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A CBCT EVALUATION OF PULP REGENERATION OF SINGLE ROOTED IMMATURE NECROTIC TEETH USING TWO DIFFERENT SCAFFOLDS

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ABSTRACT

In regenerative endodontics, scaffold developed a frame work that supports growth and development of stem cells and vasculature inside the root canal. The suggested protocol for scaffold creation entails the intentional induction of bleeding from the periapex and the formation of an intracanal blood clot. Recently the autologous platelet concentrates were used to as intra canal scaffolds. In this study PRF was compared with blood clot as a scaffold in the process of pulp regenration.17 patient with 20 non-vital immature central incisors were included in this study. The teeth were scanned with CBCT, then the teeth were accessed and the infection was controlled using irrigation with 1.25% NaOCl and TAP application during the first visit. During the second visit the teeth were grouped into two groups according to the scaffold: PRF group (n=10) and BC group (n=10). The canals were sealed at the coronal part using MTA then the access cavity was filed using bonded composite resin. The teeth were followed up for 1 year in which they were scanned using CBCT at 6 and 12 months. After 12 months the root length, dentin wall thickness, periapical radiolucency, periapical bone density and the apical foramen were compared with preoperative measurement. The data was collected, tabulates and statistically analyzed. The results showed that there was no significant difference between the study groups except with young age in PRF group and this was similar to another published research.

INTRODUCTION

Endodontic treatment of necrotic immature teeth has been always a problem due to lack of an effective apical stop [1,2]. Historically apexifecation which is defined as 'a method to induce a calcified barrier in a root with an open apex of an incomplete root in teeth with necrotic pulp was used[1,3,4]. Apexification involves the use of material such as capacity to induce the formation of a calcific barrier at the apex [5].

Calcium hydroxide and mineral trioxide aggregate (MTA) which have the Although apexification solves many clinically unsolved problems [6] but it still has some drawbacks. The apexification does not promote the formation of dentin along the entire canal wall, making the teeth more prone to fracture, and also does not induce further development of the tooth [7]. Until the introduction of MTA by Torabinejad [8] in the 1990's calcium hydroxide was the only material used for apexification. Although it was routinely used, Calcium hydroxide has the following drawbacks: firstly, it requires multiple entries during the treatment period, secondly the barrier formed is often porous and not continuous or compact further more intracanal calcium hydroxide can also make the tooth brittle because of its hygroscopic and proteolytic properties [9]. MTA on the other hand is a more osteoinductive, sets in the presence of moisture, and does not require multiple entries [3]. Recently, Tissue engineering which is a multidisciplinary science that aims at the development of clinically relevant strategies for the regeneration of tissue or organs has become prominent. It involves the

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identification of progenitor (stem) cells capable of tissue regeneration when seeded in biodegradable scaffold and is exposed to morphogenic signals (growth factors) [10]. Regenerative Endodontics which aims to replace the damaged structures including the dentin, root structure and the cells of the dentin pulp complex depends on the same principles of tissue engineering [11]. From early on research the state of the canal infection affects the ability of the body to induce root completion. The creation of a bacteria-free environment inside the root canal space through mechanical preparation and chemical disinfection is one of the essential elements for a successful endodontic regeneration protocol [12]. Disinfecting agents are used as irrigants and as intracanal dressings between appointments, and a wide variety of medicaments were commonly used. However due to complex root canal anatomy that harbor more bacteria mechanical procedures and irrigation with NaOCL has been proven that it is not enough to eradicate root canal infection [13,14,15]. Reports using antibiotics revealed that a combination of metronidazole, minocycline and ciprofloxacin can be effective against common dental pathogens in vitro and in vivo [16]. Several case reports have been published concerning revascularization procedures however the literature is lacking clinical studies which evaluating the regenerative outcomes so the aim of this study was to evaluate the efficacy of platelet rich fibrin and induced blood clot in the regeneration potential of single rooted immature teeth with necrotic pulps.

PATIENT AND METHODS

Patients Selection and Informed Consent:

The study populations comprised of 17 Patients with 20 non-vital immature incisors were selected from the endodontic clinic of the Faculty of Dental Medicine Al-Azhar University, Cairo, Egypt. Teeth with developmental anomalies, longitudinal fractures, periodontal affection of grade three mobility and non-restorable teeth were not considered for this study. Furthermore, patients with a history of systematic diseases or allergic conditions were excluded. Following initial examination and acceptance, written informed consent was taken from all patients or from the legal guardians of patients below 18 years of age following a detailed explanation of the procedures involved and the length of the treatment with emphasis on the possible outcomes.

Preoperative Assessment

Preoperative radiographic scanning of the selected immature teeth was done using cone beam computerized tomography (CBCT)[1]. Following radiographic scanning, root length, apical foramen diameter, volume of the periapical radiolucency and the periapical bone density were measured. All measurement except the apical foramen area was carried out using Romexis Viewer software[2] tools. Apical foramen measurement was carried out using Ez3D Plus Viewer[3]

Regenerative Procedures:

All the regenerative procedures were done using a surgical operating microscope[4] with magnification between x8 and x16. during the first appointment the tooth to be treated was identified followed by the administration of local anesthesia[5] followed by rubber dam application. Access cavity preparation was done using a #2 round bur in a high-speed hand piece with coolant. The tooth was built up using bonded composite resin[6] if it was required

1. Planmeca promax 3d CBCT with 90 kV ,12 mA, 284.9mGy×cm^{2}voxil size 75 μm
2. Planmeca Romixes veiwer 3.8.1 R
3. Ez3D Plus Simple Viewer Ver. 1.2.6.2
4. zumaxMedical co., Ltd.
5. Scandonest 3% plain; Septodont, Saint-Maur-Des-Fosses, France
6. 250 ZX,3MESPE,USA

to facilitate dam application. The working length was measured radiographically 1mm shorter than the furthest point of the root by placement of a #60 file in the canal that was stabilized in place using paper points. Minimal mechanical instrumentation was accomplished using circumferential filing in three cycles of eight strokes each using a #60 H-file. Irrigation with 1.5% sodium hypochlorite[1] was accomplished using a 27-gauge side vented closed end endodontic irrigating needle[2] adjusted shorter than the working length by 1 mm. The needle was attached to a 5 ml sterile plastic syringe[3]. The irrigation protocol used was as follows: Firstly 15 ml of sodium hypochlorite before mechanical preparation followed by 15 ml after the first cycle of mechanical preparation, 10 ml after the second cycle of mechanical preparation, and 10 ml after the third cycle, finally 10 ml of saline was used as a final rinse. The canal was dried using #80 paper points, and an intra canal medication of a triple antibiotic paste consisting of equal amounts(250 mg) of doxycycline[4], ciprofloxacin[5] and metronidazole[6] was injected into the canal using a 16 gauge needle attached to a 10 ml sterile plastic needle placed 2 mm from the working length after that the syringe was drawn back coronally to back fill the canal to the level of the CEJ .Then the access cavity was sealed with a resin reinforced glass ionomer[7] for a period of 3 weeks(disinfection period). Following the first appointment the patient teeth were assigned randomly and in reciprocation to one of the following groups, group BC and group PRF With a total number of 10 teeth per group. During the second appointment and following the disinfection period and positive clinical assessment, local anesthesia without vasoconstrictor was administered and rubber dam isolation was performed and the temporary glass ionomer was removed using a #2 round bur. The canal was irrigated with 20 ml of 17% EDTA liquid using a 27-gauge side vented closed end endodontic irrigating needle attached to a 5 ml sterile plastic syringe followed by dryness of the canal using a #80 paper points. Following the dryness, the regenerative procedures were done in which a pre-curved #25 k-file with a rubber stopper set 2 mm beyond the established working length was used to over instrument the canal by rotation aiming to initiate bleeding into the canal to the level of the CEJ. A piece of Teflon was inserted into the canal orifice and held there for 5-7 minutes to allow blood clot formation this was done for BC group. For PRF group a 5 mL of the blood were drawn by vein puncture using a 25-gauge needle attached to a 5 ml sterile plastic syringe and collected in a 5-mL sterile glass tube without anticoagulant. The blood was centrifuged immediately at 3000rpm for 10 minutes. A fibrin clot was obtained in the middle of the tube just between the red corpuscles at the bottom and a cellular plasma at the top. The PRF clot was collected with some straight non-toothed forceps in a sterile dish and was divided into small pieces using a sterile blade #15 on a sterile glass slab then it was introduced into the root canal with cotton pliers and carried to the apical foramen with a size #3 finger plugger[8]. PRF preparation was performed according to the PRF protocol prepared by Choukroun. For both groups the canal was sealed using MTA[9] which was introduced inside the canal for 2-3mm, and then the access opening was sealed with resin-modified glass ionomer cement.

1. Clorox; Nobel Wax Factories for Chemicals, Cairo, Egypt
2. endo-top endo irrigation needles. pphcerkamed
3. Aman disposable syringe. Egypt
4. Vibramycin; Pfizer, Cairo, Egypt
5. Egyptian group for pharma, El Obour, Egypt
6. Amriya pharm.ind, Alexanderia- Egypt.
7. Ketacfill, 3MESPE, USA
8. Dentsply Maillefer, Ballaigues, Switzerland
9. Angelus, Londrina, Brazil

Postoperative Assessment and Follow Up:

Patients were recalled for followed up at regular times 6, and 12months. Postoperative radiographic scanning of the teeth was done using (CBCT). Following radiographic scanning the change in, root length, apical foramen diameter, size of the periapical radiolucency and the periapical bone density were measured using the same parameters used for preoperative radiographic assessment. The postoperative results were compared with the preoperative results and the absolute difference was calculated. The percentage change in the root length, the apical foramen size, the volume of the periapical radiolucency and the periapical bone density were calculated according the following formulations

1-Difference in the Root Length:

$$\text{Percentage of the length difference} = \frac{\text{Postoperative length-preoperative length}}{\text{Preoperative length}} \times 100$$

2- Difference in the apical foramen size:

$$\text{Percentage of the foramen width difference} = \frac{\text{Postoperative apical size-preoperative apical size}}{\text{Preoperative apical size}} \times 100$$

3- Differance in the size of periapical radiolucency:

$$\text{Percentage of change in periapical radiolucent area} = \frac{\text{Postoperative volume-preoperative volume}}{\text{Preoperative volume}} \times 100$$

4- Difference in the Periapical Bone Density:

$$\text{Percentage of the periapical bone density difference} = \frac{\text{Postoperative periapical density-preoperative periapical density}}{\text{Preoperative apical width}} \times 100$$

The mean and standard deviation values were calculated for each group in each test (Root length, Periapical RL, Bone density, and Apical foramen). Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk tests. Data showed parametric (normal) distribution. Independent sample-t test was used to compare between two independent samples. Repeated measure ANOVA was used to compare between more than two related samples. The significance level was set at $P \leq 0.05$. Statistical analysis was performed with IBM® SPSS® Statistics Version 20 for Windows.

RESULT

Post-operative measurements were compared to pre-operative measurements and both absolute and percentage changes in root length, apical foramen diameter, size of the periapical radiolucency and the periapical bone density were measured, statistical analysis showed that there was no significant difference between **PRF** and **BC** groups throughout the entire course of the study

TABLE (1) Comparison between PRF and BC groups regarding different measurements

	Root length			**Volume of periapical radiolucency**			**Bone density**			**Apical foramen size**		
	Pre-& 6months	6 & 12months	Pre-& 12months	Pre-& 6months	6 & 12months	Pre-& 12months	Pre-& 6months	6 & 12months	Pre-& 12months	Pre-& 6months	6 & 12months	Pre-& 12months
PRF	2.33% [a]	1.84% [a]	4.28% [a]	64.61% [a]	71.15% [a]	88.65% [a]	44.47% [a]	10.80% [a]	62.23% [a]	17.70% [a]	21.59% [a]	33.89% [a]
BC	0.68% [a]	1.32% [a]	2.02% [a]	44.56% [a]	67.29% [a]	90.65% [a]	33% [a]	6.39% [a]	41.27% [a]	21.33% [a]	22.89% [a]	39.15% [a]
P-value	**0.318ns**	**0.635ns**	**0.360ns**	**0.595ns**	**0.766ns**	**0.738ns**	**0.527ns**	**0.242ns**	**0.410ns**	**0.581ns**	**0.865ns**	**0.612ns**

DISCUSSION

Regenerative Endodontics is a new born branch in Endodontics so that there is limited clinical research in this subject. This study is a randomized single blind study including a number of 20 single rooted anterior teeth. The anterior teeth are the most common teeth brown to fracture in the early childhood teeth [17]. Teeth with developmental anomalies, longitudinal fractures, periodontal affection of grade three mobility and non-restorable teeth were excluded from this study as this condition may affect the long-term viability of the teeth. Minimal mechanical preparation using Circumferential filling with only three cycles was used because of the large size of the root canal and to prevent further weakening of the dentinal walls. With regards to root canal disinfection, it was carried out using NaOCl irrigant. In this study NaOCl was used with the concentration of 1. 5% because it has the least cytotoxic effect on the stem cells (SCAP) [18,19] and this was similar to other research done in this field [20]. Furthermore, TAP was applied in between visits as was described by Hoshino in 1996 [21] to confirm root canal sterilization. Unlike Hoshino minocycline was replaced with doxycycline due to the lack of minocycline in our country and this was similar to other researches [22,23]. The disinfection period was 3 weeks which was recommended by most of the published articles [24, 25, 26, 27, 28]. Final irrigation was done by EDTA 17% during the second visit. It has been shown that EDTA-soluble factors stimulate matrix secretion, odontoblast differentiation, and tertiary dentin formation [29,30,31,32]. The use of EDTA was used by other research [33]. With regard to the scaffold used in the study blood clot is commonly used but recently PRF membrane which is a new family of platelet concentrate and was considered as a bioactive surgical additive regulating inflammation and increasing healing was used as a scaffold. PRF was prepared according to the protocol was described by Choukroun [34]. PRF was used because it is rich in platelet cytokines including transforming growth factor B1, platelet derived growth factors and insulin-like growth factors. PRF also enmeshes glycosaminoglycans which has a great capacity to support cell migrations and healing processes [35,36,37,38]. PRF was used as a scaffold in other studies [39,40]. With regards to the coronal seal MTA was used in the coronal part of the root canal to produce coronal seal as it has been shown that MTA is a bioactive material and has an excellent sealing property [2,41]. Coronal MTA is considered the golden standard in this type of research. In this study CBCT was used as the method of evaluation as it can provide better, more accurate, diagnostic information than intra oral periapical radiographs [42]. With regards to the number of CBCT scans, three scans were carried out per year at a preoperative, 6 months and after 1year to evaluate the effectivity of the treatment. There is no specific recommendation about the exact number of permissible x-ray doses per year in the literature, however the recommendations of the American Association of Endodontics and the American Academy of Oral and Maxillofacial Radiology in which the smallest possible field of view, the smallest voxel size, the lowest mA setting and the shortest exposure time in conjunction with a pulsed exposure-mode of acquisition were considered [43]. In this study our aim was to evaluate the efficacy of the PRF and the induced blood clot in the regeneration potential of single rooted immature teeth. When evaluating the effect of different scaffolds on the root length, apical diameter, size of the periapical radiolucency and bone density of the periapical region, there was no significant difference between PRF and BC groups either with the absolute values or the percentage of change over time. When evaluating root length although there was a big discrepancy between the percentage change in root length after 12 months between the PRF group (4.28%) and the BC group (2.02%), The lack of significance could be due to multiple reasons. Firstly, the presence of large periapical

radiolucency which require time to heal before root regeneration can occur. Secondly the remnant of Hertwig's epithelial root sheath which has been shown to be essential for the induction of stem cells to differentiate into odontoblast like cells [44,45] may have been affected by the persistence of infection for an extended time. Thirdly statistical significance may have been achieved if the evaluation period had been extended. Finally, due to the difficulty of acquiring a large number of patients for the study, the number of patients per group if increased may also eliminate the large standard deviation and produce statistical significance. These results are in agreement with other studies which showed no significant change within 12 months evaluation period [46, 47, 48]. On the other hand, there was a study [49] showing results that were in disagreement with this study but this may have been due to the difference in the method of evaluation in which they used intra oral periapical radiography, also the size of the periapical radiolucent areas may have been of smaller sizes. With regards to bone density when comparing the absolute values of PRF and BC groups, there was a significant difference throughout the 12 months for both groups. This can be explained by the fact that root canal disinfection and sterilization create a healthy environment for healing in the periapical area [50] and a sign of healing would be increase in bone density which may result in significance within 12 months unlike the other parameter. Finally, although PRF membrane has the ingredients and provides the adequate medium for growth and tissue repair, in our study its use did not show a significant difference when compared to blood clot scaffold.

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