Platelet-rich fibrin-mediated revitalization of immature necrotic tooth

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Abstract

Contemporary studies have shown that the regeneration of tissues and root elongation is possible in necrotic immature permanent teeth. The purpose of this case report is to add a new vista in regenerative endodontic therapy by using platelet rich fibrin for revitalization of immature non vital tooth. An 11year old boy with the history of trauma was diagnosed with the pulpal necrosis and symptomatic apical periodontitis in tooth #21. Intra oral periapical radiograph showed open apex and associated immature supernumerary tooth with respect to tooth #21. Access preparation and minimal instrumentation was done to remove necrotic debris under copious irrigation with 2.5% sodium hypochlorite. Triple antibiotic paste was packed in the canal for four weeks. During second visit, 5 mL of whole blood was drawn from the medial cubital vein of the patient and blood was then subjected to centrifugation at 2400 rpm for 12 minutes for the preparation of Platelet rich fibrin (PRF) utilizing Choukroun's method. Triple antibiotic paste was removed and canal was dried. PRF clot was pushed to the apical region of tooth #21 using hand pluggers. Three milimetres of Mineral trioxide (MTA) was placed in cervical part of the root canal and permanent restoration was done three days later. Clinical examination at 6 and 12 months revealed no sensitivity to percussion and palpation in tooth #21and it responded positively to both electric pulp and cold tests. Radiographic examination showed resolution of periapical rarefaction, further root development and apical closure of the tooth #21 and its associated supernumerary tooth. On the basis of successful outcome of the present case it can be stated that PRF clot may serve as a scaffold for regeneration of necrotic immature teeth.

Keywords: Platelet-rich fibrin, revitalization, triple antibiotic paste

Introduction

The contemporary non-surgical endodontic management of mature teeth has shown favorable outcome rates of 95% in teeth diagnosed with irreversible pulpitis[1] and 85% in necrotic cases.[2] But this management in necrotic immature permanent teeth offers limited predictability and has a questionable prognosis due to thin dentinal walls.[3,4] Moreover open apex is difficult to seal either by thermo-plasticized or lateral condensation methods.[5] Traditionally such teeth were managed with apexification (Frank AL 1966 and Steiner 1968) which entailed the long-term treatment with calcium hydroxide resulting in formation of porous hard tissue apical barrier[6,7] but it has been shown that either the short-term (Rosenberg *et al.*, 2007)[8] or long-term (Andreason *et al.*, 2002 and 2006)[9,10] use of calcium hydroxide can reduce the root strength due to denaturation of collagen. This finding is consistent with the large case series by Cvek M in 1992 which showed that major reason for tooth loss following such apexification was tooth fracture.[3] To overcome the drawbacks of patient compliance and incomplete barrier, Torabinejad[11] in 2000 and Witherspoon *et al.*,[12] in 2000 pursued the single-step apexification using MTA. Ballesio *et al.*,[13] and Felippe *et al.*,[14] in 2006 showed that one-step apexification also does not generally result in further root development and thin dentinal walls still pose a challenge to the clinicians.

The teeth with poor crown root ratio and thin dentinal walls are more susceptible to fracture by secondary injuries and are also poor candidates for the restorative procedures.[4,15]

Therefore, there was a paradigm shift in the treatment protocol of such teeth from apexification to regenerative procedures.[16,17] This approach was extrapolated from successful revascularization of avulsed immature permanent teeth after replantation in animals[18] and humans.[19] Hargreaves *et al.*, in 2008 reported that regenerative endodontic procedures are possible by application of the principles of the tissue engineering that requires the spatial orientation of stem cells, signaling molecules, and the scaffold.[20,21] It has been reported that the remnants of Hertwig's epithelial root sheath or cell rests of Mallasez are resistant to peri-apical infections.[22] Thus, the signaling networks from these remnant cells may stimulate various stem cells like stem cells from apical papilla (SCAP),[23] bone marrow,[24] and multipotent pulp stem cells[25] to form odontoblasts-like cells in non-vital, immature,

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and non-infected teeth. These newly formed odontoblasts-like cells form dentine which helps in normal root maturation.

Empty canal space does not favor the stem cell proliferation and differentiation. An appropriate scaffolding is necessary to provide a spatially correct position of stem cells with growth factors. Earlier studies on regeneration used blood clot,[26] collagen,[27] and platelet-rich plasma (PRP)[28] as a scaffold. Medline search shows that platelet-rich fibrin (PRF) has never been used for revitalization of immature necrotic permanent teeth. The purpose of this case report is to add a regenerative endodontic case to the existing literature about using PRF as a scaffold.

Case Report

An 11-year-old boy was referred to the department of conservative dentistry and endodontics for evaluation and treatment of left maxillary central incisor. The patient gave the history of bicycle fall 2 years ago. The medical history was non-contributory. The extraoral examination showed no significant changes. The intra-oral examination showed absence of any soft tissue abnormalities. Clinical examination of teeth revealed slight discoloration in tooth #21. It was sensitive to percussion and palpation tests. It did not respond to 1, 1, 1, 2-tetrafluoroethane (Endo-Ice; Hygenic Corp., Akron, OH, USA) and electric pulp test (Analytic Technology, Redmond, WA, USA). The adjacent teeth were caries free, asymptomatic, and tested positively. The periodontal examination of all teeth showed probing depths within normal limits. An intraoral radiograph was taken with paralleling technique using XCP positioner (extension cone paralleling technique) (Dentsply) showed an immature root with periapical pathology and open apex in tooth #21 and an associated immature supernumerary tooth [Figure 1a]. On the basis of clinical and radiographic findings, the pulpal diagnosis of necrotic pulp with symptomatic apical periodontitis was made for tooth #21. Various treatment options were explained and the parents gave the consent for regenerative endodontic procedures with the aid of PRF.

Local anesthesia was obtained by using 2% lignocaine with 1:200000 epinephrine. Access preparation was done in tooth #21 under rubber dam isolation. Minimal instrumentation was done and the canal was irrigated with 10 mL of 2.5% NaOCl. It was dried with paper points. Equal proportions of ciprofloxacin (Bayer, Leverkusen, Germany), metronidazole (Shionogi and Co, Ltd, Osaka, Japan), and minocycline (Aurobindo, Andhra Pradesh, India) were ground and mixed with distilled water to a thick paste consistency. This antibiotic mixture was placed in the canal using an amalgam carrier and packed with large endodontic pluggers. The access cavity was sealed with Cavit (ESPE, Chergy Pontoise, France). After 4 weeks, tooth #21 was asymptomatic and showed no sensitivity to percussion and palpation. A 5 ml of whole blood was collected from the median cubital vein of the patient for the preparation of PRF clot [Figure 2a]. Under rubber dam isolation, temporary restoration was removed. The triple antibiotic mixture was washed out with saline. After drying the canal with paper points, the fibrin membrane was pushed [Figure 2b] with Buchanan Hand Plugger Size #2 (Sybron Endo, Orange, CA) 1 mm beyond the confines of working length and coronally to the level of cementoenamel junction. Three millimetres of MTA was placed directly over the PRF clot and tooth was temporarily restored. After 72 hours, permanent restoration was done with composite (Esthet.X HD, Dentsply, UK). The patient was kept on follow-up at 6 and 12 months [Figure 1b and c] for re-evaluation. Tooth #21was asymptomatic and was not sensitive to percussion or palpation tests. Sensitivity tests with cold and Electric Pulp Tester elicited a positive response. Radiographic examination showed resolution of the periapical lesion, further root development and continued apical closure in tooth #21 and associated supernumerary tooth.

Discussion

Regenerative procedure in immature teeth was introduced in the field of endodontics by Ostby in 1961[29] and later reintroduced in 1966 by Rule and Winter.[30] It leads to apexogenesis and maturogenesis in necrotic immature permanent teeth.[31] The thickened and convergent dentinal

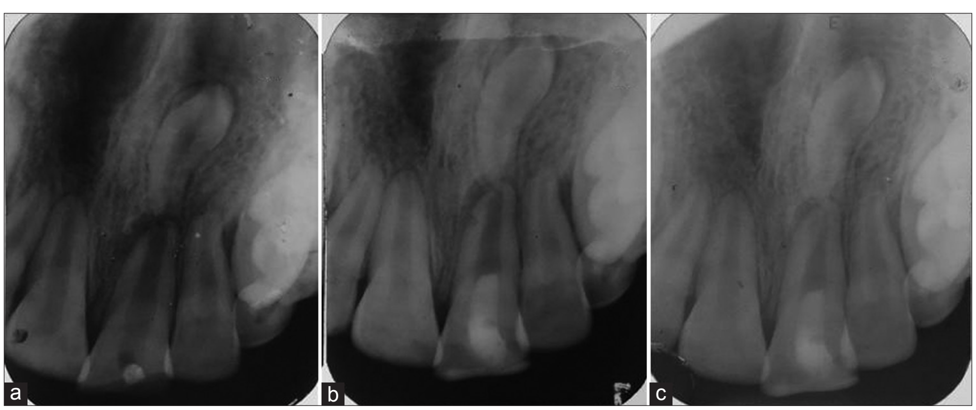

Figure 1: (a) Preoperative intraoral periapical radiograph. (b) Postoperative intraoral periapical radiograph at 6 months. (c) Postoperative intraoral periapical radiograph at 12 months

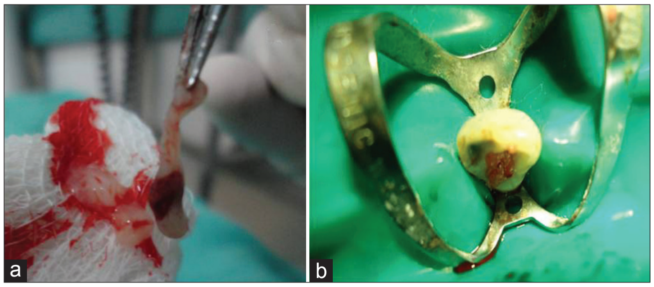

Figure 2: (a) Platelet-rich fibrin clot, (b) Platelet-rich fibrin placed in tooth #21

walls add to the long-term prognosis of the tooth by increasing its fracture resistance hence in the present case this novel technique was used.

Successful revitalization of immature teeth can be accomplished after complete disinfection, placement of a matrix in the canal for tissue in-growth and bacterial tight seal of the access opening. Minimal instrumentation and copious irrigation was done with 2.5% NaOCl, to achieve disinfection. Triple antibiotic paste (TAP)[32] a mixture of ciprofloxacin, metronidazole, and minocycline was packed in the canal to achieve further reduction of microbial load.

Appropriate scaffolding is necessary to give correct spatial location to stem cells and to regulate its differentiation, proliferation, and metabolism by different growth factors. Blood clot and platelet concentrates i.e., PRP[33] and PRF[34] serve as natural scaffolds with numerous growth factors. Platelet-derived growth factor (PDGF-AB), transforming growth factor β (TGF-β), and insulin-like growth factors have been evaluated for their ability to guide the stem cells to differentiate into odontoblasts-like cells.[35]

Lance *et al.*, in 2010 reported that platelet concentrates have increased concentration of growth factors and increase the cell proliferation over time when compared to blood clot.[36] There are several *in vitro* studies which supports that the direct dose–response influence of many growth factors like PDGF on cell migration, proliferation, and matrix synthesis.[37,38]

Marx in 2009 also emphasized that biologically compromised tissues respond optimally when concentration of growth factors is enhanced.[39]

Choukroun's PRF is defined as an autologous leukocyte- and platelet-rich fibrin (L-PRF) biomaterial.[40-42] This is prepared by collection of patient's blood in specialized glass test tubes with no anticoagulants and then it is subjected to gentle centrifugation at 2400 rpm for 12 minutes resulting in division of blood sample in three layers: A base of red cells at the bottom, acellular plasma on the top, and a clot of PRF in the middle.[43] No use of anticoagulants, thrombin and calcium chloride activators leads to formation of natural clot. PRF has the characteristic of polymerizing naturally and slowly during centrifugation. This high-density fibrin clot serves as a biological matrix by supporting cell migration and cytokine release. It is advantageous as opposed to synthetic polymers and collagen when cost, inflammation, immune response, and toxicity levels are concerned. PRF produces a 210-fold higher concentration of platelets and fibrin when compared to the initial input whole blood volume. (Lucarelli *et al.*, 2010)[44] PRF is associated with slow and continuous increase in cytokine levels.[45] Leucocytes in PRF act as anti-inflammatory agent and play key role in immune regulation. Leucocytes in clot act as anti-infectious agent,[46] immune response regulator,[47] and provides vasculoendothelial growth factor to promote angiogenesis.

This PRF clot was pressed in between two gauge pieces to form a strong membrane and it was placed in the root canal space.

It has been shown that tissue growth halts where bacteria is found.[48] Various studies have shown that MTA is superior to other materials like glass ionomer, amalgam, and composite resins with respect to marginal adaptation, bacterial leakage, and cytotoxicity. So MTA was used to provide the tight seal coronally.

Surgical contemplation of the supernumerary tooth was not undertaken considering the age of the patient. Apical closure was evident radiographically in this tooth probably due to changed microenvironment by PRF.

The successful clinical outcome in terms of regained vitality and radiographic measures of increased root length, dentinal thickness, and apical closure shows that PRF has ideal properties to be used for regenerative endodontic procedures.

Conclusions

On the basis of short-term results of present case report, it is reported that PRF is potentially an ideal scaffold for regeneration of vital tissue in necrotic immature teeth. Long-term clinical trials and studies are needed to compare its efficacy with PRP and blood clot. If these studies show a significant difference then PRF may prove a boon to regenerative endodontic therapy.

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