Case Report

Regenerative Endodontic Treatment of an Immature Incisor Tooth with a Novel Platelet-Rich Product: A Five-Year Follow-Up Case Report

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Received: 28-Oct-2022;
Revision: 27-Feb-2023;
Accepted: 01-May-2023;
Published: 21-Sep-2023

Abstract

This case report presents a five-year follow-up and the management of a necrotic, immature incisor tooth treated by a novel platelet-rich plasma/platelet extract solution and platelet-rich fibrin (PRP–PES/PRF) and the restoration by using a stress-reduced direct composite restoration (SRDC) technique. The patient with a broken maxillary lateral tooth was referred to our clinic. Extra/intra-oral examinations were within normal limits. The nonvital tooth having an apical lesion and open-apical apex was treated with a regenerative endodontic treatment procedure (REP), and further root development with continued apical closure was shown in the follow-ups up to 60 months. In conclusion, the SRDC and PRP–PES/PRF combination can be an opportunity for the teeth requiring post-restoration. In contrast to the treatment strategy susceptible to weakening or fracturing the patient's tooth root, the REP approach may lead to a better prognosis for the patient's tooth.

Keywords: *Immature necrotic tooth, platelet-rich plasma, regenerative endodontics, stress-reduced direct composite restoration*

Background

The treatment of immature teeth with necrotic pulps presents a clinical challenge, and regenerative endodontic treatment procedures (REPs) have become popular as an alternative treatment option to the previously used techniques susceptible to weakening or fracturing the teeth roots, such as apexification procedures (treatment with calcium hydroxide in long term, or immediate placement of mineral trioxide aggregate [MTA] as apical plug).[1,2] Recent research has shown that REPs are enlarging into the field of tissue engineering.[2] The presence of all three of the "stem cells, scaffolds, and growth factors" as the three components required for regeneration in REPs is considered to improve the prognosis for these teeth.[2] Blood products can be used as both a scaffold and a growth-factor source. There are three different cell types found in blood: erythrocytes, leukocytes, and platelets.[3] Leukocytes have been utilized with platelets even though the use of erythrocytes in REPs is not widely documented in the literature. The use of leukocytes has the potential to trigger an inflammatory response. This inflammation aids chemotaxis and recovery; so, it can be an advantage, while a defensive response is required. However, the protective impact, meanwhile, could potentially be detrimental to regeneration.[3,4] Therefore, it is more typical to use pure platelet-rich products.[3] Growth factors and cytokines found in platelet-rich blood products can speed up tissue repair and regeneration. However, cell membranes of the platelets have been discovered to lead to apoptosis, and some evidence indicated that the platelets with cell membranes are catabolized more; thus, the release of bioactive proteins is less than anticipated. Eventually, we aimed to produce a novel blood product called platelet-rich plasma/platelet extract solution (PRP–PES) containing pure platelets without cell membranes.[1,3-6]

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Access this article online

Quick Response Code:

Website: www.njcponline.com

DOI: 10.4103/njcp.njcp_747_22

How to cite this article: Kolcu Mİ, Yurdakul H, Belli S. Regenerative endodontic treatment of an immature incisor tooth with a novel platelet-rich product: A five-year follow-up case report. Niger J Clin Pract 2023;26:1388-92.

Also, to provide successful treatment, a tight seal via final restoration is mandatory in regeneration cases. The American Association of Endodontics (AAE) published a consideration report in 2021 and indicated that the report should be followed to apply REPs by clinicians as one source of information.[7] Also, AAE has underlined that clinicians should review new findings[7] and also recommended that the cases should not be treated via REPs requiring a post/core-restoration for the inhibition of the usage of physical space for restoration rather than regeneration.[7] A post-free restoration should be considered in these cases by using polyethylene fiber (Ribbond Inc., Seattle, WA, USA) in combination with the composite restorative material to create a stress-free build-up restoration.[8,9] Using the stress-reduced direct composite restoration (SRDC) technique as a post-restoration in REPs is a novel way to give a vital treatment opportunity to the tooth with extreme structure loss.

This five-year follow-up case report aimed to present the management of a necrotic, immature maxillary left lateral incisor tooth by using novel platelet-enriched products and restoration by using an SRDC technique.

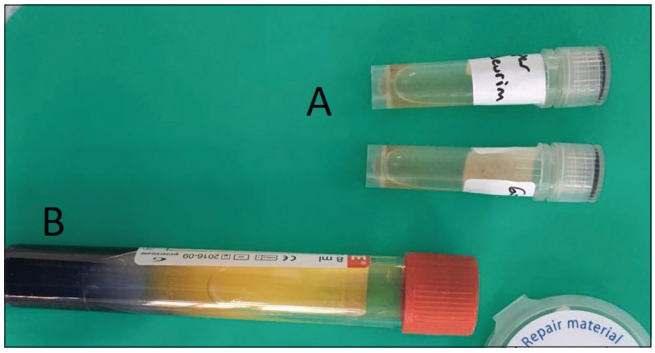


Figure 1: A indicates PRP-PES, B indicates PRF

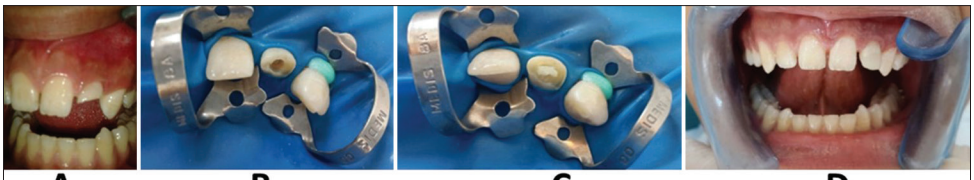
Case Report

This case report has been written under the PRICE (2020)/Care (2013) guidelines. A 16-year-old male patient was referred to our clinic on May 3, 2015, with the chief complaint of a broken maxillary lateral incisor tooth. The medical history was noncontributory. The patient reported a history of maxillary trauma eight years ago. Extra/intra-oral examinations were within normal limits, with no signs of swelling or lymphadenopathy. The responsible tooth was nonvital, and the patient had a positive response to the percussion test. A large periapical radiolucency and open apical apices were observed in the related tooth during the radiographic examination. The tooth was diagnosed with pulp necrosis and apical periodontitis. Informed consent including the treatment plan was obtained from the patient's relatives.

At the first appointment, the access was prepared under local anesthesia and rubber dam isolation. The root canal was located, and the working length was determined (1 mm short of the radiographic apex). The root canal system was gently irrigated with 1.5% of NaOCl (20 mL/canal for 5 min) followed by saline (20 mL/canal for 5 min), with an irrigating needle positioned at 1 mm far from the root end. The canals were then dried with paper points (Dentsply-Maillefer, Ballaigues, Switzerland). The triple antibiotic paste containing a mixture of Ciprofloxacin (Cipro 500 mg, Biofarma İlaç Sanayi ve Ticaret A.Ş., Istanbul, Turkiye), Metronidazole (Flagyl 500 mg, Aventis Pharma S.A., France), and Cefaclor (CEC 500 mg, Basel İlaç San veTic., Istanbul, Turkiye) was prepared with saline solution and placed into the canal using a lentulo-spiral. A cotton pellet was placed, and the cavity was temporarily sealed with a temporary sealing

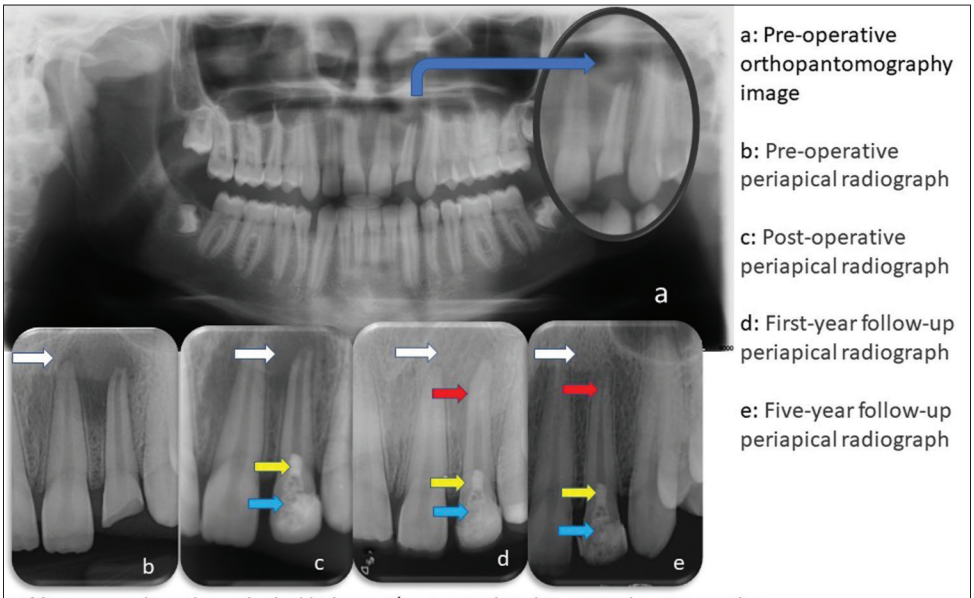
Table 1: Preparation of PRP–PES

40 cm^3 of blood was drawn into the injector with 10 cm3 of anticoagulant (citrate).
25 cm^3 were divided into 50 cm^3 conical-bottom centrifuge tubes and brought to room temperature.
10 cm^3 "density gradient media" was gently put into the tubes from the edges of the tube. It was centrifuged at 800 ×g for 15 min.
The supernatant on the "density gradient media" in both tubes was passed through a 5-µm pore filter and the platelets were separated into a 50-cm^3 conical-bottom centrifuge tube.
The filtrate was centrifuged at 1,300 ×g for 8 min. The supernatant was discarded. 15 cm^3 of 0.075 M hypotonic KCl solution was added to the top of the pellet at 37°C and homogenized with a sterile plastic Pasteur pipette. Tubes were incubated at 37°C for 15 min.
After incubation, 5 cm^3 of freshly prepared, cold (waited at −20°C for 30 min) Carnoy's fixative (1 unit of acetic acid +3 units of methanol) was added to the tube. After the tube was inverted, it was vortexed for 5 min.
All the liquid in the tube was transferred to a 50-cm^3 conical-bottom centrifuge tube by passing through a 1-µm pore filter with sufficient pressure.
The tube was centrifuged at 1,300 ×g for 16 min. The supernatant above 0.5 cm^3 was discarded.
5 cm^3 of isotonic NaCl solution was added to 0.5 mL pellet. Pasteur was homogenized with a pipette (*process repeated*). The tube was centrifuged at 1,300 ×g for 20 min. The supernatant above 0.5 cm^3 was discarded. 5 cm^3 of isotonic NaCl solution was added to 0.5 pellets. The tube was centrifuged at 1,300 ×g for 20 min. The supernatant above 0.5 cm3 was discarded.
0.5 cm^3 of PRP–PES was homogenized and transferred to an Eppendorf tube



A: Pre-operative clinical photograph, B: Cavite preparation during operation
C: Implementation of polyethylene fibres into the cavite, D: Post- operative clinical photograph

Figure 2: Clinical photographs of the treatment



White arrows *show the periapical lesion pre/postoperative changes and post operative trabecular structure in the apical region*
Red arrows *show increases in the wideness of the the root canal pre and postoperatively.*
Yellow arrows *show the placement of MTA, 3mm to cementoenamel-junction and blood clot.*
Blue *arrows show the placement of polyethylene fiber in the coronal restoration.*

Figure 3: Radiological photographs of the treatment

Table 2: Protocol for PRF

5 mL of whole venous blood is collected into a sterile vacutainer tube without anticoagulant.
The tube was centrifuged at 3,000 ×g for 10 min.
After fragmentation was completed into the three layers: (lower part as RBCs, the upper part as cellular plasma, and the middle part as fibrin clot)
The middle one was collected approximately 2 mm below the lower dividing line as PRF.[10]

material (Cavit, Dental Products of India, India). The patient was recalled after 21 days for the follow-up.

At the second appointment, the patient had no more clinical symptoms. For the PRP–PES and platelet-rich fibrin (PRF) preparation, a 40-mL sample of whole blood was drawn from the patient's right arm. In a special laboratory, PRP–PES was prepared with the following protocol given in Table 1, and PRF was prepared as presented in [Table 2 and Figure 1]. Local anesthesia was provided without any vasoconstrictor (safecaine 3%). After the rubber dam isolation, the root canal system was reopened, and the triple antibiotic paste was removed by irrigation with 17% ethylene diamine tetra acetic acid (30 mL/canal for 10 min). Bleeding was stimulated by a sterile 25 H-type file (Dentsply Maillefer, Ballaigues, Switzerland). PRP–PES were mixed with PRF, and the mixture was placed into the root canal space up to the level of the cement–enamel junction by an endodontic plugger. RetroMTA of 3 mm (BioMTA, Daejon, KOREA) was placed directly over the PRP clot to maintain a tight sealing. A moist cotton pellet was placed over the RetroMTA, and the patient was given an appointment for the next day to provide the setting of RetroMTA. During the third visit (after 24 h), the temporary restoration material was removed, and a two-step self-etch adhesive was applied to the coronal structure. After curing the adhesive for 30 s, a dual-cure resin cement (Clearfil SA Cement,

Kuraray, Japan) was injected. One piece of polyethylene fiber (20 mm long and 4 mm wide) (Ribbond, Ribbond Inc., Seattle, WA) was soaked with an unfilled resin (Ribbond adhesive, Ribbond Inc., Seattle, WA) and inserted on the REtroMTA. The free ends of the fiber were then folded,[6,7] and a build-up was created like a core restoration. It was aimed to reduce the stress of the composite restoration material. Polymerization was provided by a light curing device for 20 s with a minimum intensity of 700 mW/cm^2 (Bluephase, Ivoclar Vivadent, MV, Schaan, Liechtenstein). The rest of the crown was completed with a filled composite resin (Clearfil AP-X, Kuraray, Japan) by using an incremental technique [Figure 2].

Clinical and radiological examinations were performed in the follow-ups. The primary goal was achieved by the end of the first month, and the secondary goal was achieved by six months although it was more clearly detected at the 24th, 36th, 48th, and 60th month visits [Figure 3].

Discussion

REPs were defined as a treatment option and have been successfully performed in various clinical cases. These case reports have been crucial in developing more effective clinical regimens for REPs in both mature and immature teeth.[1,2,4] In the present case report, a new regenerative endodontic treatment protocol for the treatment of immature teeth (usage of PRP–PES) was presented. As a summary of key findings, the teeth, such as in the case needing postcore restorations, were recommended not to be treated with REPs options by AAE.[7] However, in consideration of the report of AAE released in 2021, the recommendation was to use the root canal spaces for restoration stability rather than regeneration.[7] This is one of the most important barriers to regeneration in damaged teeth, whereas the 35% etiology of necrosis in immature teeth is trauma and the remaining tooth structure is usually not sufficient.[10] Although some alternative new treatment procedures called the modified apexification technique were described in the literature,[11] the root development or maturation of the root canal walls was also reported as susceptible and might not occurred. With the improvements in dental materials like the discovery of polyethylene fibers, stability may also be provided without the use of root canal spaces, and polyethylene fibers and a combination of composite were used in this case with five years of clinical success for the first time documented in the literature.[8,9]

As another key point, a novel platelet-rich blood product was introduced in the present case report with some advantages based on the literature. To date, autologous blood products or blood clots have been used and recommended to promote regeneration capacity in REPs. Especially, compared with the other blood cells, platelets have been described as more efficient in REPs as more than 30 bioactive proteins are described in the cytoplasm of platelets.[3-6] However, the cell membranes (even in platelets) were defined to lead to apoptosis mechanism and also likely to create some disadvantages in the cases in which pure regeneration was aimed.[6] Depending on the current literature, in the PRP–PES protocol methodology presented in this report, platelets are derived, the integrity of the platelet cell membranes is disrupted the possible apoptosis mechanisms are prevented, and the bioavailability of the active cytoplasmic proteins is increased.[6] As a limitation of this study, both the restoration and the PRP–PES are sensitive to the technique and require an experienced clinician. Also, further research and clinical studies are needed to determine the application protocols of this technique.

We consider that further research should be designed and with the supportive evidence, the indications of REPs can be expanded and declared; so, more individuals should benefit from these changes.

In conclusion, in the present report, we presented a successful management of an immature necrotic tooth via modified REPs with the usage of PRP–PES and SRDC protocol.

Authors' contributions

Conception and design of the study: M.I.B.K., S.B. Managing the case: M.I.B.K., H.Y., S.B. Drafting of the manuscript: M.I.B.K., S.B., and H.Y. Revising of the manuscript: M.I.B.K., S.B. All authors have read and approved the manuscript of this case presentation.

Acknowledgement

We would like to thank Prof. Dr. Huseyin Yurdakul for his support in the PRP PES production process.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Zhujiang A, Kim SG. Regenerative endodontic treatment of an immature necrotic molar with arrested root development by using recombinant human platelet-derived growth factor: A case report. J Endod 2016;42:72-5.
2. Diogenes A, YaM H, Teixeira FB, Hargreaves KM. An update on clinical regenerative endodontics. Br Dent J 2013;215:289.
3. Dohan EDM, Pinto NR, Pereda A, Jiménez P, Del Corso M, Kang B-S, *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 2018;29:171-84.

4. Jayadevan V, Gehlot PM, Manjunath V, Madhunapantula SV, Lakshmikanth JSD. A comparative evaluation of Advanced Platelet-Rich Fibrin (A-PRF) and Platelet-Rich Fibrin (PRF) as a Scaffold in Regenerative Endodontic Treatment of Traumatized Immature Non-vital permanent anterior teeth: A Prospective clinical study. J Clin Exp Dent 2021;13:463-72.
5. Eppley BL, Pietrzak WS, Blanton M. Platelet-rich plasma: A review of biology and applications in plastic surgery. Plast Reconstruct Surg 2006;118:147-59.
6. Kile BT. The role of apoptosis in megakaryocytes and platelets. Br J Haematol 2014;165:217-26.
7. American Association of Endodontics. Clinical Considerations for a Regenerative Procedure Report. 2021. Available form: https://www.aae.org/specialty/wp-content/uploads/sites/2/2021/08/ClinicalConsiderationsApprovedByREC062921.pdf. [Last accessed on 2023 Aug 27].
8. Deliperi S, Alleman D, Rudo D. Stress-reduced direct composites for the restoration of structurally compromised teeth: Fiber design according to the "wallpapering" technique. Operative Dentistry 2017;42:233-43.
9. Eskitaşcioğlu G, Belli S, Kalkan M. Evaluation of two post-core systems using two different methods (fracture strength test and a finite elemental stress analysis). J Endod 2002;28:629-33.
10. Olivieri JG, Elmsmari F, Miró Q, Ruiz XF, Krell K V., García-Font M, *et al*. Outcome and survival of endodontically treated cracked posterior permanent teeth: A systematic review and meta-analysis. J Endod 2020;46:455-63.
11. Songtrakul K, Azarpajouh T, Malek M, Sigurdsson A, Kahler B, Lin LM. Modified apexification procedure for immature permanent teeth with a necrotic pulp/apical periodontitis: A case series. J Endod 2020;46:116-23.