Original Article

Discoloration after revascularization using calcium phosphosilicate-based bioceramic versus mineral trioxide aggregate in necrotic immature permanent anterior teeth: A Randomized clinical trial

Rana Tarek Bartaw, Mahmoud Hamdy Eid, Randa Youssef Abd Al Gawad, Ahmed Mohamed Abdel Samad[1]

Departments of Pediatric Dentistry and Dental Public Health and [1]Oral Radiology, Faculty of Dentistry, Cairo University, Giza, Egypt

Abstract

Aim: The aim of the current study is to evaluate the effect of calcium phophosilicate-based bioceramic "Totalfill bioceramic putty" and white mineral trioxide aggregate (WMTA) as the coronal plug on discoloration after revascularization of necrotic immature permanent anterior teeth.

Materials and Methods: This study was conducted on (48) necrotic young permanent central incisors in children ranging from 8 to 14 years old, that were randomly allocated to either Totalfill bioceramic (Group I = 24) or WMTA (Group II = 24) as the coronal plug. Two visits revascularization protocol was adopted in this study using 1.5% sodium hypochlorite, followed by 17% ethylenediaminetetraacetic acid, and ending with a saline flush as irrigation solution. The double antibiotic paste was used as intracanal medication. The blood clot was used as scaffold followed by the application of collagen membrane followed by coronal plud malterial. Finally, the access was sealed using resin composite restoration and composite restoration. Clinical assessment was conducted at 1, 3, 6, 9, and 12 months, while radiographic assessment was conducted at 6 and 12 months. Data were statistically analyzed using the Chi-squared test for intergroup comparisons and Cochran's Q test for intragroup comparison.

Results: Clinically, Group I exhibited a success rate of 100%, whereas Group II exhibited a success rate of 85.7%. Radiographically, both materials showed a 90.5% success rate. There was no statistically significant difference between both materials for all assessed clinical and radiographic parameters at different follow-up periods.

Conclusions: Both Totalfill bioceramic putty and WMTA can be used successfully as coronal plug in esthetic areas.

Keywords: Bioceramics; immature necrotic teeth; mineral trioxide aggregate discoloration; pulp regeneration; pulp revascularization; pulp revitalization

INTRODUCTION

The process of revascularization is a biologically based substitute for the traditional apexification treatment utilized in cases of necrotic, immature teeth. Its primary objectives are to eliminate symptoms, promote healing of periapical injuries, enhance the thickness and length of root canal walls, and restore the vitality of the pulp. The concept of revascularization suggests that, in the absence of bacteria, a suitable three-dimensional scaffold, and the presence of stem or progenitor cells, coupled with the establishment of a bacterial-tight seal within the root canal space, it is possible to repair devitalized, uninfected, immature permanent teeth.[1,2]

Address for correspondence:
Dr. Rana Tarek Bartaw,
Department of Pediatric Dentistry and Dental Public Health,
Faculty of Dentistry, Cairo University, Giza, Egypt.
E-mail: rana.tarek@dentistry.cu.edu.eg

Date of submission : 20.12.2023
Review completed : 08.01.2024
Date of acceptance : 10.01.2024
Published : 06.03.2024

Access this article online

Quick Response Code:

Website: https://journals.lww.com/jcde

DOI: 10.4103/JCDE.JCDE_337_23

How to cite this article: Bartaw RT, Eid MH, Gawad RY, Samad AM. Discoloration after revascularization using calcium phosphosilicate-based bioceramic versus mineral trioxide aggregate in necrotic immature permanent anterior teeth: A randomized clinical trial. J Conserv Dent Endod 2024;27:293-304.

The methodology includes a dual-session approach. The first visit includes minimum or no dentinal wall instrumentation, canal irrigation for disinfection, intracanal medication administration, and a temporary coronal seal. The second visit is conducted at an interval of 2–4 weeks. The procedure includes the intentional initiation of bleeding within the canal to promote the formation of a blood clot, followed by the application of hydraulic silicate cement for the purpose of capping the canal. Finally, a dependable coronal seal is established.[3,4]

The most common material used as coronal plug material in revascularization is mineral trioxide aggregate (MTA) because of its biocompatibility, sealing ability, low solubility, antimicrobial properties, induction of odontoblasts, cementoblasts, and hard tissue barrier, the capacity to set in a moist environment, radiopacity that is slightly greater than dentine and reasonable compressive strength.[5]

However, the main drawbacks of MTA include difficulties in its manipulation and the possibility of tooth discoloration, particularly when applied in the coronal region of the root, despite the utilization of a white version of MTA that contains reduced quantities of metal oxides. Various theories have been suggested regarding the discoloration caused by MTA. The suggestion indicates that it mainly arises from the interaction between bismuth oxide, utilized as a radio-opacifying agent in MTA, and the collagen that is present in the tooth structure, blood, and sodium hypochlorite, which is a standard component of disinfection in revascularization procedures.[6,7]

"EndoSequence" or "Totalfill" is a new phosphosilicate-based bioceramic. According to the manufacturer, it contains calcium silicate, monobasic calcium phosphate, zirconium oxide, tantalum oxide, and filler agents. Zirconium and tantalum oxide are radiopacifiers instead of bismuth oxide, which causes discoloration. The manufacturer claims the material is aluminum-free, non-soluble, and does not shrink during setting. It comes in paste in preloaded syringes and moldable putty for easier handling.[8,9]

Despite the high success rate of revascularization clinically and radiographically, concerns about posttreatment tooth discoloration are increasing. This outcome is very unfavorable in terms of the patient's smile esthetics and may affect patient satisfaction, despite otherwise excellent clinicians and science-based outcomes. Thus, the esthetic concern of the patient led to the need to search for solutions to overcome this drawback.[7]

Although the American Association of Endodontists Considerations 2016 revised 2018[10] stated that bioceramic materials alternatives to MTA should be considered in the esthetic area, the manufacturers claim that Totalfill does not cause discoloration *in vitro* or *ex vivo*, there is a lack of clinical studies that confirm its clinical and radiographic outcomes in revascularization procedures.

MATERIALS AND METHODS

Trial registration, study design, and grouping

The study was registered on the clinical trial.gov (https://clinicaltrials.gov/) with ID number: NCT03813433 and was verified on May 22, 2019. All the procedures completed in the present study were in agreement with the standards of the Research Ethics Committee (REC) of the Faculty of Dentistry at Cairo University (Ref. 19-07-80). The design of the current study was a triple-blinded, parallel, two-armed study with a superiority framework and 1:1 allocation ratio.

Sample size calculation

The sample size was calculated using G * Power version 3.1.9.2 (Franz Faul, Universität Kiel, Germany) for Windows based on the previous study by Chan *et al*.[11] which indicated that the probability of discoloration was 0.57 for MTA. To detect a difference of 30%, 20 teeth for each group were needed to reject the null hypothesis that the success rates for the intervention and control are equal with probability (power) 0.8. This was increased to 24 teeth in each group to compensate for losses during follow-up. The Type I error probability associated with this test of this null hypothesis is 0.05.

Eligibility criteria

Inclusion criteria

Patients:[10,12]

- The age of children ranges from 8 to 14 years
- Cooperative patient/compliant parents or guardians
- Apparently healthy children.

Teeth:

- Necrotic immature permanent maxillary central incisors
- Enough coronal portion of teeth that did not necessitate post and core for final restoration.

Exclusion criteria

Patients:

- Medically compromised children (American Society of Anesthesiologists [ASA] physical status classification system ASA III and higher)
- Allergy to antibiotics used in the study, whether from parental history or evidence of allergy during or after the antibiotic placement.

Teeth:

- Teeth with internal root resorption
- Luxative injuries
- Avulsed teeth after replantation
- Compromised remaining coronal structure
- Teeth with severe discoloration in comparison with adjacent tooth.

Recruitment

Screening of patients from the clinic of Pediatric Dentistry and Dental Public Health Department, Faculty of Dentistry, Cairo University seeking dental care continued until the target population was achieved 1 month before intervention according to the eligibility criteria. Out of 95 patients examined, 48 patients fulfilled the eligibility criteria, once the child was identified as eligible for the study by the clinical investigator, the trial procedures, benefits from the study, and expected harms were clearly discussed with the parent or the child's legal guardian. Written consent translated into Arabic, agreeing to the clinical procedures, was signed by the child's guardian. This explanation was simplified with the avoidance of any pain-promoting words when the trial was discussed with the child. Verbal assent was obtained orally from the eligible child. Figure 1 shows the flow diagram of participants in the current research.

Randomization, Sequence generation, and blinding

Eligible teeth were assigned to either Group I or Group II, where Group I represented teeth treated with Totalfill bioceramic (intervention) and Group II represented teeth treated with MTA (control). Eligible teeth were assigned to either Group I or Group II according to a sequence generated on a Microsoft Excel sheet and were simply randomized with a ratio 1:1 using (www.random.org). Sequence generation was done by a colleague not involved in the study. Allocation was concealed in opaque sealed envelopes (48 closed white sealed envelopes) which assigned the group to be followed. Those closed envelopes included papers numbered from 1 to 48 which were folded eight times in order not to show their content to assure allocation concealment and it was withdrawn by the parent or the participant child at the second visit after the blood clot formation. The current study was triple blinded to the child and his legal guardian, assessors, and statistician.

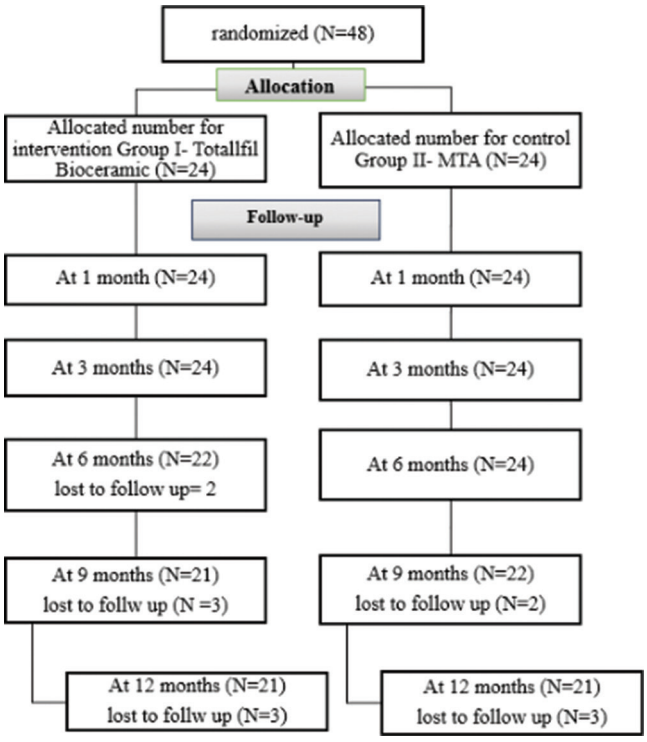

Figure 1: Flow chart of participants

Interventions

Materials used in the current study are described in Table 1. Treatment of the selected immature nonvital anterior centrals was performed according to AAE recommendations 2016 revised 2018 clinical recommendations for the regenerative endodontic procedure.[10] Revascularization procedure was applied to all the included teeth, and the only difference was the coronal plug material at the second visit, where Group I received Totalfill putty as a coronal plug while Group II received MTA as a coronal plug. Oral hygiene instructions were given to the child and his/her legal guardian, and a custom-made radiographic stent was fabricated for each child.[13]

Clinical procedures

First visit

Standardized preoperative photographs and radiographs were recorded for each child. Radiographs were taken using a size #2 digital radiographic imaging plate (KaVo, Tuusula, Finland) and X-ray machine (Sordex, Aceton Group, X-mind DC, Rome, Italy) with the following exposure parameters: 70 kVp, 10 mA, and 0.08 s exposure time. The processing of the film was done using the Digora Optime system. Each tooth was anesthetized by applying topical anesthesia (8% benzocaine) at the site of needle insertion, followed by the use of 4% articaine (Articaine (D. C. I) 40.00 mg hydrochloride, Epinephrine 0.01 mg) using a disposable short needle16 and metallic dental syringe. The involved teeth were isolated using rubber dam (Sanctuary Dental Dam System, Malaysia) then access cavity and deroofing of the pulp chamber were performed using a sterile high-speed diamond round bur (801-16-FG, 1.6 mm Meisinger, USA) and high-speed handpiece (CX207, Coxo, China) with copious water spray then flaring using a round ended taper diamond bur (TR-12, Premier solo diamond, USA) for better visibility of the root canals, as shown in Figure 2. Using side vented needles (double vented), the root canals were

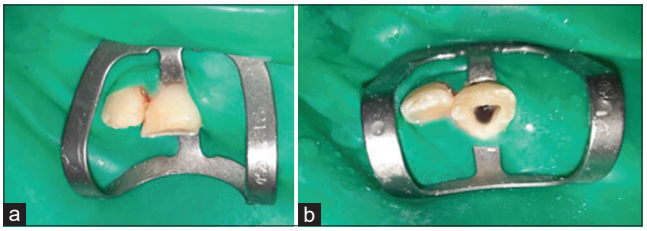

Figure 2: Showing the steps of access cavity preparation: (a) Rubber dam isolation; (b) finished access cavity

Table 1: Material used in the current study

Product	Manufacturer	Composition	Lot number
White angleus MTA	Angleus, Brazil	Powder: Tricalcium silicate, dicalcium silicate, tricalcium aluminate, calcium sulfate, and bismuth oxide Liquid: Distilled water	21381
Totafill bioceramic putty	FKG Dentaire, Swiss	Calcium silicate, monobasic calcium phosphate, zirconium oxide, tantalum oxide, and filler agents	1902BPP
Collaplug	Zimmer	Absorbable collagen plug	PDWPU200908
Riva LC	SDI	Resin-reinforced glass ionomer restorative cement	1082160EG
Coltene etch gel	Coltene	37% phosphoric acid gel	K50098
One coat bond	Coltene	HEMA, UDMA, HPMA Hydroxy-propyl-methacrylate, glycerol, methacrylates, methacrylized, polyalkenoate, amorphous silica 5% water	0164128
Neospectra composite	Dentsply composite	Matrix: Methacrylic modified polysiloxane nanoparticles, dimethacrylate resin, ethyl-4-(dimethylamino) benzoate Filler: Spherical, prepolymerized Sphere TEC fillers	1911001218

MTA: Mineral trioxide aggregate, HEMA: Hydroxyethyl methacrylate, UDMA: Urethane dimethacrylate, HPMA: Hydroxypropyl methacrylate

passively irrigated first with 1.5% NaOCl irrigant (20 mL for 5 min), followed by sterile physiological saline (5 mL), and finally with 20 mL of 17% ethylenediaminetetraacetic acid (EDTA). The irrigation needle was placed 2 mm from the root apex. The root canals were dried with large-sized paper points (#70–80) to prepare the canals to receive the intracanal medication.

A mix of double antibiotic paste consisting of 250 mg ciprofloxacin and 250 mg metronidazole was prepared.[14,15] The tablets of ciprofloxacin and metronidazole were individually grounded in mortar. The powder was freshly prepared for each patient. Using a measuring spoon, equal amounts of ciprofloxacin and metronidazole powder with a ratio of 1:1 were mixed with an equal amount of saline on a clean dry, polished glass slab or paper pad using a stainless-steel spatula to form a homogeneous paste.

The paste was then loaded inside the prepared canal using lentulo spiral size # 40. The excess paste in the access cavity was removed to a level just beneath the cemento-enamel junction by a small bond brush and the intracanal medicament was left for 2–4 weeks. A dry sterile cotton pellet was then placed to cover the root canal orifice and the access was filled with light cure resin-modified glass ionomer capsule. The capsule was first activated and immediately mixed in an amalgamator for 10 s, and then placed in the cavity to achieve a coronal seal as temporary restorative material until the second visit.

Second visit (2–4 weeks)

At the beginning of the second appointment, the shade of the tooth was recorded by the operator and another clinical assessor (blinded) using Vita shade guide 3D master shade guide (VITA, Germany). If there was a disagreement about the shade, the clinical assessor's (blinded) choice was used as a baseline shade. Clinical evaluation was done after 2 weeks for both groups. If there were signs and symptoms of persistent infection (in terms of persistence of pain, swelling, sinus, or fistula), the temporary restorative material was removed and the intracanal dressing was refreshed for another 2 weeks.[10] Otherwise, the following steps were taken. In the second visit, 3% mepivacaine without vasoconstrictor (mepivacaine HCL 3%,), was used to anesthetize the involved tooth using disposable needles and metallic dental syringes following the application of topical anesthesia. Tooth isolation with a rubber dam was done and the temporary restorative material was removed using sterile high-speed diamond round bur (801-16-FG, 1.6 mm).

Copious irrigation to remove intracanal medication was performed using side, double-vented needles and EDTA (20 mL, 5 min) followed by a flush of physiological saline (5 mL). The needle was placed 2 mm away from the root apex. The canals were then dried with large-sized paper points (#70-80). Bleeding was induced by mechanical irritation of the periapical tissues and rotational movement with a pre-bent endodontic k-file 34 (size #20).[10,12] If the k-file failed to induce bleeding, a pre-bent H-file 35 (size #15-40) was used to induce bleeding.[4,16] Blood was allowed to fill the canal till 2 mm below the gingival margin. Wet cotton was placed then in the access cavity for 10 min till the formation of blood clots, as shown in Figure 3. Blood remnants were removed from the access cavity using a small bond brush and a resorbable collagen matrix was then cut to a diameter larger than the coronal part of the root canal and a height of 2–3 mm and placed over the formed blood clot. A bond brush was then used for the gentle adaptation of the collagen matrix over the blood clot without pressure.

Placement of coronal plug

Placement of coronal plug material was done according to the tooth allocated in which group, either Totalfill bioceramic putty (Group I) or MTA (Group II) was placed over the collaplug.

Group I (Totalfill bioceramic putty): A part of premixed bioceramic material was taken from the jar and applied in the prepared canal over the collaplug using a condenser (Medentra dental company, Pakistan). A bond brush was used for the adaptation of the bioceramic over the collagen matrix. The excess was removed from the access cavity. The access cavity was then sealed with a layer of light cure resin-modified glass ionomer, leaving 2 mm for composite restoration as a final restoration for the access cavity.

Group II (mineral trioxide aggregate): Mixing was done according to manufacturer instructions; one spoon of MTA was mixed with 1 drop of distilled water for 30 s till a homogeneous mix was achieved and with a consistency similar to wet sand. The mix was placed over the collagen matrix 2 mm beneath the clinical CEJ using amalgam carrier (Medentra Dental Company, Pakistan). A bond brush was used for the adaptation of MTA over the collagen matrix without pressure. The excess was removed from the access cavity. The access cavity was then sealed with a layer of light cure resin-modified glass ionomer, leaving 2 mm for composite restoration as a final restoration for the access cavity.

Outcome assessment

All patients were planned to be recalled for clinical follow-up after 1 month, 3 months, 6 months, and 12 months. Radiographic follow-up was planned to be at 6 months and 12 months using the same radiographic parameters at the first visit.[10] Clinical assessments were carried out by the operator and another blinded assessor. Radiographic assessments were carried out by a blinded radiographic assessor. Outcomes are described in Table 2.[13,17-20]

Statistical analysis

Data were analyzed using Medcalc software, version 19 for Windows (MedCalc Software Ltd, Ostend, Belgium). Categorical data were described as frequency and percentage, intergroup comparisons between interventions were performed using the Chi-Squared test with a statistical significance level set at ($P \leq 0.05$), and intragroup comparison within each intervention was performed using Cochran's Q test followed by multiple comparisons with statistically significant level set at ($P \leq 0.005$) clinically and at ($P \leq 0.016$) radiographically. Relative risk was used to assess the clinical significance. Continuous data were explored for normality using Kolmogorov–Smirnov test and the Shapiro–Wilk test. Continuous data showed normal distribution and were described using mean and standard deviation. Intergroup comparison for continuous data was performed using independent *t*-test with a statistical significance level set at ($P \leq 0.05$), intragroup comparison was performed using repeated measures ANOVA with a statistically significant level set at ($P \leq 0.016$). Inter-observer agreement was calculated using Cohen's kappa coefficient. The confidence limit was set at 95% with 80% power and all tests were two-tailed.

RESULTS

Demographic data

The current study was conducted on (48) children with necrotic immature permanent central incisors that were randomly allocated to the intervention and the comparator arms ($n = 24$). After 12 months 42 participants completed the follow-up with an 87.5% retention rate. The mean age of the participants in the current trial was 9.35 ± 1.17 years; the mean age within the intervention group was 9.83 ± 1.09 years, while within the comparator group mean age was 9.12 ± 0.07 years, there was no statistically significant difference between both groups regarding age ($P = 0.0741$). Regarding gender, there were 34 boys and 14 girls in the current study, in calcium phosphosilicate-based bioceramic group there were 18 boys and 6 girls, while in the MTA group, there were 16 boys and 8 girls, there were no statistically significant differences between both groups regarding gender ($P = 0.5297$).

Clinical parameters

After 12 months bioceramics have shown a 100% success

Table 2: Outcomes

Outcome measure	Outcome measuring device	Outcome measuring unit
Clinical		
Discoloration	Asking the patient about his satisfaction about the color[17]	Binary (yes/no)
Postoperative pain	Asking patient[13]	Binary (present or absent)
Color stability	Operator and supervisor evaluation using shade guide (Vita shade guide 3D master)[18]	Binary (present or absent)
Pain on percussion	Percussion test[13]	Binary (present or absent)
Swelling	Visual examination by operator[13]	Binary (present or absent)
Sinus or fistula	Visual examination by operator[13]	Binary (present or absent)
Mobility	Back of the mirror and the index of operator[13]	Binary (present or absent)
Radiographic		
Root lengthening	A measuring scale in digital radiograph software (Digora® for Windows software)[13]	mm
Apical diameter closure	A measuring scale in digital radiograph software (Digora® for Windows software)[20]	mm
Healing of radiolucency	Examination of radiographs by the radiographic assessor[13]	Binary (present or absent)
Root resorption	Examination of radiographs by the radiographic assessor[13]	Binary (present or absent)
Intra canal calcification	Examination of radiographs by the radiographic assessor[13]	Binary (present or absent)

3D: Three dimensional

rate, while MTA has shown an 85.7% success rate for the tested clinical parameters. Failures in MTA were due to patient dissatisfaction and color instability (14.3%) [Table 3].

Radiographic parameters

After 12 months, both materials have shown a 90.5% success rate for the tested radiographic parameters. Failures were due to radiolucency not healed and calcification (9.5%) [Table 4 and Figures 4-7].

Inter-observer agreement

The overall inter-observer agreement for clinical and radiographic assessments has shown near-perfect agreement between observers (κ = 0.97). Inter-observer agreement for clinical assessment has shown perfect agreement (κ = 1), while inter-observer agreement for radiographic assessment has shown near-perfect agreement between observers (κ = 0.97).

DISCUSSION

In the current study, after 12 months, bioceramic and MTA have shown no statistically significant difference in all the clinical and radiographic parameters; therefore, the null hypothesis could not be rejected. Regarding the results related to the discoloration of the current study, in Group I, there were no instances of discoloration at 1, 3, 6, 9, and 12 months. The findings of Group I align with the laboratory investigation[21] on Totalfill and MTA, they found that the utilization of alternative radiopacifiers, such

Table 3: Clinical parameters

Outcomes	Follow-up	Bioceramic		MTA		*P*
		Yes, *n* (%)	No, *n* (%)	Yes, *n* (%)	No, *n* (%)	
Discoloration	Baseline	24 (100)	0	24 (100)	0	1.0000
	1 month	24 (100)	0	24 (100)	0	1.0000
	3 months	24 (100)	0	24 (100)	0	1.0000
	6 months	22 (100)	0	21 (87.5)	3 (12.5)	0.0898
	9 months	21 (100)	0	19 (86.4)	3 (13.6)	0.0829
	12 months	21 (100)	0	18 (85.7)	3 (14.3)	0.0757
	P	1.0000		0.023		RR=0.1429
Outcomes	**Follow-up**	**No, *n* (%)**	**Yes, *n* (%)**	**No, *n* (%)**	**Yes, *n* (%)**	***P***
Postoperative pain	Baseline	24 (100)	0	24 (100)	0	1.0000
	1 month	24 (100)	0	24 (100)	0	1.0000
	3 months	24 (100)	0	24 (100)	0	1.0000
	6 months	22 (100)	0	24 (100)	0	0.7681
	9 months	21 (100)	0	22 (100)	0	0.8788
	12 months	21 (100)	0	21 (100)	0	1.0000
	P	1.0000		1.0000		RR=1.0000
Swelling	Baseline	24 (100)	0	24 (100)	0	1.0000
	1 month	24 (100)	0	24 (100)	0	1.0000
	3 months	24 (100)	0	24 (100)	0	1.0000
	6 months	22 (100)	0	24 (100)	0	0.7681
	9 months	21 (100)	0	22 (100)	0	0.8788
	12 months	21 (100)	0	21 (100)	0	1.0000
	P	1.0000		1.0000		RR=1.0000
Sinus or fistula	Baseline	24 (100)	0	24 (100)	0	1.0000
	1 month	24 (100)	0	24 (100)	0	1.0000
	3 months	24 (100)	0	24 (100)	0	1.0000
	6 months	22 (100)	0	24 (100)	0	0.7681
	9 months	21 (100)	0	22 (100)	0	0.8788
	12 months	21 (100)	0	21 (100)	0	1.0000
	P	1.0000		1.0000		RR=1.0000
Pain on percussion	Baseline	24 (100)	0	24 (100)	0	1.0000
	1 month	24 (100)	0	24 (100)	0	1.0000
	3 months	24 (100)	0	24 (100)	0	1.0000
	6 months	20 (90.9)	2 (9.1)	22 (91.7)	2 (8.3)	0.9282
	9 months	21 (100)	0	22 (100)	0	0.8788
	12 months	21 (100)	0	21 (100)	0	1.0000
	P	0.075		0.075		RR=1.0000
Color stability	Baseline	24 (100)	0	24 (100)	0	1.0000
	1 month	24 (100)	0	24 (100)	0	1.0000
	3 months	24 (100)	0	24 (100)	0	1.0000
	6 months	22 (100)	0	21 (87.5)	3 (12.5)	0.0898
	9 months	21 (100)	0	19 (86.4)	3 (13.6)	0.0829
	12 months	21 (100)	0	18 (85.7)	3 (14.3)	0.0757
	P	1.0000		0.023		RR=0.1429

MTA: Mineral trioxide aggregate, RR: Respiratory rate

Table 4: Radiographic parameters

Outcomes	Follow-up	Mean±SD		*P*
		Bioceramic	MTA	
Radiographic tooth length	Baseline	10.9±1.14	10.66[a]±0.82	0.4085
	6 months	11.52±1.42	11.48[b]±1.57	0.9342
	12 months	11.48±1.57	11.66[b]±1.52	0.7001
	P	0.123	<0.001*	
Apical foramen width	Baseline	1.08[a]±0.28	1.09[a]±0.34	0.8641
	6 months	0.98[a]±0.33	0.78[b]0.56	0.1591
	12 months	0.61[b]±0.49	0.62[b]0.32	0.9337
	P	0.001*	<0.001*	

Outcomes	Follow-up	No, *n* (%)	Yes, *n* (%)	No, *n* (%)	Yes, *n* (%)	*P*
Healing of radiolucency	Baseline	22 (91.7)	2 (8.3)	18 (75)	6 (25)	0.1253
	6 months	20 (90.9)	2 (9.1)	20 (83.3)	4 (16.7)	0.4510
	12 months	19 (90.5)	2 (9.5)	19 (90.5)	2 (9.5)	1.0000
	P	1.0000		0.050		RR=1.0000
Root resorption	Baseline	24 (100)	0	24 (100)	0	1.0000
	6 months	22 (100)	0	24 (100)	0	0.7681
	12 months	21 (100)	0	21 (100)	0	1.0000
	P	1.0000		1.0000		RR=1.0000
Calcification	Baseline	22 (91.7)	2 (8.3)	24 (100)	0	0.1529
	6 months	20 (90.9)	2 (9.1)	22 (91.7)	2 (8.3)	0.9282
	12 months	19 (90.5)	2 (9.5)	19 (90.5)	2 (9.5)	1.0000
	P	1.0000		0.264		RR=1.0000

SD: Standard deviation, R.R: relative risk, *denotes statistically significant, means that do not share the same letter are statistically significant

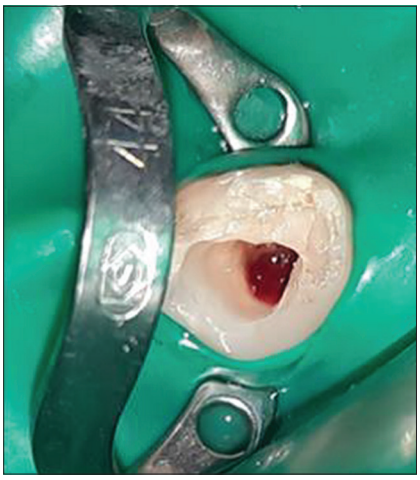

Figure 3: Blood clot formation

as zirconium oxide and tantalum oxide, instead of bismuth oxide, as well as the use of pre-mixed material in the form of ready-to-use putty, which eliminates the need for powder/water ratio variations, results in reduced interaction with the surrounding environment and consequently lowers the potential for discoloration. Furthermore, in Group II, only three patients were not satisfied with the tooth color at 6, 9, and 12 months. However, there was no statistically significant difference between Group I and Group II.[21]

The findings of the current study align with the previous trial,[22] they reported that only 3 out of 11 patients exhibited discoloration during the 12-month follow-up period. The observed result could be attributed to the common methodology employed in both studies, which involved the utilization of collagen plugs after the formation of blood clots and before the placement of coronal plug material. Placing a collagen membrane above a blood clot may reduce discoloration,[5] as the probability of discoloration due to the unset MTA being brought into direct contact with blood, increasing the risk that the blood disintegration products will penetrate inside the porosities of the MTA, causing discoloration.[23]

In contrast, a previous clinical trial found that tooth discoloration was reported in 58.33% of the participants in the MTA group.[13] This discrepancy may be attributed to not using the collagen plug or membrane before placement of MTA in contrast to the current study. The absence of collagen plug leads to direct contact between the unset MTA and the blood, and subsequently, the penetration of the blood products inside the MTA porosities increases the chance of discoloration. Another possible cause of discoloration is the level of placement of MTA, which is a critical step in decreasing the discoloration, placing the MTA below the CEJ proved to be difficult in some teeth.[24] The difficulties in the placement of MTA may have been avoided if a collagen matrix had been used. Placing the coronal plug below the CEJ will decrease the probability of tooth discoloration as the discoloration of tooth structure will be covered by bone or gingiva,[7] which was the protocol that was followed in the current study.

However, after the previously mentioned precautions in the present study, it was observed that three cases within Group II exhibited discoloration. The blood clot fibrin mesh is fragile, which could be the cause of fibrin mesh disintegration during the application of force when inserting the collagen plug, leading to the diffusion of

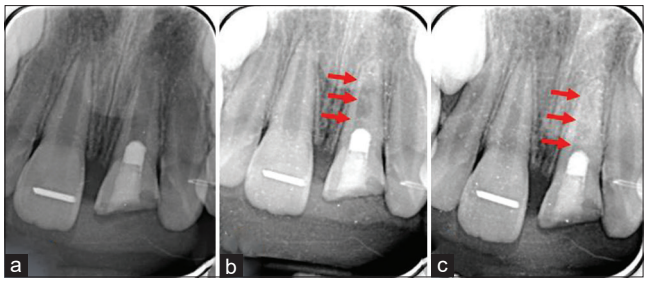

Figure 4: A representative case showing intracanal calcification in Group I: (a) Postoperative radiograph; (b) 6 months radiograph; (c) 12 months radiograph

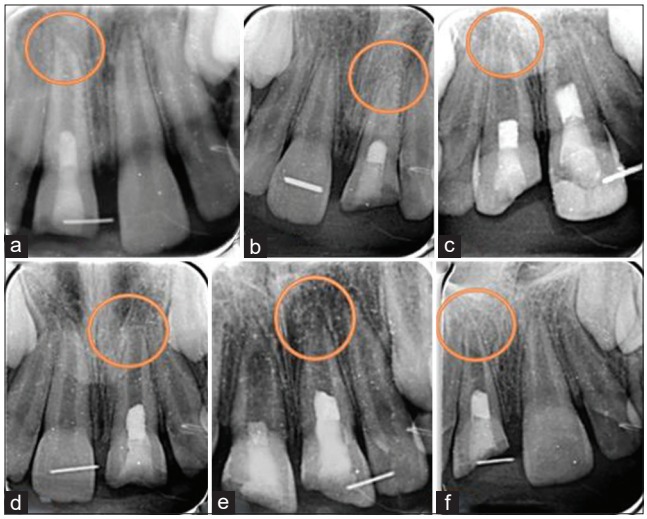

Figure 6: Radiographs show different forms of apical closure in Group I at 12 months where: (a) Complete apical closure; (b) Apical closure with canal calcification; (c) The right central represents insignificant change in apical diameter; (d) Incomplete apical closure; (e) The left central represents insignificant change in the apical diameter with the presence of osteoid triangle at the apex or maybe beginning of complete apical closure (needs more follow-up); (f) Incomplete apical closure

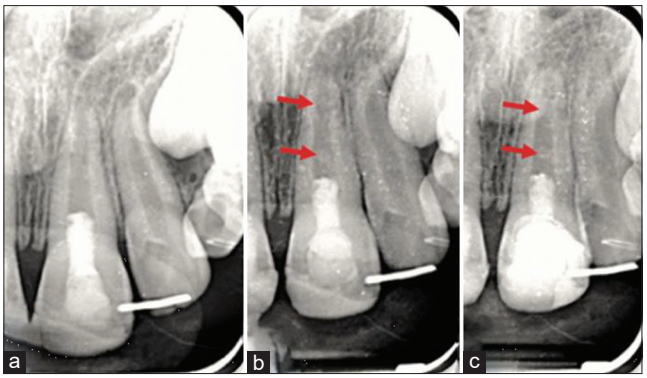

Figure 5: A representative case showing intracanal calcification in Group II: (a) Postoperative radiograph; (b) 6 months radiograph; (c) 12 months radiograph

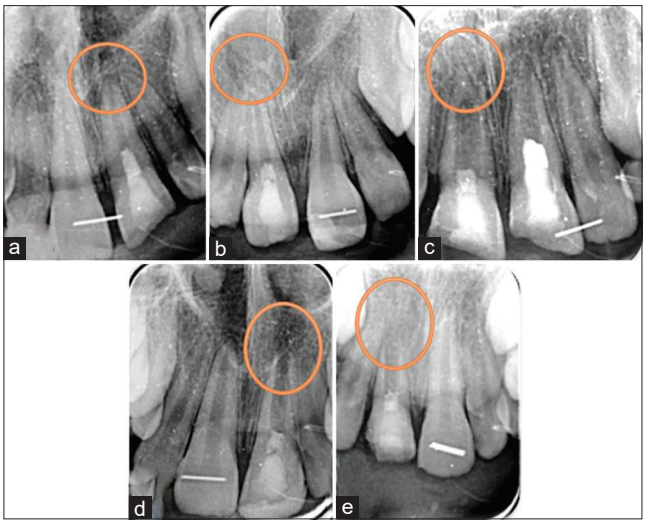

Figure 7: Radiographs show different forms of apical closure in Group II at 12 months follow-up where: (a and b) Complete apical closure; (c) Right central showing complete apical closure persistent large canal; (d) Incomplete apical closure; (e) Insignificant change in the apical foramen width

blood into the porosities of the collagen plug.[25] As a result, there has been direct interaction between MTA and the bloodstream, subsequently increasing the chance of discoloration. The results of the color stability in terms of the tooth shade evaluated by both the operator and the second blinded assessor were consistent with the findings regarding patient satisfaction toward the tooth color of the present study. No instances of discoloration were observed in Group I at 1, 3, 6, 9, and 12 months.

Within Group II, it was observed that only three patients showed tooth discoloration at the 6-, 9-, and 12-month intervals. The findings of the present study are divergent from a previous study,[22] which documented that the assessors observed discoloration at an earlier stage in comparison to the reports submitted by parents. The variation in outcomes observed may be attributed to the fact that the primary objective of the parents in the current study was not only pain elimination but also the enhancement of the esthetic appearance of their children to avoid bullying among children within the school environment.

Regarding other clinical outcomes such as postoperative pain, pain on percussion, mobility, swelling, sinus, and fistula, the results of this study showed normal clinical findings in all teeth (100%) in both groups at 1,3, 9, and 12 months. There were no statistically significant differences between Group I and Group II during all follow-up periods. These results are in conjunction with previous research.[26,27] However, at 6 months, there were 2 (9.1%) cases in Group I and 2 (9.1%) cases in Group II that were identified as clinical failure due to pain on percussion. These 4 teeth were correlated to radiographic failure in addition to pain on percussion, and alternative

treatment (apexification) was done to treat these teeth. The results of the present study were comparable to those reported by Aly *et al*.,[13] who showed clinical failure of (8.34%) of teeth treated with revascularization protocol using Angleus white MTA (WMTA) as coronal plug material due to pain on percussion.

The failure of the cases in the current study might be related to the residual infection. Bacteria and their antigens could modify stem cell differentiation into osteoblastic phenotype and hinder their mineralizing capacity. In addition, bacterial lipopolysaccharides could also remain after root canal disinfection and promote pro-inflammatory cytokines production. Furthermore, the efficacy of intracanal medicaments and irrigants against a monospecies or polyspecies biofilm shows the lack of efficacy with commonly used disinfectants. The impact of residual biofilm depends on several factors such as its location within the root canal system, the size and composition of the microbial population in the biofilm, and the availability of nutrients for the microbes. However, the root canals containing microbes in "subcritical" numbers or situated in locations where they or their products are inaccessible to periapical tissues can remain "harmless."[28,29]

In terms of radiographic outcomes, as outlined in the AAE considerations for regenerative procedures in 2016 (revised in 2018),[10] the attainment of evidence indicating bony healing is regarded as the primary aim for revascularization procedures, as it is essential for the successful outcome of cases. On the other hand, the augmentation of root length is considered a secondary goal that is desirable but not necessarily important in determining the success of cases. In relation to root length, the mean increase in root length in Group I was 0.6 mm (5.68%) at 6 months and 0.58 mm (5.38%) at 12 months, when compared to the baseline. The mean increase in root length in Group II was 0.82 mm (equivalent to 7.69%) at 6 months and 1 mm (equivalent to 9.38%) at 12 months when compared to baseline. There was no statistically significant difference observed between Group I and Group II.

The results of intragroup comparisons within Group I indicate that there is no statistically significant difference observed across various follow-up periods. However, when conducting intragroup comparisons within Group II, a statistically significant difference is observed across different follow-up periods. The findings of root length presented in the current study diverged from the outcomes of using MTA as coronal plug reported by Mittmann *et al*.,[30] which indicated a mean increase in root length of 0.96% during 22-month follow-up period with no statistical significance. The observed discrepancies in the results could potentially be attributed to the inclusion of teeth with severe luxation injuries and avulsion in the previously mentioned study, which may have had an impact on the condition of the HERs, leading to a decrease in cellular vitality.

On the contrary, the findings of the present study in terms of the increase in root length were comparable with the findings of the retrospective study,[31] they observed an average 10.2% increase in root length using MTA as a coronal plug across all cases at 17 months. Lei *et al*.[32] proposed that dental roots measuring <17 mm in length, similar to the root length of the current study, may demonstrate an increased probability of attaining a higher success rate in the process of revascularization. In contrast, Chrepa *et al*.[29] asserted that age emerged as a significant predictor for root development. There was no statistically significant difference observed between Group I and Group II in relation to the increase in root length in the current study. The key factors for optimal regeneration are an adequately debrided canal, bacterial elimination, and a reduction in inflammation, irrespective of the material used for the coronal plug.[29]

However, the results of intragroup comparisons within Group I regarding the root length indicate that there is no statistically significant difference observed in the increase of the root length across various follow-up periods. The laboratory study[33] found that Endosequence displayed a lower release of Ca2+ in comparison to MTA, which can explain this observation. The difference in Ca2+ release between the two materials may be because Endosequence has a lower amount of calcium silicate and other ingredients, such as thickening agents. Consequently, the quantity of calcium hydroxide generated following the hydration reaction may be comparatively diminished when compared to MTA. Regarding the apical closure, the mean decrease of the apical foramen width in Group I was 0.1 (9.2%) at 6 months and 0.47 mm (43.51%) at 12 months, when compared to the baseline. The mean decrease of the apical foramen width was 0.31 mm (28.44%) at 6 months and 0.47 mm (43.11%) at 12 months when compared to baseline.[33]

There was no statistically significant difference observed between Group I and Group II. The results of intragroup comparisons within Group I and Group II indicate that there was a statistically significant difference observed across various follow-up periods. The observed results related to the decrease in the apical foramen in the present study may be attributed to the particular parameters employed, such as the age group of the subjects (9–13 years). The findings of the present study confirm the conclusions established by Estefan *et al*., which indicated that individuals of younger age (9–14 years old) demonstrated a higher level of suitability for revascularization interventions in comparison to older individuals. In addition, the decrease in the apical foramen in the current study may be related to the corresponding range of initial apical foramen sizes (0.64–1.54 mm). It

has been observed that the success rates of regeneration procedures have been higher when the preoperative apical diameters fall within the range of 0.5–1 mm.[34]

However, it was noted that teeth with larger preoperative diameters (1 mm) demonstrated a more substantial increase in root thickness, length, and apical narrowing. Nevertheless, preoperative apical foramen width of <0.5 mm did not serve as a barrier to the ingrowth of vital tissue into the pulp space.[35] The apical foramen width of this study exhibited similarities to those reported by Aly *et al*.,[13] they observed a reduction of 46.15% in the apical diameter using MTA after 1 year of follow-up. Furthermore, a previous study[36] reported that 47% of the total teeth achieved complete apical closure at 6 months, and most teeth (96%) achieved apical foramen closure within 24 months. The variation in apical foramen width outcomes in the present study compared to Jiang *et al*.[36] study could potentially be attributed to several factors, including the incorporation of anterior and premolar teeth, the utilization of a collagen membrane positioned within the middle third of the root, and disparities in etiological factors such as trauma and dens evaginatus.

Furthermore, it is important to note that there was a prolonged follow-up period of 24 months. In contrast, the decrease in apical foramen width results in the current study were in contrast with Mittmann *et al*.,[30] they reported that only the 36.94% decrease in the apical foramen diameter compared to the initial situation was significant. The observed discrepancies in the results could potentially be attributed to the inclusion of teeth with severe luxative injuries and avulsion in the previously mentioned study, which may have had an impact on the condition of the HERs, leading to a decrease in cellular vitality. However, although Chen *et al*.[37] classified five types of root developmental patterns in response to regenerative endodontic therapy of immature permanent teeth with infected necrotic pulp, there is still a knowledge gap regarding factors affecting root development, which causes some confusion among researchers and clinicians with regard to the predictability of revascularization-induced root development. Regarding the healing in the current study, in Group I, the healing rate at 6 and 12 months was 90.9% and 90.5%, respectively; in Group II, the healing rate at 6 and 12 months was 83.3% and 90.5%, respectively. The healing rate in the current study was in conjunction with the study of Kahler *et al*.,[24] in which periapical healing showed that 90.3% of the cases showed resolution of the lesion.

In addition, the current study was in line with the systematic review and meta-analysis[38] which reported that the healing rate was 93.0%. However, a retrospective case series[39] that used both MTA and endosequence as coronal plug without classification found that 77% of all cases had complete healing. The discrepancy between the current study and the study by Bukhari *et al*.[39] about the rate of healing may be attributed to that 75% of the cases in the previously mentioned study had initial radiolucency. In contrast to the current study, in which Group I included only 2 (8.3%) cases with periapical radiolucency at baseline and Group II included only 6 (25%) cases with periapical radiolucency at baseline.

In relation to intracanal calcification in the present study, Group I demonstrated the initiation of calcification at baseline in 2 (8.3%) cases, at 6 months in 2 cases (9.1%), and at 12 months in 2 (9.5%) cases. In Group II, calcification was initially detected in two cases (8.3%) at 6 months and in 2 (9.5%) cases at 12 months. In contrast to the retrospective cohort study,[36] root canal calcification was found in 78% of cases; this difference may be attributed to the use of calcium hydroxide as intracanal medication and the longer follow-up period (3 years). The underlying cause of calcification in root canals has been attributed to the migration of periodontal stem cells and bone marrow stem cells from the alveolar bone into the bloodstream through the apical foramen. This migration process results in the formation of osseous and cementum tissue within the root canal.[40]

There is an association between the presence of residual biofilms and the occurrence of calcification in root canals. On exposure to residual biofilms, stem cells derived from the apical papilla exhibited strong expression of markers associated with osteoblast-like cells.[41] Furthermore, Rizk *et al*.[27] attributed the calcification observed to the osteoinductive properties of MTA. The observed calcification of the two cases in Group I at the baseline periapical radiograph in the present study can potentially be attributed to injury to the neurovascular supply of the pulp or the presence of bleeding in the canal. The formation of a blood clot may serve as a focal point for calcification, particularly if the pulp remains viable for a period of time after the trauma before undergoing degeneration and necrosis. It is imperative to note that the presence of calcification was not observed in the preoperative radiograph. This could be because a radiographic stent was not used when taking the preoperative radiograph, which caused the angulation to be different and the calcification to not be evident.[27]

The lack of tactile sensation of the focal area of calcification during the process of determining the working length can be attributed to the particular positioning of the calcification on the dentinal wall.[42] In the systematic review and meta-analysis,[38] the authors emphasized the importance of recognizing calcific barrier or any type of calcification as an indication of viable tissue within the root canal. Consequently, despite being considered undesirable, total or partial pulpal obliteration was regarded as a favorable complication. However, Rizk *et al*.[27] considered partial

or complete obliteration of the pulp canal as undesirable outcomes in revascularization because it might make it more difficult to perform root canal therapy in the future, should one be necessary.

Concerning root resorption, there was no root resorption observed in Group I and Group II during the follow-up period. These results were in conjunction with previous results,[13,22] they reported the absence of root resorption during the follow-up period (12 months). On the contrary, Mittmann *et al*.[30] found that 56.3% of teeth exhibited root resorption, this may be attributed to variations in case selection, such as the inclusion of luxative injured teeth. It is suggested that root resorption is more commonly associated with significant damage to the periodontal ligament caused by luxation injuries rather than being a direct result of revascularization treatment.

Limitations of the current study

Limitations of the present study include great variations in the clinical protocols used in earlier investigations made the comparison and interpretation of the findings challenging. The lack of clinical studies on the Totalfill bioceramic putty posed difficulties in the interpretation of the obtained results. The exact reproducibility of intraoral periapical radiographs was challenging because dentition at this age range is still growing and continuously changing. The pain during the over instrumentation, especially with the use of local anesthesia without adrenaline, could not be avoided, which affected the cooperation of some children during the second visit. The exact standardization of the manually and freshly prepared antibiotic paste and the manually mixed MTA was challenging, which may have affected the inherent properties of the material. Although radiographic evidence indicates continuous root development in the treated teeth, it was not possible to investigate histological confirmation of the success of the revascularization process.

CONCLUSIONS

1. Both Totalfill bioceramic putty and WMTA were successful coronal plug materials in the revascularization of nonvital, immature permanent teeth
2. Totalfill bioceramic putty showed less discoloration potential compared to WMTA; however, there was no statistically significant difference between both groups
3. The occurrence of coronal discoloration in revascularization is multifactorial but can be decreased by controlling the level of coronal plug and avoiding blood contamination
4. The utilization of a collagen matrix is useful for the purpose of regulating the positioning of the coronal plug at the intended level. 5. The utilization of blood clots as a scaffold has proven to be effective in the continuation of root development radiographically.

Clinical recommendations

1. Both Totalfill bioceramic and WMTA are recommended as coronal plug materials in revascularization procedures
2. Totalfill bioceramic is a promising coronal plug material that can be used for revascularization procedures in the esthetic zone to overcome the problems of the poor handling properties of WMTA
3. Further long-term randomized clinical trials are needed to evaluate the long-term color stability, and clinical and radiographic success rate of both Totalfill bioceramic and WMTA in revascularization.

Standardization of clinical protocol used in clinical trials conducted on revascularization to facilitate comparison and achieve more concise, conclusive results.

Ethical policy and institutional review board statement

The protocol of the present trial was pre-registered ClinicalTrials.gov (NCT03813433). All procedures were in agreement with the standards of the REC of the Faculty of Dentistry at Cairo University (Ref. 19-07-80).

Patient declaration of consent

All appropriate patient consent forms were obtained by the principal investigator. The participants comprehend that their identity will be kept concealed, but anonymity will not be guaranteed.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Nosrat A, Seifi A, Asgary S. Regenerative endodontic treatment (revascularization) for necrotic immature permanent molars: A review and report of two cases with a new biomaterial. J Endod 2011;37:562-7.
2. Guerra YL, da Silva EL, Gomes NV, Vivacqua FD. Clinical evidence of pulp Revascularization in necrosed and immature teeth: A literature review. J Braz J Health Rev 2022;5:12421-31.
3. Bezgin T, Sönmez H. Review of current concepts of revascularization/revitalization. Dent Traumatol 2015;31:267-73.
4. Galler KM, Krastl G, Simon S, Van Gorp G, Meschi N, Vahedi B, *et al*. European Society of Endodontology position statement: Revitalization procedures. Int Endod J 2016;49:717-23.
5. Duggal M, Tong HJ, Al-Ansary M, Twati W, Day PF, Nazzal H. Interventions for the endodontic management of non-vital traumatised immature permanent anterior teeth in children and adolescents: A systematic review of the evidence and guidelines of the European academy of paediatric dentistry. Eur Arch Paediatr Dent 2017;18:139-51.
6. Lee DS, Lim MJ, Choi Y, Rosa V, Hong CU, Min KS. Tooth discoloration induced by a novel mineral trioxide aggregate-based root canal sealer. Eur J Dent 2016;10:403-7.
7. Žižka R, Šedý J, Gregor L, Voborná I. Discoloration after regenerative endodontic procedures: A critical review. Iran Endod J 2018;13:278-84.
8. Shokouhinejad N, Nekoofar MH, Razmi H, Sajadi S, Davies TE, Saghiri MA, *et al*. Bioactivity of EndoSequence root repair material and bioaggregate. Int Endod J 2012;45:1127-34.

9. Jitaru S, Hodisan I, Timis L, Lucian A, Bud M. The use of bioceramics in endodontics – Literature review. Clujul Med 2016;89:470-3.
10. American Association of Endodontists. AAE Clinical Considerations for a Regenerative Procedure. Chicago, IL, USA: American Association of Endodontists; 2016. Available from: https://www.aae.org. [Last accessed on 2018 Apr 01].
11. Chan EK, Desmeules M, Cielecki M, Dabbagh B, Ferraz Dos Santos B. Longitudinal cohort study of regenerative endodontic treatment for immature necrotic permanent teeth. J Endod 2017;43:395-400.
12. Botero TM, Tang X, Gardner R, Hu JC, Boynton JR, Holland GR. Clinical evidence for regenerative endodontic procedures: Immediate versus delayed induction? J Endod 2017;43:S75-81.
13. Aly MM, Taha SE, El Sayed MA, Youssef R, Omar HM. Clinical and radiographic evaluation of biodentine and mineral trioxide aggregate in revascularization of non-vital immature permanent anterior teeth (randomized clinical study). Int J Paediatr Dent 2019;29:464-73.
14. El-Baz AA, Ahmed GM. Comparison of the effect of calcium hydroxide and the modified triple antibiotic paste as intracanal medication on the elimination of *Enterococcus faecalis* in retreatment cases. A randomized double blind clinical trial. Egypt Dent J 2017;63:3683-92.
15. Nazzal H, Kenny K, Altimimi A, Kang J, Duggal MS. A prospective clinical study of regenerative endodontic treatment of traumatized immature teeth with necrotic pulps using bi-antibiotic paste. Int Endod J 2018;51 Suppl 3:e204-15.
16. Neelamurthy PS, Kumar RA, Balakrishnan V, Venkatesan SM, Narayan GS, Karthikeyan I. Revascularization in immature and mature teeth with necrotic pulp: A clinical study. J Contemp Dent Pract 2018;19:1393-9.
17. Tin-Oo MM, Saddki N, Hassan N. Factors influencing patient satisfaction with dental appearance and treatments they desire to improve aesthetics. BMC Oral Health 2011;11:6.
18. Negahdari R, Pournasrollah A, Rahbar M, Bohlouli S, Pakdel SM. Comparison of shade match compatibility between vitapan classical and 3D master shade guide systems by dental students in Tabriz faculty of dentistry. J Adv Biosci Clin Med 2016;4:4-10.
19. Sood K, Kaur J. Splinting and stabilization in periodontal disease. J Int J Sci Res 2015;4:1636-9.
20. ElSheshtawy AS, Nazzal H, El Shahawy OI, El Baz AA, Ismail SM, Kang J, *et al*. The effect of platelet-rich plasma as a scaffold in regeneration/revitalization endodontics of immature permanent teeth assessed using 2-dimensional radiographs and cone beam computed tomography: A randomized controlled trial. Int Endod J 2020;53:905-21.
21. Palma PJ, Marques JA, Santos J, Falacho RI, Sequeira D, Diogo P, *et al.* Tooth discoloration after regenerative endodontic procedures with calcium silicate-based cements – An *ex vivo* study. J Appl Sci 2020;10:5793.
22. Tawfeek HA, El-Bardissy AA, Abou El-Yazeed M, Youssef R, Abd Alsamad AM. Clinical and radiographic evaluation of NeoMTA versus conventional white mineral trioxide aggregate in revascularization of non-vital immature permanent anterior teeth (A randomized controlled trial). BDJ Open 2023;9:17.
23. Shokouhinejad N, Nekoofar MH, Pirmoazen S, Shamshiri AR, Dummer PM. Evaluation and comparison of occurrence of tooth discoloration after the application of various calcium silicate-based cements: An *ex vivo* study. J Endod 2016;42:140-4.
24. Kahler B, Mistry S, Moule A, Ringsmuth AK, Case P, Thomson A, *et al*. Revascularization outcomes: A prospective analysis of 16 consecutive cases. J Endod 2014;40:333-8.
25. Sharma S, Mittal N. A comparative evaluation of natural and artificial scaffolds in regenerative endodontics: A clinical study. Saudi Endod J 2016;6:9-15.
26. Elsayyad A, Elsayed MA, Elmotayam K. Healing potential of immature permanent teeth with necrotic pulps and periapical radiolucency. Egypt Dent J 2017;63:61-6.
27. Rizk HM, Salah Al-Deen MS, Emam AA. Pulp revascularization/revitalization of bilateral upper necrotic immature permanent central incisors with blood clot versus Platelet-rich fibrin scaffolds-a split-mouth double-blind randomized controlled trial. Int J Clin Pediatr Dent 2020;13:337-43.
28. Nair PN. Endodontic biofilm, technology and pulpal regenerative therapy: Where do we go from here? Int Endod J 2014;47:1003-11.
29. Chrepa V, Joon R, Austah O, Diogenes A, Hargreaves KM, Ezeldeen M, *et al*. Clinical outcomes of immature teeth treated with regenerative endodontic procedures-A San Antonio study. J Endod 2020;46:1074-84.
30. Mittmann CW, Kostka E, Ballout H, Preus M, Preissner R, Karaman M, *et al*. Outcome of revascularization therapy in traumatized immature incisors. BMC Oral Health 2020;20:207.
31. Alobaid AS, Cortes LM, Lo J, Nguyen TT, Albert J, Abu-Melha AS, *et al*. Radiographic and clinical outcomes of the treatment of immature permanent teeth by revascularization or apexification: A pilot retrospective cohort study. J Endod 2014;40:1063-70.
32. Lei L, Liao W, Sheng P, Fu M, He A, Huang G. Biological character of human adipose-derived adult stem cells and influence of donor age on cell replication in culture. Sci China C Life Sci 2007;50:320-8.
33. Han L, Okiji T. Bioactivity evaluation of three calcium silicate-based endodontic materials. Int Endod J 2013;46:808-14.
34. Estefan BS, El Batouty KM, Nagy MM, Diogenes A. Influence of age and apical diameter on the success of endodontic regeneration procedures. J Endod 2016;42:1620-5.
35. Laureys WG, Cuvelier CA, Dermaut LR, De Pauw GA. The critical apical diameter to obtain regeneration of the pulp tissue after tooth transplantation, replantation, or regenerative endodontic treatment. J Endod 2013;39:759-63.
36. Jiang X, Dai Y, Liu H. Evaluation of the characteristics of root canal calcification after regenerative endodontic procedures: A retrospective cohort study over 3 years. Int J Paediatr Dent 2023;33:305-13.
37. Chen MY, Chen KL, Chen CA, Tayebaty F, Rosenberg PA, Lin LM. Responses of immature permanent teeth with infected necrotic pulp tissue and apical periodontitis/abscess to revascularization procedures. Int Endod J 2012;45:294-305.
38. Ong TK, Lim GS, Singh M, Fial AV. Quantitative assessment of root development after regenerative endodontic therapy: A systematic review and meta-analysis. J Endod 2020;46:1856-66.e2.
39. Bukhari S, Kohli MR, Setzer F, Karabucak B. Outcome of revascularization procedure: A retrospective case series. J Endod 2016;42:1752-9.
40. Song M, Cao Y, Shin SJ, Shon WJ, Chugal N, Kim RH, *et al*. Revascularization-associated intracanal calcification: Assessment of prevalence and contributing factors. J Endod 2017;43:2025-33.
41. Vishwanat L, Duong R, Takimoto K, Phillips L, Espitia CO, Diogenes A, *et al*. Effect of bacterial biofilm on the osteogenic differentiation of stem cells of apical papilla. J Endod 2017;43:916-22.
42. Siddiqui SH, Mohamed AN. Calcific metamorphosis: A review. Int J Health Sci (Qassim) 2016;10:437-42.