Dental Traumatology

Dental Traumatology 2016; **32**: 80–84; doi: 10.1111/edt.12189

Long-term follow up of revascularization using platelet-rich fibrin

CASE REPORT

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Key words: contact sports; dental trauma; inflammatory root resorption; permanent tooth; prognosis; treatment

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Accepted 30 January, 2015

Abstract – *Introduction*: Trauma is one of the primary causes of tooth loss and pulpal injury in adolescents and children. Prior to regenerative endodontics, treatment of necrotic, immature teeth with open apices was limited to long-term calcium hydroxide ($Ca(OH)_2$) apexification and subsequent root canal therapy or extraction. Through revascularization, retention of these teeth can be achieved and the elimination of patient symptoms and the radiographic appearance of continued root development were obtained. *Case Review*: This report illustrates a revascularization protocol through a case where platelet-rich fibrin (PRF) was utilized as an autologous scaffold for traumatized, necrotic, immature teeth with incomplete root development. Through consistent follow-up reports, comprising of both clinical examination and radiographs, marked improvement in the condition of the traumatized tooth was noted. *Discussion*: This case demonstrates the feasibility of utilizing PRF as an effective treatment protocol for traumatized teeth in lieu of traditional treatment protocols, such as long-term calcium hydroxide ($Ca(OH)_2$) apexification or extraction. The choice of utilizing PRF, as opposed to other platelet concentrates, such as platelet-rich plasma (PRP) or a blood clot, lies in PRF's ability to allow for a slow, long-term release of autologous growth factors.

Dental caries and dental trauma are the two leading causes of injury to the dental pulp (1). Although the oral cavity comprises less than 1% of the total body area, it accounts for 5% of injuries in all ages and 17% of injuries in children (2). Dental trauma in children can lead to pulp tissue damage and is of particular concern in the underdeveloped tooth, as immature and open apices limit possible treatment options.

Historical approaches utilizing long-term calcium hydroxide therapy for apexification have resulted in increased fracture rates due to loss of flexural strength as dentinal organic matrix is weakened with extended exposure to calcium hydroxide (3, 4). The process of revascularization has allowed for enhanced survivability of the tooth, alleviation of symptoms, and a radiographic confirmation of root thickening and lengthening (5, 6). Nygaard-Ostby focused the role of blood clot in endodontic therapy (7, 8) while Myers' work encompassed dental pulp regeneration through utilization of blood and blood substitutes (9).

Unlike apexification, which mainly relies on exogenous materials to create an apical barrier within the root canal system (10, 11), revascularization utilizes a combination of antibiotic paste and a biologic scaffold (12). The antibiotic paste historically consisted of a combination of metronidazole, ciprofloxacin, and minocycline (13, 14). Its purpose was to decrease and potentially eliminate bacterial strains and the pro-inflammatory response within the root canal system in preparation for the biologic scaffold (15). Concerns over tooth discoloration have been attributed to minocycline use. The survivability of stem cell progenitors within the root canal system in the face of the antibiotic treatment may also be jeopardized (16).

In traditional revascularization cases, a blood clot within the root canal system served as a biological scaffold (17, 18). Although the induction of a blood clot within the canal space is not always possible (19), its overall clinical success paved the way for the next phase of revascularization—platelet concentrates providing an autologous scaffold (20). Torabinejad and Turman reported a case where PRP, a first generation platelet concentrate, was used for revascularization (21). Recently, Pandey (2013) reported a case of an anterior traumatized permanent tooth where platelet-rich fibrin (PRF), a second-generation platelet concentrate consisting of autologous platelets and leukocytes present in a complex fibrin matrix, was utilized as a scaffold for revitalization with a follow up of 15 months (22). PRF is composed of fibrin membranes enriched with platelets, growth factors, and cytokines (23). PRF has the capability to enhance the healing potential of soft and hard tissues and slowly release growth factors, such as PDGF and TGF-B1 over the

span of 7–14 days (24), which facilitates angiogenesis, cellular growth, and differentiation over an extended period of time.

The purpose of this study was to discuss a revascularization protocol utilized for the treatment of a traumatized permanent tooth with an immature apex. The case demonstrates the viability of utilizing platelet-rich fibrin as a potential modality to treat necrotic, immature teeth with open apices.

Case review

An 11-year-old male was evaluated 4 months after sports-related trauma to the permanent maxillary left central incisor or tooth #9. This tooth was not responsive to cold testing, insensitive to percussion, but tender to palpation at initial presentation. Class II mobility was present with deep pockets noted on all sides. Previously, it was restored with a Class IV composite resin restoration to replace the missing tooth structure. The tooth was intruded 3 mm and luxated buccally. Periapical radiolucency, thin dentinal walls, and incomplete root development with an open apex were evident on the preoperative radiograph. An irregular root surface (indicative of external root resorption) was also noted (Fig. 2a). Tooth #9 was diagnosed as necrotic, with asymptomatic apical periodontitis. The treatment options presented included a conventional root canal therapy with a mineral trioxide aggregate (MTA) plug, an extraction, and revascularization procedures. The patient agreed to revascularization.

At the first treatment appointment, rubber dam isolation was used without anesthesia. The root canal space was accessed lingually and irrigated with copious amounts of an alternating rinse of 0.5% NaOCl and 17% EDTA to remove all necrotic tissue using a 27-gauge needle. The canal space was dried, and a double antibiotic paste (DAP), consisting of 200 mg ciprofloxacin and 500 mg metronidazole mixed into propylene glycol, was placed within the canal system. The tooth was temporized with a cotton pellet and Ketac Glass Ionomer temporary restoration. Post-treatment radiographs were taken. Rubber dam isolation was removed. Postoperative home care instructions were given, and a second appointment was scheduled.

At the second treatment appointment (4 weeks after the first treatment appointment), 3% mepivacaine without vasoconstrictor was used to obtain local anesthesia. The canal space was accessed under rubber dam isolation and irrigated with 0.5% sodium hypochlorite, followed by 17% EDTA with 27-gauge needle tips. Concurrently, a blood draw was performed by the Anesthesiology Department at the University of Pittsburgh School of Dental Medicine. Approximately, 7 ml of blood from the patient was obtained in non-heparinized test tubes (Becton, Dickinson, and Company Franklin Lakes, NJ). The tubes were placed in a centrifuge (Salvin Sealed Technology, Charlotte, NC) and spun for 20 min at 402 g, separating the blood sample into the plasma-poor, plasma-rich, and red blood cell components. After centrifugation, the straw-colored plasma-poor layer was decanted. The platelet-rich fibrin, which appears as a gelatinous yellow–white substance, was removed from the vial with sterile college pliers (Fig. 1a) and dragged across a sterile 4×4 gauze pad to remove excess red blood cells. The prepared PRF sample was then cut into segments approximately 2 by 5 mm (Fig. 1b). Segments were placed into the canal space to a level 3 mm below the DEJ (dentin–enamel junction) using hand pluggers following the induction of apical bleeding by passing a sterile hand file 1 mm beyond the anatomical apex of the tooth. Induction of bleeding into the canal space delivers mesenchymal stem cells into the canal space from the apical papilla (25). An MTA cap was placed over the PRF scaffold, followed by a glass ionomer base. A composite resin restoration provided a definitive seal to the access of the cavity (Figs 2a and 3).

Discussion

The presented method describes an evolving and viable alternative to the original protocol for tooth revascularization. The use of PRF addresses two parts of the

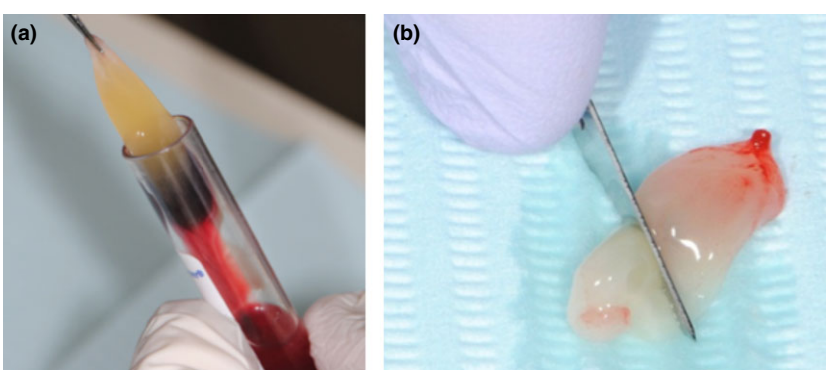

Fig. 1. (from left to right): (a) retrieval of PRP from test tube; (b) prepared PRF segments. In all subsequent follow-up appointments (up to 36 months), tooth #9 presented without any symptoms. The tooth remained negative to cold testing, but tested positive to EPT testing at 24- and 36-month recalls. Osseous healing was noted radiographically. Calibration of radiographs to adjust for orientation showed an increase in root length and an intact lamina dura, as seen on 12 months (Fig. 2b), 24 months (Fig. 2c), and 36 months (Fig. 2d). While some discoloration is evident clinically, the tooth exhibits no abnormal pocketing or probing depths (Fig. 3).

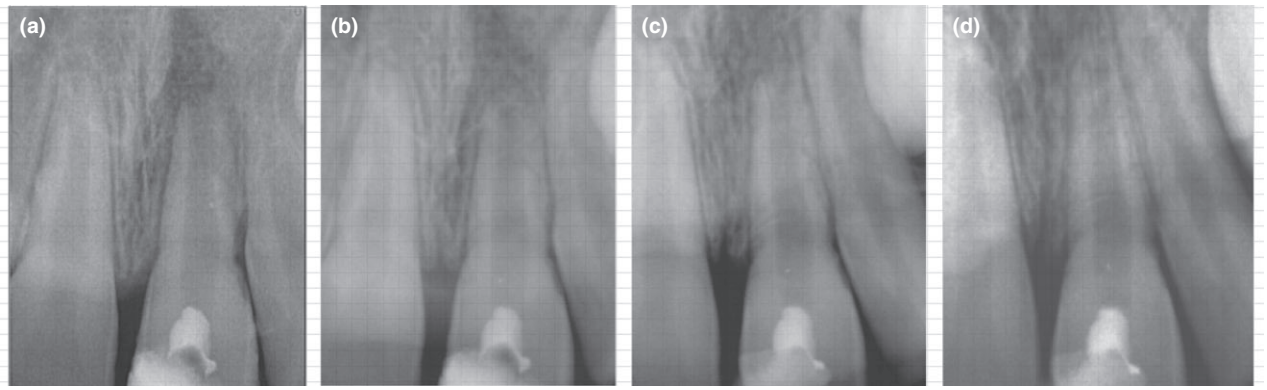

Fig. 2. (from left to right): (a) Postoperative radiograph immediately after placement of PRF and definitive restoration; (b) 12-month recall; (c) 24-month recall; (d) 36-month recall.

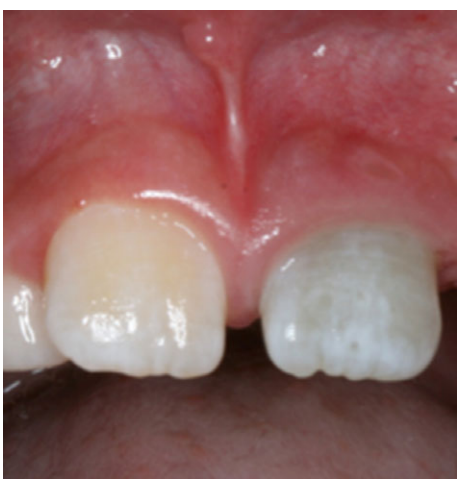

Fig. 3. Clinical photograph on 36-month follow up.

triad for tissue regeneration (26), growth factors and a scaffold; the third part of the triad is addressed with SCAP cells from the dentin papilla. The use of autologous tissues makes this both economical and excepted as safe by the patient. There have been reports of successful revascularization utilizing platelet exudates (21). Concurrently, successful use of autologous platelet concentrate has been widely described in oral and maxillofacial surgery for bone grafting (24) and promotion of soft-tissue healing (27). PRF is a fibrin biomaterial that encourages the migration of stem cells and angiogenesis, while acting as an autologous source for the controlled release of growth factors over an extended period of time (23). While there is still much to learn about the various platelet concentrates, the protocol presented illustrates the feasibility of PRF in clinical use.

Controlling infection is an important first step for revascularization procedures. The use of triple and double antibiotic paste combinations has proven efficacy against a wide variety of odontogenic bacteria. Moreover, DAP (double antibiotic paste) consisting of ciprofloxacin and minocycline has shown to minimize crown discoloration compared to TAP (triple antibiotic paste) while providing similar intracanal disinfection benefits (25). However, stem cells of the apical papilla (SCAP) experience over 80% cell death with double and triple antibiotic paste concentrations as low as 10 mg ml^{-1} (28). In retrospect, the cytotoxic concentration of antibiotics that SCAP cells were exposed to in this case may explain the lack of increase in root thickness in the presence of osseous healing, root length increase, and periodontal healing. Calcium hydroxide should be considered instead, as it is shown to be conducive to SCAP cell survival when compared to various double and triple antibiotic combinations (28). Calcium hydroxide has demonstrated promising results in continued root formation in immature necrotic teeth (19).

Moreover, higher NaOCl concentrations have also shown a negative effect on SCAP cell survival and dentin sialophosphoprotein (DSPP) expression. The use of 0.5% or 1.5% NaOCl, followed by 17% EDTA, counters these deleterious effects on stem cell viability (16, 29). SCAP cells are capable of odontoblast-like differentiation and formation of dentin *in vivo*. Their high proliferative potential makes them a likely source of odontoblasts for root dentin formation even during conditions of pulp necrosis. In a mouse model, after dentin conditioning with EDTA, SCAP cells exhibited an intimate association with dentin and differentiated into odontoblast-like cells with the expression of DSPP. Moreover, cells with sodium hypochlorite used without ETDA as a final rinse showed multinucleated giant cells and resorption lacunae (26). Therefore, disinfection of the dentinal space with the appropriate concentration of NaOCl and dentin conditioning with a final rinse of ETDA can significantly boost SCAP cell survivability and differentiation.

Platelet-rich fibrin (PRF) has been described as an immune and platelet concentrate that contains all the constituents of a blood sample considered to be favorable for healing and immunity (23). Understanding the matrix configuration of PRF is crucial for discerning the differences of biologic kinetics between PRP and PRF (23). He et al. compared the efficacy of PRF and PRP on proliferation and differentiation of rat osteoblasts. The levels of released growth factors, such as

TGF-B1 and PDGF, were markedly increased and reached the highest amount on day 14, and then decreased mildly for PRF. In contrast, PRP demonstrated an uncontrolled release of TGF-B1 and PDGF, which reached the highest amount on day 1 and then decreased rapidly (30). In short, PRF provides a delayed and prolonged release of growth factors, as opposed to the single sharp burst of growth factors provided by PRP (30, 31). Mesenchymal cells of the apical papilla, endothelial cells, osteoblasts, and fibroblasts also express receptor for these growth factors (32). Apart from growth factors, the fibrin matrix of PRF is essential to encourage angiogenesis (23), as well as osseous regeneration (33). An observation in healing associated with the cases where PRF has been used is the radiographic appearance of the canal space not being obliterated by calcification, which may be attributed to the controlled release of growth factors. These can be utilized by stem cells of the apical papilla, which migrate into the canal space as bleeding is induced. Additional clinical advantages of PRF include the provision of an autologous mechanical substrate to condense the MTA, the provision of a biodegradable fibrin scaffold, and inherent microbicidal properties. PRF can hence serve as a bioscaffold for revascularization and a reservoir for growth factors (27).

Recent studies have addressed the possibility of utilizing revascularization therapy in teeth with more developed apices. For instance, Laureys et al. suggest that the size of the apical foramen is not the decisive factor for determining whether revascularization is possible. Cases with a diameter much smaller than 1 mm did not prevent the ingrowth of new vital tissue (34). Furthermore, new vital tissue regeneration has been demonstrated in pulpectomized mature canine teeth through the use of platelet-rich plasma (35). Based on these findings, it may be possible to extend the utility of revascularization beyond teeth with immature open apices and realize the potential for a biological obturation of the root canal system.

Conclusion

This case demonstrates a clinically feasible protocol utilizing platelet-rich fibrin as an autologous scaffold for pulp revascularization. Studies have demonstrated that PRF is rich in growth factors, which enhances cellular proliferation, differentiation, and angiogenesis. It allows for the slow and continuous release of growth factors for revascularization and regulates inflammatory reactions. Further studies and continued improvements in the revascularization protocol can determine how fibrin matrix predictably increases the success of revascularization and consistently produces long-term successful clinical outcomes.

Acknowledgements

The authors would like to thank the Graduate Endodontic Department at the University of Pittsburgh School of Dental Medicine.

Conflict of interest

The authors would like to disclose no conflict of interests related to this study.

References

1. Do LG. Distribution of caries in children: variations between and within populations. J Dent Res 2012;91:536–43.
2. Glendor U, Halling A, Andersson L, Eilert-Petersson E. Incidence of traumatic tooth injuries in children and adolescents in the county of Vastmanland, Sweden. Swed Dent J 1996;20:15–28.
3. Andreasen JO, Farik B, Munksgaard EC. Long-term calcium hydroxide as a root canal dressing may increase risk of root fracture. Dent Traumatol 2002;18:134–7.
4. Cvek M. Prognosis of luxated non-vital maxillary incisors treated with calcium hydroxide and filled with gutta-percha. A retrospective clinical study. Endod Dent Traumatol 1992;8:45–55.
5. Jeeruphan T, Jantarat J, Yanpiset K, Suwannapan L, Khewsawai P, Hargreaves KM. Mahidol study 1: comparison of radiographic and survival outcomes of immature teeth treated with either regenerative endodontic or apexification methods: a retrospective study. J Endod 2012;38:1330–6.
6. Kahler B, Mistry S, Moule A, Ringsmuth AK, Case P, Thomson A, Holcombe T. Revascularization outcomes: a prospective analysis of 16 consecutive cases. J Endod 2014;40:333–8.
7. Nygaard-Ostby B, Hjortdal O. Tissue formation in the root canal following pulp removal. Scand J Dent Res 1971;79:333–49.
8. Ostby BN. The role of the blood clot in endodontic therapy. An experimental histologic study. Acta Odontol Scand 1961;19:324–53.
9. Myers WC, Fountain SB. Dental pulp regeneration aided by blood and blood substitutes after experimentally induced periapical infection. Oral Surg Oral Med Oral Pathol 1974;37:441–50.
10. Rafter M. Apexification: a review. Dent Traumatol 2005;21:1–8.
11. Rudagi KB, Rudagi B. One-step apexification in immature tooth using grey mineral trioxide aggregate as an apical barrier and autologus platelet rich fibrin membrane as an internal matrix. J Conserv Dent 2012;15:196–9.
12. Thibodeau B, Trope M. Pulp revascularization of a necrotic infected immature permanent tooth: case report and review of the literature. Pediatr Dent 2007;29:47–50.
13. Sato I, Ando-Kurihara N, Kota K, Iwaku M, Hoshino E. Sterilization of infected root-canal dentine by topical application of a mixture of ciprofloxacin, metronidazole and minocycline in situ. Int Endod J 1996;29:118–24.
14. 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.
15. Windley 3rd W, Teixeira F, Levin L, Sigurdsson A, Trope M. Disinfection of immature teeth with a triple antibiotic paste. J Endod 2005;31:439–43.
16. Martin G, Ricucci D, Gibbs JL, Lin LM. Histological findings of revascularized/revitalized immature permanent molar with apical periodontitis using platelet-rich plasma. J Endod 2013;39:138–44.
17. 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.

18. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol? J Endod 2004;30:196–200.
19. Cehreli ZC, Isbitiren B, Sara S, Erbas G. Regenerative endodontic treatment (revascularization) of immature necrotic molars medicated with calcium hydroxide: a case series. J Endod 2011;37:1327–30.
20. 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.
21. Torabinejad M, Turman M. Revitalization of tooth with necrotic pulp and open apex by using platelet-rich plasma: a case report. J Endod 2011;37:265–8.
22. 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.
23. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL et al. Platelet-rich fibrin (PRF): a second-generation platelet concentrate. Part V: histologic evaluations of PRF effects on bone allograft maturation in sinus lift. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2006;101:299–303.
24. Choukroun J, Diss A, Simonpieri A, Girard MO, Schoeffler C, Dohan SL et al. 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 2006;101:e56–60.
25. Akcay M, Arslan H, Yasa B, Kavrik F, Yasa E. Spectrophotometric analysis of crown discoloration induced by various antibiotic pastes used in revascularization. J Endod 2014;40:845–8.
26. Galler KM, D'Souza RN, Federlin M, Cavender AC, Hartgerink JD, Hecker S et al. Dentin conditioning codetermines cell fate in regenerative endodontics. J Endod 2011;37:1536–41.
27. Kang YH, Jeon SH, Park JY, Chung JH, Choung YH, Choung HW et al. Platelet-rich fibrin is a Bioscaffold and reservoir of growth factors for tissue regeneration. Tissue Eng Part A 2011;17:349–59.
28. Ruparel NB, Teixeira FB, Ferraz CC, Diogenes A. Direct effect of intracanal medicaments on survival of stem cells of the apical papilla. J Endod 2012;38:1372–5.
29. Pang NS, Lee SJ, Kim E, Shin DM, Cho SW, Park W et al. Effect of EDTA on attachment and differentiation of dental pulp stem cells. J Endod 2014;40:811–7.
30. He L, Lin Y, Hu X, Zhang Y, Wu H. A comparative study of platelet-rich fibrin (PRF) and platelet-rich plasma (PRP) on the effect of proliferation and differentiation of rat osteoblasts in vitro. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2009;108:707–13.
31. Tsay RC, Vo J, Burke A, Eisig SB, Lu HH, Landesberg R. Differential growth factor retention by platelet-rich plasma composites. J Oral Maxillofac Surg 2005;63:521–8.
32. Huang GT, Sonoyama W, Liu Y, Liu H, Wang S, Shi S. The hidden treasure in apical papilla: the potential role in pulp/dentin regeneration and bioroot engineering. J Endod 2008;34:645–51.
33. Bensaid W et al. A biodegradable fibrin scaffold for mesenchymal stem cell transplantation. Biomaterials 2003;24:2497–502.
34. Laureys WG, Cuvelier CA, Dermaut LR, De Pauw GA. The critical apical diameter to obtain regeneration of the pulp tissue after tooth transplantation, replantation, or regenerative endodontic treatment. J Endod 2013;39:759–63.
35. Zhu X, Zhang C, Huang GT, Cheung GS, Dissanayaka WL, Zhu W. Transplantation of dental pulp stem cells and platelet-rich plasma for pulp regeneration. J Endod 2012;38:1604–9.