CASE REPORT

BLOOD-SMART DENTISTRY : REVIVING TEETH USING THE BODY'S OWN RESOURCES

Dr. Palak Gupta*, Dr. Kallol Kumar Saha**, Dr. Lugu Buru Murmu***

ABSTRACT

Aim : The study aimed to evaluate the regenerative capability of advanced platelet-rich fibrin (A-PRF) in the maxillary incisor region for the regenerative treatment of necrotic immature permanent teeth (NIPT).

Study design : In this case report, three individuals with NIPT in the maxillary incisor region, age 14 to 18 years, were treated with A-PRF. Prior to beginning treatment, baseline vitality, radiographic evaluation with IOPAR, Cone beam computed tomographyand clinical tests were recorded. Following treatment, patients were monitored for three, six, and twelve months.

Results : After 3, 6, and 12 months of follow-up, all patients (100%) showed complete resolution of clinical signs and symptoms. All patients showed periradicular healing, and all patients showed a clear hard tissue bridge formation at various levels in the root canal on postoperative CBCT and radiographs.

Conclusion : Biomaterial like A-PRF has proven itself very promising in regenerative endodontic treatment. To prove its superiority over other material further studies may be planned in future.

KEY WORDS

Advanced platelet-rich fibrin, Immature permanent teeth, Necrotic pulp, Open apex, Regenerative endodontictreatment.

ABOUT THE AUTHORS

*Post Graduate Trainee, ** Professor and Head of the Department, ***Professor
Department of Conservative Dentistry and Endodontics
Dr. R. Ahmed Dental College and Hospital, Kolkata

CORRESPONDING AUTHOR

Dr. Palak Gupta
Post Graduate Trainee, Department of Conservative Dentistry and Endodontics, Dr. R. Ahmed Dental College and Hospital, 142/A, AJC Bose Road, Kolkata-700014
e-mail address: drpalak.gupta17@gmail.com
Contact no.: 9103543017

INTRODUCTION

Clinicians face difficulties when it comes to endodontic treatment of immature permanent teeth with an open apex. Their main concerns while treating teeth with "blunderbuss" canals are obturation issues, particularly with regard to regulating working length, and the effectiveness of disinfection of the canal space.[1] Many of these infected immature permanent teeth have been successfully retained by long-term apexification techniques to shut the root-end in the treatment of a non-vital pulp of an immature tooth with calcium hydroxide. However, when apexification is done, it is acknowledged that the root will no longer develop in terms of apical maturation and no dentine wall thickening.[2]

Hence these teeth have been found to be prone to cervical fracture and subsequent loss, due to their thin dentinal walls and perceived problems associated with long-term placement of calcium hydroxide.[3–5]As an alternative to using $Ca(OH)_2$ to treat diseased pulps in immature permanent teeth with regenerative endodontic techniques including pulp rejuvenation. In fact, the treatment of immature teeth with necrotic pulp may undergo a paradigm shift from conventional apexification techniques to a biologically-based regenerative endodontic approach.[6]

Regenerative endodontic treatment, or RET, has garnered a lot of interest lately and has been characterized as a paradigm change in the present arsenal of pulp therapy treatments.[7,8] It is described as a biologically based process intended to repair damaged tooth components, such as the pulp-dentin complex's cells and the dentin and root structures.9 Based on Cvek's classification of root development, RET is recommended in NIPT in stages 1, 2, and 3 with less than half, half, and two-third root formation, respectively.[7] Endodontic management of NIPT is clinically challenging as they possess thin root walls and wide open apex and are traditionally managed by apexification.[7,10] However, it is important to emphasize that apexification has no potential for root maturation and return of neurogenesis, and hence RET is considered a better treatment option in such teeth.[7,11]Amongstthe natural scaffolds utilized in RET, PRF is considered ideal as

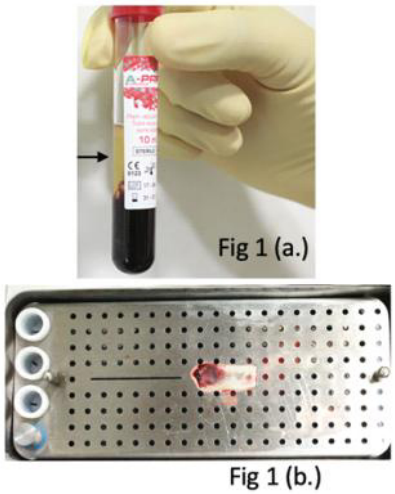

Figure 1: Advanced PRF :
a. Formation of advanced PRF after low-speed centrifugation of peripheral blood with clot (top layer) and red blood cells (bottom layer)
b. Advanced PRF membrane

it binds and localizes specific cells, contains a multitude of growth factors, and undergoes biodegradation over time leading to tissue regeneration and wound healing.[8] Based on the low-speed centrifugation concept (LSCC), Choukroun and Ghanatti[6] described two new advancements, namely A-PRF and injectable PRF, which contain more cells than conventional PRF.[12] The present study aims to assess the regenerative potential of APRF in NIPT using clinical examination, radiographic method, and vitality testing.

CASE REPORT

Patient History

3 healthy patients aged between 14-18 years were referred to the Department of Conservative Dentistry and Endodontics of Dr. R Ahmed Dental College and Hospital for the evaluation and treatment of traumatized tooth with dental abscess. On clinical examination, swelling of the upper lip was noted. Intra-oral examination revealed fractured crown on upper central incisors, tooth number 11,21,11in three different patients.

According to the American Association of Endodontists (AAE) recommendations[13] for the endodontic management of the permanent immature tooth with an open apex, the patient should fulfil the following criteria:

- Tooth with necrotic pulp and an immature apex.
- Pulp space not needed for post/core, final restoration.
- Patients not allergic to medicaments and antibiotics necessary to complete procedure (ASA 1 or 2).
- The presenting patients fulfilled the AAE requirements for the management of the traumatised anterior teeth.[14]

Informed consent

Informed consent from the parent was obtained for the following:

- Two or more appointments may be required for this treatment.

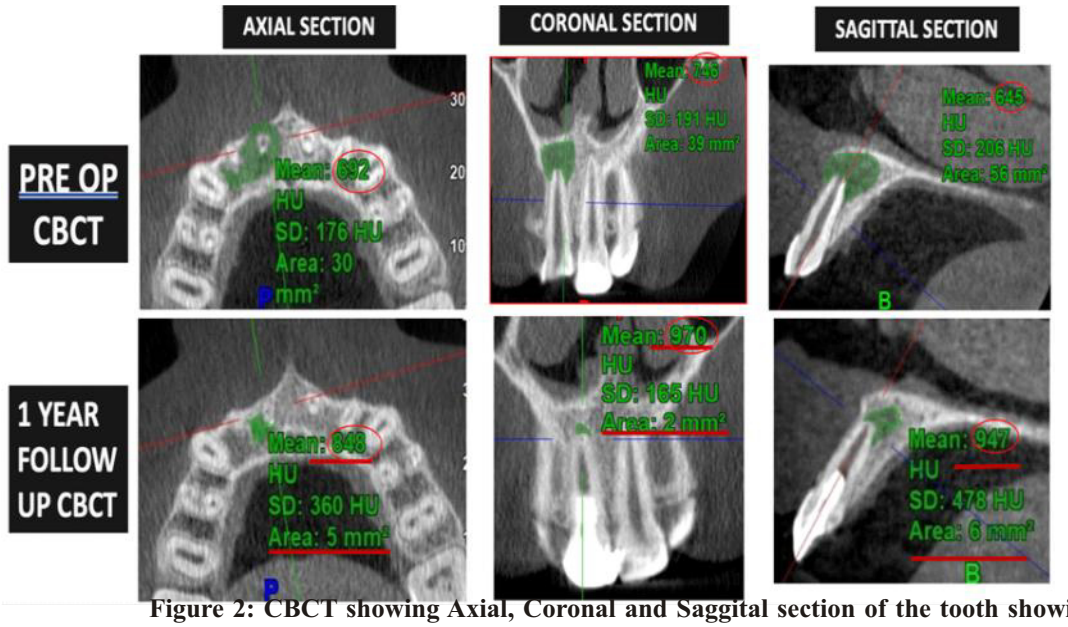

Figure 2: CBCT showing Axial, Coronal and Saggital section of the tooth showing Preoperative and 1 year follow up.

- Use of antimicrobials.
- Possible adverse effects: staining of crown/root and lack of response to treatment, pain/infection.
- Alternatives treatment options such as apexification, no treatment or extraction were presented.

First appointment

Local anaesthesia, rubber dam isolation and access into the pulp chamber were obtained. Necrotic pulp was removed and the canal space was cleaned using 20ml 1.5% NaOCl using a side-vented needle that minimized the possibility of extrusion of irrigants into the periapical area. The irrigating needle was positioned about 2 mm from root end, to reduce cytotoxicity to cells in the apical tissues. The canal was then irrigated with saline and dried with paper points. Ultracal which contains calcium hydroxide is used as intra canal medicament as used by Cachreli et al.[15] and was placed into the canal system. using a side vented needle (Canal Clean, Biodent Korea).The remaining space was restored with Cavit 3-4 mm. The patient was dismissed for 3-4 weeks.

Second appointment

The patients were assessed for signs/symptoms of persistent infection. The swelling on the gingiva was completely resolved and patients were free of any discomfort. The teeth were again isolated with a dental dam. The canal space was gently irrigated with 20ml of 17% EDTA and dried with paper points. A-PRF membrane was then sectioned into pieces using sterile scissors and condensed into the canals, using a finger plugger (Dentsply Maillefer), 1 mm beyond the confines of the working length, followed by placement of 3mm layer of Mineral trioxide aggregate (MTA; Angelus, Brazil) and the tooth was the doubly restored with RMGIC 3mm cavity (GC Gold Label, Japan) and then the rest of the cavity is filled with composite. (3M ESPE, USA).

Preparation of A-PRF

Under aseptic precautions, 10 mL of blood was withdrawn from the medial cubital vein of the patient and collected into test tubes without anticoagulants. The blood was centrifuged instantaneously using Choukroun's method of A-PRF preparation at 1500 rpm for14 minutes12with a centrifugation machine (Remi R 8C).

Usually, when blood is centrifuged, it gets divided into three distinct layers namely; straw-colored acellular platelet-poor plasma at the top level, PRF liquid at the intermediate level, and a red fraction of red blood cells at the base level. However, in the case of APRF, since the centrifugation is done at low settings for a lesser period of time, a complete demarcation of all three layers is not visible. Therefore, red blood cells being heavier, settle at the bottom, and the entire straw-colored liquid rich in platelets, and white blood cells (the top layer) is collected and used as the A-PRF clot (Fig. 1 a.) or can becompressed to form an A-PRF membrane (Fig. 1b.). In the current study, A-PRF membrane was used as a biomaterial / bioscaffold.

Follow-Up

Recall visits were scheduled at 3, 6, and 12 months postoperatively. Clinical, radiographic examination and vitality testing were done at each recall visit. The treatment was considered successful clinically when symptoms such as pain, swelling, intraoral sinustract, tenderness to apical palpation, or percussion were absent. Radiographic success was evaluated by resolution of the periapical lesion, changes in the open apex of the root canal in the form of apical closure, and further root thickening on intraoral periapical radiographs. A positive response to electric pulp testing (EPT) indicated a return of tooth vitality but it is usually seen after 18 months.

RESULTS

All patients presented with a history of trauma in the maxillary incisor region and the minimum, and maximum time elapsed between trauma and the first dental appointment was 2-5 years, respectively. All the patients tolerated the procedure well and did not complain of any immediate postprocedure discomfort. At 3-,6-, and 12-month clinical examinations, all patients (100%) were free of pain, associated intraoral swelling, and tenderness to percussion and palpation with complete healing of intraoral sinus. All patients (100%) showed periradicular healing, increase in mean bone density in hounse field unit, and apical closure. Preoperative CBCT and radiographs at 12months showed closure of the apex at the apical third of the root canal. [Fig. 2]. None of the patients (0%) responded toEPT at 3, 6, and 12 months follow-up.

DISCUSSION

Kim et al. initially introduced the term "revascularization" in endodontics. Later, Huang and Lin suggested the term "revitalization" to reflect the fact that the regenerated tissues in the canal space included not only blood vessels but also both hard and soft tissues. However, following the principles of tissue engineering, the American Association of Endodontics (AAE) adopted the term "Regenerative Endodontics" in 2007.[7]

According to AAE (2018) clinical considerations for RET; the three desired treatment outcomes are as

follows:

(1) Resolution of clinical signs and symptoms and bone healing which is essential (primary goal),

(2) Continued root maturation in the form of increased root wall thickness and length which is desirable (secondary goal), and

(3) Return of neurogenesis or positive response to vitality testing(tertiary goal).[16]

The success of regeneration is also influenced by effective disinfection of the canals and the use of an appropriate scaffold that allows apical cells to regenerate. Various studies have utilized NaOCl at concentrations ranging from 1% to 6%.[7] In the present study, based on AAE clinicalconsiderations,[16] disinfection of the canal space was obtained by using 1.5% NaOCl and ultracal to decrease the cytotoxic damage which may be caused to the stem cells ofthe apical papilla.

After disinfection, an appropriate scaffold is essential for regeneration. Among the various bioscaffolds, platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) have demonstrated clinical success in regenerative endodontic therapy (RET). However, the preparation of PRP is complex and time-consuming. Additionally, the use of bovine thrombin to activate PRP has been linked to the development of antibodies against thrombin, factor V, and factor XI, as well as adverse reactions such as hemorrhage, thrombosis, and systemic lupus erythematosus. PRF, a second-generation platelet concentrate, features a dense fibrin structure that supports cellular migration and cytokine entrapment through its trimolecular or equilateral fibrin branch junctions, while also preventing the premature invasion of unwanted cells.[8] It promotes cellular proliferation, differentiation, and angiogenesis, and provides a substantial and prolonged release of essential growth factors for up to 28 days, thereby accelerating wound closure and mucosal healing.[8,9,17] It only needs one centrifugation cycle, is simple to prepare, requires minimal placement time, and is entirely autologous since there is no biochemical processing of the blood.[18] It has been reported that the PRF causes the proliferation of human dental pulp cells and increases the protein expression of osteoprotegerin and alkaline phosphatase (ALP) activity, both of which are markers of odontoblastic differentiation and reparative dentin formation. [2]

In the current study, we have used A-PRF as a bioscaffold, an advanced form of PRF. This method involves centrifugation at a low speed to separate red blood cells from the blood, with the remaining concentrate being utilized for regeneration.[19] The varying centrifugation speeds and durations help prevent cell loss from the A-PRF matrix. As a result, A-PRF has a higher concentration of platelets, leukocytes, and growth factors, such as vascular endothelial growth factor (VEGF) and transforming growth factor ß-1, which are crucial for neovascularization and angiogenesis, compared to PRF. Additionally, A-PRF contains monocytes, which are vital for bone growth, vascularization, and the production of VEGF.[19,20] It has an improved collagen matrixsynthesis which leads to enhanced recruitment of progenitor cellsand hence greater regenerative ability.[21] Although both PRF clot and membrane have reported the same clinical success in RET, the membrane is easier to place and less time-consuming thanthe clot.[5] In the present study, all cases were treated using A-PRFmembrane, and on follow-up visits, revealed periradicular healing, which is attributed to the multitude of growth factors and tissue healing properties of A-PRF.

In the current study, Mineral Trioxide Aggregate (MTA) was directly placed over the A-PRF membrane to form a secure coronal seal, taking advantage of its improved physical, biological, and handling properties. MTA is beneficial in regenerative endodontics due to its quick setting time, high strength, and excellent marginal adaptability.[22] Root maturation after RET of NIPT is attributed to the presence of Hertwig's epithelial root sheath or cell rests of Malassez atthe apex, which is resistant to periapical infection and remains vital.[23] These stimulate stem cells present in the apical papilla, periodontal ligament, and multipotent stem cells which further differentiate into bone or dentine forming cells and help in root maturation.[23] However, several studies report that root maturation is unpredictable, and depends upon the trauma and severity of the periapical lesion both of which may disturb the biological function of Hertwigs epithelial root sheath and its interaction with mesenchymal stem cells in the dental follicle.[1] Additionally, the success of regenerative therapy is influenced by the specific clinical conditions presented.

In the current study, the recruited patients had full necrosis of pulp with chronic periapical infection of long duration. The newly formed tissue in the canal space after RET has been reported to be periodontal like tissue instead of pulptissue.[24] However, the type of tissue formed can be determined onlyby histological examination and not via conventional periapicalradiography or cone beam computed tomography.[1,24] Histologically, an animal study describes bridge formation at the apical third of the canal by ingrowth of intracanal cementum in revascularized immature necrotic teeth due to the osteoinductive activity of the MTA.[25] In the present study, MTA packed over A-PRF has been reported to stimulate odontoblastic differentiation and nodule formation during mineralization.[20] The tertiary goal of positive response to vitality testing after RET has not been reported as it requires atleast 18 months for the nerve bundle to establish.[1] In the present study, none of the teeth responded to EPT on 12-month follow-up. However, no conclusion can be drawn from this findingas a recovery of tooth sensibility may take more than 1 year. It has been suggested that the presence of thick layers of materials like MTA and

glass ionomer cement; and the fact that these materials limit the growth of new tissue ahead of it might lead to a negative response on vitality testing.[26]

LIMITATION

This case report was an exploratory observational study aimed at evaluating the impact of A-PRF in regenerative endodontic therapy (RET) of non-vital immature permanent teeth (NIPT) in maxillary incisors. Randomized trials can be conducted in the future to further determine its effectiveness in comparison to conventional and currently used biomaterials.

CONCLUSION

Advanced PRF is an effective therapeutic biomaterial in regenerative endodontic therapy (RET) of non-vital immature permanent teeth (NIPT). The resolution of clinical signs and symptoms, along with periradicular healing, was first observed at the 3-month, while the formation of a hard tissue bridge in the root canal became visible at the earliest 12 months following treatment.

There was no response to vitality testing at 12 months of follow-up

REFERENCE

1. Trope M. Treatment of the immature tooth with a non-vital pulp and apical periodontitis. Dent Clin North Am. 2010;54:313–24.

2. Huang GTJ. Apexification: The beginning of its end. Int Endod J.2009;42(10):855–66.

3. Harlamb S. Management of incompletely developed teeth requiring root canal treatment. Aust Dent J. 2016;61(S1):95–106.

4. Conde MCM, Chisini LA, Sarkis-Onofre R, Schuch HS, NörJE, Demarco FF. A scoping review of root canal revascularization: relevant aspects for clinical success and tissue formation. Int Endod J. 3 Dec 2016 doi:10.1111/iej.12711.

5. Andreasen JO, Bakland LK. Pulp regeneration after non-infected and infected necrosis, what type of tissue do we want. A review. Dental Traumatol. 2012 ;28 : 13–8.

6. Thomson A, Kahler B. Regenerative endodontics – Biologically based treatment for immature permanent teeth: A case reportand review of theliterature. Aust Dent J. 2010;55(4):446–52.

7. Kim SG, Malek M, Sigurdsson A, et al. Regenerative endodontics : a comprehensive review. Int Endod J 2018;51(12):1367–1388.

8. Hotwani K, Sharma K. Platelet rich fibrin - a novel acumen intoregenerative endodontic therapy. Restor Dent Endod 2014;39(1):1–6.

9. Murray PE, Garcia- Godoy F, Hargreaves KM. Regenerative endodontics: a review of current status and a call for action. J Endod 2007;33(4):377–390.

10. Chisini LA, Grazioli G, Francia A, et al. Revascularization versus apical barrier technique with mineral trioxide aggregate plug: a systematic review. Giornaleitaliano di endodonzia 2018; 32(1):9–16.

11. Santhakumar M, Yayathi S, Retnakumari N. A clinicoradiographic comparison of the effects of platelet-rich fibrin gel and platelet-richfibrin membrane as scaffolds in the apexification treatment of young permanent teeth. J Indian Soc Pedod Prev Dent 2018;36(1):65–70.

12. Choukroun J, Ghanaati S. Reduction of relative centrifugation force within injectable platelet-rich-fibrin (PRF) concentrates advances patients' own inflammatory cells, platelets and growth factors: the first introduction to the low speed centrifugation concept. Eur J Trauma Emerg Surg 2018; 44(1):87–95.

13. American Association of Endodontics. AAE Clinical Considerations for a Regenerative Procedure. American Association of Endodontics. 2014. Available from: www.aae.org/ Dental_Professionals/Considerations_for_Regenera tive_Procedures.aspx._and_research/research/curre nt regenerative endodontic considerations. pdf. Accessed December 2015

14. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: New treatment protocol.J Endod. 2004; 30(4):196–200.

15. Cehreli ZC, Isbitiren B, Sara S, Erbas G. Regenerative endodontic treatment (revascularization) of immature necrotic molars medicated with calcium hydroxide: a Case Series. Journal of Endodontics. 2011; 37(9): 1327-30.

16. Current considerations for regenerative procedures. American Association of Endodontists, (AAE) 2018

17. 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(11):1096–1104.

18. Sharma S, Mittal N. A comparative evaluation of natural and artificial scaffolds in regenerative endodontics: a clinical study. Saudi Endod J 2016;6(1):9–15.

19. Choukroun J. Advanced PRF&i-PRF: platelet concentrates or blood concentrates. J Periodontal Med Clin Pract 2014;1(1):3.

20. Gordon S. Alternative activation of macrophages. Nat RevImmunol 2003;3(1):23–35.

21. Shah R, M GT, Thomas R, et al. An update on the protocols and biologicactions of platelet rich fibrin in dentistry. Eur J Prosthodont Restor Dent 2017;25:64–72.

22. Tomson PL, Grover LM, Lumley PJ, et al. Dissolution of bio-active dentine matrix components by mineral trioxide aggregate. J Dent 2007;35(8):636–642.

23. Prabhakar AR, Rani NS, Yavagal C. Revascularization of immature necrotic teeth with platelet-rich fibrin and blood clot. Int J Oral Health Sci 2016;6(1):4–10.

24. Wakhloo T, Shukla S, Chug A, et al. Advanced Platelet-rich Fibrin-mediated Regeneration of Necrotic Immature Permanent Teeth: A Clinico-radiographic Observational Study. Int J Clin Pediatr Dent 2022;15(4):402–406.

25. Wang X, Thibodeau B, Trope M, et al. Histologic characterization of regenerated tissues in canal space after the revitalization/revascularization procedure of immature dog teeth with apical periodontitis. J Endod 2010;36(1):56–63. DOI: 10.1016/j.joen.2009.09.039

26. Shivashankar VY, Johns DA, Vidyanath S, et al. Platelet rich fibrin in there vitalization of tooth with necrotic pulp and open apex. J Conserv Dent 2012;15(4):395–398.