Blood Clot as a Scaffold in Pulp Tissue Regeneration of Immature Permanent Teeth by Cell Homing

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Abstract

Background: The main aim of this study is to evaluate the ability of immature permanent periapical periodontitis teeth to regenerate pulp tissue using blood clot.

Materials and Methods: Experimental study was done in Al Ain Dental Center AHS -Seha from (June/2012 - December/2015). Twelve patients with acute apical periodontitis and necrotic pulp of an immature permanent teeth, 4 anteriors, 4 premolars and 4 molars were included in this study. Informed consent was filled by patient parents. The clinical protocol involved accessing the pulp chamber; irrigation, applying a triple antibiotic paste as intracanal medicament and provisionally sealing it. After 4 weeks, the canal was cleaned, dried and the apex was irritated with a size 30 K-file to induce blood clot. MTA was used to provide a hermatic seal to pulp chamber before final restoration. All teeth were monitored clinically (mobility, palpation, percussion and sensitivity cold test) and radiographically.

Results: Two years follow-up all teeth showed resolution of periapical radiolucencies, continued root development with no response to sensitivity cold test and no discoloration notice in all teeth, only one tooth revealed a slight cervical discoloration.

Conclusion: Pulp regeneration using blood clot is recommended as endodontic treatment of necrotic pulp with open apex.

Keywords: Baicalein; MMP Inhibitor; Resin-Dentin Bond; Cross Linker

Introduction

There are several challenges when treating immature permanent teeth with a necrotic pulp and apical periodontitis [1]. Cleaning, shaping and complete obturation without extruding of the filling materials beyond the apex of blunderbuss wide canal are difficult. In addition, fracture of the root due to a thin, lateral dentinal walls during mechanical preparation and obturation [1]. Prior to 2004, apexification procedures has been used to treat immature necrotic permanent teeth, by formation an apical barrier and close the open apex [2]. However, tissue regeneration cannot be achieved with apexification, therefore; regenerative endodontic treatment is designed as a new technique to replace necrotic pulp tissue in order to restore the normal function of the pulp-dentin complex [3]. Tissue regeneration requires stem cells and growth factors in a bioactive scaffold. The 3 dimensional scaffold will support the ingrowth of new tissue from the periapical area in the empty canal space. There are two techniques which can be applied towards dental pulp regeneration, cell transplantation and cell homing. The first technique is performed by transplantation of exogenous stem cells in a scaffolds with signaling
molecules into the root canal of the host for regeneration. The transplanted cells collected from the host (autologous) or from other individuals (allogenic) and may be either processed (separated from tissues) or grown in cultures to increase their numbers. This procedure is very complicated with high cost and has the risks of immune rejection, pathogen transmission and tumorigenesis during engraftment. Dental pulp regeneration using cell transplantation is scientific valid, however it is unpractical and uncompetitive with current root canal treatment or dental implants [4]. While the second approach cell homing which is defined as an active recruitment of endogenous cells, including stem/progenitor cells, into an anatomic container. Regeneration can be occur though chemotaxis of endogenous cells to injured tissue by biological signaling molecules. Cell homing can be easily performed in the clinic without the need of isolation and manipulation of stem cell in vitro. In cell homing approach, bioactive scaffolds with growth factors are injected into empty root canals to induce the migration, proliferation and differentiation of endogenous stem cells residing around the root apex [5].

The possible cell sources are dental pulp stem cells (DPSCs), stem cells from the apical papilla (SCAP) and bone marrow stem cells (BMSCs), however; root canal disinfection without instrumentation are the principle of cell migration and regeneration [6]. Cytokines are crucial, in pulp regeneration, as they have the ability to regulate the proliferation, movement and differentiation of stem cells [7]. Therefore, a proper scaffold which can be easily manipulated and growth factors should be selected in clinical practice. Many materials like Platelet-rich plasma (PRP) and plasma rich fibrin (PRF) has been suggested as a possible scaffold for regenerative endodontic treatment [8].

Ostby [9] was the first who used blood clot for regeneration of dental pulp tissues by stimulation of bleeding from the apex which resulted in a growth of granulation tissues, fibrous tissues or cementum-like tissues into the root canals [9]. The treatment depends on the presence of viable stem cells in the apical papilla (SCAP) which are the origin of primary odontoblasts during normal root development. Stem cell of apical papilla has the ability to repopulate the root canal space and reestablish the pulp-dentin complex. Blood clot considered a scaffolds to fill intracanal spaces and help the growth of new tissues [10]. However, the blood clot is often difficult to achieve, does not have adequate mechanical properties, in addition it contains a great number of hematopoietic cells that eventually undergo cell death, releasing their toxic intracellular enzymes into the microenvironment, which may be suppress stem cell survival [11]. Nevertheless, there is demand to find appropriate therapy promoting regeneration of the pulp/dentine complex in cases of pulp necrosis since it is not exist currently [12]. Transplanted cells complex procedure with a high costs and has the risks of immune rejection, pathogen transmission and tumorigenesis during engraftment, reinfections due to coronal leakage or periapical microleakage [13] and potential contamination and development of tumorigenesis during ex vivo cell manipulation [14]. As a result, an appropriate combination of suitable scaffold and growth factors should be chosen in clinical practice. Cell homing with blood clot might be easier and cheaper to perform than stem cell transplantation clinically.

Materials and Methods

An experimental study was done in Al Ain Dental Center AHS -Seha from (June/2012-December/2015). Twelve patients with acute apical periodontitis and necrotic pulp of an immature teeth, 4 anteriors, 4 premolars and 4 molars were included in this study. Informed consent, with explanation of risks and alternative treatments or no treatment were signed by patient parents.

First visit

After anesthesia was given, rubber dam isolation was applied, 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 or canals were slowly irrigated with 1.5% 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 canals were dried with paper points. A triple antibiotic paste (TAP) (Minocycline, Metronidazole, Ciprofloxacin). (Combined total of 0.1 mg/mL) was inserted into the canal system and the access temporarily restored with glass ionomer (Fuji IX, GC America, Alsip, IL).

Second visit

Four weeks after the first visit, clinical examination was first performed to ensure that there is no sensitivity to palpation and percussion. If there is sensitivity, or a sinus tract or swelling is noted, the treatment of the first visit is repeated. After adequate local anesthesia with 3% mepivacaine (without epinephrine), rubber dam isolation was obtained. The root canal systems were accessed, the intra-canal 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. Bleeding was induced by using a precurved K-file size #30 at 2 mm passed the apical foramen with the goal of having the whole canal filled with blood to the level of the cementoenamel junction. Once a blood clot was achieved, a piece of Collaplug (Zimmer Dental Inc., Warsaw, IN) was placed on top of the blood clot to serve as an internal matrix for the placement of approximately 3 mm of white mineral trioxide aggregate (MTA) (Dentsply, Tulsa, OK). A (1-1.5 mm) and a layer of glass ionomer layer (Fuji IX, GC America, Alsip, IL) was flowed over the bioactive coronal barrier. A bonded composite resin restoration (Z-100, 3M, St Paul, MN) was placed over the glass ionomer and cured for 40 second.

Evaluations

Clinical examination

1. Inspection: To see if any change in the teeth color occur.
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 depths.
6. Recording Furcation Defects, we follow Glickman classification 1953 [16]:
   a. Class I furcation defect: The furcation can be probed but not to a significant depth.
   b. Class II furcation defect: The furcation can be entered deeply but not to perforate the opposite side.
   c. Class III furcation defect: The furcation can be probed completely and perforate the opposite side.
7. Tooth mobility, we follow Glickman classification 1953 [15].
8. Recording tooth mobility
   - +1 mobility: The movement has to be greater than normal
   - +2 mobility: Tooth movement horizontally less than 1mm
   - +3 mobility: Horizontal tooth movement more than 1 mm, with or without vertical movement.
9. Radiographical examination.

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

Results

Patient remained asymptomatic in the 24 months follow-up period.

A. Clinical results

a. No discoloration was noticed in all teeth, however only one maxillary incisor tooth revealed a slight cervical discoloration possibly related to the use of white MTA (Figure 1).

b. No pain in palpation and no tenderness to percussion was noted.

c. Sensitivity test reveals no respond to cold test.

d. Periodontal examination revealed no pocket depths over 2 mm with normal physiological mobility.

B. Radiographical results.

All teeth demonstrated evidence of periradicular bone healing with root development, maturation of the dentine and sign of apical closure with increase in root length and wall thickness (Figure 2-4).

Figure 1: Cervical discoloration of upper right central incisor after 6 months.
**Figure 2:** Periapical radiographs of the patient shows the progress of traumatized left central incisor after treatment by cell homing, A before treatment, B directly after the treatment, C after 2 years.

**Figure 3:** Periapical radiographs of patient shows the progress of root forming of traumatized lower second premolar after treatment by cell homing, A before treatment, B after 1 year, C after 2 years.

**Figure 4:** Periapical radiographs of patient shows the progress of root forming of traumatized lower first molar after treatment by cell homing, A before treatment, B directly after treatment, C after 2 year.

Discussion

In the current study none of the tested teeth became sensitive. It was concluded that sensitivity test still depend on neurological stimulation and its reliability on immature teeth is considered questionable [16]. However, Johns and Vidyanath, 2011 suggested that thick layers of MTA (3 - 4 mm) and glass ionomer cement (2 mm) might lead to negative responses to sensitivity testing [17]. During follow-up period, all patients have no symptoms, no tenderness to percussion or palpation, no pocket depths over 2 mm was noted and normal physiological mobility. This actually due to the proper disinfection and Long-term coronal seal.

Crown discoloration in this study was due to TAP and MTA [18,19], it can be overcome first by using calcium hydroxide Ca (OH)₂ instead of TAP as minocycline cause tooth discoloration [19] and TAP develop a resistance and difficulty in removal from the root canal [20]. Second by using Biodentine as an alternative to the MTA, biodentine is a bioactive material with desirable handling characteristics and less susceptibility to staining, thus biodentine appear suitable to be used in regenerative endodontic procedures [21].

An immature tooth with pulp necrosis and apical periodontitis, which has a thin divergent or parallel dentinal walls is very difficult to disinfect in clinical practice, as a result it affects the long-term prognosis of the treatment. Traditionally, calcium hydroxide and MTA had been used as an intra-canal medicament in apexification to create apical barrier.

However, this procedure will end up with a short root and thin dentinal walls which lead to root fracture [22]. Regeneration of a pulp-like tissue for dentine deposition provide a favorable alternative by the development of a longer and thicker root, less susceptible to fracture and with better long-term prognosis [23].

In this study the radiographs demonstrated periradicular bone healing, root development with maturation and increase in length, width especially in apical part of the root as compared with the preoperative radiographs.

Technically, regeneration is challenging, however; it is beneficial method to treat apical periodontitis of immature tooth. Based on a previous study, the most important step of regeneration is to disinfect the canals with minimal instrumentation to provide a sterile media for the growth of stem cells and prevent root fracture [24]. It was found according to previous study that a lower concentration (1.5%) of NaOCl should be considered as the standard irrigant for regenerative endodontic protocols (REPs) as the high concentration could kill the stem cells [25]. Hoshino., et al described, using a combination of three antibiotics (ciprofloxacin, metronidazole, and minocycline) as intra canals medicament in order to disinfect the canals and this was used in this study in the first appointment [26].

In the second appointment when there are no sign and symptoms, the clinicians should consider the use of an anesthetic without a vasoconstrictor to induce bleeding [27] and irrigate or treat the dentine with 17% EDTA solution as it has the ability to dissolve the mineral phase, liberating growth factors that orchestrate the stimulation of progenitors or stem cell differentiation [28]. Collagen matrix is useful after inducing bleeding through the canals for the controlled placement of MTA to optimal and desirable level. Research has shown formation of a blood clot in the canal space improve the regeneration outcome [24] and the bleeding in canal space which was induce from apical papilla involves the ingrowth of stem cells may help in dentin formation.

Although inducing bleeding is relatively straightforward, simplistic approach as it does not require any complicated manipulation. However, achievement of blood clot is often difficult and it does poses many characteristics of the ideal scaffold. In addition, the blood clot contains a great number of hematopoietic cells that undergo cell death, by releasing their toxic intracellular enzymes, which might limit the survival rate of stem cells [11]. Therefore, it is important to search for a scaffold alternative to blood clot regeneration of pulp tissue. Platelet rich plasma and Platelets rich fibrin were used as scaffold in tissue regeneration, as they have high concentrations of growth factors and demonstrated the reliability in improving periapical healing, apical closure and dentinal wall thickening. In addition, the mechanical properties of PRF and PRP might facilitate the condensation of overlying MTA [29].

Conclusion

Pulp regeneration treatment is minimally invasive represents a recent and viable therapy for immature teeth. It is an alternative to apexification in cases of endodontic treatment of pulp necrosis, whether or not associated with periapical lesion, but technically challenging. As a result of the experiences of this study clinicians should consider the use of Ca (OH)$_2$ to disinfect the canal rather than TAP to reduce discoloration. Biodentine is a promising materials should be used as alternative of MTA especially in anteriors. Further clinical studies with long-term follow-up need to be done to compare the efficiency of PRP, PRF as alternative to BC.

Conflict of Interest

None declared.

Bibliography


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