CONCENTRATED GROWTH FACTOR VERSUS PLATELETS-RICH FIBRIN IN REVASCULARIZATION OF IMMATURE NECROTIC MANDIBULAR FIRST PERMANENT MOLAR

Parallel Randomized Controlled Trial

Protocol

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By

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Introduction

The contemporary non-surgical endodontic management of immature teeth has shown favorable outcome rates of 95% in teeth diagnosed with irreversible pulpitis[1] and 85% in necrotic cases.[2]

There is a paradigm shift in the treatment protocol of such teeth from conventional root canal treatment to regenerative endodontic treatment (RET) [3-4]. Hargreaves et al., in 2008 reported that regenerative endodontic procedures are possible by application of the principles of tissue engineering that require the spatial orientation of stem cells, signaling molecules, and the scaffold. Such three elements of tissue engineering as seed cells, scaffold materials and growth factors have an essential impact on the efficacy of RET.[5]

A suitable scaffold can provide a suitable location for seed cells and biological conditions conducive to cell metabolism, and regulate their differentiation and proliferation. The main scaffolds for regenerative endodontic therapy are autologous blood clots, autologous platelet concentrate, biomaterial scaffolds, and so on.[6-8]

The traditional method in RET is to stimulate apical bleeding, fill the root canal with blood, and use blood clots (BC) as scaffolds for pulp revascularization. Blood clots as a scaffold in RET had a good practicability and success rate [6]. However, it was found that clinically there were quite a few cases with no or insufficient blood clots which were disable to fill the root canal or support the pressure from the crown sealing material, leading to treatment failure. Therefore, it is necessary to find scaffolds to replace blood clots. Endogenous materials replacing blood clots as scaffolds include platelet-rich plasma (PRP), platelet-rich fibrin (PRF), and concentrated growth factor (CGF).[8]

The use of platelets for regenerative medicine has increased in recent years. Platelets, which contain growth factors, play major roles in cell migration, proliferation, differentiation and angiogenesis and are associated with the tissue regeneration process. Autologous platelet concentrates (APCs) are produced by the centrifugation of venous blood at different speeds and the use or non-use of thrombin and anticoagulant. As a result of these processing protocols, a fibrin clot is formed that contains platelets and leukocytes.[9]

The main generations of APCs are platelet-rich plasma (PRP), platelet-rich fibrin (PRF) and concentrated growth factor (CGF). The efficacy of platelet concentrates in promoting wound healing and tissue regeneration has been at the center of scientific interest over the past few decades.[10]

Platelets include growth factors (GFs) such as basic fibroblast growth factor (bFGF), vascular endothelial growth factor (VEGF), insulin-like growth factor-1 (IGF-1), transforming growth factor-1 (TGF-1) and platelet-derived growth factor-BB (PDGF-BB). There are significant differences in the amounts of GFs produced using the three different APC techniques (CGF, PRF and PRP). PRF and CGF produce significantly more GFs during the procedure as compared to PRP.[11]

CGF may play an role in vascular maintenance and angiogenesis due to its inclusion of CD34-positive cells [12] Besides CGF with a stronger regeneration ability contains more growth factors and fibrin matrix than PRF on account of the preparation of CGF adopts differential centrifugation with higher separation efficiency.[12-13]

Platelet concentrate products have good biological characteristics and are easy to produce. In recent years, they have been widely used in regenerative treatment of

oral medicine, such as periodontal tissue regeneration and orthodontic tooth movement .[14-15]

Biodentine is a recently released tricalcium silicate-based material developed as a permanent dentin substitute to replace the damaged dentin.[16]

Biodentine is a two component material. The powder is mainly composed of Tricalcium silicates. It also contains Di Calcium Silicate as a second core material and Calcium Carbonate and Oxide as filler. The powder contains Zirconium oxide as a radio-opacifier. The liquid contains Calcium Chloride as a setting accelerator and a water reducing agent. The presence of a setting accelerator allows the material setting in 12 min and the presence of a water reducing agent avoids the formation of cracks within the material.[17]

Biodentine is a highly biocompatible material. This biocompatibility of the material was investigated through its direct application to human pulp cells simulating the direct pulp condition and indirectly through a dentin slice to simulate its indirect pulp capping in vivo. Under both conditions Biodentine was not found to affect target cell viability under in vivo application conditions.[18]Additionally, when Biodentine was applied onto human pulp cells to investigate its effects on their specific functions by studying expression of odontoblast specific functions such as expression of Nestin (a human odontoblast specific marker) and Dentin Sialoprotein, Bio dentine was not found to inhibit the expression of these proteins but rather induce their expression and the cells mineralization capacity.[18-19] Further investigations demonstrated the absence of toxicity of Biodentine to human MG63 human osteoblast cells with the MTT assay with properties comparable to that of MTA.

When Compared to MTA, Biodentine is easier to handle, stronger mechanically and has a shorter setting time.[20]It can be used as a temporary enamel substitute up to 6 months and in different applications as a permanent dentin substitute without any surface treatment. Additionally, while discoloration with MTA[21] and its derivatives have been reported in regenerative endodontics and seem to be mainly due to the presence of Bismuth oxide as a radio-opacifier, no discoloration of tooth crown has been reported after 48 months with Biodentine which does not contain Bismuth oxide but Zirconium oxide as a radio-opacifier.[22-23]

Aim of the study

The aim of this study is to compare and evaluate clinically and radiographically the effect of CGF and PRF with the application of biodentine on regeneration of necrotic immature lower first permanent molar and assessment of the clinical success rate over 18months.

Materials and Methods

This study will be based on the ethics committee of Minia University-Faculty of Dentistry.

Ethical regulation:

All patients and their caregivers will be informed in an easy detailed manner about the treatment procedures, expected outcome of the current treatment, possibility of any complications and assuring them to the presence of plan B in case of any complication. Verbal consent will be obtained from all children who will accept participation in the study, Parents will sign an informed consent laid down by the ethical committee of the Faculty of Dentistry / Minia University.

Study design:

Parallel Randomized Control Trial.

Two groups will be done:

Group (1): Include children who will be treated with CGF and biodentine (control group).

Group (2): Include children who will be treated with PRF and biodentine (study group).

Randomization and Concealment:

The included patients will be randomized into one of the study groups.
Randomization will be performed using a computer-generated block randomization system (Microsoft Office Excel, 2007).

Concealment: A plan of management will be sealed in closed envelopes and numbered according to the randomization tables. Packing, sealing, and numbering will all be performed by a neutral health care provider other than the investigator.

Sample size justification:

The study will be with two parallel arms (1:1 allocation ratio). The sample size per group will be calculated according to the following equation for binary outcomes; $N = (Z\alpha/2 + Z\beta)\ 2 \times p1(1 - p1) + p2(1 - p2)/\delta\ 2$ so the minimal sample size for an equal size clinical trial is 27 teeth in each group. We will recruit 30 teeth in each group for possible attrition.

Study sample:

6-8 years old will be selected with no sex predilection for the study from outpatients of the Pediatric and Community Dentistry Department, Minia University, according to the following eligibility criteria:

Inclusion Criteria:

1. Children with immature necrotic lower fitrst permanent molar.
2. No history of repeated pathosis or cellulitis.
3. Patients accept the treatment modality.
4. Patient-free from systemic, allergic or genetic problems.

Exclusion Criteria:

1. Tooth mobility.
2. Teeth with non-restorable crowns.
3. Children with facial cellulitis
4. Children with draining fistula

5. Children with a history of recurrent preapical pathosis
6. Children who will not comply to recall program.

Clinical steps:

First Appointment:

1- Child communication and psychological management.

2- Local anesthesia.

3-Rubber dam application

4- Disinfection of the tooth and the working field.

5- Access cavity preparation and remove the necrotic tissue in the pulp chamber

6- Disinfection of the root canal is accomplished by gently irrigating the root canal with a minimum of 20 mL 1.5% NaOCl for 5 minutes. It will be dispensed through a syringe and a 20-gauge needle [24-25]. NaOCl is a potent antimicrobial agent and effectively dissolves necrotic and organic tissue. [26] Its solvent potential is dependent on its concentration and the frequency of fluid exchange. [27-29]

When irrigating with NaOCl, the needle should be introduced into the root canal to a point 2 mm short of the apical foramen[28-29], and the NaOCl is slowly expressed from the syringe to prevent its introduction into the periapical tissues. .[30] Irrigation is made with ultrasonic passive activation.

7- Initial NaOCl irrigation is followed by irrigation with 20 mL sterile saline for 5 minutes, followed by drying with paper point.

8. The root canal will be filled with calcium hydroxide (UltraCal, Ultradent, Paris, France) as an intracanal medication using a hand plugger (Medesy srl, Maniago)

9. A sterile cotton pellet will placed over the root canal medication then the access cavity will be sealed with a glass ionomer temporary restoration (RIVA self-cure, SDI Ltd, Paris, France) will be placed to seal the access cavity.

10. Three weeks later, the tooth condition will be assessed. If the signs of infection still persist, the irrigation protocol and intracanal medication will be repeated.

Second Appointment (After 3 Weeks):

1. Before proceeding to the next phase, there should be no adverse clinical signs and symptoms.
2. Local anesthesia without a vasoconstrictor will be used.
3. Rubber dam will be applied.
4. The temporary filling and medicament will be removed by gently irrigating the root canal with a minimum of 20 mL of 1.5% NaOCl, 0.9% saline (5 mL), and 17% EDTA (20 mL) (Cerkamed, Dental Products, Stalowa Wola, Poland). Then, an ultrasonic noncutting tool will be passively activated for one minute. Finally, the root canal will be dried with sterile, absorbent tips.
5. Introduction of a sterile #20 K-file into the apical tissues 2 mm past the apical foramen to initiate bleeding into the root canal. .[28, 30] Bleeding should be controlled so that it does not extend beyond a point approximately 3 mm apical to the CEJ. This is done by applying intracanal pressure with a sterile saline-soaked cotton pellet until a clot is formed. The estimated mean time for the establishment of a stable blood clot is 15 minutes [28-30]

Preparation of PRF and CGF Clots:

A 10-mL blood sample will be drawn through venipuncture of the antecubital vein using a 10-mL sterile hypodermic syringe (Luer-Slip type, El Dawlia ico nos. 19 and 67). The venous blood sample will be collected into sterile, specialized plain-type single-use centrifuge tubes (Hebei Xinle Sci&Tech Co., Ltd, Shijiazhuang, China) without anticoagulant additives.

The centrifugation process will be performed using the Benchtop Low-Speed Centrifuge ET-12M device. For PRF preparation, the tubes were centrifuged at 3000 revolutions per minute (rpm) for 10 minutes.([31]) .

For CGF preparation, the centrifugation process will follow a specific sequence: acceleration for 30 seconds, 2 minutes at 2700 rpm, 4 minutes at 2400 rpm, 4 minutes at 2700 rpm, 3 minutes at 3000 rpm, and finally, deceleration for 36 seconds19. After separation of the blood components, the topmost plasma-poor layer (straw-colored layer) will be removed using a sterile syringe. The fibrin-gelatinous yellow substance will be carefully retrieved with an HTS 171c66.25 curved stainless steel college tweezer, then will be separated from the basal red blood cell fraction by a sterile scissor and dragged across a sterile 4 x 4 gauze pad to remove the excess([31])

6. The prepared CGF fractions will be inserted into the root canal space using a small hand plugger (Dentsply Maillefer, Ballaigues, Switzerland) up to 3 mm below the cementoemamel junction for group I teeth, Then Biodentine (Septodont) will be prepared according to the manufacturer's instructions and will be packed against a collagen restorable matrix (PARASORB Cone, Resorba Medical GmbH or Collacone, botiss biomaterials GmbH, Germany) using a gold-plated instrument (Nordent Dura Lite 1020, Victoria, Australia)
7. For group II the prepared PRF fractions will be the root canal space using a small hand plugger (Dentsply Maillefer, Ballaigues, Switzerland) up to 3 mm below the cementoemamel junction for group I teeth, Then Biodentine (Septodont) will be prepared according to the manufacturer's instructions and will be packed against a collagen resorbable matrix (PARASORB Cone, Resorba Medical GmbH or Collacone, botiss biomaterials GmbH, Germany) using a gold-plated instrument (Nordent Dura Lite 1020, Victoria, Australia).

8. The access will be sealed with a layer of reinforced glass ionomer base (RIVA self-cure, SDI Limited). Finally, a resin composite (Filtek z250 universal restorative, ESPE, St. Paul, MN) coronal restoration will be inserted

Follow up:

All patients will be recalled at 3, 6, 12 and 18 for clinical and digital radiographic evaluation.

Clinical assessment:

1. Absence of signs and symptoms of pain, mobility, sinus tract or periapical lesion.

2. Normal responses to percussion, palpation, and normal pocket probing depths.

Radiographical assessment:

1-Absence of periapical pathosis and furcation involvement.

2- Elongation of the roots on follow-up periods.

3- Normal PDL space width

4-Normal bone trabeculation.

Statistical methods

The collected data will be coded, tabulated, and statistically analyzed using IBM SPSS statistics (Statistical Package for Social Sciences) software version 22.0, IBM Corp., Chicago, USA, 2013. Quantitative normally distributed data will be described as mean± SD (standard deviation) after testing for normality using Shapiro-Wilk test, then compared using independent t-test if normally distributed.

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