Revascularization Induced Maturogenesis of Human Non-Vital Immature Teeth via Platelets- Rich Plasma (PRP): Radiographic Study

Introduction

Teeth with open apices present some difficulties during endodontic treatment with traditional techniques and materials. Because of their thin dentinal walls, these teeth are liable to fracture after treatment. Also, obturation increases challenges to clinician. Traditionally, these cases were treated by apexification procedure to create an apical barrier before root canals obturation but this procedure requires multiple and long-term applications of calcium hydroxide [1] and might affect the mechanical properties of dentin and make these teeth susceptible to root fracture [2].

So, an artificial apical plug of mineral trioxide aggregate (MTA) has been recommended to limit the drawbacks of apexification. Many reports proved the high success rates of this procedure [3]. However, complete root formation might not be achieved and brittleness of the root was not overcome in this procedure [4]. The ideal outcome for management of immature necrotic teeth would be the pulp regeneration into a canal capable of allowing the continuation of normal root development.

Successful regenerative endodontic procedures require eradication of bacteria from the canal space to allow interactions between the necessary three key elements for tissue regeneration [5,6]: (1) stem cells capable of proliferation and differentiation; (2) scaffold, which is a 3D structure providing support to the regenerated tissue; and (3) growth factors, which are secreted signals to control morphogenesis and differentiation. Platelet-rich plasma (PRP) has been suggested as an ideal scaffold for regenerative endodontic procedures [1,3]. The aim of the present investigation was to evaluate radiographically revascularization-induced maturogenesis of non-vital immature teeth using platelets rich plasma (PRP) compared to blood clot as a scaffold.

Material and Methods

Twenty patients with immature, non-vital maxillary anterior teeth presenting with or without signs and/or symptoms of periapical pathology were included in this study from the outpatient clinic of the Faculty of Dentistry, Minia University, Egypt. A medical and dental history was obtained from each patient’s parents or guardians. Medically free patients were included in this research. The clinical and radiographic exclusion criteria were teeth with periodontally involved teeth, vertical fractures, and non-restorable teeth. All procedures were performed after obtaining proper institutional review board
approval based on the regulations of the Ethical Committee of the Faculty of Dentistry, Minia University (Registration date 24/6/2014). The age of the patients ranged between 9 and 20 years. Informed consent was signed for each case by the patient's parents or guardians including the proposed treatment and possible outcomes or complications.

Cases were randomly divided into 2 groups (10 patients for each group):

1. Blood clot group (control group).
2. PRP group (experimental group).

Intra-oral periapical paralleling technique was used before treatment and at 6-12 months intervals after treatment. The exposure done by conventional method using standardized intra oral conventional film size #2 (D-speed Kodak film). Standardization and reproducibility done by using the followings:

1. Intra oral periapical paralleling technique.
2. XCP film holder.
3. The same periapical x-ray machine.
4. The same type of conventional film size #2 (D-speed Kodak film).
5. Fixed mA, Kvp and time of exposure at every time.
6. Fixed bite block used at exposure.
7. Processing of the films done by using automatic processing machine at the same conditions.
8. Digitalization done by using digital camera then the radiographs transferred to the computer.

Teeth were anesthetized using local anesthesia\(^\text{13}\) without a vasoconstrictor. After rubber dam isolation, access cavities were prepared, and root canals were irrigated using 20 ml of 1.5% sodium hypochlorite\(^\text{14}\) alternatively with 30 ml of 17% E.D.T.A solution\(^\text{15}\) with intermediated rinse of distilled water. The triple antibiotic paste was prepared using metronidazole\(^\text{16}\) (500 mg tablets, ciprofloxacin\(^\text{17}\) and doxycycline (100 mg) capsules\(^\text{18}\). The content of doxycycline capsule was evacuated in a sterile mortar; a tablet of metronidazole and a tablet of ciprofloxacin were crushed and ground into homogenous powder in the same mortar using a pestle. Saline drops were added and mixed using the pestle until a creamy paste was achieved. The canal space was dried using paper points, and the prepared paste was injected into the canals using a sterile plastic syringe with a 20-G needle. A sterile cotton pellet was then applied, and the access cavity was sealed using a temporary restoration\(^\text{19}\) for 3 weeks. The final visit was scheduled when the tooth was asymptomatic with no discharge was seen. After anesthesia and isolation, the temporary restoration and the cotton pellet were removed. The canal was irrigated with 10 mL NaOCl 1.5% followed by 10 mL sterile saline and dried with sterile paper points. One of the following modalities was randomly chosen.

**Blood clot group**

The root canal was over-instrumented to encourage bleeding up to 3mm from cemento-enamel junction. An MTA orifice plug was used to seal the canal orifice covered by a moist cotton pellet. After 1 week, adhesive composite resin\(^\text{20}\) was used to seal the access cavity.

**PRP group**

8 mL of blood drawn by venipuncture of the antecubital vein of patients was collected in a 10 mL sterile glass tube contained acid citrate dextrose as an anticoagulant. This was centrifuged at 2,400 rpm for 10 minutes to separate PRP and platelet-poor plasma (PPP) from the red blood cell fraction. Supernatant layer (PRP + PPP) was transferred to another tube and centrifuged again at 3,600 rpm for 15 minutes to separate the PRP from the PPP. At the end of this cycle, PRP precipitated at the bottom of the tube. This was retrieved with sterile cotton pliers and placed on a glass petridish. It was mixed with 1 mL of 10% calcium chloride to activate the platelets and to neutralize the acidity of acid citrate dextrose (7). PRP was introduced into the pulp chamber using sterile cotton pliers and carried to the apical portion with a size 40 finger plugger\(^\text{21}\). An MTA orifice plug was used to seal the canal orifice covered by a moist cotton pellet. After 1 week, adhesive composite resin was used to seal the access cavity.

**Radiographic evaluation**

Patients were recalled at 6 and 12 months. Follow up included radiographic evaluation, which included the following:

1. Change in root length.
2. Change in root canal diameter.
3. Change in apical diameter.

The three radiographs (pre-treatment, six months and one year after treatment) have been assessed radiographically at the same time by operator to avoid measurements discrepancies. Digora\(^\text{22}\) for Windows was used for measurements (Figure 1). The length of the tooth has been measured in pixels as a straight line from the most calcified point at the apical area to a fixed point at the incisal edge. The mesio-distal width of the pulp canal has been measured in pixels at the same point the three radiographs nearly at the middle of the root. The mesio-distal width of the pulp canal has been measured in pixels at the same point the three radiographs nearly at the apical end of the root canal.

Data were collected, tabulated and statistically analyzed using Wilcoxon Signed rank test for non-parametric quantitative data within each group and Mann Whitney U test for non-parametric quantitative data between the two groups.

13 (Scandonest 3% plain; Septodont, Saint-Maur-Des-Fosses, France)
14 (Clorox, HC Egyptian company, El-Asher men Ramadan city, Egypt)
15 (Sigma-aldrich corporation, St Louis, MO, USA)
16 (Flagyl 500 mg; Aventis, Cairo, Egypt)
17 (500 mg tablets [Cipromax 500 mg; El-Dwaia company, Saudi Arabia])
18 (Vibracycin; Pfizer; Cairo, Egypt))
19 (Cavit-G, 3M ESPE, St. Paul, MN, USA)
20 (Z 350, 3M ESP)
21 (Sybronendo, CA, USA)
22 Digora 2.51; Soredex Finndent Tuusula, Finland
The use of an apical plug has been recommended as a single-visit apexification by placing an artificial MTA apical barrier to obturate the apical portion of the canal [3]. This procedure has the advantage of a reduced number of visits, higher patient compliance, and a high success rate [9]. However, the problem of thin brittle roots was not solved.

Revascularization is considered a simple treatment by which pulp regeneration can be enhanced [6]. Platelets-rich plasma (PRP) is a new option in tissue regeneration. PRP is prepared from the patient’s own blood and contains growth factors. The easy application of PRP in the clinic and its possible beneficial outcome, including rapid healing of soft tissues, and bone regeneration provide promises for new treatment approaches.

Eradication of bacteria from the canal space is mandatory for successful revascularization, because the process will fail in the presence of infection [10].

### Results

#### Change in Root Length

Statistical analysis showed significant difference between the blood clot and PRP groups through the whole follow-up period (p value =<0.05*). Regarding the effect of time, there was a significant difference within each group through the whole follow up periods in both (p value =<0.05*) (Table 1).

#### Change in root canal diameter

Statistical analysis showed significant difference between PRP group and blood clotting group through the whole follow up periods (p value =<0.05*). Regarding the effect of time, there was a significant difference within each group through the whole follow up periods in both (p value =<0.05*) (Table 2).

#### Change in apical root canal diameter

Statistical analysis showed significant difference between PRP group and blood clotting group through the whole follow up periods (p value =<0.05*). Regarding the effect of time, there was a significant difference within each group through the whole follow up periods in both (p value =<0.05*) (Table 3).

### Discussion

Great challenges face clinicians in management of non-vital teeth with open apices. Traditionally, treatment of such cases was performed using calcium hydroxide apexification. However, the long-term use of calcium hydroxide has many disadvantages, including multiple visits, low patient compliance, possibility of canal contamination between visits, and increased brittleness of dentin, which increase the risk of fracture [8].
Irrigation protocol was done by 1.5% sodium hypochlorite solution alternatively with 30 ml of 17% E.D.T.A. solution with intermediated rinse of distilled water. E.D.T.A releases the growth factors from dentin, thereby increasing their bioavailability [11]. These growth factors stimulate stem cell proliferation and differentiation [12].

The intra-canal medicament used was triple antibiotic paste. The combination of the three antibiotics was proved to sufficiently eradicate bacteria from infected root canals [13].

A bacteria-free canal is a must for tissue regeneration, but tissue will not grow into an empty space [14]. So, a scaffold is essential to allow the ingrowth of new tissue into the canal space. Hence the present study compared platelets-rich plasma (PRP) to a blood clot as scaffolds.

Induction of a blood clot, with its constituent growth factors [15] from the periapical tissues, may act as a scaffold for the ingrowth of new tissue in the disinfected necrotic immature tooth. The blood clot is formed of cross-linked fibrin. It acts as a pathway for the migration of cells [16], including macrophages and fibroblasts from the periapical area. A blood clot, however, not only consists of an inactive scaffold, its cells contain several growth and differentiation factors important in the healing process [17].

PRP is a simple strategy to concentrate platelets in the natural clot for rapid healing. A natural blood clot contains 95% red blood cells (RBCs), 4% platelets and 1% white blood cells (WBCs) and many fibrin strands. A PRP blood clot is composed of 4% RBCs, 95% platelets and 1% WBCs [18]. It contains important growth factors, induce cell mitosis and differentiation, collagen production, vascular ingrowth, production of anti-inflammatory agents, attraction of other cells to the injury site and thus improve soft and hard tissue healing [19]. PRP has been widely used in dentistry. It has been advocated after oral maxillofacial [20] and endodontic surgery [19] to accelerate wound healing.

Due to the importance of a bacteria-free environment, a coronal seal with Mineral trioxide aggregate (MTA) was used in this present study, as MTA possess an excellent sealing ability [21].

The results showed that in the PRP and blood clotting groups, there was an increase in root length and a decrease in root canal diameter over the follow-up periods with significant difference between the two groups. The continued growth of the root may be attributed to many mechanisms [22].

One of the possible explanations is that few vital pulp cells remain at the apical end of the root canal [23]. These cells might able to proliferate and differentiate into odontoblasts guided by the intact epithelial root sheath of Hertwig, which is thought to be resistant to destruction even in the presence of inflammation. Another possible explanation depends on periodontal ligament stem cells [24,25], especially in case of the destruction of apical papilla tissues and epithelial root sheath of Hertwig. These cells could proliferate and grow within the apical end of the canal through the open apex.

The third possible explanation depends on the stem cells of apical papilla (SCAPs) in which instrumentation beyond the apical limit of the canal results in the migration of SCAPs into the canal lumen. SCAPs may survive infection and retain the capacity for proliferation and differentiation into bone- or dentin-forming cells [26,27].

The fourth possible mechanism involves the blood clot. The formed blood clot is rich in growth factors including platelet-derived growth factor, vascular endothelial growth factor, and tissue growth factors. This rich supply of growth factors facilitates the differentiation, growth, and maturation of fibroblasts, odontoblasts, and cementoblasts from their undifferentiated precursors. The expression of vascular endothelial growth factor in teeth with open apices has been documented [28].

In addition to all these mechanisms, the use of platelets-rich plasma (PRP) increased concentrations of growth factors that can attract stem cells present in the apical tissues (vital pulp cells, periodontal ligament, apical dental papilla, bone marrow) and even from periapical lesions [29].

Our findings concerning the increase in length and the decrease in root canal diameter and its apical diameter for both groups were in accordance with Cehreli et al. [30] Tawfik et al. [31], Nagy et al. [32], and These studies were the only studies including a quantitative analysis. They reported a marked increase in length and thickness over retrospective studies. Our findings were in agreement with lwaya et al. [33] who reported an increase in length and thickness without quantitative measurements.

Our findings were also in agreement with Jadhav et al. [34] in which they stated that PRP was more effective than blood clots in revascularization, and the authors ascribed the success of PRP to stimulation of collagen production; sustained release of growth factors; and enhanced recruitment, retention, and proliferation of undifferentiated mesenchymal and endothelial cells from the periapical area.

Our findings were in disagreement with Bezgin et al. [35] and Alagi et al. [36] who concluded that PRP successfully created a scaffold for regenerative endodontic treatment; however, treatment outcomes did not differ significantly between PRP and a conventional blood clot scaffold.

Conclusion

PRP can act as a successful scaffold for regenerative endodontic procedures with significantly higher results regarding the increase in root length and decrease in root canal width and its apical diameter than blood clot group.

References


