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The efficacy of platelet-rich fibrin and blood clot together as a scaffold in regenerative endodontic treatment: A case report

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Abstract
Introduction: *The treatment of immature teeth with regenerative endodontic procedures (REPs) has been described as a 'paradigm shift' as there is the potential for further root maturation .Regenerative endodontics has been defined as "biologically based procedures designed to replace damaged structures, including dentin and root structures, as well as cells of the pulp-dentin complex".*
Case report: *This report describes an immature nonvital 11 with apical pathology which was treated via revascularization using 3% NaOCl and 17% ethylenediamine tetraacetic acid as irrigants; Ca (OH) 2 as intracanal medicament, Blood clot and platelet‐rich fibrin (PRF) as scaffold, On follow‐up at 6 and 12 months, healing of periapical lesion, dentinal thickening, and apical closure with regaining of pulp vitality.*
Conclusion: *The clinical and radiographic evaluation after treatment revealed no signs and symptoms and root development was in progress which was an indication of successful treatment. It seems that, in the case of proper selection of the patient, this method can be an appropriate replacement for apexification in immature teeth with pulp necrosis.*
Keywords: *Platelet-rich fibrin, blood clot, regenerative endodontics and revascularization*

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I. Introduction

RET is based on the concept of tissue engineering [1], which requires the eradication of pathogens, the preservation of stem cells, and the presence of scaffolds and signal molecules [2] . To create a favorable microenvironment for stem cells to migrate, proliferate and differentiate, an ideal scaffold should facilitate spatial orientation and signal molecule release by cells. In most cases of tooth revascularization/revitalization, an endodontic explorer or file is introduced into the root canal and passes through the apical foramen to provoke bleeding from the periapical tissue into the canal to form a blood clot (BC)

The stem cells involved in the process are mainly stem cells of the apical papilla, periodontal ligament (PDL), pulp, and bone marrow. An empty canal will not support cell growth. Hence, a scaffold is essential to support cell organization and vascularization. [3]Growth factors or nutrients are supplied by the scaffold which helps in stem cell proliferation and differentiation leading to improved and faster tissue development. The important growth factors for regeneration are vascular endothelial growth factor (VEGF) and transforming growth factor‐ beta 1 (TGF‐ β1).

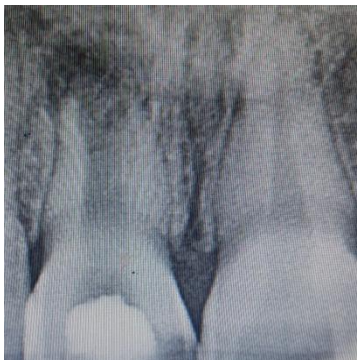
PRF is a second-generation platelet-rich concentrate that allows a sustained release in a slowly degrading fibrin matrix for multiple growth factors, such as; platelet derived growth factor; transforming growth factor-β; and vascular endothelial growth factor[4], and numerous cytokines[5].Platelet-rich concentrates are a naturally occurring cocktail of growth factors and cytokines organized in a 3D framework[5]. These bioactive factors are involved in all phases of wound healing and subsequently tissue regeneration. These properties, in addition to the ease of preparation and manipulation, make PRF an excellent candidate for RETs [6]. Additionally, the fluid obtained when centrifuging PRF (PRF-extract) can further stimulate endogenous regeneration by having synergistic action with mineral trioxide aggregate as it has been shown to enhance odontoblastic differentiation of human dental pulp stem cells [4]. Herein, we describe the successful use of PRF and its extract in conjunction with induction of bleeding, or as an alternative scaffold, for the regenerative endodontic management of challenging case.

II. Case Report

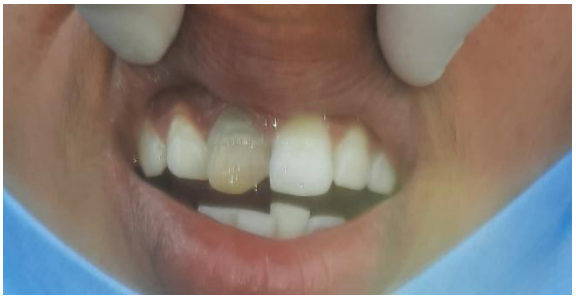
A 24 year female patient was referred to the Department of Conservative Dentistry and Endodontics at K D DENTAL COLLEGE MATHURA for the management of a non-vital tooth with open apex. According to the patient's clinical record, she had experienced pain in his upper front region of jaw since 2 months. She visited a general dentist for the treatment and the tooth was treated with extirpation of inflamed pulp tissues to relieve the symptoms and referred to higher center for further treatment. Her medical history was not significant. On extraoral examination, no significant findings were noted. Intraoral examination revealed zinc oxide eugenol restoration on maxillary right central incisor. The periodontal examination of all teeth showed probing depths within normal limits. There was no response to cold test or electric pulp test on #11, and the intraoral periapical radiograph revealed maxillary central incisor with an open apex, periapical radiolucency's and thin dentinal walls. (Fig 1)

Asymptomatic apical periodontitis with necrotic pulp was the diagnosis. Various treatment options were explained to the patient who consented for REPs with the aid of autologous platelet‐ rich fibrin (PRF) as scaffold. The methodology followed was based on the guidelines of the American Association of Endodontics and the European Society of Endodontology.

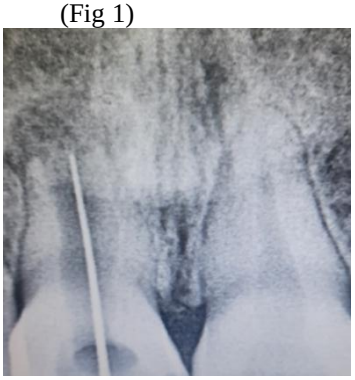
After local anaesthesia and rubber dam isolation of tooth #11, the pulp chamber was accessed. Pus was drained from the canal and flushed with copious saline irrigation. Working length was determined using digital x-rays and the canal was minimally instrumented (Fig 2). Copious irrigation using 20 mL of 1.5%sodium hypochlorite for 5 min was done. Finally, the canal was dried with sterile paper points followed by filling the root canal with Calcium Hydroxide paste (Ultra-Cal XS, Ultradent, United States), was placed as an intracanal medicament, sealed with 3–4 mm of Cavit (3M ESPE, Germany). Patient dismissed for 1–4 weeks and was asked to report in case of any complaint.



Pre-operative IOPA
(Fig 1)



Initial clinical picture

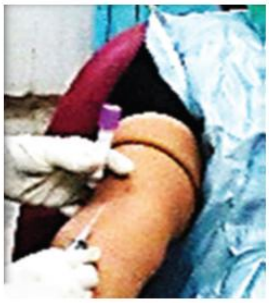


Working length determination
(Fig 2)

On the second appointment, three weeks later, the tooth was asymptomatic the teeth were re‐ accessed. Calcium hydroxide was removed via the same irrigation protocol. The tooth was anesthetized with 2% lignocane without vasoconstrictor, the canal was found to be dry and then it was copiously irrigated with 1.5% Sodium Hypochlorite followed by 10 mL of sterile saline. After drying the canal with paper points, the dentin was conditioned with 17% EDTA (Glyde) for one minute then it was rinsed with saline irrigation. The canal was dried and intra-canal bleeding was provoked using a K-file #25 extending 2 mm beyond the working length.

Meanwhile, PRF was prepared. A volume of 5 mL of patient's blood was drawn from the patient's cubital vein (Fig 3), collected in a glass test tube and centrifuged at 3000 rpm for 15 min in a tabletop centrifuge

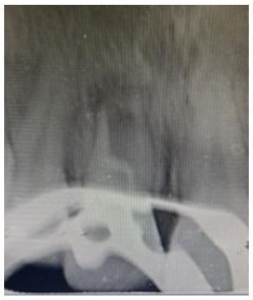
machine. PRF was obtained in the test tube as the middle layer with acellular plasma at the top and red blood cell (RBC) at the bottom. Using sterile tweezers (fig 4), the fibrin clot was removed and was placed on gauzepieces (to drive out the fluids trapped in the fibrin matrix), and autologous fibrin membrane was obtained. The freshly prepared PRF membrane was then placed into the canal opening, and then pushed below the level of cementoenamel junction (CEJ) using finger and hand pluggers such that it reached to the apical end (Fig 5). 3 mm of MTA was placed over the PRF followed by placement of moist cotton pellet (Fig 6), The patient was recalled 24 h later for removal of cotton and replacement of the temporary coronal seal with a permanent restoration.



Blood drown (Fig 3)

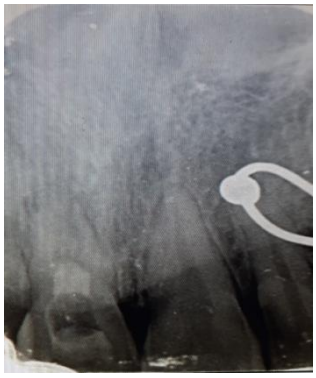
PRF (Fig 4)

PRF pushed in canal (Fig 5)

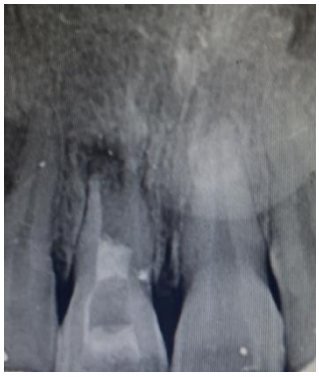


MTA placed over PRF (Fig 6)

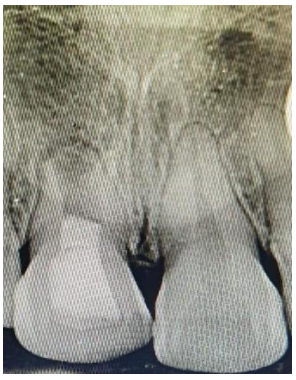
The patient was followed up at 1, 6 and 12 months revealing closure of apex along with thickened dentinal wall (Fig 7 ,8, 9,) . The follow up visit could not confirm the tooth response to either thermal or electric stimulus when tested. To reconfirm the response, the restorative materials were removed from the access opening and the calcified structure exposed. The tooth was then tested for responsiveness with the help of cold test and electric pulp sensibility tester, and the tooth responded.



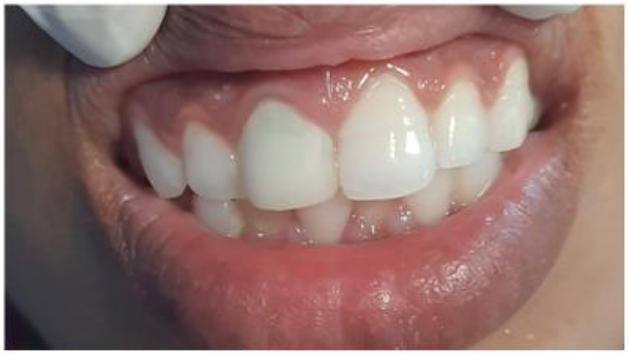
IOPA after 1 month (Fig 7)



IOPA after 6 month (Fig 8)



12 month follow up (Fig 9)



Final clinical picture

III. Discussion

The promise and potential of regenerative endodontic therapies in necrotic teeth was first explored by Nygaard-Ostby in 1961 who investigated the potential for repair when bleeding was induced by over-instrumentation beyond the apex prior to partial root filling of the canal with limited success [(7).] Forty years later, in 2001 Iwaya et al. [(8)] reported a case using a procedure termed 'revascularization',on an infected necrotic immature premolar tooth that showed continued root maturation and thickening of root canal walls with mineralized tissue.

The American Association of Endodontists (AAE) clinical considerations for regenerative endodontic procedures define success by three measures [(9)]: Primary goal(essential): The elimination of symptoms and the evidence of bony healing Secondary goal(desirable):Increased root wall thickness and/or increased root length Tertiary goal: positive response to vitality testing [(10)] Whilst the primary goal is an objective for all endodontic treatments, it is the secondary goal of increased width and/or length that is pertinent in the immature tooth. The assumption is that further root maturation often assessed in conjunction with apical closure of the immature root apex may minimize the incidence of root fracture. Another assumption is in the tertiary goal that are turn of neural capacity may indicate a more organized vital pulp tissue [(9)].

In this study we provoke periapical bleeding before PRF placement, periapical radiolucency resolution and root development were caused by the presence of the PRF scaffold. Our result is consistent with most previous studies [11, 12, 13] in which Platelet-rich plasma (PRP) served as the only bioscaffold in the treatment protocols. The superior performance in our study might be due to the synergistic effects of a BC and PRF in root development.

The stem cell population in periapically-induced BC should be significantly higher than that in PRF, which is prepared from peripheral blood. On the other hand, compared to a BC, PRF contains a much higher concentration of platelets [14], which might continuously release various growth factors, thereby contributing to tissue regeneration. In a pilot study, Narang et al. also compared the efficacy of PRF scaffold with that of a BC in RET. It was found that PRF achieved comparable effects in apical closure, dentinal wall thickening and even better results in periapical healing and root lengthening than a BC did [15].

The effectiveness of disinfection needed for RET is more critical than that needed in traditional endodontics, hence, in these cases, we used lower concentrations of NaOCl to avoid killing the stem cells[16,17]. In addition, calcium hydroxide was selected as an intracanal medicament because it could enhance the attachment, viability and proliferation of apical papilla cells in contrast to other medicaments [17].

In this case double seal of MTA,(3mm apically to CEJ) and glass ionomer cement is has been placed. Ideally, the coronal edge of MTA should be placed 1 to 2mm apical to the CEJ versus 3 to 4mm as recommended by Banchs and Trope to allow for more root development [18].Nosrat et al. suggested the use of calcium enriched mixture (CEM) in place of MTA [19]. CEM might promote the process of differentiation of stem cells and induce the formation of dentin-like hard tissue.

In this case, signs of root maturation with apex closed partially along with thickened root dentinal wall, the tooth responded to cold test and electric pulp tester after 12 months of follow up period. The radiographic evidence of increased root thickness might be due to dentin, cementum or bone [20] and the present findings are insufficient to distinguish among these possibilities. Studies had also shown that the tooth regaining its responsiveness to electric pulp sensibility testing even after 18-month follow up period after remaining asymptomatic all the time [21]. Further, in spite of the possibility of pulp tissues to re-enter the canal, histological examination is required to confirm it [22].

IV. Conclusion

Regenerative endodontics presents a new era in biological and clinical endodontics. Currently, this biologically based treatment is being recognized as the treatment of choice for immature teeth with pulp necrosis. The outcome of this case report suggests that the conservative treatment approach can create a suitable environment for pulp regeneration and result in root maturation via regaining the vitality of the tooth. This leads to an added advantage of increased strength of the tooth, with lesser financial burden as well as better psychological well-being to the patient

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