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Use of platelet-rich plasma for regeneration in non-vital immature permanent teeth: Clinical and cone-beam computed tomography evaluation

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Abstract

Objective: This study was performed to assess the clinical and radiological outcomes of a revascularization procedure in immature teeth with apical periodontitis using platelet-rich plasma (PRP). The PRP protocol and conventional revascularization protocol, which used a blood clot as the scaffold, were compared.

Methods: Thirty non-vital immature permanent teeth were randomly categorized into two groups. After disinfecting the root canal space with triple antibiotic paste (1:1:1 ciprofloxacin, metronidazole, and cefaclor), a tissue scaffold was created using either PRP or a blood clot (control) and covered with white mineral trioxide aggregate. All cases were followed up clinically and radiographically for 12 months. Differences in bone density, root length, and lesion size were calculated using preoperative and postoperative computed tomography images. The means of the differences in individual parameters in the blood clot and PRP groups were compared using the Mann–Whitney U test.

Results: After 5 months, sensitivity tests (cold and electric pulp tests) elicited a delayed positive response in 23 sites. At 12 months, cone-beam computed tomography revealed resolution or a decrease in lesion size and an increase in bone density in all 30 (100%) teeth. Additionally, continued root development was observed in 22 (73%) teeth and early root growth was observed in the test group (mineral trioxide aggregate with PRP).

Conclusions: The results of this study suggest that PRP can serve as a successful scaffold for regenerative endodontic treatment. With the exception of a significant increase in root length, the

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results of treatment with PRP were not significantly different from those of the conventional protocol using a blood clot as the scaffold.

Keywords

Regenerative endodontics, blood clot, platelet-rich plasma, mineral trioxide aggregate, cone-beam computed tomography, immature permanent teeth

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Introduction

Management of permanent teeth with necrotic pulp, periapical pathology, and arrested root development poses a great challenge for dentists. The results of halted root development include weak root dentin, open apices, and stunted root growth; hence, such teeth are prone to root fracture.[1] Moreover, such abnormalities make them unsuitable for debridement and filling with traditional techniques and obturation materials.[2,3] Earlier apexification with calcium hydroxide,[4] which is used to form an apical hard tissue barricade after multiple visits, was historically the treatment of choice in such cases. However, with the advent of mineral trioxide aggregate (MTA), a synthetic wall can be promptly formed in the apical third of root with a very high success rate as proven by many milestone studies.[5–7] This has also reduced the number of required clinical sessions. A disadvantage common to both calcium hydroxide and MTA, however, is that they do not allow for continued root development, resulting in thin dentin walls and hence a feeble root structure.[8,9]

The clinical management of such a perplexing clinical challenge is to stimulate the regenerative tissues of the pulp–dentin complex to complete apical development.[3] Recent advances in tissue engineering have focused upon three key factors for tissue regeneration: adult stem cells, signaling molecules, and a three-dimensional (3D) physical scaffold that can sustain cell growth and differentiation. Various authors have recently reported on the concept of regenerative endodontics.[9–11] According to these studies, stem cells from the apical papilla of immature teeth are accountable for continued apex formation in a sterile environment.[11] Unlike apexification, regeneration allows for rapid continuation of root development, increased wall thickness, and natural healing of periapical tissues.[10,11] Previously published case studies using a blood clot as a scaffold for regeneration have established the radiographic signs of continued thickening of root dentin and ensuing apex formation in teeth with periapical lesions.[12,13] Platelet-rich plasma (PRP) has recently been described as a source of growth factors and a potentially ideal scaffold for regenerative endodontic treatment regimens because of its ability to maintain the vitality of pulp tissues by promoting cell growth and transport of growth factors in a disinfected environment.[14–17] Treatment protocols that make use of this innate mechanism of stimulated healing and regeneration of tissues in combination with thorough disinfection of the root canal system are useful to manage non-vital immature teeth with periapical pathology. Various case series and in-vivo studies of regeneration procedures with PRP have been published.[15,16,32,36–39] However, a lack of sufficient evidence has prevented the extensive use of such treatment protocols in the clinical setting. The aim of the present study was to determine the efficacy of a regenerative protocol using PRP in non-vital young permanent teeth.

Materials and methods

In this split-mouth-designed study (approved by the University of Dammam Institutional Review Board: IRB-2014-02-029), 16 healthy patients with young permanent single-rooted teeth were selected among patients attending the outpatient dental clinics at the College of Dentistry, University of Dammam. None of the teeth had been previously treated for necrosis. Written informed consent was acquired from the patients and their guardians. Institutional ethical clearance was obtained for this study.

The inclusion criteria were as follows:

(1) Pulp necrosis with or without periapical lesions and an immature apex
(2) Probability of tooth restoration
(3) No pathologic mobility, ankylosis, root fracture, or probing depths of >3 mm
(4) No allergy to medications or antibiotics necessary to complete procedure[29,30]

Pulp necrosis was tentatively diagnosed by the patient's dental history and clinical examination findings, which included electric pulp testing and cold testing. Clinical signs and symptoms such as pain, swelling, fistulas, and sensitivity to percussion and palpation were also noted. Among the 16 patients, 32 non-vital young permanent teeth with either apical periodontitis or abscesses showing a negative response on pulp testing were included in the study. All teeth were randomly divided into either the control group, in which a blood clot was used as the scaffold, or the test group, in which PRP was used as the scaffold. Teeth with periapical lesions were grouped according to the size of the lesion as follows:

Score 0 Intact periapical bone structures
Score 1 Periapical radiolucency of >0.5 to 1 mm
Score 2 Periapical radiolucency of >1 to 2 mm
Score 3 Periapical radiolucency of >2 to 4 mm
Score 4 Periapical radiolucency of >4 to 8 mm
Score 5 Periapical radiolucency of >8 mm

Treatment procedures

Regenerative treatment was performed according to the protocol established by the American Association of Endodontists. Under rubber dam isolation, an access opening was made followed by copious, gentle irrigation with 1.5% sodium hypochlorite, and the working length was determined with a size #20 sterile K-file. Canals were not instrumented but copiously irrigated with 2.5% NaOCl (20 mL), sterile saline (20 mL), and 0.12% chlorhexidine (10 mL) and dried with sterile paper points. After sealing the pulp chamber with a dentin-bonding agent to minimize the risk of staining, a 1:1:1 mixture of metronidazole, ciprofloxacin, and minocycline was pulverized and mixed with distilled water to a final concentration of 0.1 mg/ml[18,19,29] until a creamy paste was formed. This paste was placed in the canal below the cementoenamel junction (CEJ) to minimize crown staining using a lentulo spiral in a slow-speed handpiece. Canals were sealed with reinforced zinc oxide–eugenol cement. The patients were followed up at 3 weeks, the success of which to disinfect the root canal has been as proved by previous studies.[2,8,12] The triple antibiotic paste was applied again for patients with any persistent signs or symptoms of infection, such as purulent drainage, failed pain resolution, swelling, fistulas, or sensitivity to percussion and palpation.

The response to the initial treatment was assessed after 3 weeks; if the patient was asymptomatic, the antibiotic paste was removed by copious irrigation with 20 ml of 17% EDTA and normal saline followed by drying of the canals with sterile paper points. Scaffolds were then created according to the allocated group. In the blood clot group, a size #20 K-file was rotated 2 mm past the apical foramen under local anesthesia to induce bleeding, filling the root canal up to the level of the CEJ.[20,22–28] After blood clot formation, the final restoration was completed with white MTA (Dentsply Tulsa Dental, Tulsa, OK), glass ionomer cement (Fuji VII; GC, Tokyo, Japan), and composite resin (Filtek Z350; 3M ESPE, St. Paul, MN) during the same visit to achieve a coronal seal.

In the PRP group, no anesthetic was used. PRP was prepared according to the description by Dohan et al.[31] PRP was combined with equal volumes of sterile saline solution containing 10% calcium chloride and sterile bovine thrombin (100 U/mL) to achieve coagulation. The PRP was then injected into the root canal up to the level of the CEJ. Final restoration was completed as described for the blood clot group.

Clinical and radiographic follow-up

Clinical and radiographic follow-up examinations were performed every 3 months during a 12-month follow-up period. The same pediatric dentist performed all follow-up examinations.

Treated teeth were examined for vitality using both electric pulp and cold testing. They were recorded as responding positively to vitality testing only if they responded positively to both tests. A post-treatment computed tomography (CT) scan was performed at 3 months and then repeated at least every 3 to 12 months depending on the patient's condition. At the end of 12 months, cone-beam CT (CBCT) images were independently evaluated with respect to lesion size, periapical healing, and apical closure. The same pediatric dentist re-examined all radiographs 1 month after the preliminary examination, and intra-examiner validity was assessed using kappa statistics. Kappa values ranged from 0.8 to 1.0, signifying good reliability.

For all cases, CBCT (I-Cat; Imaging Sciences International, Hatfield, PA) was used to take images of the maxillofacial area

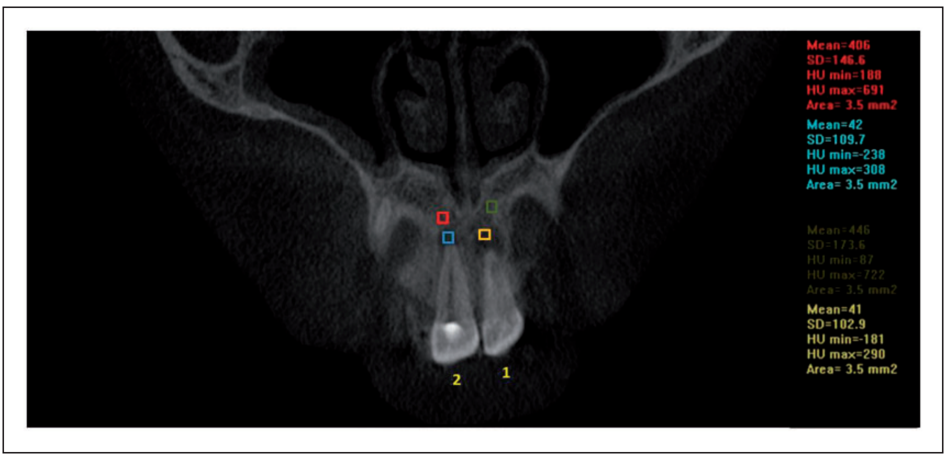

Figure 1. Preoperative cone-beam computed tomography image showing periapical radiolucencies (decreased bone density compared with adjacent normal bone) and root length measured for both the control site (blood clot[1]) and test site (platelet-rich plasma[2]).

at a setting of 120 kVp and 3 to 7 mA. Images were obtained with an exposure time of 9 s. Each scan was taken over 360° with a voxel size of 0.3 mm. The lesion size, bone density (Hounsfield units), and root length were measured.

In the pretreatment CBCT images, the bone density was recorded in the coronal plane with a 3.5-mm^2 area in regions containing periapical lesions and normal adjoining bone regions (Figure 1). The final postoperative measurements were made 1 year after the endodontic treatment using CBCT images taken with the same parameters (Figure 2). The mean initial and final bone density measurements are shown in Table 1.

All CBCT measurements were obtained with Dolphin 3D Imaging software (Dolphin Imaging Systems, Chatsworth, CA) on a computer workstation running under

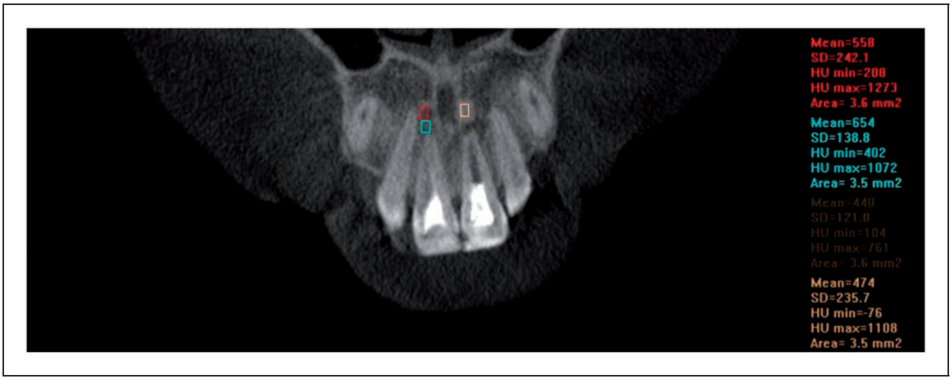

Figure 2. Postoperative cone-beam computed tomography image showing significant increase in bone density in periapical region and increase in root length of test site (mineral trioxide aggregate + platelet-rich plasma).

Table 1. Pre- and post-treatment comparison of parameters in both study groups (control and test).

	Initial	Follow-up	
Parameters studied	Mean (SD)	Mean (SD)	P value
Blood clot (Control)			
Lesion size (mm)	4.02 (1.59)	1.95 (1.16)	.001*
Bone density (HU)	129.00 (58.27)	445.44 (153.54)	.001*
Root length (mm)	11.30 (3.10)	11.80 (3.28)	.001*
PRP (Test)			
Lesion size (mm)	3.92 (1.46)	1.50 (1.08)	.001*
Bone density (HU)	120.56 (61.47)	485.88 (154.15)	.001*
Root length (mm)	11.08 (3.02)	12.14 (3.32)	.001*

*Statistically significant
SD, standard deviation; HU, Hounsfield units

Figure 3. Sagittal scan used for measurement of diameter and depth using the built-in measurement tool.

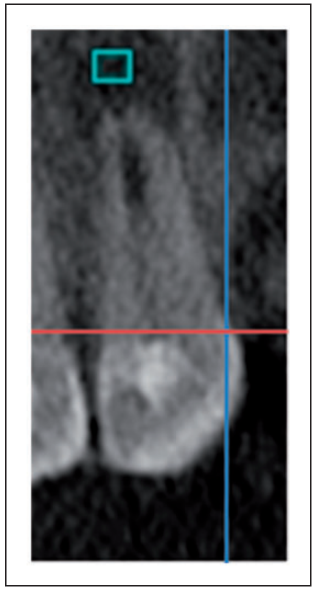

Figure 4. Identification of mesial and distal cementoenamel junctions on axial plane using coronal view of cone-beam computed tomography image.

Microsoft Windows XP professional SP-1 (Microsoft Corp., Redmond, WA). Sagittal-view CBCT images were evaluated to measure lesion size using the built-in measurement tools (Figure 3).

To standardize the root length measurements, the alignment for each tooth was attuned using the coronal view; the sagittal and axial planes were adjusted to intersect on the CEJ (Figure 4). The root length was measured along the long axis of each tooth from the most apical point of the root perpendicular to the line connecting the mesial and distal CEJs as a measure of standardization[34] to determine the change in the length postoperatively.

Statistical analysis

The data were analyzed using the Statistical Package for Social Sciences, version 13 (SPSS Inc., Chicago, IL). Clinical and radiographic data were assessed using a paired t-test, and a P value of $<.001$ was considered statistically significant. Comparison between the mean of the differences in individual parameters in the control group (blood clot) and test group (PRP) was performed using the Mann–Whitney U test, with a P value of $<.005$ considered statistically significant.

Results

Preoperative clinical and radiographic findings

Of 16 patients (6 girls, 10 boys) selected for the study, only 1 patient did not return for the postoperative follow-up visits. Of 30 necrotic teeth (PRP, 15; blood clot, 15) in 15 children (6 girls, 9 boys), 24 were maxillary incisor teeth exhibiting pulp necrosis in conjunction with a history of traumatic injury (either crown fracture or luxation) and 6 were premolar teeth (1 maxillary first premolar, 3 mandibular first premolars, and

2 mandibular second premolars) exhibiting pulp necrosis due to deep dentin caries or secondary caries.

Preoperative acute symptoms including night pain, spontaneous pain, and extreme sensitivity to percussion were observed in 26 teeth (PRP, 14; blood clot, 12), and preoperative apical abscesses were recorded in 9 teeth (PRP, 4; blood clot, 5). Moreover, four teeth assigned to the PRP group and five teeth assigned to the blood clot group required two sessions with triple antibiotic paste until the teeth were symptom-free. With regard to periapical lesions, 8 teeth (PRP, 3; blood clot, 1) had no lesions, 10 teeth (PRP, 3; blood clot, 4) had a score 1 periapical lesion, 9 teeth (PRP, 1; blood clot, 4) had a score 2 periapical lesion, and 3 teeth (PRP, 3; blood clot, 1) had a score 3 periapical lesion.

Postoperative clinical and radiographic findings

The clinical and radiographic findings of both groups are shown in Table 3. All 15 patients (30 teeth: PRP, 15; blood clot, 15) who underwent this endodontic treatment protocol exhibited exceptional clinical and radiographic responses. All patients were followed up for 12 months. After 1 month, all teeth were clinically asymptomatic with comprehensive resolution of associated soft tissue lesions. After 5 months, sensitivity tests (cold and electric pulp tests) elicited a delayed positive response close to that found in adjacent teeth in 23 sites. At the 12-month follow-up visit, CBCT findings exhibited resolution of or a decrease in the lesion size and an increase in bone density, depicting periapical healing with resolution of signs and symptoms (pain, swelling, fistula, and/or sensitivity to percussion and palpation) of pathology in all 30 teeth (100% of cases). In addition, continued root development or apical closure was seen in 22 teeth (73% of cases; 14 in the PRP group and 8 in the blood clot group), whereas a delayed positive response to pulpal sensitivity testing was seen in 19 cases (63.3% of cases; 13 in the PRP group and 6 in the blood clot group). Data derived from pretreatment and postoperative CT evaluations within the control and test groups showed significant differences in the study parameters ($P < .001$) (Table 1). In the comparison of each parameter (lesion size, bone density, and root length) between the control group (MTA) and test group (MTA + PRP), only the mean difference in the root length was found to be statistically significant ($P < .004$) (Table 2)

Discussion

Numerous previous studies[2,3,20,22–28] have investigated different scaffolds for use in revascularization protocols. In the present

Table 2. Comparison of mean of the differences in individual parameters in control group (blood clot) and test group (platelet-rich plasma).

	Blood clot	Platelet-rich plasma	
Parameters studied	Mean (SD)	Mean (SD)	P value
Lesion size (mm)	2.06 (0.84)	2.41 (0.80)	.157
Bone density (HU)	316.4 (123.31)	365.3 (127.50)	.132
Root length (mm)	0.502 (0.42)	1.06 (0.62)	.004*

*Statistically significant
SD, standard deviation; HU, Hounsfield units

Table 3. Preoperative symptoms and treatment outcomes after 12-month follow-up.

Sample No.	Patient age/sex	Tooth	Etiology	Preoperative signs and symptoms	Preoperative lesion size	Months to complete healing of periapical lesions	Positive response to vitality testing	Complete apical closure
PRP								
1	9/M	Premolar	Caries	Yes	2	6	Yes	Yes
2	11/M	Incisor	Trauma	Yes	2	6		
3	9/F	Incisor	Trauma	Yes	3	12	No	No
4	9/M	Incisor	Trauma	Yes	1	6	Yes	Yes
5	10/M	Incisor	Trauma	Yes	1	6	Yes	Yes
6	9/F	Incisor	Trauma	No	–	–	Yes	Yes
7	11/F	Incisor	Trauma	Yes	3	6	Yes	Yes
8	9/M	Incisor	Trauma	Yes	1	6	Yes	Yes
9	10/M	Incisor	Trauma	Yes	2	6	Yes	Yes
10	9/F	Incisor	Trauma	Yes	3	9	No	Yes
11	8/M	Incisor	Trauma	No	–	–	Yes	Yes
12	9/M	Incisor	Trauma	Yes	2	6	Yes	Yes
13	9/F	Premolar	Caries	No	–	–	Yes	Yes
14	9/F	Premolar	Caries	Yes	1	9	Yes	Yes
15	11/M	Incisor	Trauma	Yes	2	6	Yes	Yes
BC								
1	9/M	Incisor	Trauma	Yes	2	6	No	Yes
2	11/M	Incisor	Trauma	Yes	3	9	Yes	Yes
3	9/F	Incisor	Trauma	Yes	1	6	Yes	No
4	10/M	Incisor	Trauma	Yes	1	3	No	No
5	9/F	Incisor	Trauma	Yes	1	6	No	Yes
6	9/M	Incisor	Trauma	Yes	1	3	No	Yes
7	10/M	Incisor	Trauma	Yes	1	6	Yes	No
8	9/M	Premolar	Caries	Yes	2	3	Yes	Yes
9	11/F	Incisor	Trauma	Yes	2	3	Yes	No
10	9/F	Incisor	Trauma	Yes	2	9	No	No
11	8/M	Incisor	Trauma	No	–	–	No	Yes
12	9/M	Incisor	Trauma	Yes	2	6	Yes	Yes
13	9/F	Premolar	Caries	Yes	2	6	No	No
14	9/F	Premolar	Caries	No	–	–	No	Yes
15	11/M	Incisor	Trauma	Yes	2	12	No	No

PRP, platelet-rich plasma; BC, blood clot; M, male; F, female

study, we explored the effect of PRP in healing and regeneration of pulpal and periapical tissues. The results of this study suggest that the PRP can act as a successful scaffold for regenerative endodontic treatment. However, with the exception of the increase in root length, the effects of treatment with PRP were not significantly different from those of the conventional protocol using a blood clot as the scaffold.

Previous studies[30,32,35] have revealed that the outcomes of regenerative protocols may vary between teeth showing partial necrosis (good prognosis) and those exhibiting full necrosis (poor prognosis), and a separate protocol and scaffold may be required for each of these types of teeth.

In the present study, we evaluated the efficacy of PRP as a scaffold in teeth with complete pulp degeneration. At the end of 12 months, all teeth in the PRP group showed resolution of signs and symptoms (pain, swelling, fistulas, and/or sensitivity to percussion and palpation), and 93% of teeth in the PRP group showed various degrees of increased root length or apical closure.

This study did not include collection of tissue samples for histologic examination because of ethical considerations. However, the results of the present study are consistent with those of recent histologic studies that reported that PRP alone cannot significantly affect treatment outcomes.[32,36–39] Bezgin et al.[32] concluded that PRP successfully created a scaffold for regenerative endodontic treatment; however, treatment outcomes did not differ significantly between PRP and a conventional blood clot scaffold. In another study, Martin et al.[36] found that PRP may enhance wound healing only if parenchymal tissue has not been completely destroyed; it may not otherwise induce tissue regeneration. This would explain the similar outcomes between PRP and the blood clot in the present study because only totally necrotic teeth were included.

Zhu et al.[37] used PRP and dental pulp cells in a canine study and found a lower rate of dentin wall thickening and less mineralized tissue formation when PRP was used alone. This result was attributed to more rapid degradation of growth factors in the PRP.

In the present study, 3D CBCT was used to examine periapical and root length changes based on the fact that previous studies[40,41] have revealed 3D CBCT to be more sensitive than periapical radiographs in detecting periapical lesion treatment outcomes. Estrela et al.[33] effectively used 3D CBCT and periapical index scoring to measure radiolucent areas. In the present study, bone density was quantitatively measured using the tools within the software. A significant decrease in lesion size and increase in bone density were observed.

Narang et al.[38] also concluded that blood clots and PRP show comparable results in terms of apical closure, root lengthening, dentinal wall thickening, and periapical healing. In another study by Jadhav et al.,[39] PRP was more effective than blood clots in revascularization, and the authors ascribed the success of PRP to stimulation of collagen production; sustained release of growth factors; and enhanced recruitment, retention, and proliferation of undifferentiated mesenchymal and endothelial cells from the periapical area.

At the time of this writing, all patients were still being monitored to determine the long-term clinical success of PRP as a successful scaffold for regenerative protocols.

Conclusion

The results of this study suggest that PRP can act as a successful scaffold for regenerative endodontic treatment. However, the results of treatment with PRP were not significantly different from those of the conventional protocol using a blood clot as a scaffold.

What this paper adds

- Platelet-rich plasma can act as a successful scaffold for regenerative endodontic treatment.
- Platelet-rich plasma results in an early and significant increase in root length compared with the conventional protocol using a blood clot as a scaffold.

Declaration of conflicting interest

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