A comparison of two pulp revascularization techniques using platelet-rich plasma and whole blood clot

Naren Ramachandran, Shishir Singh, Rajesh Podar, Gaurav Kulkarni, Roshan Shetty, Padmini Chandrasekhar
Department of Conservative Dentistry and Endodontics, Terna Dental College and Hospital, Navi Mumbai, Maharashtra, India

Abstract

Aim: The aim of this preliminary study was to compare outcomes of two regenerative endodontic protocols in necrotic teeth with open apices.

Methodology: Forty teeth with open apices in patients with an age range of 15-54 were randomly distributed in two groups: group 1, with whole blood clot in the canal (n=20) and group 2, whole blood with Platelet rich plasma (PRP) in the canal (n=20). Clinical and radiological follow up assessments were undertaken over a period ranging from 6 months to one year. Radiographic Root Area (RRA) was measured using the freeware ImageJ. Statistical analysis was performed using the independent samples t test and the Chi-squared test, the significance level was set at p=0.05.

Results: There was no difference in the percentage change in RRA between the PRP group and whole blood-clot + PRP group.

Conclusion: Whole blood clot and PRP are comparable on the grounds of percentage change in RRA and there is no statistically significant difference between the two in a follow-up period of up to one year.

Keywords: Platelet-rich plasma, pulp revascularization, regenerative endodontics

INTRODUCTION

Regenerative endodontics is considered as a “paradigm shift” in the management of immature necrotic teeth. What was considered to be an experimental treatment protocol a few years previously has become more established, with a concurrent increase in the published literature in this field. The American Association of Endodontists and the European Society of Endodontontology have released position statements and clinical guidelines for regenerative endodontic procedures (REPs) in recent years. Necrotic immature teeth have been conventionally treated with calcium hydroxide apexification. Owing to the long treatment duration as well as the dentin-weakening effects of calcium hydroxide, a one- or two-step apical barrier technique using mineral trioxide aggregate (MTA) is preferred. However, neither of these procedures leads to an increase in the root length or width, and the tooth continues to remain weak. Regenerative endodontics has been defined as “biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentine complex.” Besides requiring disinfection of the canal system, a suitable scaffold, and growth factors, the regenerative endodontic protocol also requires the introduction of stem cells in the canal space. Studies have demonstrated equally good outcomes for REPs, as well as MTA apexification.

Whole blood clot has been typically used as a scaffold and is created by deliberate overinstrumentation of the periapical...
tissues to induce bleeding in the canal. An alternative to using whole blood is to use blood drawn by venepuncture and injected in the canal space. Autologous fibrin matrices such as platelet-rich plasma (PRP)\cite{11,12} and platelet-rich fibrin (PRF)\cite{13} have been used as an alternative or adjunct to whole blood. Essentially, these platelet concentrates provide a higher concentration of growth factors that are released over a prolonged period of time as compared to whole blood. The platelet concentrates differ in their method of preparation, activation, and duration of release of growth factors. The difference in outcome of REPs when different platelet matrices are used is not clear. When whole blood and a combination of blood clot and PRP were compared, after 1 year, the latter had a more favorable response in terms of periapical healing and dentinal wall thickening.\cite{13} When PRP, PRF, and whole blood clot were compared, all three were found to be comparable on the grounds of root lengthening, thickening, and response to vitality testing; periapical healing was better in the PRP group; however, it was concluded that whole blood clot is preferable to using PRP.\cite{14} Studies have usually analyzed the radiographic outcome qualitatively,\cite{13} as it is difficult to standardize radiographs and quantify the root growth. One study used a digital ruler to measure the increase in root length,\cite{14} whereas another used a computer-based image measurement tool that calculated percentage changes in root length and dentin wall thickness.\cite{15} The aim of this study was to compare two REPs, both employing different scaffolds – whole blood and a combination of whole blood and PRP, and to quantify the change in radiographic root area (RRA) over a 1-year follow-up using a computer-based measurement method. The null hypothesis was that there is no difference in the change in RRA over a 1-year follow-up between the whole blood clot group and the PRP and blood clot combination group.

**MATERIALS AND METHODS**

The study was approved by the institutional ethics committee (Number TDC/IRB-EC/31/2012). The sample consisted of 40 patients who reported to the outpatient section of the Department of Conservative Dentistry and Endodontics. Out of the 40 patients, 27 were male and 13 were female, with an age ranging from 15 to 54. Relatively healthy individuals with a noncontributory medical history and having immature permanent teeth with necrotic pulps and apical periodontitis were included. Patients with a significant medical history such as bleeding disorders and those with teeth having poor periodontal and restorative prognoses were excluded from the study. Patients with known allergies to antimicrobial drugs were also excluded from the study. The entire treatment procedure, possible outcomes, and alternate treatment options were explained to the patient or their legal guardian in case of young patients. They were informed about the lengthy follow-up and the necessity to assess the outcome of the treatment at subsequent visits. The participation in the study was voluntary. After obtaining signed informed consent forms, the patients were randomly allocated to one of the two treatment arms – REP using whole blood (n = 20) and REP using a combination of blood clot and PRP (n = 20). A web page (www.randomizer.org) was used for the randomization process.

**Preoperative steps**

Preoperative radiographs were taken, and vitality tests were performed with Endo Ice® refrigerant spray (Coltene, Ohio, USA) and an Electric Pulp Tester (EPT) (Denjoy® New Vitality Pulp Tester, Hunan Province, China) for all patients. A preoperative radiograph was taken using the paralleling technique with a digital radiograph system (Schick by Sirona, NY, USA) and sensor holder (Sensibles universal sensor holding device, Sylmar, USA) for all cases to establish a baseline.

**First appointment**

For all 40 patients, this part of the protocol was common. Local anesthesia with vasoconstrictor (lignocaine 2% with 1:100,000 epinephrine, Lignox, Indoco Remedies Ltd., India) was administered by suprapriosteal infiltration injection, and the tooth was isolated with a rubber dam. The rubber dam sheet, clamp, and the tooth were swabbed with 5% sodium hypochlorite solution (NaOCl).\cite{16} Access was gained into the tooth with a sterile diamond point (Endo Access Bur, Dentsply Maillefer). Working length was established using an electronic apex locator (Propex II, Dentsply) and verified radiographically. Removal of the necrotic tissue from the canal space was accomplished using gentle irrigation with 20 mL of 1% NaOCl solution (Prime Dental, Mumbai, India). This was followed by irrigation with 5 mL of sterile water (Ranbaxy Laboratories, India). The canals were then dried with presterilized paper points (Dentsply Maillefer, Ballaigues, Switzerland).

**Canal disinfection using triple antibiotic paste**

Triple antibiotic paste (TAP) was prepared by scraping off the coating on tablets of ciprofloxacin, metronidazole, and minocycline using a sterile surgical blade and crushed to a powder using a mortar and pestle. The powder and sterile water were mixed in a ratio of 1:1:1 to form a loose paste with a runny consistency. The paste was carried on a sterile K-file premeasured at the working length and gently coated on the canal walls. The paste was placed below the cementoenamel junction (CEJ) to avoid the discoloring effect of minocycline. A double coronal seal was placed using Cavit G (3M ESPE, St. Paul, MN, USA) followed by type 2 restorative glass-ionomer cement (GIC) (GC Corp., Japan). The medicament was left in the canal for a minimum of 3 weeks.
Second appointment
Using deliberate overinstrumentation to create a whole blood clot – Group 1
It was ensured that all clinical signs and symptoms had subsided. The persistence of symptoms was taken as inadequate disinfection, and the disinfection protocol was repeated as previously explained. Anaesthesia was achieved using plain lignocaine 2% without vasoconstrictor (Lignox, Indoco Remedies Ltd., India) before isolation with rubber dam. After regaining access, the medicament was gently irrigated out of the root canal with 20 mL of 17% ethylenediaminetetraacetic acid (EDTA) (Prime Dental, Mumbai, India) followed by drying with paper points. A sterile 20-K file was used 2 mm beyond the working length of the tooth, and with sharp strokes, it was pushed beyond the canal to intentionally induce bleeding into the canal. A small, sterile cotton pellet was held inside the canal for 6–7 min to allow the formation of a blood clot. Following this, white MTA (Angelus, Londrina, Brazil) was placed in a 3–4 mm thick layer directly over the clot. Type 2 GIC was placed as a coronal seal. A postoperative radiograph was taken to confirm the placement of the MTA, and the patient was scheduled for recall.

Using platelet-rich plasma – Group 2
Venous blood was obtained using an intravenous cannula set from the median cubital vein. The blood was directly collected in 2 sterile vacutainers with acid citrate dextrose as an anticoagulant. Nine milliliters of blood was withdrawn in each tube; the blood collection in the vacutainer ended as soon as the vacuum inside the tube had been exhausted, ensuring that exactly 9 mL of blood was collected for each sample. The tubes were placed in a centrifuge (REMI 8M, Mumbai, India), ensuring that the tubes were counterbalanced, and an initial spin was performed at 1201for 10 min. At the end of this spin, two well-defined columns were evident in the vacutainers – a supernatant clear plasma and buffy coat, along with a bottom layer of red blood cells. The upper layer along with the buffy coat was aspirated with a sterile spinal needle and dispensed into a plain vacutainer with no additives. The second tube was subjected to a spin at 2000 RPM for 10 min. At the end of the second spin, the platelet-poor plasma remained as a supernatant and highly concentrated PRP collected at the bottom of the tube. The supernatant was discarded, and the PRP was decanted onto a sterile glass container. It was activated by adding 0.1 mL of calcium chloride to neutralize the anticoagulant. A sterile 20-K file was used beyond the canal to deliberately injure the periapical tissues and induce bleeding within the canal space before injecting the PRP into the canal.[12] Subsequently, a collagen sponge soaked with the PRP was placed in the apical third of the canal, and the scaffold was allowed to gel. Finally, MTA was placed on top of the PRP till below the CEJ, and the access cavity was sealed with Type 2 GIC.

Follow-up and evaluation
Twelve patients were unavailable for recall, one of them being deceased. Four cases were considered as failed because of the presence of pain. The remaining patients were recalled at 6 months and 12 months. Three patients did not report for the 6-month follow-up but were available for the 12-month follow-up. At the follow-up, clinical examination was undertaken to check for signs that indicated the persistence of periapical disease – including palpation of soft tissues and percussion testing of the teeth. Pulp tests using Endo Ice® as well as EPT were also performed. A periapical radiograph was taken at each follow-up appointment to compare with the baseline. The radiographs are shown in Figures 1 and 2 for both groups.

Measurement and calculation of change in root surface area
The radiographs were imported in the freeware ImageJ v 1.47 (U. S. National Institutes of Health, Bethesda, Maryland, USA), and the RRA was calculated according to the protocol mentioned in a previous study, as shown in Figure 3.[17] A blinded endodontist performed two measurements 1 week apart, and the mean of the two scores was selected as the final score. The percentage change in RRA was calculated for each patient.

RESULTS

Group 1 (blood clotting group)
The mean preoperative RRA in the 14 samples in this group was 20791.33 as measured by the polygon tool in the ImageJ software. The mean postoperative RRA at the 6-month follow-up was 21,351.07 and at the 12-month follow-up was 22,492.20. The percentage change in RRA at the 6-month appointment was 3.036% at the 6-month follow-up and 9.843% at the 12-month follow-up.

Group 2 (platelet-rich plasma group)
The mean preoperative RRA in the 10 samples in this group was 21,555.79 as measured by the polygon tool in the ImageJ software. The mean postoperative RRA at the 6-month follow-up was 22,415.29 and at the 12-month follow-up was 23,377.14. The percentage change in RRA at the 6-month appointment was 4.467% at the 6-month follow-up and 9.564% at the 12-month follow-up.

Intergroup comparison was performed using the unpaired Student t-test. An $\alpha = 0.05$ was considered statistically significant. The $P$ value for difference in RRA at the 6-month period was 0.616 ($P > 0.05$). The $P$ value for percentage change in RRA at the 6-month period between the two groups was 0.663 ($P < 0.05$). At the 12-month period between the two groups, $P$ values for difference in RRA and percentage change in RRA were 0.927 ($P > 0.05$).
and 0.973 (P > 0.05), respectively. Although the PRP group showed increased RRA, this was not statistically significant.

None of the cases in either group showed an increase in periapical radiolucency, and none of them responded to vitality testing on the follow-up visits.

**DISCUSSION**

Both calcium hydroxide apexification and placement of an apical MTA plug have predictable outcomes,[18] but neither has the potential to increase the length and width of the thin roots. Revascularization in an immature necrotic tooth was reported by Iwaya et al. in 2001.[19] It was reported that the surviving pulp cells in the apical portion of the canal can proliferate if the root canal infection is eliminated.

The causal relationship between microorganisms and root canal infection is well established.[20,21] Root canal infection is a polymicrobial biofilm-based disease.[20,22,23] Root canal treatment is a series of steps consisting of mechanical debridement with files and chemical disinfection using irrigating solutions such as NaOCl. The “biocompatible disinfection” strategy in REPs is different from conventional root canal treatment.[24] Mechanical instrumentation has traditionally not been performed in REPs, and the published guidelines advocate the use of chemical disinfection in the form of irrigation with 1% NaOCl. The use of 17% EDTA is recommended during the second appointment of REP as it enables release of growth factors directly from within the dentin.[25] Iwaya et al. reported using a mixture of ciprofloxacin and metronidazole in the canal to obtain disinfection of the canal space,[19] and subsequently, Banchs and Trope[26] added minocycline to this mixture which came to be known as TAP, which has shown good antimicrobial efficacy in *in vitro* studies.[27] Problems associated with the usage of TAP are (i) development of antibiotic resistance, (ii) discoloration of teeth, and (iii) incomplete removal from the canal.[28] The ESE clinical guidelines recommend using calcium hydroxide as an alternative to obviate the problems associated with the usage of TAP.[29] Alternatively, a double antibiotic paste avoiding minocycline or a TAP with cefaclor substituted in place of minocycline may be used to avoid discoloration. At the time, the cases in this study were performed, the
Ramachandran, et al.: Pulp revascularization with PRP

Guidelines recommended using TAP in the canal, and therefore, TAP was used as the intracanal medicament. Updated guidelines also suggest using a thinner mix and a lower concentration of TAP delivered through a syringe. TAP was placed below the CEJ to avoid discoloration, but discoloration was still seen in 12 patients in this study. Subsequent studies have reported that a layer of bonding agent can be applied in the access cavity walls and light cured to prevent this.\[30\]

The role of a blood clot in wound healing and its experimental use in endodontics was recognized as early as 1961 by Ostby in his histological study involving a case series of 9 patients.\[31\] The blood clot acts as a scaffold for subsequent tissue ingrowth. The bleeding can be unpredictable as there could be little or no bleeding on laceration of the periapical tissues.\[32-34\]

In 2011, a case report of an REP using PRP was added to the growing endodontic literature in this field. This was the first time in the literature that PRP was used as a scaffold for an REP.\[11\] PRP has been suggested as an alternative for whole blood owing to its many advantages – as an autologous fibrin matrix, it is naturally high in growth factors and has many adhesion molecules required for cell migration.\[35,36\] The preparation of PRP also eliminates erythrocytes which have no role to play in the healing process and tend to disintegrate shortly after placement. Calcium chloride

Figure 2: (Group 2): Periapical radiographs of patients treated using platelet-rich plasma. Cases (a-h) representing (1) preoperative radiograph, (2) immediate postoperative radiograph after the second appointment, (3) 6-month follow-up, and (4) 12-month follow-up of regenerative endodontic procedure using platelet-rich plasma. (e) Immediate postoperative radiograph after placing triple antibiotic paste in the first appointment

Figure 3: Representative periapical radiographs indicating the digital alignment and measurement procedure for calculation of radiographic root area. (i1, i2) Geometric alignment of radiographs (j1, j2) Outlining of total root area and canal space using the polygon tool
and thrombin are added to the PRP to neutralize and activate the platelets, respectively. In this study, bleeding was induced in the canal before placing the PRP to avoid using bovine thrombin owing to concerns associated with allergies and religious sentiments. In this way, the patient’s own thrombin present in their blood activated the PRP placed in the canal.

Two patients returned for a follow-up 4 years after the treatment. One of them showed creation of a calcific barrier within the root canal, and one case showed persistence of the periapical lesion. The possible treatment outcomes have been described by Chen et al.[37] The primary objective of elimination of signs and symptoms was observed in 24 patients. None of the patients in this study showed complete root apex closure within the time frame of the study. Although few studies have reported the return of pulp vitality on EPT testing,[11,19] it has not been consistently reported in other studies. The possibility of a pulpless tooth with MTA in the coronal third, responding to EPT or Endo Ice,[6] is questionable. No tooth in this study responded to vitality testing with EPT as well as Endo Ice[6]. There is no defined cutoff period after which a case may be considered as a failure, and Nosrat et al. reported a case where maturogenesis had not happened even after 6 years.[38] The results of this study are in contrast to those of Jadhav et al.[12] which does not necessarily reflect a true difference in outcomes but reinforces the fact that the results obtained with REPs are not always consistent.

CONCLUSION

Within the limitations of this study, there was no difference in percentage RRA change between whole blood clot and PRP used for REPs. The revascularization procedure provides several potential benefits over conventional root canal treatment because it allows for continued root development. However, not much is known about the multiple variables that may account for different outcomes. More long-term clinical outcome studies and randomized clinical trials on REPs are required for identifying whether any particular technique is better than the other.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Kim SG, Malek M, Sigurdsson A, Lin LM, Kahler B. Regenerative endodontics: A comprehensive review. Int Endod J 2018;51:1367-88.
2. AAE Position Statement: Scope of Endodontics: Regenerative endodontics. J Endod 39:561-3.
3. Galler KM, Krast G, Simon S, Van Gorp G, Meschi N, Vahedi B, et al. European society of endodontology position statement: Revitalization procedures. Int Endod J 2016;49:717-23.
4. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: A review of current status and a call for action. J Endod 2007;33:377-90.
5. Bezug D, Sönmez H. Review of current concepts of revascularization/revitalization. Dent Traumatol 2015;31:267-73.
6. Diogenes A, Henry MA, Teixeira FB, Hargreaves KM. An update on clinical regenerative endodontics. Endod Topics 2013:28:2-23.
7. Galler KM. Clinical procedures for revitalization: Current knowledge and considerations. Int Endod J 2016;49:926-36.
8. Law AS. Considerations for regeneration procedures. J Endod 2013;39:S44-S66.
9. Alobaid AS, Cortes LM, Lo J, Nguyen TT, Albert J, Abu-Melha AS, et al. Radiographic and clinical outcomes of the treatment of immature permanent teeth by revascularization or apexification: A pilot retrospective cohort study. J Endod 2014;40:1063-70.
10. Kahler B, Rossi-Fedeley L, Chugul N, Lin LM. An evidence-based review of the efficacy of treatment approaches for immature permanent teeth with pulp necrosis. J Endod 2017;43:1052-7.
11. Torabinejad M, Turman M. Revitalization of tooth with necrotic pulp and open apex by using platelet-rich plasma: A case report. J Endod 2013;39:1063-70.
12. 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;38:1581-7.
13. Keswani D, Pandey RK. Revascularization of an immature tooth with a necrotic pulp using platelet-rich fibrin: A case report. Int Endod J 2013;46:1096-104.
14. Shivashankar VY, Johns DA, Maroli RK, Sekar M, Chandrasekarar K, Karkikeyan S, 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:ZC34-9.
15. Kahler B, Mistry S, Moule A, Ringsmuth AK, Case P, Thomson A, et al. Revascularization outcomes: A prospective analysis of 16 consecutive cases. J Endod 2014;40:333-8.
16. Ng YL, Spratt D, Sriskantharajah S, Gulaibivala K. Evaluation of protocols for field decontamination before bacterial sampling of root canals for contemporary microbiology techniques. J Endod 2003;29:317-20.
17. Flase NK, Gibbs JL, Diogenes A, Hargreaves KM, Khan AA. A standardized novel method to measure radiographic root changes after endodontic therapy in immature teeth. J Endod 2014;40:46-50.
18. Chala S, Abouqal R, Rida S. Apllication of immaturity teeth with calcium hydroxide or mineral tioxide aggregate: Systematic review and meta-analysis. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2011;112:e36-42.
19. Iwaya SI, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with apical periodontitis and sinus tract. Dent Traumatol 2011;27:185-7.
20. Möller AJ, Fabricius L, Dahlén G, Ohman AE, Heyden G. Influence on periapical tissues of indigenous oral bacteria and necrotic pulp tissue in monkeys. Scand J Dent Res 1981;89:475-84.
21. Kakeshi S, Stanley HR, Fitzgerald RJ. The effects of surgical exposures of dental pulps in germ-free and conventional laboratory rats. Oral Surg Oral Med Oral Pathol 1985;20:340-9.
22. Ramachandran Nair PN. Light and electron microscopic studies of root canal flora and periapical lesions. J Endod 1987;13:29-39.
23. Fabricius L, Dahlén G, Holm SE, Möller AJ. Influence of combinations of oral bacteria on periapical tissues of monkeys. Scand J Dent Res 1982;90:200-6.
24. Kishen A, Peters OA, Zehnder M, Diogenes AR, Nair MK. Advances in endodontics: Potential applications in clinical practice. J Conserv Dent 2016;19:199-206.
25. Galler KM, Buchhalla W, Hiller KA, Federlin M, Echt A, Schierersteiner M, et al. Influence of root canal disinfectants on growth factor release from dentin. J Endod 2015;41:363-8.
26. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: New treatment protocol? J Endod 2004;30:196-200.
27. Hoshino E, Kurihara-Ando N, Sato I, Uematsu H, Sato M, Kota K, et al. In vitro antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. Int Endod J 1996;29:125-30.
28. Berkhoff JA, Chen PB, Teixeira FB, Diogenes A. Evaluation of triple antibiotic paste removal by different irrigation procedures. J Endod 2014;40:1172-7.
29. Segura-Egea JJ, Gould K, Shen BH, Jonasson P, Cotti E, Mazzoni A, et al. European society of endodontology position statement: The use of antibiotics in endodontics. Int Endod J 2018;51:20-5.
30. Bansal R, Jain A, Mittal S. Current overview on challenges in regenerative endodontics. J Conserv Dent 2015;18:1-6.
31. Ostby BN. The role of the blood clot in endodontic therapy. An experimental histologic study. Acta Odontol Scand 1961;19:324-53.
32. Ding RY, Cheung GS, Chen J, Yin XZ, Wang QQ, Zhang CF. Pulp revascularization of immature teeth with apical periodontitis: A clinical study. J Endod 2009;35:745-9.
33. Petrin JA, Boda KK, Shambarger S, Bowles WR, McClanahan SB. Challenges in regenerative endodontics: A case series. J Endod 2010;36:536-41.
34. 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;37:562-7.
35. Marx RE. Platelet-rich plasma (PRP): What is PRP and what is not PRP? Implant Dent 2001;10:225-8.
36. Marx RE. Platelet-rich plasma: Evidence to support its use. J Oral Maxillofac Surg 2004;62:489-96.
37. Chen MY, Chen KL, Chen CA, Tayebat F, Rosenberg PA, Lin LM. Responses of immature permanent teeth with infected necrotic pulp tissue and apical periodontitis/abscess to revascularization procedures. Int Endod J 2012;45:294-305.
38. Nosrat A, Homayounfar N, Oloomi K. Drawbacks and unfavorable outcomes of regenerative endodontic treatments of necrotic immature teeth: A literature review and report of a case. J Endod 2012;38:1428-34.