Regenerative Potential of Immature Permanent Teeth with Necrotic Pulps after Different Regenerative Protocols

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Abstract

Introduction: Regenerative endodontics is a promising alternative treatment for immature teeth with necrotic pulps. The present study was performed to assess the regenerative potential of young permanent immature teeth with necrotic pulp after the following treatment protocols: (1) a mineral trioxide aggregate (MTA) apical plug, (2) the regenerative endodontic protocol (blood clot scaffold), and (3) the regenerative endodontic protocol with a blood clot and an injectable scaffold impregnated with basic fibroblast growth factor. **Methods:** Immature necrotic permanent maxillary central incisors (n = 36) of patients 9–13 years old were divided into 3 groups according to the treatment protocol: the MTA group (MTA apical plug), the REG group (regenerative endodontic protocol [blood clot]), and the FGF group (regenerative endodontic protocol [blood clot + injectable scaffold]). Follow-up was done up to 18 months. Standardized radiographs were digitally evaluated for an increase in root length and thickness, a decrease in the apical diameter, and a change in periapical bone density. **Results:** After a follow-up period of 18 months, most of the cases showed radiographic evidence of periapical healing. Groups 2 and 3 showed a progressive increase in root length and width and a decrease in apical diameter. **Conclusions:** The regenerative endodontic procedure allowed the continued development of roots in teeth with necrotic pulps. The use of artificial hydrogel scaffold and basic fibroblast growth factor was not essential for repair. *(J Endod 2014;40:192–198)*

Key Words

Basic fibroblast growth factor, hydrogel scaffold, mineral trioxide aggregate, regeneration, revascularization

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The treatment of immature permanent teeth with necrotic pulp constitutes a challenging situation facing endodontists. Such conditions present difficulty in root canal debridement and obturation because of the open apex. Moreover, they are more prone to fracture because of thin weak dentinal root canal walls. Such cases were traditionally treated by apexification procedures using calcium hydroxide (1). Such management requires long-term placement of calcium hydroxide inside the root canal to induce the formation of an apical hard tissue barrier. Recently, many authors advocated the placement of an orthograde apical plug (2–4). Mineral trioxide aggregate (MTA) proved to be an excellent candidate; however, apical plugs do not solve the problem of the thin and weak dentinal root canal walls (5, 6).

Periapical tissues in immature teeth are rich in blood supply and contain stem cells that have the potentiality for tissue regeneration (7). Under suitable conditions, stem cells can be programmed for self-regeneration to restore the lost part. Hence, the concept of regeneration of immature nonvital teeth was advocated. Eradication of bacteria from the canal space is mandatory for successful regenerative endodontic procedures. Research with topical antibiotics showed that a combination of metronidazole, minocycline, and ciprofloxacin could be effective against common endodontic pathogens *in vitro* and *in vivo* (8, 9). However, a disinfected empty canal space cannot support the ingrowth of new regenerated tissues on its own so a scaffold is needed for support. Advances in tissue engineering research focused on 3 key elements for tissue regeneration (10, 11): (1) stem cells that have the ability for proliferation and differentiation; (2) scaffold, which is a 3-dimensional structure that supports the regenerated tissue integrity; and (3) growth factors, which are secreted signals governing morphogenesis and differentiation.

The regenerative endodontic protocol depends on the regenerative capacity of periradicular tissues, which act as an endogenous source of the key elements of regeneration. Several case reports and series were published (12–18) concerning revascularization procedures; however, the deficiency of prospective studies and clinical randomized trials prevents the widespread application of this promising treatment protocol. The aim of the present investigation was to assess the regenerative potential of young permanent immature teeth with necrotic pulps after the following treatment protocols: (1) an MTA apical plug, (2) the regenerative endodontic protocol (blood clot scaffold), and (3) the regenerative endodontic with a blood clot and an injectable scaffold impregnated with basic fibroblast growth factor (bFGF).

Materials and Methods

Thirty-six patients with immature, nonvital 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, Ain Shams University, Cairo, Egypt. A detailed medical and dental history was obtained from each patient's parents or guardians. Only medically free patients were included in this research. The clinical and radiographic exclusion criteria were teeth with vertical fractures, periodontally involved teeth, and nonrestorable 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, Ain Shams University. Intraoral periapical radiographs revealed immature apices. The age of the patients ranged between 9 and 13 years. Informed consent was signed for each case by the patient's parents or guardians including the proposed treatment and possible outcomes or complications.

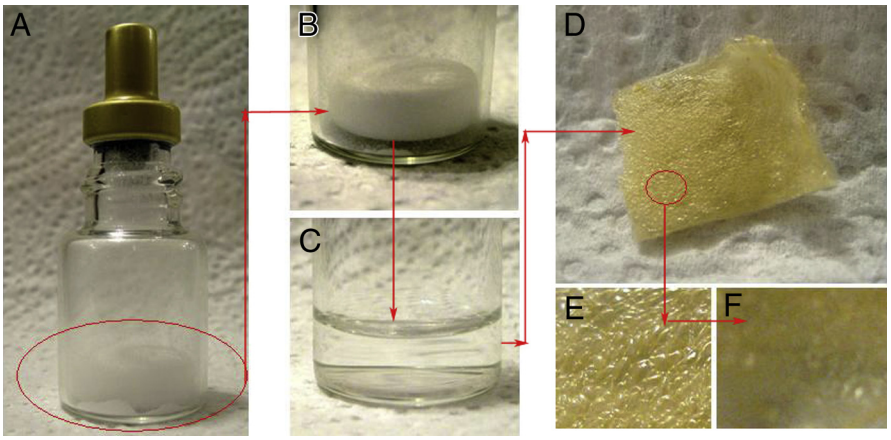

Figure 1. Preparation of the injectable scaffold: (*A*) bFGF in tablet form, (*B*) a magnified photograph of the tablet, (*C*) the growth factor after saline activation, (*D*) a dried gelatin hydrogel sheet, (*E*) the magnified area before the application of the growth factor, and (*F*) the magnified area after impregnation of the growth factor (gelation).

Cases were randomly divided into 3 groups (12 patients for each group):

1. *The MTA group:* MTA apical plug
2. *The REG group:* The regenerative endodontic protocol (blood clot scaffold)
3. *The FGF group:* The regenerative endodontic with a blood clot and an injectable hydrogel scaffold impregnated with bFGF

Preoperative radiographs were taken using the standardized paralleling technique with the Rinn XCP alignment system (Rinn Corporation, Elgin, IL). Periapical radiographs were digitized using a transparency scanner (HP Scanjet G3110; Hewlett-Packard Development Co, Palo Alto, CA) for further comparison.

Teeth were anesthetized using local anesthesia without a vasoconstrictor (Scandonest 3% plain; Septodont, Saint-Maur-Des-Fosses, France). After rubber dam isolation, access cavities were prepared, and root canals were irrigated using 10 mL 2.6% sodium hypochlorite with minimal preparation. The triple antibiotic paste was prepared using metronidazole (500-mg tablets [Flagyl 500 mg; Aventis, Cairo, Egypt]), ciprofloxacin (250-mg tablets [Ciprocin 250 mg; EPICO, Cairo, Egypt]) and doxycycline (100-mg capsules [Vibramycin; Pfizer, Cairo, Egypt]).

The doxycycline capsule content 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 1 mL 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 (Coltosol F; Coltene Whaledent, Altstatten, Switzerland) for 3 weeks.

The final visit was scheduled when the tooth was asymptomatic with no signs of discharge. In cases of persistent infection, 1 or more visits were scheduled for further drainage and chemical disinfection. After anesthesia and proper isolation, the temporary restoration and the cotton pellet were removed. The canal was irrigated with 10 mL NaOCl 2.6% followed by 10 mL sterile saline and dried with sterile paper points. One of the following treatment modalities was randomly chosen.

MTA Group

MTA (MTA Angelus, Londrina, PR, Brazil) was mixed and inserted into the canal using a suitable-sized amalgam carrier and packed using a suitable-sized plugger filling the apical third of the canal (4–5 mm). The MTA plug was verified radiographically using a standardized radiographic platform. A moist cotton pellet was inserted at the canal orifice, and the access cavity was then sealed using a temporary restoration.

After 1 week, the rest of the canal was filled using thermoplasticized gutta-percha. Adhesive composite resin (Z100 Restorative; 3M ESPE, St Paul, MN) was used to seal the access cavity.

REG Group

A sterile hand file size #80 was used with sharp strokes into the periapical tissue 2 mm beyond the apex until bleeding was evident at the cervical portion of the canal. 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.

FGF Group

A gelatin hydrogel incorporating bFGF (Kaken Pharmaceutical Co, Tokyo, Japan) was used in this group (19). Preparation of the hydrogel was done by mixing 150 μg bFGF with 300 μL phosphate-buffered saline to form a suspension. The suspension was dropped onto a 2-mg dried gelatin hydrogel sheet (Nitta Gelatin Co, Osaka, Japan). The mixture was left for 1 hour at 37°C (Fig. 1*A–F*). The induction of bleeding was done as described in group 2, and then the prepared hydrogel was inserted into the canals using a suitable-sized plugger. MTA was placed over the blood clot and sealed the same as in group 2.

Evaluation

Patients were recalled at 3, 6, 12, and 18 months. Follow-up included the clinical assessment of pain and/or swelling and standardized radiographic assessment, which included the following:

1. An increase in root length
2. An increase in root thickness
3. A decrease in apical diameter
4. A change in periapical bone density

All measurements were performed blindly. The second and third authors performed the measurements, and their average was calculated while the first author performed the treatment.

Increase in Root Length. A measuring scale was set in the ImageJ software (ImageJ v1.44; US National Institutes of Health, Bethesda, MD)