LASER-PLATELET RICH FIBRIN AS A NOVEL REGENERATIVE PULPOTOMY TECHNIQUE FOR TREATMENT OF IRREVERSIBLE PULPITIS IN PERMANENT MOLARS: A CASE SERIES STUDY

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ABSTRACT
INTRODUCTION: The main objective of pulp therapy is to maintain the integrity and health of the treated teeth and their surrounding supporting structures. Recently laser has been applied in various dental procedures, among which were the vital pulp therapy procedures.


MATERIALS AND METHODS: Three lower permanent mature molars with irreversibly inflamed pulp were treated by regenerative coronal pulpotomy technique. After access opening, inflamed coronal pulp tissues were removed to the canal orifices level. Hemostasis in all teeth was achieved using diode laser 970nm application. Radicular pulp was then capped using PRF then covered with Biodentine and the cavities were permanently restored immediately. Patients were scheduled for clinical and radiographic follow-up after three, six and 12 months.

RESULTS: All the three cases showed clinical and radiographic success throughout the follow up period with resolution of the pretreatment clinical signs and/or symptoms.

CONCLUSION: Laser pulpotomy together with PRF and Biodentine showed favorable clinical and radiographic outcomes when used for regenerative treatment of irreversible pulpite in mature permanent molars.

KEYWORDS: Diode Laser, Platelet-rich fibrin, Regenerative Pulpotomy, Permanent teeth, Irreversible pulpitis

INTRODUCTION
Patients attending to the dental clinics with extensive caries are not considered to be related to an irreversible pattern of pulpal inflammation unconditionally. Moreover, the severe/spontaneous pain is not always indicative that the pulp is not repairable (1,2).

Two different approaches have been advocated to dealing with deep carious pulp exposures: the vital pulp therapy (VPT) as the conservative method and the consistent but more invasive approach in the form of conventional root canal therapy (3). Pulpotomy is a single visit vital pulp therapy procedure in the form of excision of the coronal part of the pulp, performed as a means of preserving the vitality as well as the function of the remaining radicular pulp (4).

Coronal Pulpotomy (CP) has been indicated to be used as the final treatment in deciduous teeth as well as immature permanent teeth (5) with normal/reversible inflamed pulp tissue accompanied with a carious lesion or post-traumatic pulp exposure. Although pulpotomy as a final treatment in permanent teeth is of great interest, but still only few clinical research studies have been conducted.

Literature shows that in search for ideal medicament for pulpotomy, several materials have been advocated. Also techniques like lasers and electro surgery had been evaluated with varying histological, clinical and radiological success for pulpotomy in primary as well as permanent teeth (6).

Calcium hydroxide (Ca(OH)₂) was considered to be of the most widely used...
biomaterials for pulp capping and pulpotomy. It is a high-alkaline (pH = 12.5-12.8), slightly soluble salt that dissociates into calcium and hydroxyl ions (7). However, Ca(OH)₂ is associated with certain limitations such as leakage and dissolution over time.

Torabinejad introduced Mineral trioxide aggregate (MTA) in 1993 (8) and considered it a reliable alternative to Ca(OH)₂ for vital pulp therapy procedures due to its good biocompatibility and improved sealing. It also possesses good physical and mechanical properties with excellent marginal adaptation (9). Nowadays MTA is considered the optimum material for vital pulp therapy in permanent teeth (5,9). However, some practitioners reported difficulty in its handling properties as well as postoperative tooth discoloration resulting in dissatisfaction of the patients.

Biodentine is a newer calcium silicate–based material introduced. It is maintaining the optimum properties of the MTA but with easier handling properties, cause no discoloration of the tooth and with shorter setting time (10-12mins) than MTA (10).

Platelet-Rich Fibrin (PRF) is considered as an ideally pure autologous biodegradable scaffolding material used for regeneration; to maintain the pulp vitality, minimize pulpal inflammation, increase tooth longevity and help in regenerating the lost pulp dentin complex in the coronal pulp chamber (11). PRF is second-generation platelet concentrates and was introduced by Choukroun J in 2001. It is composed of concentrates of cytokines and platelets that are widely used to enhance healing of the hard/soft tissues and is considered as ideal material needed for regeneration. It serves as a scaffolding and as a reservoir of certain growth factors important for pulp-dentin-complex regeneration (12).

Lasers have been numerously applied in the clinical dental field such as in oral surgery, preparation of the restorative cavities etc. The Diode lasers are soft-tissue laser of 810-980 nm wavelength. This wavelength is eagerly engrossed by soft-tissues and has poor penetration in dental hard structures which makes it suitable for soft-tissue applications such as pulpotomy in closeness to the tooth dentine and enamel. They act by excision of the damaged pulp tissues in the immediate contact of the beam, disinfects the remaining radicular pulp tissue by causing lysis of the cell-wall of the available bacteria. It also acts by bio-stimulation of adjacent pulp tissue, which enhances healing (13). They also provide deep penetration into biological tissues resulting in good coagulation. Moreover, they are less expensive than the other laser-systems and they save space.

Various researches had been conducted to study the effect of diode laser on vital pulp. Studies concerned with vital pulp healing (14) reported reparative (secondary) dentine formation due to laser application (15). Other studies found that it induces calcification within 30 to 45 days, resulting in the formation of a continuous and organized reparative dentine bridge to a greater extent than that accomplished by traditional methods. They also found that laser application result in milder tissue reaction with steady rise in macrophage count in the remaining pulp tissue which is considered a good sign of pulp tissue healing (16). The combined use of Laser and bioactive capping materials would provide better and faster healing of the pulp-dentine complex and better post-operative conditions.

To our knowledge, no study has explored the effect of Diode laser together with PRF and Biodentine for regenerative pulpotomy in mature permanent teeth. This case series study shows the management of three symptomatic deeply decayed permanent mature molars with symptoms suggesting irreversible pulpsitis. Clinical and radiographic follow-up was conducted over regular follow-up periods to evaluate the prognosis of the treatment outcomes of these cases when managed using a new pulpotomy technique using Diode Laser and PRF.

MATERIALS AND METHODS

This case series study was conducted in accordance with the CARE guidelines for case reports. This case series was done after the approval of the “Research Ethical Committee (IORG 0008839) – 0011-03/2019”; on 17/3/2019 at the Faculty of Dentistry, Alexandria University. The new treatment protocol was simply explained to the participating candidates as an alternate to conventional RCT, explaining all the advantages and expected drawbacks of the treatment and all candidates signed the informed consents to participate in this study.

Case 1: A 27 years old male patient presented to the “Outpatients clinics, Faculty of Dentistry, Alexandria University” complaining of severe pain in the lower right region with cold drinks and room-temperature water. Dental history was taken regarding the history of the complaint. Extra-oral examinations showed no swelling nor tenderness related to that area. Intraoral clinical examination showed large carious lesion in the occlusal surface of tooth #46 (Fig. 1.a). Upon applying cold stimuli, the symptoms were reconfirmed with deep intense pain that remained for few minutes after the removal of the stimuli. Clinical examination also showed absence of sinus tract related to the affected tooth, the periodontal probing around the offended tooth was found to be within the normal limits however there was mild pain on vertical percussion. The radiographic examination was done using digital intraoral
radiographs and showed that the carious lesion was extending through enamel/dentin/pulp. The pre-operative radiograph also showed normal periapical bone trabeculae around the offended tooth with slight periodontal widening around the mesial root (Fig. 2.a). According to the clinical/radiographic findings, together with sensitivity test examinations, the case was diagnosed as “Symptomatic irreversible pulpitis with mild apical periodontitis”.

The medical history was discussed thoroughly and tests for the bleeding time/clotting time/platelets count were performed to confirm being within the normal range.

Clinical intervention: Platelet Rich Fibrin (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 g force 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. The PRF obtained was placed on the perforated pad in the PRF cassette and left to drain its exudate to get thick small plug of PRF, this allowed easily placement of the membrane on top of the remaining radicular pulps.

After local anesthesia administration and rubber dam application, the tooth crown and the surrounding rubber dam was disinfected with 2% chlorhexidine solution. A sterile high-speed hand-piece with a round bur was used for caries removal. After pulp exposure, a sterile Endo-Z bur was used for complete de-roofing and adjustment of the access cavity. The coronal pulp tissues was excised to the canal orifices level using a large sharp round bur on high-speed hand-piece under continuous cooling and then large sharp spoon excavator was used to confirm removal of any remnants of the inflamed pulp tissue from the pulpal tissue. The pulp chamber was washed with saline and initial homeostasis was achieved using a saline wet cotton pellet with light pressure over the perforated pad in the PRF cassette and left to drain its exudate to get thick small plug of PRF, this allowed easily placement of the membrane on top of the remaining radicular pulps.

The cavity was then prepared according to the manufacturer regulations and placed over the PRF to cover all the pulpotomized canals (Fig. 1.f). Biodentine (Septodont, St-Maur-des-Fosses, France) was then prepared according to the manufacturer regulations and placed over the PRF with approximately 2-mm thickness and was allowed to set initially for about 12 min (Fig. 1.g). The cavity was then immediately permanently restored using resin-modified glass ionomer cement (RMGIC) (Riva LC, SDI Limited, Victoria, Australia) then covered with composite resin restoration (Fig. 1.h) (Filttek Z250 XT, 3m ESPE, St. Paul, MN, USA).

Case 2: A 19-years old female 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 left posterior region. The patient was medically free of any systemic diseases. Extra-oral examinations showed absence of any palpable lymph nodes in the head and neck regions or swelling. Intraoral examinations showed that the tooth #36 showed a deep caries cavity involving the occlusal and distal walls. Sensibility test (cold thermal test) showed sharp pain lasting minutes after the removal of the stimulus. There was slight tenderness on vertical percussion and no pain with horizontal percussion with no sinus tract opening near to the tooth, periodontal probing depth of the tooth was within normal limits, with absence of tooth mobility. Radiographic examination showed deep occluso-distal carious lesion approaching the pulp in tooth #36 with periodontal widening around apical area of both roots (Fig. 3.a). Based on the examination, the diagnosis was “Symptomatic irreversible pulpitis with mild apical periodontitis”. The patient was informed of Laser-PRF regenerative pulpotomy procedure and consent form was signed from her.

After thorough medical history taking, blood test was performed to confirm normal ranges. The patient was then treated with the same regenerative pulpotomy procedure as in case 1 using Diode laser and PRF.

Case 3: A 40 years old male patient was presented to the out-patients clinics, Faculty of Dentistry, Alexandria University, complaining of moderate spontaneous pain in the lower right side that gets exaggerated to be severe with cold water. The patient was medically free. Extra-oral examinations of the head and neck region revealed absence of palpable lymph nodes or swelling. Intraoral examinations showed that the tooth #46 showed a large occlusal amalgam filling with broken distal marginal ridge. There was mild pain on vertical percussion. The cold pulp sensibility test resulted in severe pain that stayed for minutes after removal of the stimulus. The periapical radiographic examination showed deep caries extending to the distal wall approaching the axial wall of the pulp chamber in tooth #46, with slight widening in the

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PDL space around the mesial root (Fig. 4.a). Based on the examination, the diagnosis was “Symptomatic irreversible pulpitis with symptomatic apical periodontitis”. Laser-PRF regenerative pulpotomy procedure was thoroughly explained to the patient and consent form was signed from him for acceptance. The patient was treated using the same Laser-PRF regenerative pulpotomy technique as in case 1 and 2.

After treatment, immediate post-operative periapical digital radiograph was taken for each of the three cases and the patients were recalled for initial clinical evaluation after 1 week then they were scheduled for follow up after three, six and 12 months for clinical and radiographic evaluations.

Follow up: The 1-week clinical follow up showed resolution of the pre-operative complaint to a great extent in all the three cases. The three, six and 12-months follow ups of the three patients were asymptomatic showing clinical and radiographic success of the treatment. The clinical examination showed the treated teeth to be normally functioning with total patient satisfaction in all the three cases all through the follow up period. There were no swellings/sinus tracts related to the treated teeth. All of the treated patients reported normal response to percussion (axial/vertical), apical palpation and had no response to sensibility tests.

On radiographic examination and follow-up till 12-months, all teeth showed normal pattern of bone trabeculae around the roots and mild resolution of the pre-operative periodontal space widening in case 1 (Fig. 2.e) and case 3 (Fig. 4.e) but with total resolution of the pre-operative periodontal widening noticed in case 2 (Fig. 3.e). There was no signs of internal/external resorptions or intra-canal calcifications. All patients in this case series were still under annual systematic follow-up.

Fig. 1: Case 1: Clinical Pictures, 1(a) Preoperative carious lower right 6, 1(b) Carious cavity floor, 1(c) Access opening cavity and coronal pulpotomy performed, 1(d) Pulp chamber showing Laser application over the remaining radicular pulp, 1(e) showing radicular pulp after laser hemostasis, 1(f) PRF membrane placed over the radicular pulp, 1(g) Biodentine application, 1(h) Final Composite filling.

Fig. 2: Case 1: Periapical Radiographs, 2(a) Preoperative radiograph showing deep occlusal carious lesion in lower right 6 approaching the pulp, with mild periodontal widening and normal bone trabeculae, 2(b) Immediate postoperative radiograph showing the PRF membrane maintaining the pulp chamber space and the Biodentine over the PRF, 2(c) Three-months follow-up radiograph showing normal bone trabeculation around the tooth with continuation of the appearance of periodontal widening, 2(d) Six-months follow up radiograph showing normal bone trabeculae with less periodontal widening, 2(e) Twelve-months follow up radiograph showing maintained pulp canals space, pulp chamber height, with normal bony trabeculation around the tooth and with continuation of slight periodontal widening.

Fig. 3: Case 2: Periapical Radiographs, 3(a) Preoperative radiograph showing deep occluso-distal carious lesion in lower left 6 approaching the pulp, with mild periodontal widening around both roots and normal bone trabeculae, 3(b) Immediate postoperative radiograph showing the PRF membrane in the pulp chamber space, 3(c) Three-months follow-up radiograph showing normal bone trabeculation with continuation of the appearance of periodontal widening, 3(d) Six-months follow up radiograph showing normal bone trabeculae with resolution of the periodontal widening, 3(e) Twelve-months follow up radiograph showing normal bony trabeculation around the tooth with normal periodontal ligament space around both roots.
In this study, pulp hemostasis and disinfection were achieved using 970nm Diode-Laser. The use of the diode laser in vital pulp therapy has been recently supported by several studies in laser-assisted pulp capping (20,21). This was further supported by a meta-analysis conducted by Deng Y et al (22) in 2016 who concluded that lasers effectively improved the direct pulp capping prognosis for permanent teeth.

The Diode laser has further been considered as a good non-chemical alternative in pulpotomy of primary teeth (4). This was also supported by Sivadas et al (16) where they found that the Laser-assisted pulpotomy formed dentine bridge with a better quality then the control group. A recent clinical trial also concluded that laser pulpotomy showed advanced clinical and radiographic success rate when combined with MTA or Biodentine as pulp capping materials (23).

Although there are no clinical trials studying the laser-assisted pulpotomy in permanent teeth, however Mathur et al (13) used the diode laser for pulpotomy in a traumatically exposed pulp of an immature permanent tooth and found it to show success for 10-months follow up with complete root formation.

To avoid the adverse heat effect of laser on the pulp, the diode laser output parameters in our study cases was precisely adjusted to avoid overheating. This, together with its short application time, the removal of the coronal pulpal tissue using high speed hand-piece under cooling and using proper saline irrigation, all together reflected the improvement in understanding laser method of application and prevented excessive heat generation and is in accordance with previous successful laser pulpotomy study (23).

The main advantages of laser-supported vital pulp therapy is due to its triplet effect in decontamination, hemostasis as well as Biostimulatory effect to the remaining pulp tissues. The hemostatic efficacy of diode laser is due to the significant absorption of laser light by hemoglobin and melanin, providing deeper penetration and hemostasis of the area exposed to laser. The hemostasis area created by the diode laser is in the form of a thin layer of necrosis below which is a place for the migration of inflammatory cells and the fibroblasts that donate to the dentine bridge formation (20).

Furthermore, the diode laser is poorly absorbed by the dental hard tissues. This criteria allows the use of the diode laser on pulp in proximity to the dentine causing no hard tissue damage.

Having a sterile field is considered a crucial phase of the VPT. Being near the infrared wavelengths, the diode laser and Nd: YAG lasers penetrate deeply into the dentine and they also have a significant scattering ability. These criteria are...
bioactivity as well as its maneuverability. Biodentine in terms of its biocompatibility, the Biodentine and the results were in the favor of et al (34) conducted a review about the MTA versus reduction of inflammation and pain (25).

Biodentine was selected to be used as the pulp capping material in all the three cases of this study, showing improved properties than MTA with more efficient characteristics such as better bioactivity and biocompatibility. It has been the material of choice for successful pulp capping in multiple studies (26,27) due to its easier handling properties and it doesn’t cause the tooth discoloration caused by MTA. It also shows improvement in setting time and mechanical properties than MTA. Other studies showed favorable pulp cells response to Biodentine when used in direct contact with pulp tissue (28,29). It has the advantages of being biocompatible and the induction of reactionary dentine apposition by stimulation of activity of odontoblasts, and also the induction of cell differentiation in the pulp that result in reparative dentine formation(30).

Biodentine was also found to significantly increase growth factors secretion from pulp cells like transforming growth factor beta1(TGF \( \beta_1 \)) (31) and significantly increased the proliferative, migratory and adhesion of stem cells when placed directly in contact with the pulp (32).

The long-term failure of vital pulp therapy was found to be mainly due to the presence of leakage through the process of tissue healing. Biodentine showed excellent sealing as well as resistance to micro-leakage (33). Furthermore, Kaur et al (34) conducted a review about the MTA versus the Biodentine and the results were in the favor of Biodentine in terms of its biocompatibility, bioactivity as well as its maneuverability.

With the increasing interest in the VPT, there is a crucial need for a biologically-based autologous material for radicular pulp capping in order to neutralize mild/moderate cytotoxicity accompanied with freshly-mixed calcium silicate based cements, thus minimizing the pulpal inflammation and enhancing faster wounded pulp healing (35).

PRF was used in this study as an autologous scaffold considering its advantages of excellent biocompatibility and bioactivity. PRF exerts no cytotoxic effect on DPSCs maintaining their original morphology (36). It also enhances the migration/attachment/proliferation/ differentiation of the DPSCs needed for the of the pulp-dentin structure regeneration and also promotes hemostasis (35). The 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 pulpal 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 of this study aiming to help stabilizing and protecting the membranes against the restorative procedure’s trauma. By this technique we could gain the advantages of the ideal scaffolding and GFs from the PRF membranes and the excellent sealing capability of Biodentine, together with the laser, accelerating the healing of the irreversible inflamed pulpal tissues. The three cases of this study were non-responsive to thermal pulp tests. This is supported with the idea that the tooth with complete coronal pulpotomy can be non-responsive to the sensibility tests. A negative response is not an indication of pulp necrosis. Absence of symptoms and continuation of the vitality of the pulp after a least one-year was considered the indication of successful treatment (37).

All cases in our study showed clinical as well as radiographic success even after one year follow-up. These findings are in accordance with the results of Mobarak et al 2020 (38) who conducted Case series studies using PRF and Biodentine as the pulp capping material in pulpotomy of permanent teeth with irreversible pulpitis and showed that the success rate was comparable to that of MTA pulpotomy previously mentioned in the literature. This is also in accordance with the clinical trial of Patil N et al (39) and the results of case series done by Taha et al 2018(40) using Biodentine as the material of choice for pulp capping in regenerative pulpotomy. They related the favorable results to the Biodentine biocompatibility as well as its superior sealing ability. The successful outcomes of the cases in our study might be attributed to that the radicular pulp might still have DPSCs with stem cells potentials similar to uninflamed healthy pulpal stem cells, therefore they might be resources for autologous pulp regeneration, as seen in previous study published by Wang et al (11). Moreover, it might be also attributed to the use of PRF as a scaffold over the radicular pulp together with the laser bio-stimulatory effect. This actively participates in the healing of the pulp by the release of suitable GFs such as: TGF \( \beta_1 \) and PDGF which play a major role in differentiation and proliferation of DPSCs (12).

The limited number of cases is considered one of the important limitations of this study. So larger number of cases with longer follow up periods may be needed to clearly evaluate this new pulpotomy technique, as long-term follow up is always recommended to expose late failures of any treatment modality.
CONCLUSION
Within the limitation of our study, together with the limited use of the Diode laser in pulpotomy of mature permanent teeth, the outcome of our case series study using Diode laser for hemostasis, disinfection and biostimulation together with PRF and Biodentine have shown to have favorable clinical and radiographic outcomes. However, further clinical trials as well as histological studies are needed to confirm a more detailed image of its effect on the pulp tissues.

ABBREVIATIONS
VTP: Vital Pulp Therapy; RCT: Root Canal Treatment; CP: Coronal Pulpotomy; Ca(OH)₂: Calcium Hydroxide; MTA: Mineral Trioxide Aggregate; GFs: Growth Factors; TGFB1: Transforming Growth Factor Beta1; LASER: Light Amplification by Stimulated Emission of Radiation; PDL: Periodontal Ligament; PRF: Platelet Rich Fibrin; DPSCs: Dental Pulp Stem Cells; PDGF: Platelet Derived Growth Factor

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AUTHORS’ CONTRIBUTION
DM participated in the clinical dental care of the patient and have continued performing regular clinical and radiographic follow-up and writing the paper. NAL, NAM and RAS participated in writing the paper, revising and editing the manuscript. All authors read and approved the final manuscript.

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CONSENT FOR PUBLICATION
Written informed consent was obtained from the patients when they registered at the hospital regarding future publication of the case and any accompanying images.

COMPETING INTERESTS
The authors declare that they have no competing interests.

REFERENCES


