REGENERATIVE PULPOTOMY AS A NOVEL TECHNIQUE FOR TREATMENT OF PERMANENT MATURE MOLARS DIAGNOSED WITH IRREVERSIBLE PULPITIS USING PLATELET-RICH FIBRIN: A CASE SERIES STUDY

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ABSTRACT

INTRODUCTION: Management of teeth with clinical signs/symptoms suggestive of irreversible pulpitis was conventionally invasive, but the emerging evidence suggested successful treatment outcome using the less invasive vital pulp procedures such as coronal pulpotomy, leading to preservation of the remaining pulp in a vital and functioning state.

OBJECTIVES: Evaluation of the clinical/radiographic success of novel regenerative coronal pulpotomy technique using Platelet-Rich Fibrin (PRF) and Biodentine.

MATERIALS AND METHODS: Three irreversibly inflamed permanent molars with mature roots in three patients were treated by regenerative pulpotomy technique. Access openings were done, and coronal pulps were removed to the level of root canal orifices. Hemostasis in all teeth were done by the compression with cotton pellets moistened with 2.5 % sodium hypochlorite (NaOCl). PRF membranes were prepared by drawing 10 ml of patients’ own blood, then they were immediately centrifuged using a table top centrifuge at 400 gforce for 12mins. The PRF membranes were placed over the remaining radicular pulps followed by Biodentine preparation and placement over the PRF. The cavities were then immediately restored. Patients were scheduled for clinical/radiographic evaluations after three-, six- and 12-months.

RESULTS: Throughout the follow-up periods, the three teeth demonstrated clinical/radiographic success with complete resolution of clinical signs/symptoms.

CONCLUSIONS: All presented cases showed favorable clinical/radiographic outcomes of “Regenerative pulpotomy” procedure in treating irreversibly inflamed permanent molars using PRF and Biodentine combination.

KEYWORDS: Regenerative Pulpotomy, Mature Teeth, Platelet-Rich Fibrin, Irreversible Pulpitis, Case series.

INTRODUCTION

The main aim of restorative procedures was to preserve the viability of dental pulp tissues whenever possible. Over the years, minimally-invasive endodontic techniques (MIE) have been reintroduced, receiving wider acceptance over the conventional treatments in treating patients with inflamed pulps(1).

When there is no restriction on time or cost factors, root canal therapy(RCT) could be the choice of treatment in many clinical situations with a high success rates as shown in many literature studies(2). However, it is a relatively time-consuming expensive non-conservative treatment, and is considered as a non-biological therapy. Also, in some clinical scenarios the quality of treatment done by general dentists was poor(3). Therefore, alternative treatments such as vital pulp therapies (VPTs) could be a less-invasive, viable treatment options preventing dental extractions and dental neglects(4).

Preserving the vitality of the pulp tissues aids in retaining all its functions, including: vascularity, innervation, neurosensory, immuno-competency and proprioceptive functions of teeth(4). Also, many studies had found that the long-term prognosis of endodontically-treated teeth was not the same as teeth with vital pulps(5,6).

The main rationale behind the use of coronal pulpotomy(CP) is based on the ability of radicular pulps to survive after removing the infected inflamed coronal pulp tissues followed by placing the suitable medicaments(7). This changed the understanding of the clinical diagnosis of irreversible pulpitis and changed its management, especially in the era of better

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understanding the healing process and the regenerative ability of dental pulp tissues in the presence of biocompatible materials(8).

Many materials have been introduced to be used in CP based on their properties as: its biocompatibility, ability to create an adequate efficient seal, induction of mineralization, its antimicrobial efficiency and having good bioactive properties stimulating the formation of the reparative dentin and inducing the regenerative abilities of the remaining radicular pulps(9).

Mineral trioxide aggregate (MTA) is considered as one of the most commonly used and evidenced-based calcium silicate cements used for such purpose showing positive clinical outcomes. Though, due to some characteristic drawbacks of MTA, as long setting time, difficult handling properties and its liability to cause tooth discoloration(10). Therefore, there is a critical demand for the development of new materials that fulfill the requirements of CP and could overcome the drawbacks with MTA cements.

Recently, new bioactive calcium silicate-based cement was introduced as Biodentine, it is considered as second generation bioceramic dental material. It was primarily and specifically introduced to act as a dentine-replacement biomaterial. It is mainly invented using the same MTA cements technology with added improvements in properties of these cements, such as improved mechanical, physical and handling properties with shorter setting time (10-12 min) and without tooth discoloration(11).

However, despite these growing enhancements in material science, researches still document mild to moderate cytotoxic effects of the different materials used for CP, when they are placed directly on the remaining radicular pulp tissues(12). Moreover, the conventional CP treatments never lead to the regeneration of pulp and dentin that were removed from the coronal portion, as the inflamed pulp tissues in the pulp chamber were removed surgically, and the radicular remaining pulps is treated with medicaments at the level of the root canal orifices to promote the formation of dentine bridges(13).

Therefore, it was crucial to develop autologous biocompatible treatments focused to maintain pulpal vitality, minimizing pulpal inflammation, increasing tooth longevity and regenerating the lost pulp dentin complex in the coronal pulp chamber(14).

Platelet-Rich Fibrin (PRF) is second-generation platelet concentrates that was introduced by Choukroun J in 2001. PRF is considered as an ideally pure autologous biodegradable scaffolding material used for regeneration. It is composed of concentrates of platelets and cytokines, that is widely used to accelerate healing of the hard/soft tissues and is considered as ideal material needed for regeneration(15). It favors the development of micro-vascularity and guides epithelial cells migration on its surface, while being easily prepared without biochemical handling of blood(16). It would not only serve as a scaffolding material but it would also serve as a reservoir to deliver certain bioactive growth factors at the site of application essential for pulp-dentin-complex regeneration(17).

This study described a new management for three carious pulpally-involved permanent mature teeth with symptoms suggesting irreversible pulpsitis. The clinical/radiographic outcomes of these cases were evaluated over regular follow-up periods to gauge the prognosis of the treatment performed using a novel regenerative coronal pulpotomy technique using PRF and Biodentine.

MATERIAL AND METHODS

This case series was done after the approval of the “research ethical committee” (IRB NO 00010556- IORG 0008839) at the Faculty of Dentistry in Alexandria University. All cases received oral/written information about the new treatment protocol then signed the informed consents to participate in this study. This case series was conducted in accordance with the CARE guidelines for case reports.

Case 1:

A 28 year old female patient presented to the “Department of Conservative Dentistry, Faculty of Dentistry, Alexandria University” complaining of acute spontaneous pain in the lower right posterior region. Dental history was recorded emphasizing on history of the present illness. Extra oral examinations showed neither swelling nor tenderness related to this area. Clinical examination revealed large carious lesion in the occlusal surface of tooth number #46 (Fig. 1A). The patient’s symptoms were reconfirmed with deep intense pain that remained after the removal of the thermal stimuli. No tenderness on percussion or associated sinus tract were found around the affected tooth, periodontal probing of the tooth was found to be within the normal limits. Radiographic examination showed carious lesion extending through enamel/dentin/pulp with normal periapical tissues in tooth #46. Based on the clinical/radiographic/pulp sensibility examinations, the case was diagnosed with “symptomatic irreversible pulpsitis with normal apical tissues”.

CP using PRF/Biodentine combination was explained to the patient as an alternate to conventional RCT. The medical examination and tests for the bleeding time/clotting time/platelets count were done and were within the normal range.

After local anesthesia and rubber dam application, the crown and the surrounding rubber dam was disinfected with 2% chlorhexidine solution. A sterile high-speed round bur was used for caries excavation; after exposure, a sterile Endo-Z bur was used to refine the access cavity. The coronal pulp was removed at the level of canal orifices using large sharp spoon excavator to remove remnants of pulp tissue in the pulp chamber (full pulpotomy). Irrigation was done for the pulp chamber space using 2.5% NaOCl then hemostasis was achieved (Fig. 1B) by the application of a cotton pellet moistened with 2.5% NaOCl for 2-3 min and repeated if required up to 10 min. PRF was prepared by drawing 10 mL of the patient own blood into two 5mL test tubes without the addition of any anticoagulants. Tubes were placed in two opposing cylinders of a table-top centrifuge device (800D centrifuge, Makaad, Shanghai, China) for balance. Then they were immediately centrifuged at 400 gforce for 12mins. After centrifugation, the resultant product exhibited following three layers; platelet-poor-plasma at the surface, PRF in the middle, and RBCs at the bottom. Sterile artery forceps was inserted into the test tube to retrieve the PRF clot (Fig. 2A), which was separated from the underlying RBC layer using scissors and then placed it in the PRF box (Fig. 2B). The PRF...
clot was placed in one of the cylinders of the PRF box (Fig. 2C) then compressed with the piston gently (Fig. 2D), to get thick small plug of PRF (Fig. 2E), this allowed easily placement of the membrane ontop of the remaining radicular pulps (Fig. 1C). Biodentine was then prepared and placed over the PRF plug with a thickness of 2 mm approximately and was allowed to initial set for about 12 min (Fig. 1D). The cavity was restored using resin-modified glass ionomer cement (RMGIC) then covered with composite resin restoration (Fig. 1E and Fig. 1F).

An immediate postoperative periapical digital radiograph was taken, and the patient was recalled for follow-up after three, six and 12 months for clinical and radiographic evaluation.

Figure 1: A: Showing a carious lesion on the occlusal surface of tooth #46; B: Removal of coronal pulpal tissues and hemostasis achieved; C: PRF plug was placed over the remaining radicular pulp; D: Biodentine was placed over the PRF plug; E: RMGIC placed over the Biodentine; F: Composite restoration as a final restoration.

Figure 2: A: Showing a sterile artery forceps inserted into the test tube to retrieve the PRF clot; B: PRF clot placed in the PRF box; C: PRF clot was placed in one of the cylinders of the PRF box; D: The piston was compressed gently to pack the PRF clot to form PRF plug; E: PRF plug at the base of the PRF box cylinder.

Case 2:
A 35 year old male patient was presented to the post graduate clinic at “Faculty of Dentistry, Alexandria University” complaining of sharp spontaneous pain lasting for minutes in the mandibular right posterior region. The medical history was noncontributory. Extra-oral examinations revealed absence of swelling or palpable lymph nodes in the head and neck regions. Intraoral examinations showed that the tooth #46 showed a deep carious lesion involving the occlusal surface. Sensibility test was done and showed sharp pain lingering after the removal of the stimulus. There was slight tenderness on percussion with no sinus tract opening near to the tooth, periodontal probing depth of the tooth was within normal limits, with normal physiological mobility. Radiographic imaging showed large deep carious lesion extending through the enamel/dentin/pulp in tooth #46 with slight widening in the PDL space around both mesial and distal roots. Based on the clinical/radiographic/pulp sensibility examinations, the diagnosis was “symptomatic irreversible pulpitis with symptomatic apical periodontitis”. The patient was informed of regenerative pulpotomy treatment and consent was obtained from him. The patient was treated with the same regenerative pulpotomy procedure using PRF as in case 1, and he was recalled for follow up after three, six and 12 months for clinical/radiographic evaluations.

Case 3:
A 40 year old male patient was presented at the Conservative Dentistry Department at the “Faculty of Dentistry, Alexandria University” complaining of sever spontaneous pain lasting for minutes which awaken him at night, and only relieved by analgesics in the mandibular right posterior region. The patient did not have any significant medical history contradictory to regenerative endodontic procedures. In extraoral examination, neither swelling nor palpable lymph nodes were noted. Intraoral examination showed a large carious lesion involving the occlusal surface of tooth #46. The tooth responded positively to pulp sensibility tests. Periodontal probing measurements of the tooth were within normal limits, with normal physiological mobility, showing no tenderness on vertical/horizontal percussion. Radiographic imaging showed large deep carious lesion approaching the pulp in tooth #46 with slight widening in the PDL space round the mesial root. Based on the clinical/radiographic/pulp sensibility examinations, the diagnosis was “symptomatic irreversible pulpitis with asymptomatic apical periodontitis”. The patient was informed of regenerative pulpotomy treatment using PRF and the consent was obtained from him. The patient was treated with the same regenerative pulpotomy procedure using PRF as in case 1 and 2, and he was recalled for follow up after three, six and 12 months for clinical/radiographic evaluations.

Follow up:
The follow-up periods for all clinical cases presented were at three-, six- and 12-months period. The three patients were asymptomatic showing clinical and radiographic success. On clinical examination, the three teeth were still functional with no pain on chewing, lack of swellings and sinus tracts adjacent to the treated teeth. All patients reported negative response to
axial percussion, apical palpation and responded normally to pulp sensibility tests.

On radiographic examination, all teeth showed normal trabecular pattern of bone around the roots, with normal periodontal ligament (PDL) space, even in cases (2 and 3); where they showed preoperative widening in PDL space. All teeth showed no internal/external resorptions or intracanal calcifications (Fig. 3A-3E, Fig. 4A-4E and Fig. 5A-5E). All patients in this case series were still under annual systematic follow-up.

**Figure 3:**
A: A pre-operative radiograph with a large carious lesion approaching the pulp; B: Immediate post-operative radiograph showing maintenance of the pulp chamber space using PRF; C: Three-month follow-up radiograph showed normal trabeculation of bone around the tooth; D: Six-month follow-up radiograph showed normal trabeculation of bone around the tooth; E: Twelve-month follow-up radiograph showed normal trabeculation of bone around the tooth.

**Figure 4:**
A: A pre-operative radiograph with a large carious lesion approaching the pulp with widening of the PDL space around both roots; B: Immediate post-operative radiograph showing maintenance of the pulp chamber space using PRF; C: Three-month follow-up radiograph showed normal trabeculation of bone around the tooth with normal PDL space around both roots; D: Six-months follow-up radiograph showed normal trabeculation of bone around the tooth with normal PDL space around both roots; E: Twelve-months follow-up radiograph showed normal trabeculation of bone around the tooth with normal PDL space around both roots.

**Figure 5:**
A: A pre-operative radiograph with a large carious lesion approaching the pulp with widening of the PDL space around the mesial root; B: Immediate post-operative radiograph showing maintenance of the pulp chamber space using PRF; C: Three-month follow-up radiograph showed normal trabeculation of bone around the tooth with normal PDL space around both roots; D: Six-months follow-up radiograph showed normal trabeculation of bone around the tooth with normal PDL space around both roots; E: Twelve-months follow-up radiograph showed normal trabeculation of bone around the tooth with normal PDL space around both roots.

**DISCUSSION**

Vital pulp therapy (VPT) has been increasingly considered as a minimally invasive endodontic (MIE) approach for management of teeth with inflamed pulps compared to conventional RCT(18).

Full pulpotomy in adult mature apices has been studied to a much lesser extent than in primary or immature teeth and related debates still found in literature (19). However, many recent clinical trials and systematic reviews have been published showing that CP procedures was able to treat irreversibly inflamed mature molars with high success rates which were comparable to the success rates of pulpotomy treatment for immature and primary teeth(20,21).

The justification behind the use of CP procedures in treating inflamed teeth is based on the removal of the whole coronal part of the inflamed pulp to the level of canal orifices while preserving the health of the remaining radicular pulps, providing a better chance for removing the irreversibly inflamed tissues compared to other VPTs(21).

Clinical signs/symptoms of irreversible pulpitis do not always demonstrate that the pulps were inflamed or damaged beyond repair. Pain was present in 40 % of cases with histologically savable pulp and partial pulpitis was present in 88 % of painful cases(22). Furthermore, 15.4 % of cases with clinical signs/symptoms of irreversible pulpitis showed histological features of reversible pulpitis. Even cases that showed irreversible changes and/or necrosis, these changes were found to be limited in the coronal pulpal tissues, reactions were found to be less severe in the rest of the coronal tissues, and it was frequent to observe normal un-inflamed pulpal tissue in the contralateral pulp horn as well as in the roots(23).
This required the need to review the currently used diagnostic terminology for the different pulpal conditions. For this reason, a new diagnostic system for pulpal diseases has recently been proposed to assess pulpitis, which includes: initial/mild/moderate/severe pulpitis, and implementing minimally invasive treatment strategies to treat these conditions instead of the conventional root canal treatment (RCT)(18). This has changed the general understanding of the clinical diagnosis of irreversible pulpitis and its management. Preventing the invasion of bacteria and its toxins into the pulp is a vital factor for favourable prognosis of VPTs. So in this case series pre-operative and per-operative decontaminations were achieved using complete isolation by rubber dam, preventing the bacterial invasion from the oral cavity and saliva, also the clinical crowns and the surrounding rubber dam were disinfected before excavation using 2% chlorhexidine-glucocanate as adopted by Patil T et al(24) and Dammaschke T et al(25).

When dealing with cariously exposed pulps, it is hard to assess the actual condition of the pulp, which plays an important role in the success/failure of VPTs. There is no reliable tool to help evaluate how deep the inflammation has advanced in to the pulp(26). Many studies recommended observing the pulpal bleeding and the ability to control it, rather than depending on the preoperative clinical signs/symptoms(4,26). However, the profuse bleeding which is difficult to control confirms severe pulp inflammation(27). The solution used for hemostasis in this study was 2.5% of NaOCl, as it aids in disinfecting and rapid control of bleeding of the access cavity, as recommended by many previous recent clinical trials (21,28). This might also be why the recent consensus statement in a prominent endodontic journal recommended the use of NaOCl for hemostasis, as most of the articles of this publication specialized in the field of endodontology(9). The solution used for hemoastasis in this study was 2.5% of NaOCl, as it aids in disinfecting and rapid control of bleeding of the access cavity, as recommended by many previous studies(21,28). The rapid proper hemostasis of bleeding induced after the pulp tissues removal is a critical step during all VPTs procedures, as the blood clot that might form at the material/pulp interface might lead to treatment failure as proposed by Solomon R et al(29) and Taha and Khazali(30). It is believable that the blood clot formed between the capping material/pulp surface would inhibit the effect of the capping material on the exposure site and it might also act as a substrate for bacteria if there is a defect in the restoration(31).

Latest calcium silicate-based bioactive cements such as MTA and Biodentine were considered as an ideal material to be used after CP showing excellent bioactivity, biocompatibility and efficient sealing abilities(32). Biodentine was selected to be used in this study, showing improved properties than MTA with more efficient characteristics such as better bioactivity and biocompatibility(33). Calcium silicate-based cement was chosen depending on its hydrophilic natures, requiring some moisture for setting, which is an important property especially when there is a potential moisture in the clinical setting, as recommended by Kaur M(10).

Literature reported mild/moderate cytotoxicity of the freshly-mixed calcium silicate based cements due to their high initial pH(34). So, there is a constant need for an autologous biologically based biomaterial to be placed over the remaining radicular pulp to neutralize these side effects, minimizing pulpal inflammation, inducing faster wound healing and regenerating the lost pulp tissues in the coronal pulp chamber(12,13).

Therefore, the present case series adopted the novel “Regenerative pulpotomy” approach that locally regenerates the lost pulp-dentin complex in the pulp chamber instead of the conventional coronal pulpotomy. This novel strategy is based on the induction of out-growth of dental pulp stem cells (DPSCs), blood capillaries and neurons from the remaining radicular pulp, using an ideal scaffold(35).

PRF membrane which was used in this case series is considered superior to platelet-rich-plasma (PRP); first generation blood matrices, in many ways. Unlike the PRP, the method for preparation is simple, economical and the biochemical handling of blood and the addition of anticoagulants wasn’t required. Its slow polymerization helped efficient migration/attachment/proliferation/differentiation of the DPSCs needed for pulp-dentin complex regeneration. Also, It supports the immune system and promotes hemostasis (12,29). PRF was used in this study considering its advantages of excellent biocompatibility and bioactivity, PRF exerts no cytotoxic effect on DPSCs, maintained their original morphology(36). PRF membrane acts as an ideal scaffold; maintaining the space of the pulp chamber, acting as a reservoir for the suitable growth factors (GFs) needed to induce the formation of new pulp tissues, blood vessels and nerves into the defect area and dentine-like hard tissue over them.

PRF plugs was covered with a layer of Biodentine in all cases, that would help stabilizing and protecting the membranes from the forces of the restorative procedures; added to the other advantages of Biodentine, as recommended by Solomon R et al(29). By this technique we could gain the advantages of the ideal scaffolding and GFs releasing potential of PRF membranes and the excellent sealing capability of Biodentine, utilizing them as a dual-sword accelerating the healing of the irreversible inflamed pulp tissues. All cases in this case series showed both clinical and radiographic success. the results of this study were in accordance to previous case reports and clinical trials done by Hiremath H et al (12) Solomon R et al (29), Patil T et al (24) and Prasanthi N et al(19). The potential theory for the successful outcomes of the present cases might be attributed to that DPSCs residing in the radicular pulp; which was diagnosed clinically with pulpitis, might still have stem cells potentials similar to uninfamed healthy pulp stem cells, therefore they might be resources for autologous pulp regeneration, as seen in previous studies published by Wang et al(14) and Li Y et al(37). Moreover, it might be also attributed to the use of PRF as a scaffold over the radicular pulps, as it actively participates in the pulp healing by the release of suitable GFs such as: transforming-growth-factor Beta-1 (TGF β1) and platelet-derived-growth-factor (PDGF) which play a key role in proliferation and differentiation of DPSCs(17).

Also, PRF releases healing cytokines such as Interleukin-4 (IL-4) due to activation of subpopulation of T-cells. This IL-4 supports healing by moderating inflammation. It also inhibits...
Interleukin-1β (IL-1β), mediated stimulation of matrix metalloproteinase-1 (MMP-1) and metalloproteinase-3 (MMP-3), and synthesis of prostaglandin E2. Thus, PRF membrane could be of added advantage in treating cases with inflamed pulp, as it might be possible to utilize this controlled inflammation as a modulating factor to induce regeneration in the presence of GFs and cytokines released by PRF(38). Even cases that showed preoperative widening in the PDL space (cases 2 and 3) showed normal trabecular pattern of bone with normal PDL space during the follow up period, that might be attributed to that the apical inflammation always preceede the total pulpal necrosis. That resulted from neurogenic inflammation related to bacterial biproducts extending into the periapical areas causing complex interactions of inflammatory mediators and neuropeptides and from afferent nerve fibers suppling the periapical and pulpal tissues. Healing after VPTs for permanent mature teeth with periapical radiolucency had been reported in several studies(20,21,30). Intra-oral periapical radiograph were chosen to be used during the follow up periods, that was in accordance with recommendations for radiological follow-up durations and frequency recommended by the European Society of Endodontology and Patel & Saunders, which specified a radiograph at 1 year after completion of the treatment, at which point a decision can be taken whether the treatment was successful or there is a need for an intervention (39). These findings suggest exciting opportunities for biologically based approaches for dental tissues repair. Also provided valuable insights in how the natural regeneration process might be operating in the tooth. While six months follow-up has been considered by many studies as sufficient for teeth treated with CP (4,40), long-term follow-up is always desirable to reveal late failures; all cases treated in this case series were followed up for 1 year and were scheduled for a yearly follow up.

CONCLUSIONS
Within the limitations of this study and based on its positive outcomes, we concluded that clinicians could be advised to go for non-invasive regenerative approach to improve the standard of dental care offered to the patients. But these positive results recommended the need for more studies and clinical trials with bigger sample size to evaluate the effectivenss of such procedures and also the histological evaluation of such procedure is needed to confirm the fate of the PRF membrane before considering it as a mainstay of treatment.

Conflict of interest :
The authors declare that they have no conflict of interest.

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