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

Mohamed M. Nagy, BDS, MSc, PhD, Hosam E. Tawfik, BDS, MSc, PhD,* Ahmed Abdel Rahman Hashem, BDS, MSc, PhD,*† and Ashraf M. Abu-Seida, BVSc, MVSc, PhD‡*

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

From the *Department of Endodontics, Faculty of Dentistry, Ain Shams University, Cairo; †Department of Endodontics, Faculty of Dentistry, Future University, Cairo; and ‡Department of Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Cairo University, Giza, Egypt.

Address request for reprints to Dr Ahmed Abdel Rahman Hashem, Endodontic Department, Faculty of Dentistry, Ain Shams University, Abassia, Cairo, Egypt. E-mail address: endohashem@gmail.com

0099-2399/$ - see front matter

http://dx.doi.org/10.1016/j.joen.2013.10.027

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.

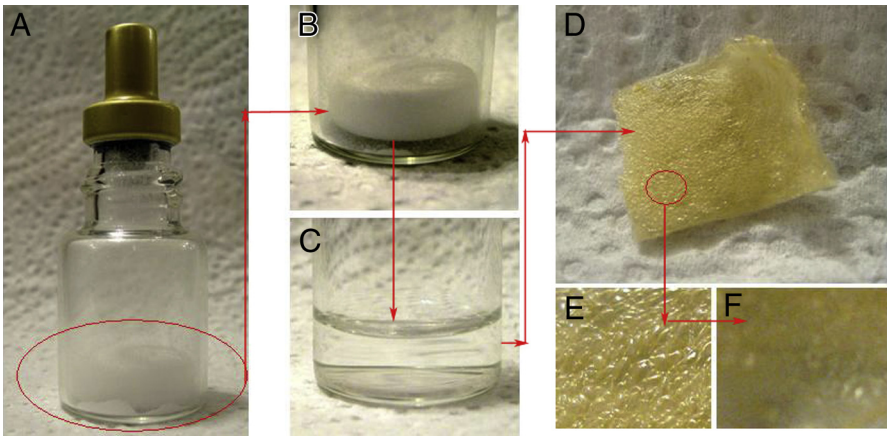

Figure 1. Preparation of the injectable scaffold: (*A*) bFGF in tablet form, (*B*) a magnified photograph of the tablet, (*C*) the growth factor after saline activation, (*D*) a dried gelatin hydrogel sheet, (*E*) the magnified area before the application of the growth factor, and (*F*) the magnified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

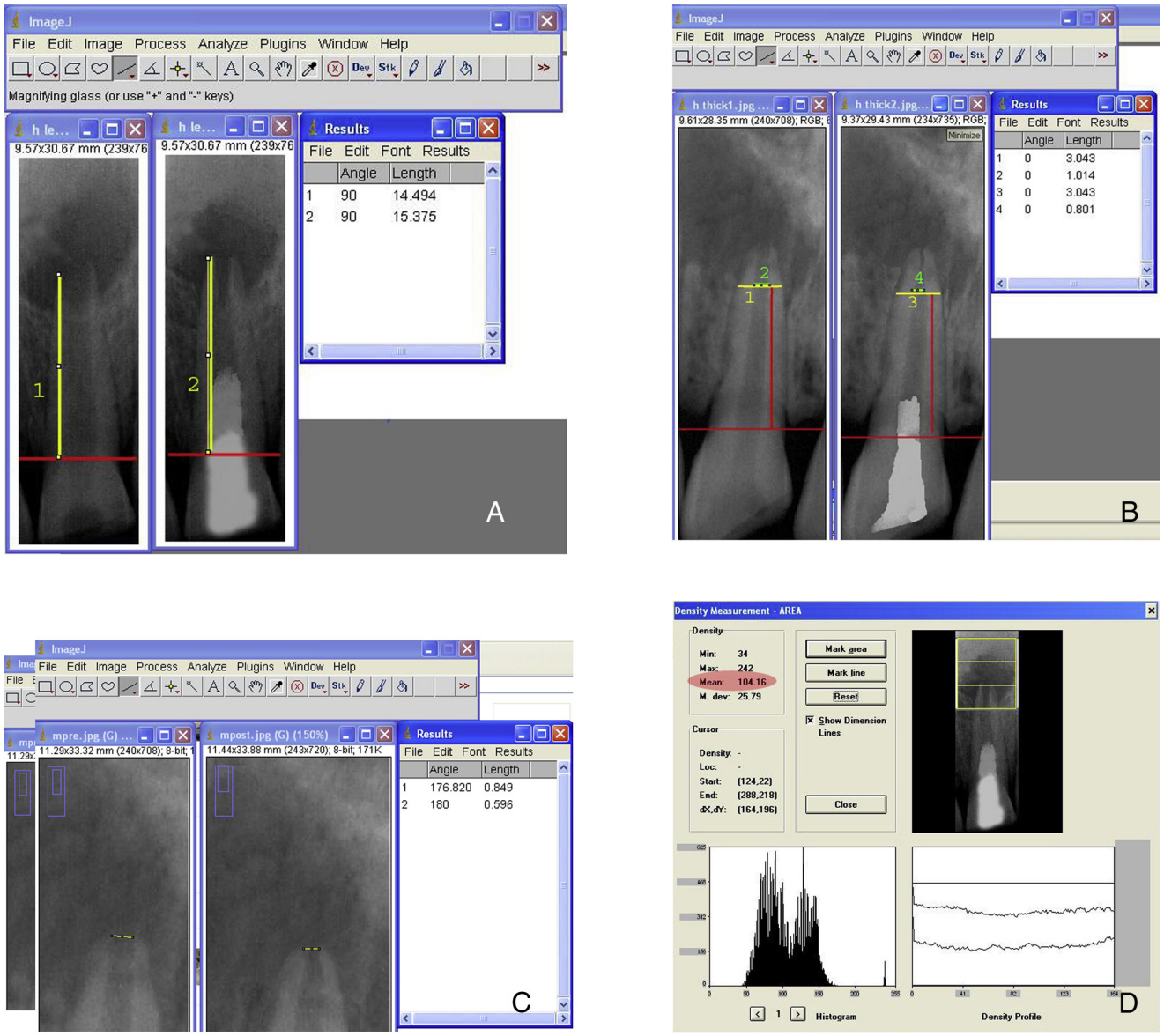

Figure 2. Digital measurements using ImageJ software of (*A*) root length in pre- and postoperative radiographs, (*B*) root thickness at the apical third in pre- and postoperative radiographs, (*C*) the apical diameter in pre- and postoperative radiographs, and (*D*) bone density measurements in pre- and postoperative radiographs using Digora for Windows software.

TABLE 1. Summary of Patients' Demographic Data

	MTA group			REG group			FGF group		
#	Tooth no.	Age/sex	Follow-up	Tooth no.	Age/sex	Follow-up	Tooth no.	Age/sex	Follow-up
1	7	9/M	Excluded	9	13/M	18 mo	8	12/F	18 mo
2	7	9/M	18 mo	8	9/M	18 mo	9	12/F	18 mo
3	8	13/F	Excluded	9	10/F	18 mo	7	13/M	Excluded
4	10	12/M	18 mo	10	12/M	18 mo	10	10/F	18 mo
5	10	10/M	18 mo	7	11/F	Excluded	8	9/M	18 mo
6	8	11/F	18 mo	8	12/M	18 mo	8	10/M	18 mo
7	9	9/F	18 mo	8	10/F	18 mo	9	9/F	18 mo
8	8	10/M	18 mo	7	9/M	Excluded	10	9/F	18 mo
9	8	9/F	Excluded	10	13/F	18 mo	7	10/M	Failed (2 mo), shifted to MTA
10	8	12/F	18 mo	10	9/M	Failed (6 months), shifted to MTA	8	12/F	Failed (3 mo), shifted to MTA
11	9	13/M	18 mo	7	13/M	18 mo	9	11/M	Excluded
12	8	11/M	18 mo	8	10/F	18 mo	10	13/F	18 mo

F, female; FGF, fibroblast growth factor; M, male; MTA, mineral trioxide aggregate; REG, regenerative endodontic protocol.

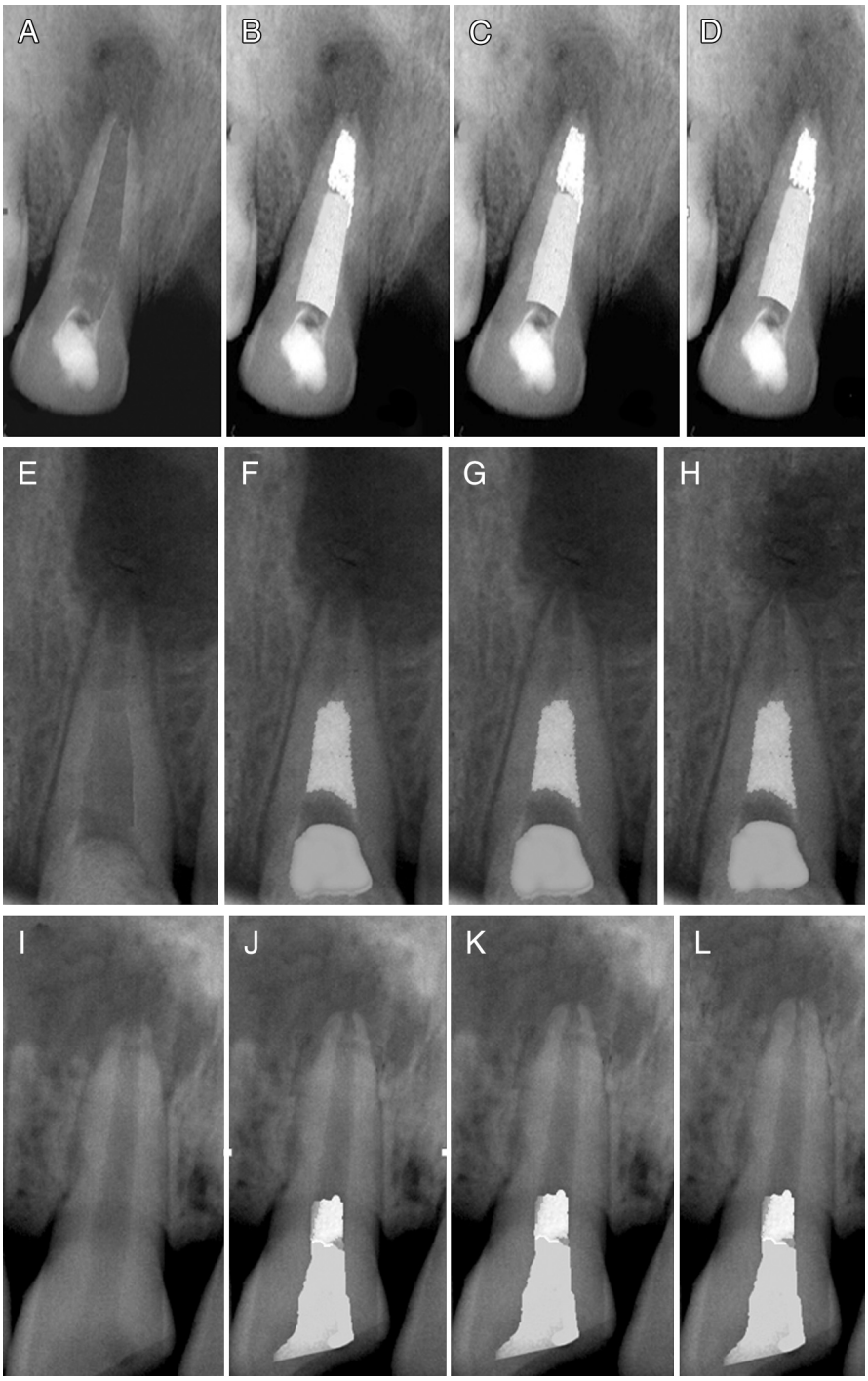

Figure 3. The first row shows a representative case of the MTA group: (*A*) the preoperative radiograph, (*B*) 6-month postoperative radiograph, (*C*) 12-month postoperative radiograph, and (*D*) 18-month follow-up radiograph. The second row shows a representative case of the REG group: (*E*) the preoperative radiograph, (*F*) 6-month postoperative radiograph, (*G*) 12-month postoperative radiograph, and (*H*) 18-month postoperative radiograph. The third row shows a representative case of the FGF group: (*I*) the preoperative radiograph, (*J*) 6-month postoperative radiograph, (*K*) 12-month follow-up radiograph, and (*L*) 18-month follow-up radiograph.

by measuring a known clinical dimension to its radiographic dimension. The scale was calculated as the number of measured pixels per mm length. Root lengths were measured as a straight line from the cementoenamel junction to the radiographic apex of the tooth in millimeters (20). Pre- and follow-up root lengths were measured as shown in Figure 2*A*, and the difference in root length was calculated. The percentage of increase in length was calculated as follows: percentage of increase in length = (postoperative length − preoperative length/preoperative length) × 100.

Increase in Root Thickness. Using the preset measurement scale, the level of the apical third was determined and fixed from the cementoenamel junction. The root thickness and the pulp width were measured at this level in millimeters. Dentin thickness was measured by subtracting the pulp space from the whole root thickness.

TABLE 2. The Increase in Length in Millimeters and the Percentage of Increase of All Groups in the 4 Evaluation Periods

	MTA group (mean ± SD)	REG group (mean ± SD)	FGF group (mean ± SD)	*P* value
3 months (n, %)	$0 \pm 0\ (0 \pm 0)^{Aa}$	$0.3 \pm 0.2\ (3 \pm 3.5)^{Ba}$	$0.3 \pm 0.2\ (3 \pm 2)^{Ba}$	<.001
6 months (n, %)	$0 \pm 0\ (0 \pm 0)^{Aa}$	$0.6 \pm 0.3\ (5.3 \pm 2.6)^{Ba}$	$0.6 \pm 0.3\ (6.1 \pm 3)^{Ba}$	<.001
12 months (n, %)	$0 \pm 0\ (0 \pm 0)^{Aa}$	$0.8 \pm 0.5\ (7.6 \pm 4.7)^{Ba}$	$1 \pm 0.5\ (9.9 \pm 4.9)^{Ba}$	<.001
18 months (n, %)	$0 \pm 0\ (0 \pm 0)^{Aa}$	$1.2 \pm 0.5\ (11.8 \pm 4.9)^{Bb}$	$1.3 \pm 0.5\ (12.4 \pm 4.7)^{Ba}$	<.001
P value	—	.0137	.0137	

FGF, fibroblast growth factor; MTA, mineral trioxide aggregate; REG, regenerative endodontic protocol; SD, standard deviation.
Significant at $P \leq .05$. Different capital letters indicate a significant difference between groups within the same follow-up period. Different small letters indicate a significant difference between periods within the same group.

Measurements were performed pre- and postoperatively at the same fixed level (20) as shown in Figure 2*B*. The difference in thickness was calculated. The percentage of increase in dentin thickness was calculated as follows: dentin thickness = root thickness − pulp width. The percentage of increase in thickness = (postoperative thickness − preoperative thickness/preoperative thickness) × 100.

Decrease in Apical Diameter. Using the preset measurement scale, the diameter of the apical foramen was measured in millimeters. Measurements were performed pre- and postoperatively as shown in Figure 2*C*. The difference in apical diameter was calculated. The percentage of apical closure was calculated as follows: percentage apical closure = postoperative apical diameter − preoperative apical diameter/preoperative apical diameter × 100.

Periapical Bone Density. Periapical bone density was estimated using Digora image analysis software (Digora for Windows 1.51; Soredex Finndent, Tuusula, Finland) as follows:

The periapical area was located and analyzed for bone density as shown in Figure 2*D*. The average area density was measured in scale from 0 (black) to 255 (white) and recorded for each radiograph. The same area was then measured in subsequent radiographs, and the average densities were recorded for the follow-up radiographs. The difference between densities was calculated between subsequent radiographs. The percentage of change in density was calculated from the original preoperative radiograph density as follows: percentage of change in density = postoperative bone density − preoperative bone density/preoperative bone density × 100.

Data were collected, tabulated, and statistically analyzed using statistical analysis software SPSS (Statistical Packages for the Social Sciences 19.0; IBM, Armonk, NY). Two-way analysis of variance was performed. The Tukey post hoc test was used in case of significance.

Results

Patients' demographic data are summarized in Table 1. A total of 7 patients were excluded from the study because of inadequate compliance and failure to recall. Three cases were excluded from the MTA group, 2 cases were excluded from the REG group, and 2 cases were excluded from the FGF group. The percentages of recall for the MTA, REG, and FGF groups were 75%, 83%, and 83% respectively. Clinical and radiographic examination during the follow-up period showed signs and symptoms of failure in 3 of the 29 recalled cases. Two cases belonged to the FGF group, and 1 case belonged to the REG group. The 3 failed cases were re-evaluated, and the treatment plan was shifted to MTA apexification. The success rates for the MTA, REG, and FGF groups were 100%, 90%, and 80%, respectively. The representative cases of all groups are shown in Figure 3*A–L*.

Increase in Root Length

Statistical analysis showed no significant difference between the REG and FGF groups through the whole follow-up period. Regarding the effect of time, a statistically significant difference was found after 18 months of follow-up for the REG group (Table 2).

Increase in Root Thickness

Statistical analysis showed no significant difference between the REG and FGF groups. Regarding time, a significant difference was evident at 18 months for both groups (Table 3).

Decrease in Apical Diameter

Statistical analysis showed no significant difference between the REG and FGF groups at 3, 6, 12, and 18 months. The MTA group was significantly different than the REG and FGF groups at 12 and 18 months (Table 4).

Periapical Bone Density

A significant improvement in bone density was found after 12 months of follow-up in all groups. No significant difference was found between all groups through the whole follow-up period (Table 5).

Discussion

The management of immature necrotic teeth has been considered a great challenge in endodontics. Historically, the treatment of such cases was performed using calcium hydroxide apexification. However, the long-term use of calcium hydroxide has several drawbacks (21), including multiple patient visits, low patient compliance, probability

TABLE 3. The Mean Increase in Root Thickness in Millimeters and the Percentage of Increase of All Groups in the 4 Evaluation Periods

	MTA group (mean ± SD)	REG group (mean ± SD)	FGF group (mean ± SD)	*P* value
3 months (n, %)	$0 \pm 0\ (0 \pm 0)^{Aa}$	$0.04 \pm 0.03\ (1.8 \pm 1.3)^{Ba}$	$0.06 \pm 0.01\ (2.3 \pm 1.4)^{Ba}$	<.001
6 months (n, %)	$0 \pm 0\ (0 \pm 0)^{Aa}$	$0.14 \pm 0.03\ (5.8 \pm 1.2)^{Ba}$	$0.11 \pm 0.04\ (4.5 \pm 1.6)^{Ba}$	<.001
12 months (n, %)	$0 \pm 0\ (0 \pm 0)^{Aa}$	$0.21 \pm 0.08\ (8.4 \pm 3.2)^{Bb}$	$0.2 \pm 0.07\ (8.3 \pm 2.9)^{Bb}$	<.001
18 months (n, %)	$0 \pm 0\ (0 \pm 0)^{Aa}$	$0.32 \pm 0.12\ (12.7 \pm 4.7)^{Bb}$	$0.29 \pm 0.09\ (11.6 \pm 3.6)^{Bb}$	<.001
P value	—	<.001	<.001	

FGF, fibroblast growth factor; MTA, mineral trioxide aggregate; REG, regenerative endodontic protocol; SD, standard deviation.
Significant at $P \leq .05$. Different capital letters indicate a significant difference between groups within the same follow-up period. Different small letters indicate a significant difference between periods within the same group.

TABLE 4. The Mean Decrease in the Apical Diameter in Millimeters and the Percentage of Apical Closure in All Groups in the 4 Evaluation Periods

	MTA group (mean ± SD)	REG group (mean ± SD)	FGF group (mean ± SD)	*P* value
3 months (n, %)	$0 \pm 0\ (0 \pm 0)^{Aa}$	$0.08 \pm 0.04\ (6.4 \pm 3.2)^{Ba}$	$0.06 \pm 0.04\ (5.7 \pm 3.7)^{Ba}$	<.001
6 months (n, %)	$0 \pm 0\ (0 \pm 0)^{Aa}$	$0.22 \pm 0.11\ (17.6 \pm 8.8)^{Bab}$	$0.15 \pm 0.08\ (12.6 \pm 6.7)^{Ba}$	<.001
12 months (n, %)	$0 \pm 0\ (0 \pm 0)^{Aa}$	$0.67 \pm 0.04\ (34.6 \pm 2)^{Bb}$	$0.59 \pm 0.41\ (29.9 \pm 20.7)^{Bab}$	<.001
18 months (n, %)	$0 \pm 0\ (0 \pm 0)^{Aa}$	$0.8 \pm 0.3\ (50.5 \pm 18.9)^{Bc}$	$0.9 \pm 0.2\ (44.3 \pm 9.8)^{Bb}$	<.001

FGF, fibroblast growth factor; MTA, mineral trioxide aggregate; REG, regenerative endodontic protocol; SD, standard deviation.

Significant at $P \leq .05$. Different capital letters indicate a significant difference between groups within the same follow-up period. Different small letters indicate a significant difference between periods within the same group.

of canal contamination between visits, and increased dentin brittleness, which increase the risk of fracture (21).

The technique of an immediate apical plug has been advocated as a single-visit apexification by placing an artificial apical barrier to obliterate the apical portion of the canal (2–5). MTA proved to be an excellent candidate for this protocol. This protocol has the advantage of a reduced number of visits, higher patient compliance, and a high success rate (5, 6, 22). However, the problem of thin brittle roots was not solved.

Recently, regenerative endodontics has gained much attention as a biologically based alternative. Regenerative approaches gained the advantage over apexification because they can allow for further root maturation in length and thickness by regenerated vital tissue (23, 24). Revascularization is considered a simple protocol by which pulp regeneration can be enhanced (11, 25).

An injectable scaffold is one of the treatment alternatives for regenerative endodontics. The model of using an injectable scaffold and hydrogel incorporating bFGF aimed to enhance the regenerative endodontic process via a drug delivery system. Gelatin hydrogel acts as a resorbable scaffold and is a preferable candidate for a protein carrier because of its biosafety and high inertness toward protein drugs (9). The growth factor was prepared in a basic form in order to be electrostatically attached to the acidic gelatin. Thus, delivery of the growth factor is controlled via degradation of the hydrogel carrier and not by simple diffusion. The use of growth factor was thought to enhance root development and maturation.

The aim of our study was to evaluate the treatment outcomes of immature necrotic teeth via an MTA apical barrier and using the regenerative endodontic protocol alone and with an injectable scaffold. Several case reports and series dealing with regenerative endodontics are already published in the literature; however, there is an increasing demand for higher levels of evidence for determining the efficacy of a given treatment modality.

The triple antibiotic paste consists of 3 antibiotics: metronidazole, ciprofloxacin, and doxycycline. The combination of the 3 antibiotics was proved to sufficiently eradicate bacteria from infected root canals (8, 9).

Radiographic evaluation was dimensionally standardized using a radiographic platform (Rinn XCP alignment system) to allow for quantitative assessment in terms of root length and thickness through the whole follow-up period. Radiographic images were standardized in terms of brightness and contrast using image-editing software to allow for standard measurement of bone density.

The results showed that in the MTA group most of the cases did not show any increase in length or thickness. This may be caused by the cessation of growth after MTA orthograde plugging. In the REG and FGF groups, there was an increase in root length and thickness over the follow-up period. However, there was no significant difference between the 2 groups. The continued root growth may be attributed to many mechanisms (26).

One possible mechanism postulates that few vital pulp cells remain at the apical canal end (27). These cells might have the ability 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 hypothesized mechanism depends on periodontal ligament stem cells (28, 29). This hypothesis is valid in case of the destruction of epithelial root sheath of Hertwig and apical papilla tissues. These cells have the ability to proliferate and grow within the apical end of the canal lumen through the open apex.

The third possible mechanism relies on stem cells of apical papilla (SCAPs) in which instrumentation beyond the apical limit of the canal leads to the transplantation of SCAPs into the canal lumen. SCAPs may survive infection and retain the capacity for proliferation and differentiation into bone- or dentin- forming cells (30, 31).

The fourth possible mechanism involves the blood clot itself. The formed blood clot is considered a reservoir of growth factors including platelet-derived growth factor, vascular endothelial growth factor, and tissue growth factors. This rich supply of growth factors stimulates the differentiation, growth, and maturation of fibroblasts, odontoblasts, and cementoblasts from their undifferentiated precursors. The expression of vascular endothelial growth factor in immature teeth has been documented (32).

Our findings concerning the increase in length and thickness for regenerative endodontic cases were in agreement with Cehreli et al (14), Bose et al (20), and Tawfik et al (25). Of the current available data, these studies were the only studies including a quantitative analysis

TABLE 5. The Mean Decrease in Bone Density in All Groups in the 4 Evaluation Periods

	MTA group (mean ± SD)	REG group (mean ± SD)	FGF group (mean ± SD)	*P* value
3 months (%)	4.47 ± 5.6^{Aa}	2.21 ± 1.9^{Aa}	2.51 ± 2.1^{Aa}	.2664
6 months (%)	8.71 ± 3.2^{Aa}	6.43 ± 2.1^{Aa}	5.26 ± 3.7^{Aa}	.1941
12 months (%)	11.25 ± 7.8^{Ab}	10.91 ± 6.2^{Ab}	9.56 ± 5.8^{Ab}	.8073
18 months (%)	14.61 ± 7.1^{Ab}	12.77 ± 5.5^{Ab}	12.21 ± 6^{Ab}	.6191
P value	.0021	<.001	<.001	

FGF, fibroblast growth factor; MTA, mineral trioxide aggregate; REG, regenerative endodontic protocol; SD, standard deviation.

Significant at $P \leq .05$. Different capital letters indicate a significant difference between groups within the same follow-up period. Different small letters indicate a significant difference between periods within the same group.

of regenerative endodontic outcomes. They reported a marked increase in length and thickness over retrospective studies. Our findings were in agreement with many authors (12–14, 25) who reported an increase in length and thickness without quantitative measurements.

Regarding the FGF group, the absence of a significant difference from group 2 can be explained by the fact that the injectable scaffold did not play a significant role in the regeneration process. Kimura and Tabata (33) did not support the present findings. They found that the use of hydrogel incorporating bFGF enhanced angiogenesis and regeneration in ischemic tissues. This conflict may be caused by the difference in substrate; their medical use of that drug delivery system was concentrated on ischemic tissues, which are still vital and already supported by collateral circulation, and cellular supply is guaranteed. However, in the present study, the pulp space was empty and enclosed in hard tissue deprived from collateral circulation with minimum cellular infiltration (18).

The bone density profile for group 1 clearly improved over the 18-month follow-up period. This is linked to the healing progress after MTA application. MTA is known for its excellent biocompatibility and capacity to induce repair and regeneration. Our findings were consistent with many authors (2–6) who reported healing of periapical radiolucencies although no quantitative data had been available except for some studies (2, 34) in which the periapical index score was used as a quantitative method.

Regarding the REG and FGF groups, the bone density results showed an increase in the bone density of previously rarefacted areas. This indicates a favorable healing response to the proposed treatment, which was initiated by canal disinfection via the triple antibiotic paste and terminated by the induction of bleeding and the establishment of a coronal seal (MTA plug). No significant difference was found between the 3 groups over the follow-up period, which was related to the favorable tissue response to the 3 treatment protocols.

Conclusions

Under the circumstances of this study, it can be concluded that both treatment protocols (ie, an MTA apical plug and the regenerative endodontic procedure) were successful treatment options with regard to the closure of open apices. Regenerative endodontic procedures induced an increase in root length, thickness, and apical closure. The use of an artificial hydrogel scaffold and bFGF was not essential for repair.

Acknowledgments

The authors thank Professor Yasuhiko Tabata for providing the hydrogel gel scaffold and bFGF used in this study.

The authors deny any conflict of interest related to this study.

References

1. Cvek M. Treatment of non-vital permanent incisors with calcium hydroxide: I—follow-up of periapical repair and apical closure of immature roots. Odont Revy 1972;23:27–44.
2. Simon S, Rilliard F, Berdal A, et al. The use of mineral trioxide aggregate in one visit apexification treatment: a prospective study. Int Endod J 2007;40:186–97.
3. Pace R, Giuliani V, Pinni Prato L, et al. Apical plug technique using mineral trioxide aggregate: result from a case series. Int Endod J 2007;40:478–84.
4. D'Arcangelo C, D'Amario M. Use of MTA for orthograde obturation of non vital teeth with open apices: a report of two cases. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2007;104:e98–101.
5. Holden DT, Schwartz SA, Kirkpatrick TC, et al. Clinical outcomes of artificial root end barriers with mineral trioxide aggregate in teeth with immature apices. J Endod 2008;34:812–7.
6. Witherspoon DE, Small JC, Regan JD, et al. Retrospective analysis of open apex teeth obturated with mineral trioxide aggregate. J Endod 2008;34:1171–6.
7. Lovelace T, Henry M, Hargreaves K, et al. Evaluation of the delivery of mesenchymal stem cells into the root canal space of necrotic immature teeth after clinical regenerative endodontic procedure. J Endod 2011;37:133–8.
8. Sato I, Kurihara-Ando N, Kota K, et al. Sterilization of infected root canal dentine by topical application of a mixture ciprofloxacin, metronidazole and minocycline *in situ*. Int Endod J 1996;29:118–24.
9. Hoshino E, Kurihara-Ando N, Sato I, et al. *In vitro* antibacterial susceptibility of bacteria taken from infected root dentine to a mixture of ciprofloxacin, metronidazole and minocycline. Int Endod J 1996;29:125–30.
10. Tabata Y. Tissue regeneration based on tissue engineering technology. Congenit Anom (Kyoto) 2004;44:111–24.
11. Murray PE, Garcia-Godoy F, Hargreaves KM. Regenerative endodontics: a review of current status and a call for action. J Endod 2007;33:377–90.
12. Iwaya S, Ikawa M, Kubota M. Revascularization of an immature permanent tooth with periradicular abscess after luxation. Dent Traumatol 2011;37:55–8.
13. Nosrat A, Seifi A, Asgary S. Regenerative endodontic treatment (revascularization) for necrotic immature permanent molars: a review and report of two cases with new biomaterial. J Endod 2011;37:562–7.
14. Cehreli ZC, Ishitiren B, Sara S, et al. Regenerative endodontic treatment (revascularization) of immature necrotic molars medicated with calcium hydroxide: a case series. J Endod 2011;37:1327–30.
15. Torabinejad M, Faras H. A clinical and histological report of a tooth with an open apex treated with regenerative endodontics using platelet-rich plasma. J Endod 2012;38:864–8.
16. Jadhav G, Shah N, Logani A. revascularization with and without platelet-rich plasma in nonvital, immature, anterior teeth: a pilot clinical study. J Endod 2012;38:1581–7.
17. Wigler R, Kaufman AY, Lin S, et al. Revascularization: a treatment for permanent teeth with necrotic pulp and incomplete root development. J Endod 2013;39:319–26.
18. Soares A, Lins FF, Nagata JY, et al. Pulp revascularization after root canal decontamination with calcium hydroxide and 2% chlorhexidine gel. J Endod 2013;39:417–20.
19. Fujihara Y, Koyama H, Ohba M, et al. Controlled delivery of bFGF to recipient bed enhances the vascularization and viability of an ischemic skin flap. Wound Repair Regen 2008;16:125–31.
20. Bose R, Nummikoski P, Hargreaves K. A retrospective evaluation of radiographic outcomes in immature teeth with necrotic root canal systems treated with regenerative endodontic procedures. J Endod 2009;35:1343–9.
21. Andreasen J, Farik B, Munksgaard E. Long term calcium hydroxide as a root canal dressing may increase risk of root fracture. Dent Traumatol 2002;18:134–7.
22. Shabahang S, Torabinejad M, Boyne PP, et al. A comparative study of root end induction using osteogenic protein-1, calcium hydroxide and mineral trioxide aggregate in dogs. J Endod 1999;25:1–5.
23. Huang GTJ. Apexification: the beginning of its end. Int Endod J 2009;42:855–66.
24. Hargreaves K, Law A. Regenerative endodontics. In: Hargreaves K, Cohen S, eds. *Pathways of the Pulp*, 10th ed. St Louis: Mosby Elsevier; 2011:602–19.
25. Tawfik H, Abu-Seida AM, Hashem AA, et al. Regenerative potential following revascularization of immature permanent teeth with necrotic pulps. Int Endod J 2013;46:910–22.
26. Shah N, Logani A, Bhaskar U, et al. Efficacy of revascularization to induce apexification/apexogenesis in infected nonvital immature teeth: a pilot clinical study. J Endod 2008;34:919–25.
27. Banchs F, Trope M. Revascularization of immature permanent teeth with apical periodontitis: new treatment protocol. J Endod 2004;30:196–200.
28. Lieberman J, Trowbridge H. Apical closure of non vital permanent incisor teeth where no treatment was performed: case report. J Endod 1983;9:257–60.
29. Nevin A, Wrobel W, Valachovic R, et al. Hard tissue induction into pulpless open-apex teeth using collagen-calcium phosphate gel. J Endod 1977;3:431–3.
30. Krebsbach P, Kuznetsov SA, Satomura K, et al. Bone formation *in vivo*: comparison of osteogenesis by transplanted mouse and human marrow stromal fibroblasts. Transplantation 1997;63:1059–69.
31. Gronthos S, Mankani M, Brahim J, et al. Postnatal human dental pulp stem cells (DPSCs) *in vitro* and *in vivo*. Proc Natl Acad Sci U S A 2000;97:625–30.
32. Wang Q, Lin XJ, Lin ZY, et al. Expression of vascular endothelial growth factor in dental pulp of immature and mature permanent teeth in human. Shanghai Kou Qiang Yi Xue 2007;16:2859.
33. Kimura Y, Tabata Y. Experimental tissue regeneration by DDS technology of biosignaling molecules. J Dermatol Sci 2007;47:189–99.
34. Mente J, Hage N, Pfefferle T, et al. Mineral trioxide aggregate apical plugs in teeth with open apical foramina: a retrospective analysis of treatment outcome. J Endod 2009;35:1354–8.