Long-term follow-up of traumatized immature necrotic permanent teeth treated with regenerative endodontic protocol using platelet-rich fibrin: A prospective case series

Ruchika Roongta Nawal, Shivani Utneja, Vivek Sharma, Sudha Yadav, Sangeeta Talwar
Department of Conservative Dentistry and Endodontics, Maulana Azad Institute of Dental Sciences, New Delhi, India

Abstract
The purpose of this prospective case series was to report the long-term clinical and radiographic outcomes of regenerative endodontic procedures (REP) using platelet-rich fibrin (PRF), in traumatized immature permanent teeth with necrotic pulps. Six immature necrotic permanent maxillary central incisors from six patients were treated with REP supplemented with PRF. Patients were recalled at 6, 12, 18, 24, 36, 48, and 60 months. Radiographic changes in the radiographic root area (RRA), apical diameter, root thickness, and root length were quantified. Clinically, tooth survival in terms of tooth retention in the arch and absence of any sign and symptoms of endodontic pathology were noted. All the teeth survived and met the clinical criteria for success throughout the follow-up period. Follow-up radiographs at 5 years revealed complete resolution of the periapical lesions in all patients and the average apical diameter decrease was 30.96%. An average increase of 13.18% for root length, 40.20% for root thickness, and 26.35% for RRA were noted.

Keywords: Immature teeth; long-term follow-up; platelet-rich fibrin; revascularization

INTRODUCTION
Traumatized immature permanent teeth with pulp necrosis have been conventionally treated with either calcium hydroxide or Apexification. However, these treatment modalities do not promote further root development. Regenerative endodontic procedure (REP) has emerged as a promising treatment modality for such cases. Contrary to Apexification, REP encourages the development of longer and thicker dentinal root wall decreasing the propensity of long term root fracture.1-2

The conventional revascularization involves the disinfection of the root canal system followed by inducing bleeding via over-instrumentation. This results in the formation of a blood clot within the root canal acting as scaffold. However, bleeding cannot be evoked in every case.3-4 Recently, three-dimensional scaffolds such as platelet-rich plasma and platelet-rich fibrin (PRF) with improved efficacy and increased concentration of growth factors have been developed to promote dental tissue regeneration. The PRF membrane releases high quantities of growth factors for at least 1 week and up to 28 days making it an ideal scaffold material to be utilized in REP.5-7

This case series demonstrates the outcome of revascularization supplemented with PRF in traumatized permanent immature necrotic anterior teeth.

CASE REPORT
Six patients (13–28 years of age; 02 females, and 04 males; Ethnicity-South Asian) with immature necrotic permanent...
maxillary anterior teeth with infected necrotic pulps and apical periodontitis/abscess were referred to the department of Conservative dentistry and endodontics. All of the patients were healthy with no underlying systemic disorders. All the teeth had a history of traumatic injury followed by necrosis of the pulp. The patients presented with either fractured or discolored anterior teeth and were tender to percussion. Cold test and electric pulp test gave a negative response in all the cases suggestive of pulp necrosis. Radiographically, the teeth had an incompletely formed apex, either in the form of a wide canal with parallel walls or blunderbuss. A common final diagnosis of pulp necrosis with symptomatic apical periodontitis was made. Considering the wide-open apices and incomplete root development, REP with PRF was considered. Ethical clearance was obtained from the institute's ethical committee board. Informed consent was obtained from patients before initiating treatment.

**Revascularization protocol**

All patients were treated by a single endodontist using a similar treatment protocol. On the initial visit, the diseased tooth was anesthetized with lignocaine (1:100,000 adrenaline) and access preparation was done under rubber dam isolation. After confirming the working length radiographically, each root canal was thoroughly irrigated with 20 ml of 1.5% sodium hypochlorite (NaOCl) (Novo Dental Product, India) using a side vented needle. Triple antibiotic paste (TAP) was used as an intracanal medicament. TAP was mixed in a thin consistency in a proportion of 1:1:1 in a concentration of 0.1 mg/ml. The canal was dried with sterile paper points and TAP was placed in the canal. The access was sealed with a temporary restoration (Cavit™ (3M ESPE) and the patients were called after 3 weeks.

In the subsequent visit, if the tooth was asymptomatic, revascularization was performed as follows. The tooth was isolated with rubber dam and re-accessed. TAP was removed under a dental operating microscope with copious amounts of 1.5% NaOCl as irrigant for 15 min, thereafter 20 mL of 17% EDTA (Prevest Denpro, Jammu, India) irrigant was used and final rinsing was accomplished with sterile saline.

A 10 ml sample of whole blood was drawn intravenously from the patient’s right antecubital vein and immediately centrifuged at 3000 rpm for 10 min to make PRF. It was gently pressed into a membrane with sterile gauze. PRF membrane was cut into small pieces and condensed into the canal up to 2–3 mm below the cementoenamel junction (CEJ). ProRoot White MTA (Dentsply Tulsa, UK) was mixed according to manufacturer instructions and was adapted over PRF. A moist cotton pellet was placed over Mineral Trioxide Aggregate (MTA), and the access was then provisionally sealed with glass ionomer cement.

The patient was recalled for final coronal restoration after 1 week. The final restoration was done with composite restoration (Z100 Restorative; 3M ESPE, St Paul, MN).

**Follow up evaluation**

Patients were recalled at 6, 12, 18, 24, 36, 48, and 60 months. Follow-up included clinical and radiographic analysis. Preoperative and postoperative periapical radiographs were evaluated for the presence or absence of periapical radiolucency and standardized radiographic assessment included the following:

An increase in radiographic root area (RRA), root length, root thickness and decrease in apical diameter.

Clinical and radiographic follow-up examinations were performed by 2 experienced endodontists and inter examiner validity was assessed by using kappa statistics.

**Image registration and analysis**

Preoperative and postoperative images of digitized radiographs were opened in the Image-J software program (National Institutes of Health, Bethesda, MD). Preoperative and recall radiographs were aligned using the TurboReg plug-in (Biomedical Imaging Group, Swiss Federal Institute of Technology, Lausanne, VD, Switzerland). After alignment, RRA, length, width, and apical diameter were measured. Figure 1 shows sample images demonstrating digital measurements using Image J software (V. 1.44p; National Institutes of Health, Bethesda, MD). RRA was measured using the methodology described by Flake et al. All the data were presented as a percentage.

**Figure 1:** Sample images demonstrating digital measurements using ImageJ software (a) root length (b) root thickness at the apical third (c) inner canal space thickness at the apical third (d) apical diameter (e) radiograph showing outlining of the total root area radiograph (f) showing outlining of the canal space
change from preoperative values rather than the actual measurements to minimize one potential source of systematic error.

**Clinical findings**

Clinical findings were evaluated based on both survival and clinical success criteria as used by Saoud et al. Clinically, the presence/absence of sign and symptoms of endodontic pathology such as tenderness to percussion or palpation, swelling, sinus tract, and spontaneous pain were noted. Survival was defined as the tooth remaining present in the arch throughout the study period. Clinical success was defined as a tooth that survived and did not require another endodontic intervention during the recall period.

**Statistical analysis**

The data were analyzed using SPSS 16.0 (IBM, Armonk, NY, USA) statistical software. Two-way analysis of variance (ANOVA) with the mixed model was applied to find the intraclass correlation coefficient between the two observers. The percentage change from the preoperative was determined and reported with a mean percentage change and its standard deviation. \( P < 0.05 \) was considered to be statistically significant. The repeated measures ANOVA test was used to test for significant changes in RRA, root length, root thickness, and apical closure at the baseline versus different follow-up periods.

**RESULTS**

**Clinical outcomes**

All six teeth survived and met the criteria for clinical success at the end of the follow-up period. None of the teeth regained responsiveness to pulp vitality tests.

**Radiographic outcome**

All patients with an initial periapical radiolucency showed complete resolution of radiographic signs in the 4–5 year follow-up [Figure 2]. The resolution of the periapical lesion was evident in a 1–2 year follow-up for all cases. A significant decrease in the mean apical diameter from baseline to follow-up period was observed. At 4–5 year follow-up interval, the average apical diameter decrease was 30.96% and the mean reduction in apical diameter was 0.476 mm [Table 1]. Significant changes in the average root length, root thickness, and RRA in the study population were observed between baseline and 4–5-year intervals. An average increase of 13.18% for root length, 40.20% for root thickness, and 26.35% for RRA were noted [Table 1].

**DISCUSSION**

Previous studies have shown that revascularization of immature necrotic permanent teeth has a high clinical success rate (78%–100%) in the initial 1–2.5 years. However, none of the studies have given a long-term estimate of clinical success in such cases. To the author’s knowledge, this is the first prospective case series using PRF as a scaffold to evaluate the clinical and radiographic outcomes of REP in immature permanent necrotic teeth over a 5-year follow-up period.

In revascularization, the most important factor during case selection is a compliant patient or parent as the treatment involves multiple appointments and stringent follow-up visits. In long-term studies, patients have to be regularly motivated to come for regular follow-up by educating them about the advantages of the prescribed treatment modality.

Most of the studies on revascularization procedures have been conducted on adolescent patients mostly aged 8–16 years. Patients in this study included a mixture of adolescents and adults in the age range of 13–28 years. Compared to other studies in which traditional revascularization protocols were employed, we used autologous PRF membrane. Recently, many investigators have found remarkable results with the use of this immune concentrate in regenerative endodontics. It was speculated that owing to the trimolecular architecture of PRF, it can support cytokine enmeshment and cellular migration. In addition, physiological thrombin available in PRF creates equilateral junctions in polymerized fibrin, which results in long-term growth factor release (up to 28 days). Furthermore, the presence of leukocytes and cytokines in PRF reduces infection and inflammation. Angiogenesis is increased because of the release of vascular endothelial growth factor, which plays an important role in revascularization.

No postoperative complications and failures such as discoloration and persistence of pain or swelling was noted in any of the case. It can be attributed to the strict disinfection protocol, use of additional scaffold (PRF) and bacteria tight coronal seal provided by MTA and composite resin. Discoloration is often reported with the use of TAP as intracanal medicament. However, it can be easily avoided by placing TAP below CEJ and by sealing the pulp chamber with dentin bonding agent. Furthermore, thorough removal of TAP should be ensured in second appointment under dental operating microscope as TAP has detrimental effect on survival of stem cells and also results in discoloration.

None of the teeth gained responsiveness to pulp sensibility tests at the end of 5-year recall. The lack of response might be because REP is less likely to result in regeneration of the dentin-pulp complex; rather there is repair because the canal is generally filled with tissue resembling fibrous connective tissue, bone, and cementum.

All the teeth demonstrated radiographic evidence of apical closure in the form of a blunt closed apex at the end of the study period. The average decrease in apical
diameter in the individual cases over a period of 4–5 years was 30.96% which was statistically significant. Apical closure of revascularized teeth has been observed in other studies at various follow-up times as the frequency and time of apical closure after REP varies owing to differences in age, sex, and treatment protocols.\textsuperscript{[3,10]} In this study, the longer time taken for apical closure might be attributed to the low stem cell population found in older patients.

The most consistent radiographic change observed in the patients treated was the increase in root thickness. To estimate the proportion of cases with a clinically meaningful change in root thickness, we applied a threshold of 20%...
change.[15] Using these criteria, we found 5 out of 6 cases met these criteria at the 3–4 year follow-up visit and all the 6 cases were well above the cut-off at the 4–5 year follow-up. An average of 27% increase at 21-month, 12.7% increase at 18 months, and 10% increase at 15-month was reported by Jeeruphan et al., Nagy et al. and Alobaid et al. respectively.[2,10,15]

The average change in RRA found in this study increased from 7.2% to 12.4%, 15.4%, and 26.3% at 0.5–1 year, 1–2 year, 2–3 year, and 4–5 year follow-up interval, respectively [Table 1]. The variation in the values of root thickness and RRA in different studies involving REP of immature permanent necrotic teeth is probably related to the difference in contributing etiology in various study populations.[2,10,15] Traumatized teeth may be less likely to achieve clinically meaningful continued root development as compared to immature teeth caused by other reasons. In the present study also trauma leading to pulpal necrosis was the contributory etiology; still, the results for the radiographic outcome are better than the previously reported changes in root thickness and RRA.

Out of all the radiographic parameters assessed, the least change was observed in root lengthening which increased from 3.23% to 6.12%, 8.23%, 13.18% at 0.5–1 year, 1–2 year, 2–3 year, and 4–5 year follow-up interval, respectively. These results, however, corroborate with other studies reporting a root length increase of 5%–15%.[2,9,15]

**CONCLUSION**

Although the level of evidence provided by the case series is quite low, well-documented case series can recognize potentially important factors affecting the efficacy of a treatment modality and can pave the way for well-designed future clinical trials/research. The findings reported here suggest that revascularization treatment supplemented with PRF membrane leads to continued root development of permanent immature necrotic teeth associated with periapical pathology even in older individuals. The long-term follow-up done in this case series gives better evidence of the success of REP compared to previously reported short-term studies.

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**Conflicts of interest**

There are no conflicts of interest.

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