Grover LM, Lumley PJ, et al. Dissolution of bio-active dentine matrix components by mineral trioxide aggregate. *J Dent* 2007;35(8):636–642. DOI: 10.1016/j.jdent.2007.04.008.