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The ability of platelet-rich plasma to regenerate a non-vital immature permanent teeth

Capacidade do plasma rico em plaquetas de regenerar dentes permanentes imaturos não vitais

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ABSTRACT

Objective: To test the ability of platelet-rich plasma clinically and radiologically for pulp regeneration of immature teeth with apical periodontitis. **Material and Methods:** An experimental study was conducted From (March/2018-July/2020) 12 upper central immature incisors with acute apical periodontitis and necrotic pulp from six patients receiving regenerative endodontic treatment using concentrated platelets rich plasma were performed by the same endodontist at Mediclinic Middle East Hospitals. Informed consent, including explanation of risks and alternative treatments or no treatment were prepared and filled by the patient parents. The therapeutic protocol was involved accessing the pulp chamber; irrigation copiously with sodium hypochlorite; applying calcium hydroxide as intracanal medicament and a provisionally sealing it after 4 weeks. The canal was cleaned, dried and injected with concentrated platelets rich plasma which serve as a scaffold for pulp regeneration. MTA was used to seal the chamber before final filling with composite. **Evaluations:** All teeth were monitored clinically (mobility, palpation, percussion, and sensitivity cold test) and radiographically. **Results:** Twenty months follow-up all teeth showed resolution of periapical radiolucencies, continued root development with positive response to sensitivity cold test and no discoloration. **Conclusion:** The results of this study confirmed the previous finding that pulp regeneration can be gained by using cPRP successfully.

KEYWORDS

Immature teeth; Necrosis; PRP; Regeneration.

RESUMO

Objetivo: Testar a capacidade do plasma rico em plaquetas clinicamente e radiograficamente para a regeneração pulpar em dentes imaturos com periodontite apical. **Material e Métodos:** O estudo experimental foi realizado em Março/2018 e Julho/2020, 12 incisivos centrais imaturos com periodontite apical aguda e necrose pulpar em 6 pacientes recebendo tratamento endodôntico regenerativo usando concentrado de plasma ricas em plaquetas. Foram realizadas pelo mesmo endodontista no Hospital Mediclinic Middle East. O consentimento informado incluindo explicação do risco e tratamentos alternativos ou de nenhum tratamento foi preenchido pelos responsáveis do paciente. O protocolo terapêutico envolveu acesso à câmara pulpar, irrigação abundante com hipoclorito de sódio, aplicação de hidróxido de cálcio como medicação intracanal e selado intracanal por 4 semanas. O canal foi limpo, seco e injetado concentrado de plasma rico em plaquetas que servem como um scaffold para a regeneração pulpar. Usou-se MTA para selar a câmara antes do preenchimento final com compósitos. **Avaliações:** Todos os dentes foram monitorados clinicamente (mobilidade, palpação, percussão e teste de sensibilidade com frio) e radiograficamente. **Resultados:** Após 20 meses de acompanhamento, todos os dentes apresentaram a resolução das radioluscências periapicais, desenvolvimento contínuo da raiz com resposta positiva ao teste de sensibilidade ao frio e sem descoloração. **Conclusão:** O resultado do estudo confirmou descobertas anteriores que a regeneração pulpar pode ser obtida usando cPRP com sucesso.

PALAVRAS-CHAVE

PcPRP; Dente imaturo; Necrose; Regeneração.

INTRODUCTION

Regenerative endodontic procedure focus upon three key factors for tissue engineering, adult stem cells, signaling molecules and a three-dimensional (3D) physical scaffold that can sustain cell growth and differentiation [1]. Several studies have used stem cells from the apical papilla by provoking apical bleeding into the pulp space as a possible source of stem cells and creating a blood clot (BC) that act as a biologic scaffold for immature teeth to continue their apex formation in a sterile environment [2,3]. However; sometimes it is very difficult to achieve adequate volume of blood within the canal space via the apical foramen [4-6]. In addition, the blood clot contains large number of hematopoietic cells that eventually undergo cell death, releasing their toxic enzymes which may be detrimental to stem cell survival [7].

Recently, Platelet-rich plasma (PRP) and platelet-rich fibrin (PRF) [6,8,9], are used in the field of regenerative endodontics as a scaffold with a high success rate [10,11]. PRP is an autologous first-generation platelet concentrate with a rich source of growth factors which forms a 3D fibrin matrix that entrap the growth factors [12]. It recruit other cells to the site of injury, produce anti-inflammatory agents, initiate vascular ingrowth and induce cells. PRP considered a potential substitute scaffold [13], it contains platelets that exceed 2 million/μL and 5 times more than the platelets count in the natural human blood clot [14]. Thrombin and calcium chloride were added during centrifuging of the blood to prepare concentrated platelets gel [15-17]. PRF is an autogenous biomaterial consists of cytokines, glycan chains, and structural glycoproteins trapped in the free-polymerizing fibrin grid. The slow mode of fibrin polymerization in PRF provides cytokines with increased viability, which significantly affects the healing process [15,18]. Clinicians who treat hundreds of traumatized immature teeth with regenerative endodontic protocols (REPs), may find that using PRP and PRF will slightly increase the rate of procedure success. The disadvantages of PRP and PRF Vs BCR are time consuming, child fear from the needles and costly.

The main aim of this study was to test the ability of concentrated platelet-rich plasma clinically and radiologically to regenerate pulp of immature teeth with apical periodontitis.

MATERIAL AND METHODS

An experimental study was conducted in Mediclinic Middle East Hospitals from (March/2018-July/2020). 12 upper central immature incisors from six patients with age ranged between 8-10 years having acute apical periodontitis and necrotic pulp, were included in this study (Figure 1). Informed consent, including explanation of risks and alternative treatments or no treatment were prepared and signed by the patient parents.

Figure 1 - At presentation; the tooth shows sinus and chronic apical abscess (Feb 2019).

Clinical procedures

First visit

After anesthesia was secured, rubber dam isolation placed, the root canal systems were accessed and working length determined (radiograph of a file loosely positioned at 1 mm from root apex). The root canal was slowly irrigated with 1.5% sodium hypochlorite (NaOCl) (20 mL/canal, 5 min) firstly followed by normal saline (20 mL/canal, 5 min). Irrigation

was performed using a needle positioned about 1 mm from root apex and the canal was dried with paper points. Calcium hydroxide was delivered to the canal system as intra canal medicament and the access temporarily restored by glass ionomer (Fuji IX, GC America, Alsip, IL).

Second visit

Four weeks after the first visit, clinical examination was performed to ensure the absence of moderate or severe sensitivity to palpation and percussion (Figure 2). If sensitivity, sinus tract, and swelling is present, the treatment of the first visit is repeated. Therefore, we conducted the second part of the regeneration protocol.

Figure 2 - The tooth after removal of $Ca(OH)_2$ and application of PRP (March 2019).

Preparation of PRP

PRP was obtained by drawing blood from the patient into sterile tubes with 3.8% sodium citrate that were centrifuged for 5 minutes at a speed of 4,000 rpm in a standard laboratory centrifuge (plasma fill-DrPRP-USA). Subsequently, in a laminar flow chamber, the obtained fractions were carefully separated with a 500 μL sterile pipette to isolate the PRP in the middle part of the tube, distinct from the red blood cells at the bottom and the platelet-poor plasma at the top of the tube which were transferred to the activator tube to obtain the cPRP. cPRP was transferred to a special syringe and incubated in Dr PRP-USA machine to obtain cPRP gel. Finally the gel cPRP was loaded into 10 mL hypodermic syringe to be injected into the canal.

Application of PRR

Following local anesthesia (3% mepivacaine without epinephrine), rubber dam isolation was obtained. The root canal was accessed; intracanal medicament removed by irrigating with 17% ethylenediaminetetraacetic acid (EDTA) (30 mL/canal, 5 min), flushed with saline (5 mL/canal, 1 min) and dried with paper points. PRP gel was injected into the canal to full length and plugger (Hu-Friedy, Chicago, IL, USA) was used for condensation to the orifice of the canal and sealed with a 3-mm-thick layer of MTA Pro Root (Dentsply Maillefer). The MTA coronal barrier was sealed with (2 to 3 mm) layer of glass ionomer (Fuji IX, GC America, Alsip, IL,). A bonded composite resin restoration was placed (Filtek Z 250, 3M ESPE) over the glass ionomer and cured for 30 seconds.

Evaluations

Clinical examination

1. Inspection: To see any change in the teeth color.

2. Palpation: To check if there is any pain during palpation of the tooth

3. Percussion: To notice if the tooth is tender to percussion

4. Sensitivity test: cold test using 1,1, 1, 2-tetrafluoroethane was performed to check patient response to cold application

5. Measuring pocket depth

6. Tooth mobility, we follow Glickman classification 1953 [19] in measuring tooth mobility.

Recording Tooth Mobility

1. +1 mobility: The first distinguishable sign of movement greater than normal

2. +2 mobility: Horizontal tooth

movement no greater than 1mm

3. +3 mobility: Horizontal tooth movement greater than 1 mm, with or without the visualization of rotation or vertical depressability

Radiographical examination

To see if there is periradicular bone healing, closure of root apex and if there is an increase in root length and wall thickness.

RESULTS

During the 20 months follow-up period all the patients were remained asymptomatic.

Clinical results

1. No discoloration was notice in all teeth.

2. Sensitivity test revealed that all the teeth were respond to cold test after 10 months till the 20 months.

3. Periodontal examination revealed no pocket depths over 2 mm and normal physiological mobility.

Radiographical results

All the teeth demonstrated evidence of periradicular bone healing and significant root development with maturation of the dentine and sign of apical closure with increase in root length and wall thickness (Figure 3-6).

Figure 3 - The tooth after 2 months of PRP application.

Figure 4 - The tooth after 6 months of PRP application.

Figure 5 - The tooth after 9 months of PRP application.

Figure 6 - The tooth after one year and a half of PRP application.

DISCUSSION

Different studies have shown the success of REPs using BC as a scaffold [20,21]. However, the ability to get adequate apical bleeding may not always be possible [4,6], and the adequate concentration of growth factors in the BC is limited [22]. Several studies have compared the clinical and radiographic outcomes of PRP or PRF with BC [23,24], and were not been able to show the superiority of PRP and PRF over the BC approach. This may be due to a relatively shorter follow-up time of 12–18 months. The success of regenerative endodontic procedure depend mainly in adequately disinfect the root canal space and proper seal of the coronal tooth structure in order to minimize bacterial contamination as well regeneration is possible when a suitable scaffold for a new tissue growth exists [25]. The continuation of the root development, thickening of the dentinal wall with closure of the apex and resolution of periapical lesion, all are the results of the regenerative treatment [26,6]. Intracanal irrigants (NaOCl and chlorhexidine) and antibiotics such as the ciprofloxacin/metronidazole/minocycline were used for several weeks to disinfect the immature teeth with apical periodontitis [27]. In the first visit of this study 1% of NaOCl and Ca(OH)2 were used for 4 weeks to disinfect the immature acute apical periodontitis teeth. Although Ca(OH)2 appears to be less effective against some intracanal bacterial species than antibiotic paste formulations [28], but it has lower cytotoxicity to the stem cells [29]. It releases bioactive growth factors from the treated dentin [30], and increases the survival and proliferation of the stem cells [31]. Ca(OH)2 as intracanal medicament can be removed from the canal space easier than the triple antibiotics [32]. On the second visit, 17% EDTA was used as a final rinse to promote the survival and increase the attachment of the stem cell of apical papilla (SCAP) to the dentinal walls of the root canal, it release growth factors from demineralized dentin [33], therefore EDTA is recommended as a final rinse for revascularization [33].

The cPRP gel used in the current clinical study as a scaffold for pulp regeneration was the cause of our clinical and radiographic success as it is rich of natural growth factors such as transforming growth factor, vascular endothelial growth factor, and platelet-derived growth factor [34]. The white MTA was gently placed over our scaffold without using a collagen barrier, it should be emphasized that a collagen barrier is a safe and reliable way to control the placement of coronal MTA or bioceramic barriers. However; MTA was preferred due to its sealing ability and biocompatibility [35]. It was placed inside the root canal after cPRP to provide a tight coronal seal as it is hydrophilic and needs moisture to set as well MTA obtains signaling molecules for the growth of the stem cells [36]. In this study cPRP gel was collected using 1-step centrifugation, to save time, and the presence of activator tube increased the concentrations of collected platelets with proper 3 dimension scaffold. Healing was achieved, with apical closure and resolution of the periapical lesion observed at 20 months can be attributed to the high concentration of platelets, which are known to be the key factors in wound healing. It release a variety of growth factors that induce, support healing and tissue formation. However; cPRP has some disadvantages, special equipments , drugs, drawing blood from young patients are needed as well the treatment is costly.

PRF has many advantages over PRP, its platelets and leukocytes entrapped inside fibrin gel, liberating growth factors which sustained for a long time, beside it does not require the addition of anticoagulant and counteract infection [18]. For necrotic immature permanent teeth, revascularization/revitalization utilizing PRP/PRF is a highly successful method and showed no significant difference [8,24,37]. In this study we overcome the limitation of PRP by convert it to cPRP gel.

CONCLUSION

After 20-months follow up, clinical and radiographic observations showed that, cPRP

gel could be the first choice used as scaffolding material in regenerative endodontic treatment

Limitations: Pulp vitality testing that evaluate blood supply should have been used which done by Laser Doppler Flowmetry & Pulse Oximeter. The histology of tissues formed inside root canal could not be assessed due to ethical reasons.

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Conflict of interest

There is no type of financial and non-financial conflicts of interest from the authors

Regulatory Statement

None. Each parent sign the informed consent.

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