Regenerative Endodontic Therapy with Platelet Rich Fibrin: Case Series

Kandemir Demirci G*/ Güneri P **/ Çalışkan MK***

Regenerative endodontic therapy (RET) provides a novel treatment modality for the immature teeth with pulp necrosis. The aim of this case series was to evaluate RET of immature permanent teeth using platelet rich fibrin (PRF) at 36-month follow-up periods. In the present case series, three immature maxillary incisors diagnosed with pulp necrosis and apical periodontitis were treated with RET. The root canals were irrigated with 1.5% sodium hypochlorite (NaOCl) and medicated with triple antibiotic paste(TAP). At the second visit, TAP was removed and root canals were conditioned with 17% EDTA. PRF was used as a scaffold. MTA was placed over PRF and the teeth were restored with composite resin. Periapical radiographs and cone beam computerized tomography(CBCT) were used to evaluate the healing. At the end of the 36-month follow-up periods, there was no response to pulp sensibility tests with cold and electric pulp tester, but all teeth showed decreased periapical lesions or evidence of healing.

***Keywords**: immature permanent tooth, platelet rich fibrin, regenerative endodontic therapy*

INTRODUCTION

Regenerative endodontic therapy (RET) applies principles of biology and engineering technology in order to regenerate the pulp-dentin complex of teeth which has been damaged by caries, trauma, or dental anomalies. It has been suggested as the treatment of choice for immature permanent teeth with necrotic pulp and apical periodontitis.[1] An ideal regenerative endodontic procedure of immature permanent teeth with necrotic pulp should be able to remove clinical symptoms/signs and to resolve periapical lesion, to promote root maturation and to increase the thickness of the canal walls and the root length, similar to the vital pulp treatment outcomes of apexogenesis.

From the School of Dentistry, Ege University, İzmir, Turkey.
*Gözde **Kandemir Demirci**, DDS,PhD, Department of Endodontology.
.Pelin **Güneri, DDS,PhD, Department of Oral and Maxillofacial Radiology.
*** Mehmet Kemal Çalışka**n,** Department of Endodontology.

Send all correspondence to:
Gözde Kandemir Demirci
Department of Endodontology, School of Dentistry,
Ege University,
Izmir, 35100, Turkey
Phone: +90 232 3114608
E-mail : dt.gozdekandemir@hotmail.com

In most studies of RET, a blood clot (BC) within the root canal system served as a biological scaffold and demonstrated successful outcomes.[2,3] Additionally, platelet preparations such as platelet-rich plasma (PRP)[4,5] and platelet rich fibrin (PRF)[6-9,] have been used in RET to provide an autologous 3-dimensional physical scaffold to support tissue regeneration.[10] PRF is the second generation platelet concentrate,[11] which is totally autologous in nature[12] and includes platelets, growth factors, and cytokines that might continuously increase the healing potential of both soft and hard tissues.[13,14]

The aim of the present clinical case series was to investigate the efficacy of PRF as an adjunct to BC by assessing the clinical and radiological outcomes of RET in immature permanent teeth with necrotic pulps and apical periodontitis.

Case Reports

Three patients, two 14 years old males (Cases A and B) and one 13 years old female (Case C) were referred to the Department of Endodontics Outpatient Clinic at Ege University, School of Dentistry for root canal treatment of three immature permanent teeth with periapical lesions. Case A, an upper lateral incisor (#12) had *dens invaginatus* with fractured tubercle, resulting in pulp infection and apical periodontitis (Figure A1). Cases B and C, two upper central incisors (#11) had a history of trauma 5 years ago and subsequently developed pulp infection and apical periodontitis (Figures B1, C1). In case C, root canal treatment was initiated by referring dentist before the patient was admitted to Endodontic Outpatient Clinic (Figure C1).

The patients' dental and medical histories were non-contributory. Extra-oral and intra-oral examinations showed no teeth discoloration, swelling, or draining sinus tract. All teeth did not respond to pulp sensibility tests with cold and electric pulp tester, and were also not sensitive to percussion and palpation. Probing of the periodontal pockets of all teeth was within normal limits (3-4 mm in depth). Periapical radiographs of all teeth were taken with paralleling technique device (Hawe Super-Bite, Kerr Hawe SA, Switzerland). The radiographs revealed that all teeth had periapical radiolucent lesions and open root apices (Figures A1, B1, C1). Based on clinical and radiographic examinations, dental histories and patients' chief complaints, cases were diagnosed as pulp necrosis and chronic apical periodontitis. For all teeth, treatment options including apexification, RET, extraction, and observation were presented to the patients and the patients' parents. The advantages and disadvantages of all treatment options were also explained. RET was selected as the treatment of choice by all patients, and written informed consents were obtained from the patients' parents prior to treatment procedures.

First treatment visit

All three patients were treated with the same protocol, which was presented within the RET guideline of The American Association of Endodontists (AAE).[15] The teeth were isolated with rubber dam. Access cavities were prepared and working lengths of canals were determined radiographically by placing a hand #80 K-file (Dentsply Maillefer, Ballaigues, Switzerland) into the root canal. The patients did not perceive any sensibility. Without mechanical instrumentation, the pulp chambers and the root canals were gently irrigated with 20mL of 1.5% sodium hypochlorite (NaOCl) (Merck, Darmstadt, Germany) and 17% EDTA (Merck, Darmstadt, Germany). The canals were dried with sterile paper points. Equal proportions of metronidazole, ciprofloxacin and minocycline were ground to powder and mixed with distilled water into a creamy consistence to form a triple antibiotic paste (TAP) in order to be used as an intracanal medication. TAP was placed in the canals below the cemento-enamel junction to minimize crown staining. The access cavities were sealed temporarily with glass ionomer cement (Ketac-Molar, 3M ESPE, Seefeld, Germany), and a sterile cotton pellet. The patients were scheduled for a second visit after 3 weeks.

Figures A1, B1, and C1: Preoperative periapical radiographs of case A (#12), case B (#11) and case C (#11).

Figures A2, B2, and C2: Immediate postoperative periapical radiographs of teeth A (#12), case B (#11) and case C (#11).

Figures A3, B3, and C3: Periapical radiographs of teeth at 36-month follow-up. At 36-month follow-up, Case A still has a small periapical radiolucent lesion but has decreased in size considerably (Fig. A3) as compared to immediate postoperative radiographs (Fig. A2). Case B shows periapical healing (Fig. B3). However, there is no thickening of the canal walls and apical closure of cases A and B (Figs. A3, B3). Case 3 shows resolution of periapical lesion, formation of hard tissue barrier adjacent to the coronal MTA plug and presence of foci of calcification within the apical third of the canal (Fig. C3).

Second treatment visit

All patients did not have symptoms/signs within 3 weeks. Following administration of local anesthetic solution without epinephrine (Safecaine, VEM, İstanbul, Turkey), the teeth were isolated with rubber dam and the access cavities were reopened. Final irrigation was done with 17% EDTA (Merck, Darmstadt, Germany) and the root canals were dried with paper points. Under dental loupe (EyeMag Pro, Carl Zeiss, Oberkochen, Germany), bleeding into the canal space was induced by provoking periapical tissues with a precurved #30 K-file introduced into the canal through the immature apex. The presence of bleeding in apical one third of the canal was verified with paper points. Then, PRF was used as a scaffold. To prepare the PRF, 5 ml of whole blood was drawn from the median cubital vein of the patient. The blood sample was collected in a test tube with no anticoagulant and was centrifuged immediately using a tabletop centrifuge (Hettich Laboratories, Zentaifugeni D-78532, Tuttlingen, Germany) at 2400 revolutions per minute for 12 minutes, according to the method described by Choukroun *et al* [11] Three different layers formed in the tube: a base of red blood cells at the bottom, platelet-rich fibrin clot in the middle and acellular plasma on the top. PRF layer was separated from the other layers and then compressed in a PRF box. The freshly prepared PRF membrane was fragmented and fragments inserted into the root canal and condensed apically with an endodontic hand plugger (Dentsply Maillefer, Ballaigues, Switzerland). A 3-mm thickness of white mineral trioxide aggregate (MTA) (ProRoot MTA; Dentsply, Tulsa, OK, USA) was placed directly over the PRF scaffold and the teeth were temporarily restored. Three days later, permanent restorations of teeth were finished with bonded resin composite (Filtek Z250, 3M ESPE, St. Paul MN, USA) (Figures A2, B2, C2). The patients were scheduled for recall visits.

Follow-up examinations

The postoperative clinical and radiological outcomes of teeth were evaluated according to The American Association of Endodontists (AAE) criteria.[15] The treated teeth were followed up for 36 months, all teeth were asymptomatic and did not respond to pulp sensibility tests with cold and electric pulp tester. Periapical radiographs showed that periapical lesions had decreased in size (case A) or revealed evidence of healing (cases B & C). However, in all cases, there were no obvious changes in root wall thickness and/or root length (Figs. A3, B3, C3). In case C, complete hard tissue-like

Figures A4, B4, and C4: Sagittal view of CBCT of 3 treated teeth.

Figures A5, B5, and C5: Coronal view of CBCT of 3 treated teeth. Cases A and B still have a small periapical radiolucent lesion and thickening of the canal walls and apical closure is not noted in CBCT. Case C shows complete healing of periapical lesion, hard tissue barrier formation adjacent to the coronal MTA plug and foci of calcification presence within the apical third of the canal.

barriers were formed in the canal; one adjacent to the coronal MTA plug and another one near to the root apex (Fig. C3). CBCT images (sagittal and coronal views) of all teeth were obtained. Case A still had a small periapical lesion but the lesion had decreased considerably in size. Also, changes in in root wall thickness and/or root length were not noted on sagittal and coronal images of CBCT (Figs. A4, A5). Case B appeared to have a very small periapical lesion on CBCT image (Fig. B4, B5), which was not obvious in conventional periapical radiograph (Fig. B3). There were no changes in root wall thickness and/or root length in case B. Case C had complete healing of periapical lesion, but did not show changes in root wall thickness and/or root length (Figs. C4, C5). The hard tissue-like barrier formed in the canal was not complete in sagittal and coronal views of CBCT image (Fig. C4, C5).

DISCUSSION

In the present case series, clinical and radiographic outcomes of immature permanent teeth with necrotic pulp and apical periodontitis after RET using PRF and BC showed efficacy of the treatment to achieve the primary goal suggested by AAE.[15]

There are many preoperative, intraoperative and postoperative factors that can influence the outcome assessment of RET. Patient age is considered as one of the important preoperative factors, since it is related to the regenerative capacity and functionality of stem cells.[16] In the present study, the patients (13 to 14 years) were older than the patients (7 to 11 years) who were reported in past PRF studies.[6-9] Although the treated teeth in present case series revealed periapical healing similar to previous reports of young patients,[6-9] there were no increase in the root wall thickness and/or root length of the teeth. Additionally, severity and duration of apical periodontitis[17] and trauma are influential factors for the success of RET.[18,19] The presence of trauma and apical periodontitis in two cases may have damaged the residual cells of the Hertwig's epithelial root sheet which is needed to regenerate pulp tissue.

Secondly, intraoperative factors can affect the outcome of RET. The studies reporting successful treatment outcomes with RET have different intraoperative protocols regarding the concentrations of irrigation solutions and the intracanal medicaments.[2] Since higher concentrations of NaOCl are known to possess negative effects on survival and differentiation of stem cells of the apical papilla, concentration of 1.5% NaOCl was preferred in our cases.[20] With the same rationale,[21] chlorhexidine utilization was avoided, as well. Some authors have recommended the use of 17% EDTA for final irrigation due to its cell survival facilitating effect[21] and release of growth factors from dentin which is necessary for stem cell migration, proliferation, and differentiation[22] as well as tertiary dentin.[23,24] Considering that dentin contains many growth factors fossilized in the dentin matrix during initial dentinogenesis[22], dentin matrix derived growth factors are more specific and important than PRF for the regeneration of dentin-pulp complex. Thus, 17% EDTA was utilized during final irrigation. These intraoperative variations indicate that there is no consensus regarding the clinical protocol of RET. Different treatment protocols can result in different treatment outcomes. In the present case series, 1.5% NaOCl and 17% EDTA were used as suggested by AAE.[15] Additionally, a triple antibiotic paste was utilized as recommended by AAE[15] and many other studies.[3,9,25-27] In most RET of immature permanent teeth with necrotic pulp, BC is used as the source of bioactive growth factors and scaffold since it has favorable outcome in terms of increased thickness of the canal walls and/or continued root development.[3,26,27] However, PRP or PRF with or without BC also have been used in RET, especially when induction of intracanal bleeding is a problem.[4,7,8] PRF has been shown to have several advantages over BC and PRP[28], such as elimination of anticoagulants, thrombin, calcium chloride activators and two-step centrifugation procedure,[12] providing self-regulation of inflammatory phenomenon[29], increasing the proliferation of various cell types, stimulating cellular differentiation[14] and supplementing the angiogenesis[11] and the slow release and continuous increase in cytokine levels.[6] In our cases, due to the insufficient bleeding, PRF was used to augment the healing process. Initially, bleeding induced BC served as a matrix and PRF was used as an adjunct. Although each of them can act as a scaffold on their own merit, their combined use can provide the compensation of their inherent limitations.

Additionally, there is no standard postoperative evaluation protocol for the healing assessment for RET.[2] The responses to pulp vitality tests after RET were reported only in %50-60 of the case reports,[30] probably because of the assumption that the nature of coronal restoration[31] and the thickness of MTA[4,32] could affect the response to cold and electric pulp tests. In our cases, no vital response could be obtained and this finding was in accordance with the comments of the studies in the literature.

In RET studies, except a few case reports which utilized parallel technique[3,7] and CBCT[33,34] for radiographic evaluation, the radiographs were not obtained by using a standard technique which may affect the accuracy of periapical bone healing and root length assessment. Recently, Linsuwanont *et al.*[35] also showed that root maturation observed in conventional radiographs was not exactly similar to that observed in CBCT of immature permanent teeth with necrotic pulp after revitalization. Therefore, objective assessment of root maturation of immature permanent teeth with necrotic pulp after RET is necessary by using standard conventional radiographs and CBCT. In our cases, CBCT was used to determine the final periapical status of the teeth at the end of the follow-up period so that more accurate evaluation can be performed.

Even though the guideline of AAE[15] was utilized throughout the treatment protocol, the main limitation of the present study is the small number of cases. This also limited the standardization of the cases according to the ages of the patients and also to the etiology and to the duration of the periapical lesions. All these factors have been attributed as contributors to the negative outcome of RET .[16,17,18]

In recent years, RET has attracted great interest in the field of endodontics; but it reserves many unanswered questions.[19] It must be emphasized that response of revascularized/revitalized tooth to pulp sensibility testing does not essentially indicate that a more organized vital pulp tissue is regenerated in the canal. Many animal and human studies have shown that RET failed to encourage regeneration of the pulp-dentin complex; rather, it stimulated formation of cementum-, bone- and periodontal ligament-like tissue in the canal space.[36-39] Considering that vital tissues including regeneration or repair tissues can response positive to pulp sensibility testing and additionally, the radiological

appearance of root prolongation may be because of the hard tissue apposition within the root canal, a simple question still requires answers: does RET enhance the actual regeneration or repair the pulp tissue in the canal space of immature permanent teeth with necrotic pulp/apical periodontitis?

CONCLUSIONS

Within the limitations of this case series, using PRF with BC achieved the primary goal of RET of immature permanent teeth with periapical lesions after 3 years of follow-up period. More prospective randomized clinical trials and histological studies are necessary to determine the benefit of PRF in RET of immature teeth with necrotic pulp/apical periodontitis on a long-term follow-up with larger sample size and standardized treatment protocol.

REFERENCES

1. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: A review of current status and a call for action. J Endod; 33: 377-90. 2007.
2. Kontakiotis EG, Filippatos CG, Tzanetakis GN, Agrafioti A. Regenerative endodontic therapy: A data analysis of clinical protocols. J Endod; 41; 146-54. 2015.
3. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: New treatment protocol? J Endod 30; 196-200, 2004.
4. Torabinejad M, Turman M. Revitalization of tooth with necrotic pulp and open apex by using platelet-rich plasma: A case report. J Endod; 37:265-8. 2011.
5. Bezgin T, Yılmaz AD, Celik BN, Kolsuz ME, Sonmez H. Efficacy of platelet-rich plasma as scaffold in regenerative endodontic treatment. J Endod; 41, 36-44. 2015.
6. Keswani D, Pandley RK. Revascularization of an immature tooth with a necrotic pulp using platelet-rich fibrin: a case report. Int Endod J; 46: 1096-104. 2013.
7. Mishra N, Narang I, Mittal N. Platelet rich fibrin mediated revitalization of immature necrotic tooth. Contemp Clin Dent; 4: 412-5. 2013.
8. Ray HL, Marcelino J, Braga R, Horwat R, Lisien M, Khaliq S. Long-term follow up of revascularization using platelet-rich fibrin. Dent Traumatol; 32: 80-4. 2016;
9. Shivashankar VY, Johns DA, Vidyanath S, Kumar MR. Platelet rich fibrin in the revitalization of tooth with necrotic pulp and open apex. J Conserv Dent; 2012:15: 395-8.
10. Nurden AT, Nurden P, Sanchez M, Andia I, Anitua E. Platelets and wound healing. Front Biosci; 13: 3532-48. 2008.
11. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL, Dohan AJ, Mouhyi J, Dohan DM. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part IV: Clinical effects on tissue healing. Oral Surg Oral Med Oral Pathol Oral Radiol Endod; 101: e56-60. 2006.
12. Dohan DM, Choukroun J, Diss A, Dohan SL, Dohan AJ, Mouhyi J, Gogly B. Platelet-rich fibrin (PRF): A second-generation platelet concentrate. Part I: Technological concepts and evolution. Oral Surg Oral Med Oral Pathol Oral Radiol Endod; 101: e37-44. 2006.
13. Safi L, Ravanshad S. Continued root formation of a pulpless permanent incisor following root canal treatment: A case report. Int Endod J; 38:489-93. 2005.
14. Huang FM, Yang SF, Zhao JH, et al. Platelet-rich fibrin increases proliferation and differentiation of human dental pulp cells. J Endod; 36: 1628-32. 2010.
15. American Association of Endodontics. AAE Clinical considerations for a regenerative procedure. American Association of Endodontists; 2016. Available from: http://www.aae.org/uploadedfiles/publications_and_research/research/currentregenerativeendodonticconsiderationns.pdf.
16. Estefan BS, El Batouty KM, Nagy MM, Diogenes A. Influence of age and apical diameter on the success of endodontic regenerative procedures. Endod J; 42: 1620-5. 2016.
17. Saoud TMA, Zaazou A, Nabil A, Moussa S, Lin LM, Gibbs JL. Clinical and radiographic outcomes of traumatized immature permanent necrotic teeth after revascularization/revitalization therapy. J Endod; 40: 1946-52. 2014.
18. Lin J, Zeng Q, Wei X, Zhao W, Cui M, Gu J, Lu J, Yang M, Ling J. Regenerative endodontics versus apexification in immature permanent teeth with apical periodontitis: a prospective randomized controlled study. J Endod: 43: 1821-7. 2017.
19. Kim SG, Malek M, Sigurdsson A, Lin LM, Kahler B. Regenerative endodontics: a comprehensive review. Int Endod J; 51: 1367-1388. 2018
20. Martin DE, Almeida JF, Henry MA, Khaing ZZ, Schmidt CE, Teixeira FB, Diogenes A. Concentration-dependent effect of sodium hypoclorite on stem cells of apical papilla survival and differentiation. J Endod ; 40: 51-5. 2014.
21. Trevino EG, Patwardhan AN, Henry MA, Perry G, Dybdal-Hargreaves N, Hargreaves KM, Diogenes A. Effect of irrigants on survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. J Endod; 37:1109-15. 2011.
22. Torabinejad M, Faras H, Corr R, Wright KR, Shabahang S. Histologic examinations of teeth treated with 2 scaffolds: a pilot animal investigation. J Endod; 2014:40: 515-20.
23. Smith AJ, Duncan HF, Diogenes A, Simon S, Cooper PR. Exploiting the bioactive properties of the dentin-pulp complex in regenerative endodontics. J Endod; 42: 47-56. 2016.
24. Galler KM, Buchalla W, Hiller KA, Federlin M, Eidt A, Schiefersteiner M, Schmalz G. Influence of root canal disinfectants on growth factor release from dentin. J Endod ;2015:41: 363-8.
25. Windley W, Teixeira F, Levin L, Sigurdsson A, Trope M. Disinfection of immature teeth with a triple antibiotic paste. J Endod ; 31:439-43. 2015.
26. Ding RY, Cheung GSP, Chen J, Yin XZ, Wang QQ, Zhang CF. Pulp revascularization of immature teeth with apical periodontitis: a clinical study. J Endod; 35: 745–9. 2009.
27. Petrino JA, Boda KK, Shambarger S, Bowles WR, McClanahan SB. Challenges in regenerative endodontics: a case series. J Endod; 36: 536–41. 2010.
28. 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 Clinic Dent; 56:63-8. 2011.
29. Toffler M, Toscano N, Holtzclaw D, Corso M, Ehrenfest DD. Introducing Choukroun's platelet rich fibrin (PRF) to the reconstructive surgery milieu. J Implant Adv Clin Dent; 1: 21–30, 2009.
30. Diogenes A, Ruparel NB. Regenerative endodontic procedures: clinical outcomes. Dent Clin North Am; 61; 111-25. 2017
31. Caliskan MK. Success of pulpotomy in the management of hyperplastic pulpitis. Int Endod J ;1993:26: 142- 8.
32. Johns DA, Vidyanath S. Revitalization of tooth with necrotic pulp and open apex by using platelet-rich plasma. a case report. J Endod 37: 743-4, 2011.
33. Becerra P, Ricucci D, Loghin S, Gibbs JL, Lin LM. Histologic study of a human immature permanent premolar with choronic apical abscess after revascularization/revitalization. J Endod ; 40: 133-9. 2014.
34. Bakhtiar H, Esmaeili S, Fakhr Tabatabayi S, Ellini MR, Nekoofar MH, Dummer PMH. Second generation platelet-concentrate (Platelet-rich fibrin) as a scaffold in regenerative endodontics: a case series. J Endod: 43: 401-8. 2017.
35. Linsuwanont P, Sinpitaksakul P, Lertsakchai P. Evaluation of root maturation after revitalization in immature permanent teeth with nonvital pulp by cone beam computed tomography and conventional radiographs. Int Endod J; 50: 836-846. 2017
36. Zhu W, Zhu X, Huang GTJ, Cheung GSP, Dissanayaka WL, Zhang C. Regeneration of dental pulp tissue in immature teeth with apical periodontitis using platelet-rich plasma and dental pulp cells. Int Endod J ; 46: 962-70. 2013.
37. Martin DE, Almeida JS, Henry MA, Khaing ZZ, Schmidt CE, Teixeira FB, Diogenes A. Concentration-dependent effect of sodium hypoclorite on stem cells of apical papilla survival and differentiation. J Endod; 2014:40: 51-5.
38. Zhang DD, Chen X, Bao ZF, Chen M, Ding ZJ, Zhong M. Histological comparison between platelet-rich plasma and blood clot in regenerative endodontic treatment: an animal study. J Endod; 2014:40: 1388-93.
39. Torabinejad M, Milan M, Shabahang S, Wright KR, Faras H. Histological examination of teeth with necrotic pulps and periapical lesions treated with 2 scaffolds: an animal investigation. J Endod ; 41: 846-52. 2015.