International Journal of Dental Sciences and Research, 2018, Vol. 6, No. 3, 78-82
Available online at http://pubs.sciepub.com/ijdsr/6/3/5

DOI:10.12691/ijdsr-6-3-5

Tissue Engineering: An Analogous Platelet Rich Fibrin for Coronal Pulpotomy in Revival of Pulp Tissue Vitality in Adult Molar with Pulpitis, a Case Report

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Abstract Aim: management of a clinical case with irreversible pulpitis for young adult molar with closed apices using analogous blood derivative PRF and evaluating the clinical and radiographic success for coronal pulpotomy. Summary: A 18 years old male patient reported to the screening clinic, was referred to endodontic clinic with acute pulpitis diagnosed in left first molar tooth. Deep occlusal caries involving pulp, with history of spontaneous lingering pain associated with sign and symptoms of pulpitis. Standard clinical care protocol was strictly implemented, rubber dam isolation was followed by coronal pulpotomy. Blood drawn from cuboidal vein. Platelet rich fibrin membrane placed over the remaining pulp and restored with MTA and finally with Glass ionomer cement. Follow-up results clinically and radio-graphically were encouraging and successful at day 1 to 24 months. No post-operative pain, the treated tooth responded to pulp sensibility test and radiographs revealed periapical healing. However, for standardization of the clinical procedure well designed research study is further needed.

Keywords: *pulpotomy, tissue engineering, pulpitis, analogous PRF, growth factor*

Cite This Article: Abdul Aziz Aleid, "Tissue Engineering: An Analogous Platelet Rich Fibrin for Coronal Pulpotomy in Revival of Pulp Tissue Vitality in Adult Molar with Pulpitis, a Case Report." *International Journal of Dental Sciences and Research*, vol. 6, no. 3 (2018): 78-82. doi: 10.12691/ijdsr-6-3-5.

1. Introduction

Caries and dental pulpitis are the most common oral diseases resulting from the bacterial invasion. [1]. Gram - negative bacteria are the more common microorganism associated with tooth caries and dental pulpitis. Lipopolysaccharide, the main component of the bacterial cell wall that acts as the infection source, it induces the expression of pro-inflammatory cytokines and chemokines such as the matrix metalloproteinase 2 and 9, the tumor necrosis factor alpha, and interleukin 1 and 8, they trigger a variety of immune responses in the odontoblasts, fibroblast and monocytes on dental tissue stimulating inflammatory response and facilitating the healing process. [2,3].

Root canal treatment is the choice of treatment for irreversible pulpitis. The outcome of conventional root canal therapy will be dictated by reducing or eliminating the infectious micro-organisms. It will facilitate the healing process and that is the major goal of clinical research. [4]

Treatment strategies for diseases of dental pulp are direct and indirect pulp capping, pulpotomy in early stage, or pulpectomy in later stage. Maintaining pulp vitality is considered important for formation of dentin, for providing nutritive support to the vital tooth, defence function and unique reparative capacity of dental pulp. [5]

In the early stage of pulp injury pulpotomy is performed as vital pulp therapy, the coronal pulpal tissue is surgically excavated from the pulp chamber and the remaining vital radicular pulp tissue is covered with a suitable biocompatible material for pulp protection from any injury and initiate healing and promote repair. [6]

Calcium hydroxide was used after pulpotomy in 1929 by Hess. [7]

pulp cells with dentinogenic potential induce dentinal bride formation following the placement of suitable biomaterial over the vital pulp, after removal of diseased infected coronal pulp. Various materials that are biocompatible with pulp, have good sealing ability and have antimicrobial property. [8]

Biomaterials like Calcium hydroxide, Mineral Trioxide aggregate (MTA), Tri-calcium silicate (Bio-dentine) are used in clinical practice with material inherent drawbacks, mild to moderate cytotoxicity and other body reactions, hence compared to synthetic products, biologically based natural products are more favorable for healing.

Autologous: derived from same individual, that is; donor and recipient is same individual (Merriam-Webster.com). Exclusively obtained from human own blood, topical haemostatic blood derivatives are biological material that possess tissue healing and hemostatic properties and some of these derivatives can also stimulate cell growth and differentiation generating more research interest for both medical clinical uses and stem cell theapy. These autologous derivatives are physiologically compatible with own tissues, readily allows colonization

by cells, will not cause tissue necrosis or body reactions and are totally biodegradable in few days to weeks. These blood derivatives are the tissue engineering tools that influence to improve the cellular environment in In-Viva or In-Vitro to improve and enhance soft and hard tissue successful grafting. [9]

Platelet rich fibrin PRF) introduce by Choukroun et al is a second-generation autologous platelet concentrate with blood sample constituents that helps wound healing and immunity [10].

PRF can be called as an ideal regenerative biomaterial for pulp-dentin complexes as it has multitude of growth factors like platelet derived growth factor, transforming growth factor b1and insulin-like growth factor and like-wise have various potent favorable local properties that includes cell migration, cell attachment, cell proliferation and cell differentiation necessary for tissue regeneration [11,12]. Furthermore, PRF is ease to prepare and without any biochemical added to blood. A case report, presented here, describes the preparation technique and management of pulp for the carious permanent molar diagnosed as acute irreversible pulpitis in a young adult male.

2. Case Report

In the screening clinic a 18-years age young Saudi male patient reported of acute pain and requested for dental treatment for his lower left back region of the mandible bone. Patient was referred to endodontic clinics. Relevant medical and dental history was recorded.

On intra oral clinical examination, in the fourth quadrant there was a large deep occlusal caries with tooth #36. Slight Tenderness to percussion was present with no any swelling or tenderness of left mandibular molar region. Pulp sensibility was performed with *Endo-frost* Endo-ice (Coltnene/Whaledent, Langenau, Germany) and *Digitest II* electric pulp tester (Parkell, NY, USA). Intra oral peri-apical radiograph (Soredex, Tuusula, Finland) was made that revealed extensive deep occlusal extending from enamel and dentin to the pulp. Periapical radiolucency at the apical third region of mesial root of periodontal ligament space following root shape was detected on radiograph (Figure 5). Diagnosis of acute symptomatic irreversible pulpitis was established based on clinical assessment, history of spontaneous pain, Radiographic examination, pulp sensibility test associated with lingering pain to Endo ice cold stimuli.

The treatment procedure was informed to the patient. The difference between conventional root canal therapy and an alternative treatment modality, the blood derived PRF as a pulp-like tissue repair was explained the patient. A written consent was obtained from the patient. Blood test, the bleeding time, clotting time and platelet count was done and was in the normal range as in healthy individuals.

2.1. PRF Preparation Procedure

PRF is prepared by venous blood from same patient is collected from the cubital vein. The required amount of blood is drawn into a 10ml vacuum blood collection test-tube that does not require any addition of anticoagulant, however blood will begin to coagulate when it comes in contact with the glass test-tube, therefore blood sample is centrifuged immediately using laboratory centrifugal machine *Labofuge 200* (Thermo Scientific, Germany) before the blood clotting cascade is triggered. Centrifugation was performed at 3000 RPM for 12 minutes to obtain the standard PRF. The PRF was obtained in the middle of the test-tube, between the clear acellular plasma layer on the top and the red blood corpuscles at the bottom of the tube. The PRF was segregated using fibrin cloth and obtained in the membrane form (Figure 3a).

2.2. Clinical Procedure

Tooth No.36 was anesthetized, mandibular inferior alveolar nerve block using Octocaine 100 (lidocain HCL 2% and epinephrine 1:100,000 injection, (Novocal pharmaceutical, Ontario, Canada) and rubber dam was placed for single tooth isolation Blossom Dental Dam (Malaysia) and (Hu-Friedy, USA). Occlusal access to the carious lesion was obtained with round bur and Endo-z bur (Dentsply , Malliefer, S Switzerland) and pulpotomy for coronal pulp from the pulp chamber till canal orifices was performed using round bur in air-rotor compact torque hand-piece (Kavo, Germany) with water spray (Figure 2). Sterile saline was used for pulp chamber irrigation and hemostasis was attained with small cotton pellets dampened with saline. Clean pulp wound stump, free of blood-clot, was covered and capped with sufficient amount of PRF (Figure 3b). Mineral trioxide aggregate MTA (Produits Dentaries SA, Vevey, Switzerland) was placed over the PRF and final restoration (Figure 6) was done with glass ionmer cement(ChemFil, Dentsply DeTrey GmbH, Konstony, Germany). Patient was given post-operative instructions and recalled next day for clinical and radiographical examination and post-operative pain. On recall day patient was without any signs and symptoms. There no pain, discomfort or any swelling for the treated tooth. Patient was advice for long term follow-up. At 1,3,6,12,18 and 24 months follow up and the treated tooth was clinically asymptomatic, responded positively to the vitality pulp sensibility tests and the radiographs (Figure &) showed normal trabecular bone pattern and periapical radiolucency, at the mesial root apex, resolved with periodontal ligament space reducing to the normal width.

3. Discussion

Cells, matrix and the tissue inducing substances are the three main essential components for tissue engineering to regenerate tissue that was proposed by Langer and Vacanti in 1993 [13]. Stem cells, scaffolds, and signaling molecules the triad of tissue engineering that function as biological concept of regenerative endodontics. Also, a patent blood supply is essential and crucial for continuity of regenerative process [14].

Tissues engineering technique n for pulp regeneration use two main basic approaches, a cell based and one that is cell-free [15]. Cell based approach is used for regeneration of pulp-dentin like tissue [16].

Chouukroun's innovative PRF has fibrin matrix that dissolves slowly, not like PRP that dissolves very fast. After application of PRF it will be slowly remolded just like natural blood clot. The technique is highly efficient method to harvest platelets and leucocytes, preserves leucocytes throughout. Also, it is easy, simple and low cost that allows to obtain PRF concentrates quickly and by natural means. Hence it can be said that in daily clinical practice it is a most suitable method [17] Platelet rich fibrin and platelet rich plasma differs in preparation technique and other aspect. For PRP production is a two-step centrifugation, anticoagulant is added for blood collection, and biochemical's like calcium chloride bovine thrombin is added for artificial polymerization of platelet concentrate, but for PRF is one step centrifugation, polymerizing naturally and slowly, does not require any addition of biochemical or elaborate procedure. Furthermore, PRF is elastic membrane highly resistant [18]. that continuously releases cytokines like transforming growth factor (TGF ß1), Platelet derived growth factor (PDGF), and Vascular -endothelial growth factor (VEGF), the peak level of their release coincides with the cell growth around 14th day. Whereas 81.4 % of TGF ß1 released on the first day from PRP and thereafter it decreases at 3,7 and 14 days [19]. The PRF accumulates platelets and release cytokines that enhances proliferation of multiple cell types, stimulates cell differentiation and helps angiogenesis and combines healing and immunity promoters [20]. Hence in the present case of a young patient with symptomatic irreversible pulpitis and closed molar apex, PRF was used as a autologous bandage for repair and regeneration of diseased pulp.

With PRP no cytotoxic effect was exhibited by pulp cells, and also gingival fibroblast, periodontal ligament cells, osteoblast cells and dermal pre-keratinocytes. Dental pulp cells have also shown to maintain its original morphology and were observed to be attached at the edge of PRF under phase contrast microscopy. [21,22,23]

Vital pulpotomy is an universally accepted therapy for incompletely form roots [24].

Pulpotomies in young patients within the range from 16 to 28 years histological evaluation by Eghbal et al revealed in all the sample evaluated shows radicular pulp remaining vital, free from inflammation and was covered by complete dentinal bridge [25].

Pulpotomy therapy in teeth with mature apex is scanty in literature, less explored and associated with the existing controversies. In a systematic review of Vital pulp treatment for permanent teeth with closed apices has shown to have a very high success rate for partial pulpotomy 99.4%, similarly 99.3% success for full pulpotomy [26].

With innovations, researchers using technology advancement in clinical, histological and histobacteriologcal techniques have concluded that 84% of the time clinical diagnosis of irreversible pulpit matched with histological diagnosis [27]. In current case the molar tooth exhibited signs and symptoms that of irreversible pulpitis, probably the coronal pulp, near minute carious lesion exposure site, was irreversibly inflamed and the radicular pulp remained vital with reversible pulpitis, however, the accurate histological diagnosis will remain uncertain for the case. Diseased pulp may not be completely damaged and some of the pulp cell might have capacity as stem cell potential, similar to healthy cells for autologous regeneration of pulp tissue [28].

Mineral trioxide aggregate is a recent biomaterial for use in vital pulp treatment for permanent teeth [29]

It is postulated that the Biocompatible MTA provides an impenetrable barrier against all future microbial leakage into remaining vital pulp [30].

Clinical success of MTA is because it induces more amount od reparative dentin, high quality dentin formation and provides long term sealing ability [31]. In this study, with favourable properties of MTA a thick layer was placed over the PRF membrane, similarly high strength glass ionomer cement was placed for impenetrable double coronal seal, over MTA. Both materials are hydrophilic requires moisture during setting, hence suitable in clinical cases where possibility of moisture contamination is high. Final set GIC restoration is protected with coca-butter for long term success. Patient's periodic recalls till 24 months of follow-up visits the treated tooth was a asymptomatic and responded positively top pulp vitality sensibility cold Endo-ice test and the radiographs revealed good healing resolved radiolucency associated with mesial root tip. Thus a successful clinical and radiographic outcome results for this presented case is encouraging for further more standardization of each clinical step and protocols.

Conclusion: PRF and vital puplotomy for young mature teeth can successfully be employed in clinical practice as an alternative to complete pulpectomy treatment, for pulp-dentin complex regeneration, to improve patient care quality. For fully validating potential PRF treatment modality random clinical trials as well as sophisticated advanced histological studies are advocated for regenerative endodontics.

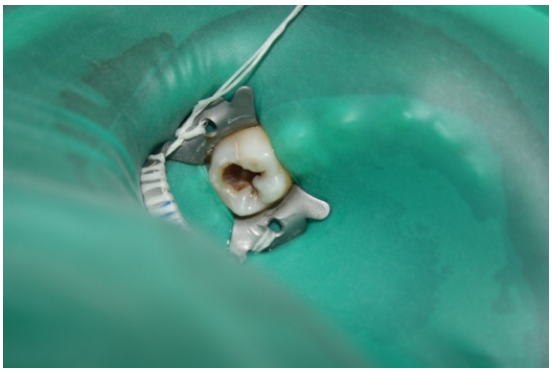

Figure 1. Deep occlusal caries mandibular molar

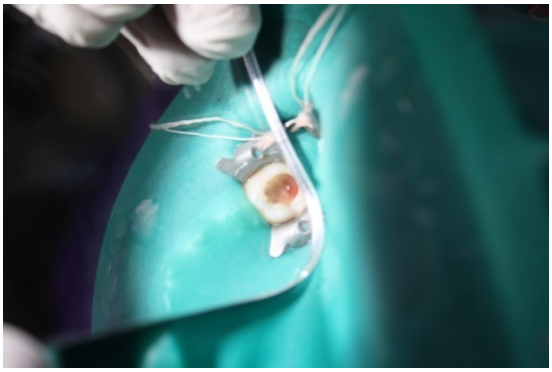

Figure 2. Coronal Pulpotomy on mandibular molar

Figure3a. Autologous Plasma rich fibrin (PRF) obtained with centrifuge blood sample

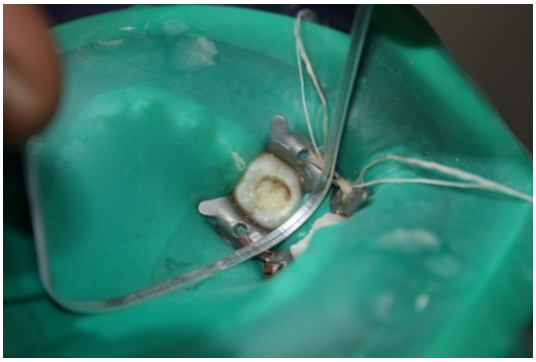

Figure3b. PRF placed over pulpotomized pulp.

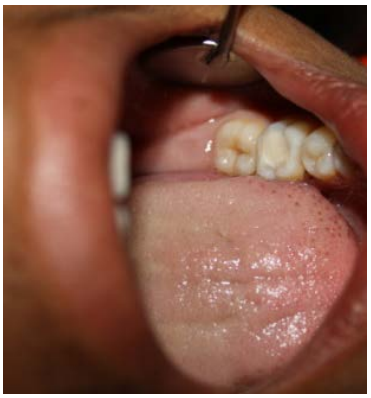

Figure 4. final restoration for mandibular molar

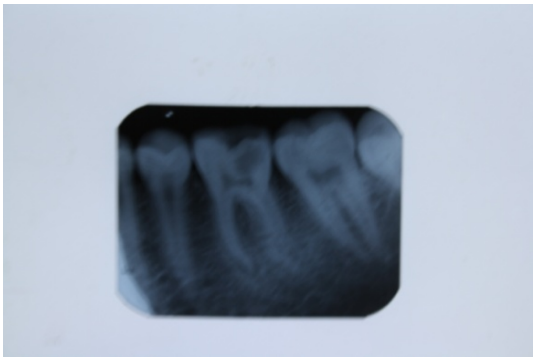

Figure 5. Pre operative intra oral radiograph of left mandibular molar with deep occlusal caries

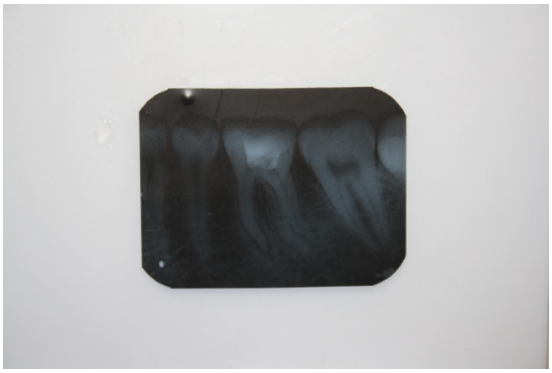

Figure 6. Post-Operative IOPA radiograph with PRF, MTA and GIC restoration

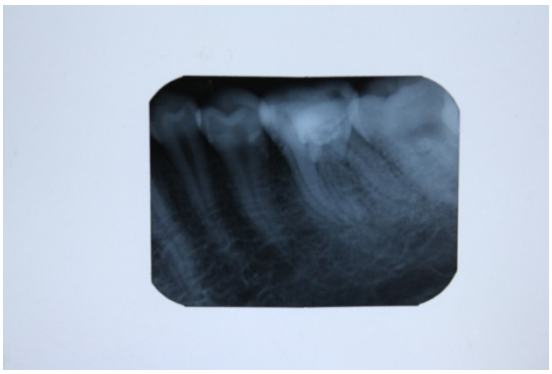

Figure 7. twenty-four months Follow-up IOPA radiograph with periapical healing

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