Review Article

Pulp regeneration treatment using different bioactive materials in permanent teeth of pediatric subjects

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Abstract

Background and Objectives: The present systematic review aims to assess the success rate of the pulp regeneration treatment, according to the American Association of Endodontists (AAE) criteria, using different bioactive materials in permanent teeth of pediatric subjects (6–17 years of age).

Materials and Methods: The study protocol was registered on PROSPERO and adhered to the Preferred Reporting Items for Systematic Reviews and Meta-analyses statement. The question formulation was accomplished using the PICO model, and an electronic search was carried out on Scopus, MEDLINE/PubMed, Web of Science, and Cochrane databases till April 1, 2023. A total of 30 studies were established to fulfill the inclusion criteria of this systematic review.

Results: A total of 273 teeth have been treated with pulp regeneration treatment. By comparing different biomaterials and the success criteria defined by the AAE, the material associated with a higher success rate was found to be the white mineral trioxide aggregate. However, the overall success rate of pulp regeneration treatment was reported for 248 out of 273 teeth (91.20%).

Conclusions: Data obtained support the potential that regenerative endodontics aids in continuing root development in permanent immature teeth. Further studies are needed for a more extensive evaluation of the use of different biomaterials and the success rate in regenerative endodontics.

Keywords: Bioactive materials; bioceramic sealers; dental pulp regeneration; mineral trioxide aggregate; pulp regeneration; regenerative endodontics

INTRODUCTION

The treatment of infected immature permanent teeth is one of the most complex procedures that have to be overcome in endodontics due to the anatomy of these teeth.[1,2] In particular, they have wide canals, thin-shaped dentin walls, and open apices, making it difficult for the endodontists to perform a correct instrumentation procedure, determine the working length, and properly control the irrigants and filling material.[3,4]

Conventional treatments for permanent immature necrotic teeth are apexification through calcium hydroxide ($Ca(OH)_2$) to stimulate apical barrier formation or apical mineral trioxide aggregate (MTA) plugs.[5-7] However, apexification with $Ca(OH)_2$ has drawbacks, such as requiring multiple visits over a longer time, increased tooth fragility and root fracture, coronal leakage, unpredictable apical hard-tissue formation, and apex closure.[6,8] Therefore, using an MTA

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apical plug can reduce treatment time and can induce biological sealing and hard-tissue formation. Nevertheless, attention should be paid to not over-extruding it, not to affect the periapical healing.[9-12] Moreover, the apical plug technique does not induce root development or increase the crown/root ratio,[13] enhancing a future risk of root fracture despite being lower when compared to $Ca(OH)_2$ treatment.[14] Moreover, the treatment with apexification does not allow tooth vitality, nor does it allow for root maturation in necrotic permanent immature teeth.[15]

The introduction of the concept of "revascularization" is due to Iwaya *et al*.,[16] who proposed the treatment of an immature permanent tooth with apical periodontitis and sinus tract, which resulted in symptom elimination and healing from apical periodontitis.[16] Since then, another term emerged, "revitalization," as more proper to indicate that blood vessels and soft and hard tissues could be regenerated in the canal space.[17] Accordingly, "regenerative endodontics" was characterized as a biologically based approach that determines the replacement of damaged dental structures, such as dentin and root structures and cells from the pulp–dentin complex.[18] Regenerative endodontics is based on the principle of tissue engineering: stem cells, scaffolds, and bioactive growth factors.[19] Furthermore, this term also includes the concepts of revascularization and revitalization to describe the treatment of necrotic immature permanent teeth.[20]

Importantly, to perform pulp regenerative treatment, a precondition is to carry out an efficient root canal disinfection,[21] which can be achieved by minimal or no mechanical instrumentation and the use of sodium hypochlorite (NaOCl) as an irrigant, as reported by the American Association of Endodontists (AAE) guidelines.[22]

To lower the bacterial load in pulp regeneration treatment, different types of intracanal medicament have been proposed, such as triple antibiotic paste (TAP), double antibiotic paste (DAP), and $Ca(OH)_2$.[23]

As mentioned above, regenerative endodontics involves using a scaffold[19] that regulates cell proliferation, differentiation, and metabolism.[19] Blood clots, autologous platelet concentrates such as platelet-rich fibrin (PRF) and platelet-rich plasma (PRP), and synthetic biomaterials could act as a scaffold during pulp regeneration procedures.[24]

To carry out pulp regeneration treatment, current guidelines suggest using bioactive materials, including MTA or bioceramics cement such as Biodentine.[22,25]

According to the AAE,[22] a successful regenerative endodontics treatment provides the accomplishment of three main goals: a primary goal – that is essential – which consists of symptom elimination and bone healing; a secondary goal that is an increase of root lengthening or thickening – which is preferable but not essential – and a tertiary goal which consists in a positive response to vitality tests, that if obtained may suggest a better organized vital pulp tissue.

The present systematic review aims to assess the success rate in pulp regeneration treatment according to the AAE criteria using different bioactive materials in permanent teeth of pediatric subjects (between 6 and 17 years of age).

MATERIALS AND METHODS

Study protocol

The study protocol, registered on the PROSPERO systematic review register (registration number ID: CRD42023449254), was developed before the literature search and data analysis and followed the Preferred Reporting Items for Systematic Reviews and Meta-analyses statement,[26] focusing on dental pulp regeneration treatment in immature teeth using different scaffold or bioactive materials and evaluating the posttreatment outcomes after follow-up.

Question formulation, search strategy definition, and study selection criteria were accomplished using the PICO model.[27]

The study question focalized on the following:

- P - Population: Pediatric subjects (between 6 and 17 years of age)
- I - Intervention: Pulp regeneration treatment with Biodentine with scaffolds (natural/synthetic)
- C - Comparison: No pulp regeneration treatment with MTA and scaffolds (natural/synthetic)
- O - Outcome(s): Pulp regeneration success according to the AAE success criteria.

Search strategy

An electronic search was carried out for articles in English on Scopus, MEDLINE/PubMed, Web of Science, and Cochrane databases till April 1, 2023, by two independent reviewers (GDB and AI), applying filters concerning the year of publication, involving studies from 2013, because, in this year, the AAE[22] published treatment considerations and guidelines about regenerative endodontics and using the following keywords combined with Boolean operators:

(Regenerative endodontics OR pulp regeneration OR dental pulp regeneration OR pulp revascularization OR dental pulp revascularization OR pulp revitalization OR dental pulp revitalization) AND (MTA OR mineral trioxide aggregate OR bioceramics OR bioceramic sealers OR bioceramic endodontic OR PRP OR PRF OR dental mesenchymal cells OR platelet-rich plasma OR platelet-rich fibrin OR EMD OR dental stem cell).

Study selection and eligibility criteria

Two independent reviewers (MP and GDB) performed the study selection and resolved disagreements by discussing and involving a third reviewer (FG) if necessary.

After deleting duplicates, all titles and abstracts achieved from the electronic search were screened. Full texts were obtained for those deemed potentially relevant, and if they were missing, study authors were contacted. References were exported and achieved using Mendeley Reference Manager software.

Inclusion criteria are the following: data from prospective, retrospective, and case–control studies, as well as case series, case reports, and letters to the editor, accepted or published in the English language from 2023 to April 1, 2023, with no restrictions of gender or sample size, describing pulp regeneration protocol and follow-up in pediatric subjects (between 6 and 17 years old). This age range has been set up considering that it is not recommended to deliver regenerative endodontics to deciduous teeth.[21,28] Since the youngest age at which a permanent element erupts is six,[29,30] it represents the minimum subjects' age of choice. In addition to that, although pulp regeneration was carried out in subjects older than 17 years old,[31] there is evidence of a greater risk of complications or failure of the procedure,[18,32,33] along with the knowledge that regenerative potential is very limited in adult permanent teeth.[34,35] This led to the choice of setting 17 as the maximum age.

Exclusion criteria were as follows: *in vitro* and preclinical *in vivo* studies, systematic and narrative reviews, conference papers, oral communications, and books/chapters; non-English studies; participants <6 years of age or >17 years old, or subjects who did not receive any pulp regeneration treatment.

Data extraction and collection

Data extraction and collection were independently performed on a standardized form, developed following the models proposed for intervention reviews on RCTs and non-RCTs[27] by two reviewers (DA and MP), and in case of disagreements, a third reviewer (GDB) has been involved. From included studies only data that met eligibility criteria were extracted and analyzed; therefore, data from individuals who were <6 years old or >17 years old or not treated with pulp regeneration treatment were not detailed.

The following data were collected from each study included in the present systematic review:

- First author, year, study quality, journal, and funding
- Design and number of studies recorded; sample size, gender ratio, age, and comorbidities of the population examined
- Total number of teeth treated with pulp regeneration, tooth type, and root development stage
- Pretreatment signs and symptoms, including pulp tests, percussion examination, palpation examination, presence of periapical radiolucent lesions, and definitive diagnosis
- Number of visits, instrumentation, irrigation, irrigant activation, type of medication, temporary sealer, scaffold and bioactive material used, and final restorative material
- Follow-up, clinical success, response to vitality test, periapical healing, apex closure, root lengthening, root thickening, and coronal discoloration.

Data synthesis

A narrative synthesis focused on the considered population, intervention, comparison, and outcome.

Descriptive statistical analyses synthesized data from the included studies through Microsoft Excel software 2019 (Microsoft Corporation, Redmond, WA, USA):

- Evaluating the different procedures of pulp regeneration
- Comparing different materials for the treatment of the root in pulp regeneration
- Estimating the most successful procedures in relation to the material used
- Estimating the most successful treatment in response to vitality test, periapical healing, apex closure, root lengthening, root thickening, and coronal discoloration
- Evaluating the material associated with the highest success rate according to AAE criteria through percentage calculation.

Quality assessment

The risk of bias (RoB) concerning the included studies of the present systematic review was evaluated by three independent reviewers (FG, GDB, and AI).

The RoB for the nonrandomized studies of interventions which not use randomization to allocate individuals or clusters to comparison groups was assessed through the RoB Instrument for Nonrandomized Studies of Exposures,[36] which is a modified form of "Risk Of Bias In Nonrandomized Studies of Interventions" (ROBINS-I) tool (freely available online on:. ROBINS-I tool | Cochrane Methods).

The RoB for any type of randomized studies of interventions was assessed through the revised Cochrane RoB tool for randomized trials (RoB-2) (freely available online on: RoB 2: A revised Cochrane risk-of-bias tool for randomized trials | Cochrane Bias).

The RoB for the case report and case series was assessed using the critical appraisal tools of the Joanna Briggs Institute (JBI) for case report and for case series,

respectively (freely available online on: JBI Critical Appraisal Tools | JBI).

RESULTS

Study selection

The electronic search found a total of 276 records, precisely 140 from MEDLINE/PubMed, 66 from Scopus, 48 from Web of Science, and 23 from Cochrane Library; 58 duplicates were removed afterward. Through screening of the titles and abstracts of the 217 left, 61 were specifically excluded because 6 were *in vitro* studies, 6 were preclinical *in vivo* studies, 5 did not include 6–17 years subjects, 19 were reviews, 9 were systematic reviews, 4 included book chapters or conference papers, 1 was not in the English language, 2 did not include a pulp regeneration treatment, and for 9 studies, it was not possible to extract data from subjects between 6 and 17 years of age.

A total of 30 studies[37-66] from the electronic search were established to fulfill the inclusion criteria of this systematic review.

No additional manual research was carried out for the present systematic review.

Data from 30 studies[37-66] regarding pulp regeneration treatment in pediatric subjects (6–17 years) were collected and synthesized.

Of the 30 studies[37-66] assessing the inclusion criteria, 16 were case reports, 5 were case series, 4 were comparative studies, 3 were randomized controlled trials, and 2 were prospective cohort studies.

Figure 1 shows the Preferred Reporting Items for Systematic Reviews and Meta-analyses 2020 flowchart of the included studies in the present systematic review.

Study characteristics and qualitative synthesis

The qualitative synthesis of the included studies is itemized in Table 1.

Mineral trioxide aggregate

To carry out pulp regeneration treatment, the use of MTA as bioactive material has been reported by 13 studies.[39,42-45,50,51,54-56,60,61,63]

Furthermore, the sample of the 13 studies[39,42-45,50,51,54-56,60,61,63] was 124 subjects, with a mean age of 9.6 years old and an age range between 7 and 16 years.

Eleven studies[39,42,44,45,50,54-56,60,61,63] reported the male-to-female ratio, comprised of 52 males and 38 females (male:female = 1.36:1).

Five studies[43,45,55,56,61] reported the absence of comorbidities.

A total of 93 teeth[39,42-45,50,51,54-56,60,61,63] underwent pulp regeneration treatment, in particular: 10[42,43,56] were maxillary right central incisors; 13[42-45,50,51,55,60] were maxillary left central incisors; 19 were permanent anterior teeth;[39] 25 were permanent central incisors;[61] 5 teeth[56] were maxillary right lateral incisors; 8[50,56,63] were maxillary left lateral incisors; 1 tooth[50] was maxillary left second premolar; 1[50] was mandibular left second premolar; 5[43,51] were mandibular left first molars; and 6[43,51,54] were mandibular right first molars.

Incomplete root development and open apices were reported by five studies[54-56,60,61] in 51 teeth in total; immature root and open apices were reported by three studies[50,51,63] in 10 teeth; incomplete root formation was reported by two studies[44,45] in 2 teeth, and Cvek's development stages in two studies[39,43] as follows: 2 teeth[60] belong to Stage II; 5[60] Stage III; 2[60] Stage IV; and 19[39] teeth belong to Stages from I to IV.

Four studies[42,44,55,60] reported a negative response to both the cold pulp test and electric pulp test for five teeth; one study[51] a negative cold pulp test on five teeth; one[45] negative response to electric pulp test on one tooth; and two[50,54] negative response to cold, electric, and heat pulp tests on five teeth. Sensibility to percussion was proved on three teeth, as mentioned by three studies;[44,54,55] no sensibility to percussion in two teeth was reported by two studies.[45,60] Palpation examination showed sensibility in one tooth,[55] slight sensibility in one tooth[60] and no sensibility in two teeth;[44,45] no sensibility to both percussion; and palpation was stated in two studies,[42,50] for a total of six teeth.

Ten studies[39,42,43,45,50,51,54,55,60,63] reported periapical radiolucid lesions in 27 teeth in total, whereas one study[39] found no lesions in 11.

The diagnoses reported were as follows:

- Pulp necrosis for 38 teeth[43,51,56]
- Pulp necrosis and apical periodontitis for seven teeth[45,50,54,55]
- Pulp necrosis and trauma for 18 teeth[39]
- Caries for one tooth[39]
- Pulp necrosis and enamel-dentin-pulp fracture with intrusive luxation for one tooth[44]
- Pulp necrosis, apical periodontitis, and external root resorption for one tooth[22]
- Enamel-dentin-pulp fracture for 18 teeth[61]
- Enamel dentine fracture for seven teeth[61]
- Apical periodontitis and dens invaginatus for one tooth[63]
- Sinus tract, multiple horizontal fractures, chronic apical abscess, external resorption, and pulp necrosis for one tooth.[42]

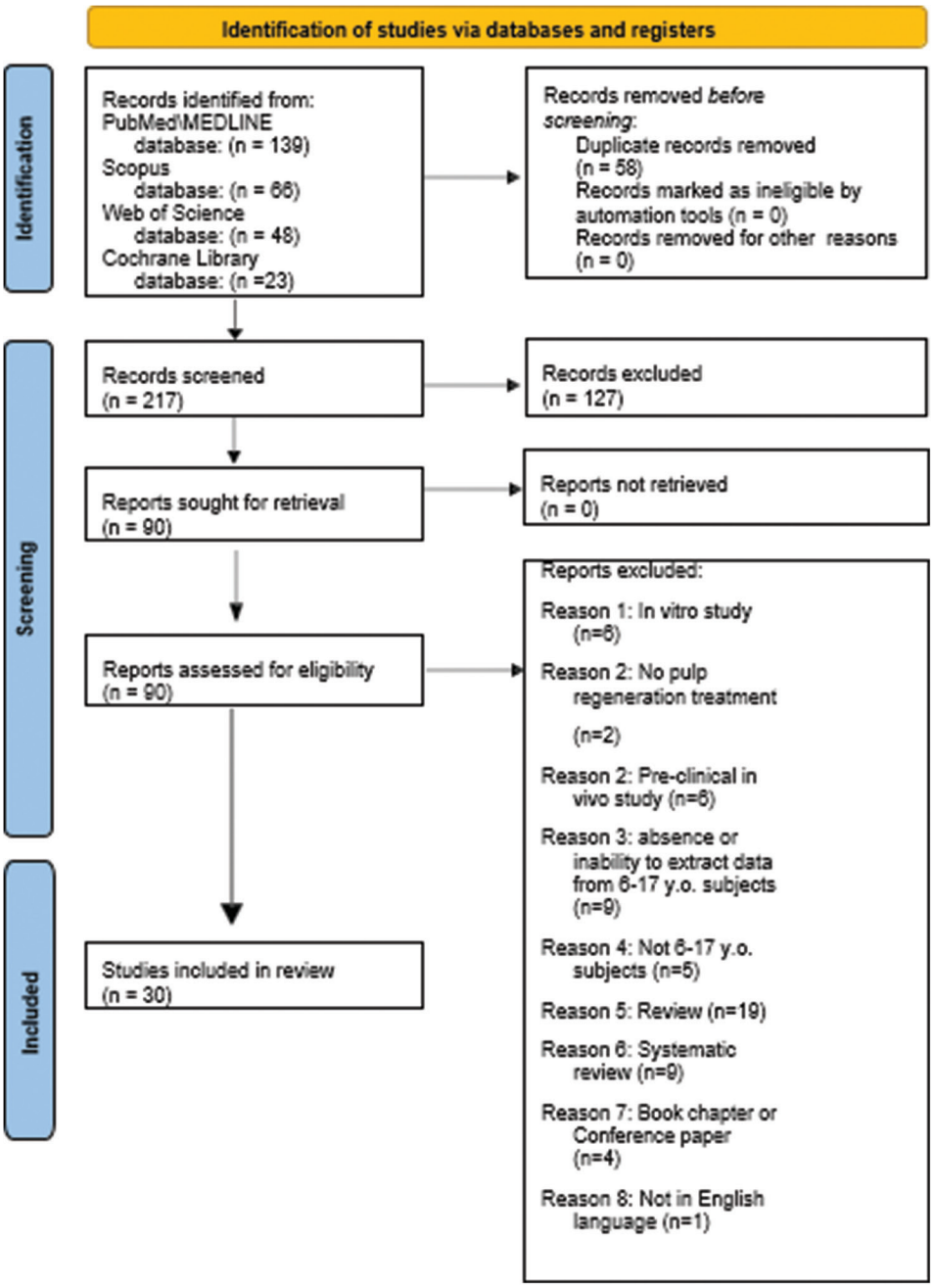

Figure 1: The flow chart of the records obtained through electronic research

Pulp regeneration was carried out in a single visit on one tooth,[55] whereas a two-visit approach was reported by 11 studies[42-45,50,51,54,56,60,61,63] on 75 teeth; a multivisit approach in a range between 2 and 5 visits was performed on 19.[39]

Minimal instrumentation was conducted on 30 teeth, as reported by three studies;[51,56,63] no instrumentation was carried out on two teeth;[42] minimal or no instrumentation was performed on 19 teeth;[39] and k-file instrumentation was done on 27 teeth as mentioned by three studies.[44,54,61]

As irrigant, NaOCl and EDTA were used by four studies[43,55,60,61] on 36 teeth in different volumes and concentrations; NaOCl was used by itself in different volumes and concentrations on 37 teeth, as reported by six studies;[42,50,51,54,56,63] chlorhexidine and sterile saline were employed on one tooth;[44] NaOCl, chlorhexidine, and EDTA were used on 19 teeth;[39] NaOCl, sterile saline, and chlorhexidine were used on one tooth.[45]

Irrigant activation was reported in two studies[55,63] on two teeth through ultrasonic activation.

Table 1: Data extracted and collected from the studies included in the present systematic review

Studies	Population	Teeth characteristics	Signs and symptoms	Treatment of root(s) with ret	Posttreatment outcomes
Alagl *et al.*, 2017[37] Comparative study J Endod No funding	Sample size: *n*=16 Mean age: 9.46 years old; range 8–11 years old Gender ratio: 10 males/6 females Comorbidities: None	Number of treated teeth: *n*=30 Tooth type(s): *n*=24 of maxillary incisors; *n*=6 of premolars (*n*=1 of maxillary first premolar; *n*=3 of mandibular first premolar; *n*=2 of mandibular second premolars) Root development stage: Incomplete root development with open apices (*n*=30)	Pulp tests CPT: Negative (*n*=30) HPT: MD EPT: MD Percussion examination: Sensitivity (*n*=26) Palpation examination: MD Periapical radiolucid lesion(s): Yes (*n*=22); no (*n*=8) Diagnosis: PN (*n*=30)	Number of visits: 2 First visit: Instrumentation: MD; irrigant: 20 mL of 2.5% NaOCl; 20 mL of sterile saline; 10 mL of 0.12% CHX; irrigant activation: MD; type of medication: TAP (*n*=4 of PRP group and *n*=5 to blood clot required two sessions of TAP); temporary sealer: Reinforced zinc-oxide eugenol cement (*n*=30) Second visit: Irrigant: 20 mL of 17% EDTA; irrigant activation: MD; scaffold used: blood clot (*n*=15); PRP (*n*=15) Bioactive material: White MTA (*n*=30) Final restorative material: Glass ionomer cement, composite resin (*n*=30)	Follow-up: 3; 6; 9; 12 months (*n*=15) Clinical success: Yes (*n*=30) Response to vitality tests: Yes (*n*=13 of PRP group; *n*=6 of blood clot group); no (*n*=2 of PRP group; *n*=9 of blood clot group) to EPT and CPT; no sensitivity to percussion or palpation (*n*=30) Periapical healing: Yes (*n*=30) Apex closure: Yes (*n*=30) Root lengthening: Yes (*n*=30) (higher in PRP group-mean 0.62 mm -than blood clot group -mean 0.42 mm) Root thickening: MD Coronal discoloration: MD
Alsofi, 2019[38] Case report J Contemp Dent Pract. No funding	Sample size: *n*=1 Mean age: 8 years old Gender ratio: 1 female Comorbidities: MD	Number of treated teeth: *n*=2 Tooth type(s): 11; 21 Root development stage: Incomplete root development with parallel root walls	Pulp tests CPT: Negative (*n*=2) HPT: MD EPT: MD Percussion examination: No sensibility (*n*=2) Palpation examination: No sensibility (*n*=2) Periapical radiolucent lesion(s): No (*n*=2) Diagnosis: PN (*n*=2)	Number of visits: 1 First visit: Instrumentation: None (*n*=2); irrigant: 10 mL of 2% CHX gluconate; physiological saline (*n*=2); irrigant activation: MD; scaffold used: Blood clot (*n*=2); Bioactive material: White MTA, wet cotton pellet (*n*=2); Temporary sealer: Cavit temporary material (*n*=2); final restorative material: Bonded resin restorations (*n*=2)	Follow-up: 6; 24; 36 months (*n*=1) Clinical success: Yes (*n*=1) Response to vitality tests: MD Periapical healing: No periapical lesions at base-line Apex closure: Yes (*n*=1) Root lengthening: Yes (*n*=1) Root thickening: Yes (*n*=1) Coronal discoloration: MD
Alobaid *et al.*, 2014[39] Comparative study J Endod. No funding	Sample size: *n*=31 EG (*n*=19) CG (*n*=12) Mean age: 8.8±1.6 years old; range 6–16 years old Gender ratio: 9 males/10 females Comorbidities: MD	Number of treated teeth: *n*=23 Tooth type(s): Permanent anterior teeth *n*=19 for group I; *n*=9 for group II Root development stage: Immature root apex (Cvek's stage I–IV)	Pulp tests CPT: MD HPT: MD EPT: MD Percussion examination: MD Palpation examination: MD Periapical radiolucent lesion(s): yes (*n*=8); no (*n*=11) Diagnosis: PN; Trauma (*n*=18); caries (*n*=1)	Number of visits: From 2 to 5 visits First visit: Instrumentation: Minimal or none; irrigant: Yes (*n*=19): NaOCl (*n*=N/D), CHX (*n*=N/D), and/or EDTA (*n*=N/D); irrigant activation: MD; type of medication: DAP or TAP and/or Ca(OH)2 (*n*=19); temporary sealer: IRM (*n*=19) Last visit: Irrigant: N/A; irrigant activation: MD; scaffold used: Blood clot (*n*=19); Bioactive material: MTA (*n*=19); final restorative material: Composite resin-bonded (*n*=19)	Follow-up: Mean of 15 months Clinical success (yes/no): Yes (*n*=15); no (*n*=3 because of reinfection I; *n*=1 because of re-traumatization) Response to vitality tests: MD Periapical healing: MD Apex closure: MD Root lengthening: Yes (*n*=15) Root canal thickening: Yes (*n*=15) Coronal discoloration: Yes (*n*=2 of the failed cases)
Aly *et al.*, 2019[40] RCT Int J Pediatric Dent. No funding	Sample size: *n*=24 Mean age: 9±0.89 years old for group I- biodentine; 8.92±1.26 for group II- MTA Gender ratio: 69.23% male/30.77% female for group I; 61.54% male/34.46% female in group II Comorbidities: None	Number of treated teeth: *n*=26 EG (*n*=13) CG (*n*=13) Tooth type(s): permanent anterior teeth Root development stage: Incomplete root formation, immature root apex (>1 mm of apical opening)	Pulp tests CPT: MD HPT: MD EPT: MD Percussion examination: MD Palpation examination: MD Periapical radiolucent lesion(s): MD Diagnosis: PN (*n*=26)	Number of visits: 2 First visit: Instrumentation: MD; irrigant: 20 mL 1.5% NaOCl; 20 mL sterile saline (*n*=26); irrigant activation: MD; type of medication: DAP (*n*=26); temporary sealer: Dry cotton pellet and IRM (*n*=26) Second visit: Irrigant: 20 mL of 17% EDTA (*n*=26); irrigant activation: MD; scaffold used: Blood clot (*n*=26); Bioactive material: Biodentine (*n*=13), white MTA (*n*=13); final	Follow-up: 3; 6; 9; 12; months (*n*=25) Clinical success: Yes (*n*=24) Biodentine (*n*=13); MTA (*n*=12); no MTA (*n*=1) Response to vitality tests: MD Periapical healing: MD Apex closure: MD Root lengthening: Yes (*n*=25) mean increase: 0.7±0.32 mm (*n*=13, Biodentine) versus 0.7±0.23 mm (*n*=12, MTA)

Contd...

Table 1: Contd...

Studies	Population	Teeth characteristics	Signs and symptoms	Treatment of root(s) with ret	Posttreatment outcomes
				restorative material: Composite resin (*n*=26)	Root canal thickening: MD Coronal discolouration: Yes (*n*=1 Biodentine), (*n*=7) MTA
Alencar *et al.*, 2022[41] Case report Iran Endod J No funding	Sample size: *n*=1 Mean age: 6 years old Gender ratio: 1 female Comorbidities: MD	Number of treated teeth: *n*=1 Tooth type(s): 21 Root development stage: Incomplete root formation (*n*=1)	Pulp tests CPT: Negative HPT: MD EPT: MD Percussion examination: Sensibility Palpation examination: Sensibility Periapical radiolucent lesion(s): Yes Diagnosis: ST (*n*=1), PN (*n*=1)	Number of visits: 2 First visit: Instrumentation: MD; irrigant: 20 mL of 1.5% NaOCl; 20 mL of 17% EDTA (*n*=1); irrigant activation: MD; type of medication: Ca(OH)2 (*n*=1); temporary sealer: Coltosol and composite (*n*=1) Second visit: Irrigant: 20 mL of 17% EDTA (*n*=1); irrigant activation: MD; scaffold used: Blood clot (*n*=1); bioactive material: MTA repair HP (*n*=1); final restorative material: Glass ionomer cement and composite (*n*=1)	Follow-up: 6; 12; 18; 36 months (*n*=1) Clinical success: Yes (*n*=1) Response to vitality tests: Negative (*n*=1) Periapical healing: Yes (*n*=1) Apex closure: MD Root lengthening: Yes (*n*=1) Root canal thickening: Yes (*n*=1) Coronal discoloration: No (*n*=1)
Arango-Gómez *et al.*, 2019[42] Case report Restor Dent Endod No funding	Sample size: *n*=1 Mean age: 9 years old Gender ratio: 1 male Comorbidities: MD	Number of treated teeth: *n*=2 Tooth type(s): 11; 21 Root development stage: MD	Pulp tests CPT: Negative (*n*=2) HPT: MD EPT: Negative (*n*=2) Percussion examination: No sensibility (*n*=2) Palpation examination: No sensibility (*n*=2) Periapical radiolucent lesion(s): Yes (*n*=2) Diagnosis: ST, multiple horizontal root fracture, CAA, ERR, PN (*n*=2)	Number of visits: 2 First visit: Instrumentation: None (*n*=2); irrigant: 10 mL of 1.25% NaOCl (*n*=2); Irrigant activation: MD; Type of medication: TAP (*n*=2); Temporary sealer: Glass ionomer cement (*n*=2) Second visit: Irrigant: 5 mL of 5.25% NaOCl and 5 mL of sterile saline solution to remove TAP and 17% EDTA (*n*=2); irrigant activation: MD; scaffold used: PRP (*n*=1, #11)/blood clot (*n*=1, #21); bioactive material: MTA Pro Root (*n*=2); final restorative material: Resin composite (*n*=2)	Follow-up: 7; 12; 36; 48 months (*n*=2) Clinical success: Yes (*n*=2) Response to vitality tests: Positive (*n*=1, #11), negative (*n*=1, #21) Periapical healing (yes/no): Yes (*n*=2) Apex closure: MD Root lengthening: MD Root canal thickening: Yes (*n*=2) Coronal discoloration: MD
Caleza-Jiménez *et al.*, 2022[43] Comparative study Eur Arch Paediatr Dent No funding	Sample size: *n*=18 EG (*n*=9) CG (*n*=9) Mean age: 8±1, 04 years old; range 7–10 years old Gender ratio: MD Comorbidities: None (*n*=18)	Number of treated teeth: *n*=9 Tooth type(s): *n*=1 of 11; *n*=1 of 21; *n*=3 of 36; *n*=3 of 46 Root development stage: Cvek's stage II (*n*=2); Cvek's stage III (*n*=5); Cvek's stage IV (*n*=2)	Pulp tests CPT: MD HPT: MD EPT: MD Percussion examination: MD Palpation examination: MD Periapical radiolucent lesion(s) (yes/no): Yes (*n*=9) Diagnosis: PN (*n*=9)	Number of visits: 2 First visit: Instrumentation: MD; Irrigant: 1.5%–2.5% of NaOCl; 17% of EDTA (*n*=9); irrigant activation: MD; type of medication: TAP (*n*=9); temporary sealer: IRM (*n*=9) Second visit: Irrigant: MD; irrigant activation: MD; scaffold used: Blood clot (*n*=9); bioactive material: MTA (*n*=9); final restorative material: Composite resin (*n*=9)	Follow-up: 12–66 months, mean 31±23, 77 months (*n*=9) Clinical success: Yes (*n*=9) Response to vitality tests: MD Periapical healing: Yes (*n*=9) Apex closure: Yes (*n*=9) Root lengthening: Yes (*n*=9), range of change from 4.28% to 35.29% Root canal thickening: Yes (*n*=9), range of change from 5.55% to 63.63% Coronal discoloration: MD
Soares Ade *et al.*, 2013[44] Case report J Endod No funding	Sample size: *n*=1 Mean age: 9 years old Gender ratio: 1 female Comorbidities: MD	Number of treated teeth: *n*=1 Tooth type(s): 21 Root development stage: Incomplete root formation (*n*=1)	Pulp tests CPT: Negative (*n*=1) HPT: MD EPT: Negative (*n*=1) Percussion examination: Sensibility (*n*=1) Palpation examination: No sensibility (*n*=1) Periapical radiolucent	Number of visits: 2 First visit: Instrumentation: Manual endodontic K-files sizes #55, 50, and 45 and Gates Glidden drills sizes #5, 4, 3, 2 (*n*=1); irrigant: 2% of CHX; sterile saline (*n*=1); Irrigant activation: MD; type of medication: Ca(OH)2, 2% of CHX gel (*n*=1); temporary	Follow-up: 1, 3, 6, 9, 12, 15, 24 months (*n*=1) Clinical success: Yes (*n*=1) Response to vitality tests: Not responsive to CPT, HPT, percussion, and palpation (*n*=1) Periapical healing: MD Apex closure: Yes (*n*=1) Root lengthening: Yes (*n*=1)

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Table 1: Contd...

Studies	Population	Teeth characteristics	Signs and symptoms	Treatment of root(s) with ret	Posttreatment outcomes
			lesion(s): MD Diagnosis: PN, enamel-dentin-pulp fracture, intrusive luxation (*n*=1)	sealer: Zinc oxide cement, composite resin (*n*=1) Second visit: Irrigant: Sterile saline; 3 mL of 17% EDTA (*n*=1); irrigant activation: MD; scaffold used: Blood clot (*n*=1); bioactive material: MTA (*n*=1); final restorative material: Zinc oxide cement and composite resin (*n*=1)	Root canal thickening: Yes (*n*=1) Coronal discoloration: MD
Dhiman *et al.*, 2018[45] Case report Contemp Clin Dent No funding	Sample size: *n*=1 Mean age: 9 years old Gender ratio: 1 male Comorbidities: None	Number of treated teeth: *n*=1 Tooth type(s): 21 Root development stage: Incomplete root formation (*n*=1)	Pulp tests CPT: MD HPT: MD EPT: Negative (*n*=1) Percussion examination: No sensibility (*n*=1) Palpation examination: No sensibility (*n*=1) Periapical radiolucent lesion(s): Yes (*n*=1) Diagnosis: PN, AP (*n*=1)	Number of visits: 2 First visit: Instrumentation: MD; irrigant: 20 mL of 5.25% NaOCl; 5 mL of sterile saline; 10 mL of 2% CHX (*n*=1); irrigant activation: MD; type of medication: TAP (*n*=1); temporary sealer: Sterile cotton pellet and Cavit G (*n*=1) Second visit: Irrigant: 5 mL of sterile saline; 10 mL of 17% EDTA (*n*=1); irrigant activation: MD; scaffold used: blood clot, collocate barrier (*n*=1); bioactive material: MTA (*n*=1); final restorative material: Cavit G and adhesive resin (*n*=1)	Follow-up: 1; 30 months (*n*=1) Clinical success: Yes Response to vitality tests: Not responsive to vitality tests; no sensibility to percussion and palpation (*n*=1) Periapical healing: Yes (*n*=1) Apex closure: Yes (*n*=1) Root lengthening: Yes (*n*=1) Root canal thickening: Yes (*n*=1) Coronal discoloration: MD
D'Mello and Moloney[46] Case report Aust Dent J No funding	Sample size: *n*=1 Mean age: 7 years old Gender ratio: 1 female Comorbidities: MD	Number of treated teeth: *n*=1 Tooth type(s): 11 Root development stage: Immature root with open apex (*n*=1)	Pulp tests CPT: MD HPT: MD EPT: MD Percussion examination: No sensibility (*n*=1) Palpation examination: No sensibility (*n*=1) Periapical radiolucent lesion(s): MD Diagnosis: AP associated with facial cellulitis (*n*=1)	Number of visits: 1 First visit: Instrumentation: MD; irrigant: 1% NaOCl; 17% EDTA (*n*=1); irrigant activation: MD; scaffold used: Blood clot, absorbable adhesion barrier (*n*=1); bioactive material: White MTA (*n*=1); Final restorative material: Glass ionomer cement, composite resin restoration (*n*=1)	Follow-up: 7; 15; 27; 36 months (*n*=1) Clinical success: No (*n*=1) Response to vitality tests: MD Periapical healing: MD Apex closure: No (*n*=1) Root lengthening: MD Root canal thickening: Yes (*n*=1) Coronal discoloration: Yes (*n*=1)
Johns *et al.*, 2014[47] Case report J Conserv Dent No funding	Sample size: *n*=1 Mean age: 17 years old Gender ratio: 1 male Comorbidities: Noncontributory	Number of treated teeth: *n*=2 Tooth type(s): 11; 21 Root development stage: Immature roots with open apices (*n*=2)	Pulp tests CPT: Negative (*n*=2) HPT: MD EPT: Negative (*n*=2) Percussion examination: No sensibility (*n*=2) Palpation examination: Slight sensibility (*n*=2) Periapical radiolucent lesion(s): Yes (*n*=2) Diagnosis: PN (*n*=2)	Number of visits: 1 First visit: Instrumentation: K files (*n*=2); irrigant: 20 mL of 5.25% NaOCl; physiological saline; 17% EDTA (*n*=2); Irrigant activation: Diode laser and 10 mL sterile saline solution (*n*=2); scaffold used: Choukroun's PRF (*n*=2); bioactive material: Gray MTA (*n*=2); final restorative material: Coltosol and composite (*n*=2)	Follow-up: 6–10 months (*n*=2) Clinical success: Yes (*n*=1); no (*n*=1) Response to vitality tests: Not to EPT; no to palpation and percussion tests (*n*=2) Periapical healing: Yes (*n*=1, #11); no (*n*=1, #21) Apex closure: Yes (at 10 months) (*n*=2) Root lengthening: Yes (*n*=2) Root canal thickening: Yes (*n*=2) Coronal discoloration: MD
Kahler *et al.*, 2014[48] Comparative study J Endod No funding	Sample size: *n*=12 Mean age: 10.5 years old; range 7–12 years old Gender ratio: MD; gender ratio of treated teeth: 5 males/11 females Comorbidities: MD	Number of treated teeth: *n*=16 Tooth type(s): Mandibular second premolars (*n*=3); central incisors (*n*=13) Root development	Pulp tests CPT: MD HPT: MD EPT: Positive (*n*=5); negative (*n*=11) response Percussion examination: MD Palpation examination:	Number of visits: 2 First visit: Instrumentation: MD; irrigant: 1% NaOCl (*n*=16); irrigant activation: MD; type of medication: TAP (*n*=16); temporary sealer: Cavit and glass ionomer cement (*n*=16) Second visit: Irrigant: 1% NaOCl (*n*=16); irrigant activation:	Follow-up: 18, 36 months (*n*=16) Clinical success: Yes (*n*=16) Response to vitality tests: MD Periapical healing: Yes (*n*=13) Apex closure: Yes (*n*=16) Root lengthening: Yes range

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Table 1: Contd...

Studies	Population	Teeth characteristics	Signs and symptoms	Treatment of root(s) with ret	Posttreatment outcomes
		stage: Incomplete root maturation and open apex (>1.1 mm in diameter) (*n*=16)	MD Periapical radiolucent lesion(s): Yes (*n*=13) Diagnosis: Chronic periapical abscess (*n*=5); AP (*n*=8); PN (*n*=2); IRR and AP (*n*=1)	MD; scaffold used: Blood clot (*n*=16); bioactive material: Pro Root white MTA (*n*=16); final restorative material: glass ionomer cement (*n*=16)	between−2.7% and 25.3% (*n*=9) Root canal thickening: Yes range between−1.9% and 72.6% (*n*=9) Coronal discoloration: Yes (*n*=13)
Kandemir *et al*., 2020[49] Case series J Clin Pediatr Dent No funding	Sample size: *n*=3 Mean age: 13.6 years old; range 13–14 years old Gender ratio: 2 males/1 female Comorbidities: MD	Number of treated teeth: *n*=3 Tooth type(s): 11 (*n*=2); 12 Root development stage: immature (*n*=3)	Pulp tests CPT: Negative (*n*=3) HPT: MD EPT: Negative (*n*=3) Percussion examination: No sensibility (*n*=3) Palpation examination: No sensibility (*n*=3) Periapical radiolucid lesion(s): Yes (*n*=3) Diagnosis: PN and AP (*n*=3)	Number of visits: 2 First visit: Instrumentation: None (*n*=3); irrigant: 20 mL of 1.5% NaOCl; physiological saline; 17% EDTA (*n*=3); irrigant activation: MD; Type of medication: TAP (*n*=3); temporary sealer: Glass ionomer cement (*n*=3) Second visit: Irrigant: 17% EDTA (*n*=3); irrigant activation: MD; scaffold used: PRF (*n*=3); bioactive material: White MTA (*n*=3); final restorative material: Bonded resin composite (*n*=3)	Follow-up: 36 months (*n*=3) Clinical success: Yes (*n*=3) Response to vitality tests: No (EPT, CPT) (*n*=3) Periapical healing: Yes or decreased size (*n*=3) Apex closure: Yes (*n*=3) Root lengthening: No (*n*=3) Root canal thickening: No (*n*=3) Coronal discoloration: MD
Lin *et al*., 2018[50] Case series Aust Endod J No funding	Sample size: *n*=3 Mean age: 10.6 years old age range 10–12 years old Gender ratio: 3 males Comorbidities: N/A	Number of treated teeth: 4 Tooth type(s): 21; 22; 25; 35 Root development stage: Wide open apex (*n*=3); short roots open apices (*n*=1)	Pulp tests CPT: Negative (*n*=4) HPT: Negative (*n*=4) EPT: Negative (*n*=4) Percussion examination: No sensibility (*n*=4) Palpation examination: No sensibility (*n*=4) Periapical radiolucid lesion(s): Yes (*n*=4) Diagnosis: PN and AP (*n*=4)	Number of visits: 2 First visit: Instrumentation: MD; irrigant: 20 mL of 3.25% NaOCl (*n*=4); irrigant activation: MD; type of medication: Ca(OH)2 (*n*=4); temporary sealer: IRM (*n*=4) Second visit: Irrigant: 3.25% NaOCl; 17% EDTA (*n*=4); irrigant activation: MD; scaffold used: Blood clot and absorbable adhesion barrier (*n*=4); bioactive material: Pro Root MTA (*n*=4); final restorative material: Glass ionomer cement (*n*=4)	Follow-up: 3, 6, 12, 24 months. The scheduled follow-up failed to attend due to complications at 2 months (*n*=1, #25), 4 months (*n*=1, #21), 14 months (*n*=1, #25), 22 months (*n*=1, #22) Clinical success: No (*n*=4) Response to vitality tests: MD Periapical healing: No (*n*=4) Apex closure: Yes (*n*=4) Root lengthening: Yes (*n*=4) Root canal thickening: Yes (*n*=4) Coronal discoloration: MD
Carmen *et al*., 2017[51] Case series Case Rep Dent No funding	Sample size: *n*=4 Mean age: 7.75 years old range 6.5–8.5 years old Gender ratio: MD Comorbidities: MD	Number of treated teeth: 5 Tooth type(s): 21; 36 (*n*=2); 46 (*n*=2) Root development stage: Immature roots with open apices (*n*=5)	Pulp tests CPT: Negative (*n*=5) HPT: MD EPT: MD Percussion examination: MD Palpation examination: MD Periapical radiolucent lesion(s): Yes (*n*=5) Diagnosis: PN (*n*=5)	Number of visits: 2 First visit: Instrumentation: Minimal (*n*=5); irrigant: 5% NaOCl (*n*=5); irrigant activation: MD; type of medication: TAP (*n*=5); temporary sealer: IRM and glass ionomer cement (*n*=5) Second visit: irrigant: 5% NaOCl (*n*=5); irrigant activation: MD; scaffold used: Blood clot (*n*=5); bioactive material: MTA (*n*=5); final restorative material: Glass ionomer cement, composite (*n*=2), metal crown (*n*=3)	Follow-up: 6, 12 months (*n*=5) Clinical success: Yes (*n*=5) Response to vitality tests: MD Periapical healing: Yes (*n*=5) Apex closure: Yes (*n*=2); not completely (*n*=2); no (*n*=1) Root lengthening: Yes (*n*=1), MD (*n*=4) Root canal thickening: Yes (*n*=5) Coronal discoloration: Yes (*n*=1); N/A (*n*=4)
Loroño *et al*., 2021[52] Case report J Dent (Shiraz) No funding	Sample size: *n*=1 Mean age: 8 years old Gender ratio: 1 male Comorbidities: MD	Number of treated teeth: 1 Tooth type(s): 21 Root development stage: Cvek's stage 2 (*n*=1)	Pulp tests CPT: Negative (*n*=1) HPT: MD EPT: Negative (*n*=1) Percussion examination: Sensibility (*n*=1) Palpation examination: Sensibility (*n*=1)	Number of visits: 2 First visit: Instrumentation: None (*n*=1); irrigant: 20 mL of 1.5% NaOCl; physiological saline; 17% EDTA (*n*=1); irrigant activation: MD; type of medication: Ca(OH)2 (*n*=1); temporary sealer: Sterile cotton pellet and Cavit G (*n*=1)	Follow-up: 3, 6, 12, 24, 48 months (*n*=1) Clinical success: Yes (*n*=1) Response to vitality tests: MD Periapical healing: Yes (*n*=1) Apex closure: Yes (*n*=1) Root lengthening: No (*n*=1)

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Studies	Population	Teeth characteristics	Signs and symptoms	Treatment of root(s) with ret	Posttreatment outcomes
			Periapical radiolucent lesion(s): Yes (*n*=1) Diagnosis: Complicate crown fracture; ST, IRR (*n*=1)	Second visit: Irrigant: 20 mL of 17% EDTA (*n*=1); irrigant activation: MD; scaffold used: Blood clot and collagen sponge (*n*=1); bioactive material: White MTA (*n*=1); final restorative material: Glass ionomer cement and light cured composite resin (*n*=1)	Root thickening: Yes (*n*=1) Coronal discolouration: Yes (*n*=1)
Maniglia-Ferreira *et al*., 2020[53] Case report Braz Dent J No Funding	Sample size: *n*=1 Mean age: 7 years old Gender ratio: 1 female Comorbidities: None	Number of treated teeth: 2 Tooth type(s): 11, 21 Root development stage: Immature teeth with wide-open apex (*n*=2)	Pulp tests CPT: Negative (*n*=2) HPT: MD EPT: MD Percussion examination: Sensibility (*n*=2) Palpation examination: Sensibility (*n*=2) Periapical radiolucent lesion(s): MD Diagnosis: Intrusion, PN, acute apical abscess (*n*=1), intrusion, PN, acute apical abscess, and not complicated enamel fracture (*n*=1)	Number of visits: 3 First visit: Instrumentation: Hand files #140 (*n*=2); irrigant: 2.5% NaOCl; 5 mL of17% EDTA; 20 mL of saline solution (*n*=2); irrigant activation: MD; type of medication: 2% CHX gel (*n*=2); temporary sealer: Cotton pellet and resin Modified Glass Ionomer (*n*=2) Second visit: Irrigant: 2.5% NaOCl; 5 mL of17% EDTA; 20 mL of saline solution (*n*=2); irrigant activation: MD; type of medication: DAP, Ca(OH)2 paste and 2% CHX gel with Zinc oxide (*n*=2); temporary sealer: Resin modified glass ionomer (*n*=2) Third visit: Irrigant: MD; irrigant activation: MD; scaffold used: Blood clot (*n*=2); bioactive material: White MTA (*n*=2); final restorative material: Modified glass ionomer (*n*=2)	Follow-up: 12; 60; 144 months (*n*=2) Clinical success: No (*n*=2) Response to vitality tests: No sensibility to palpation/ percussion (*n*=2) Periapical healing: MD Apex closure: Yes (*n*=2) Root lengthening: MD Root thickening: MD Coronal discoloration: No (*n*=2)
Martin *et al*., 2013[54] Case report J Endod No funding	Sample size: *n*=1 Mean age: 9 years old Gender ratio: 1 male Comorbidities: MD	Number of treated teeth: 1 Tooth type(s): 46 Root development stage: Incomplete root development, open apex (*n*=1)	Pulp tests CPT: Negative (*n*=1) HPT: Negative (*n*=1) EPT: Negative (*n*=1) Percussion examination: Sensibility (*n*=1) Palpation examination: MD Periapical radiolucent lesion(s): Yes (*n*=1) Diagnosis: PN, AP (*n*=1)	Number of visits: 2 First visit: Instrumentation: distal canal #40 K-file and mesial canals #30 K-file (*n*=1); Irrigant: 10 mL of 5.25% of NaOCl (*n*=1); Irrigant activation: MD; Type of medication: TAP (*n*=1); Temporary sealer: Cotton pellet and IRM (*n*=1) Second visit: Irrigant: 10 mL of 5.25% of NaOCl (*n*=1); Irrigant activation: MD; Scaffold used: Blood clot + PRP (*n*=1); Bioactive material: MTA (*n*=1); Final restorative material: Bonded resin (*n*=1)	Follow-up: 7, 14, 48 months (*n*=1) Clinical success: No (*n*=1) Response to vitality tests: MD Periapical healing: No (*n*=1) Apex closure: MD Root lengthening: MD Root thickening: MD Coronal discoloration: MD
McCabe, 2015[55] Case report Int Endod J No funding	Sample size: *n*=1 Mean age: 7 years old Gender ratio: 1 female Comorbidities: None	Number of treated teeth: 1 Tooth type(s): 21 Root development stage: Incomplete root development, open apex (*n*=1)	Pulp tests CPT: Negative (*n*=1) HPT: MD EPT: Negative (*n*=1) Percussion examination: Sensibility (*n*=1) Palpation examination: Sensibility (*n*=1) Periapical radiolucent lesion(s): Yes (*n*=1) Diagnosis: PN, AP (*n*=1)	Number of visits: 1 First visit: Instrumentation: MD; irrigant: 30 mL of 5% NaOCl; 3 mL of 17% EDTA (*n*=1); irrigant activation: Suprasson ultrasonic handpiece and irritate files size 25 (*n*=1); scaffold used: Blood clot (*n*=1); bioactive material: MTA (*n*=1); final restorative material: Glass ionomer cement (*n*=1)	Follow-up: 3, 6, 12, 24 months (*n*=1) Clinical success: Yes (*n*=1) Response to vitality tests: MD Periapical healing: Yes (*n*=1) Apex closure: Yes (*n*=1) Root lengthening: Yes (*n*=1) Root canal thickening: Yes (*n*=1) Coronal discoloration: MD

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Studies	Population	Teeth characteristics	Signs and symptoms	Treatment of root(s) with ret	Posttreatment outcomes
Nagy *et al.*, 2016[56] RCT J Endod No funding	Sample size: *n*=36 EG (24) CG (*n*=12) Mean age: 11 years old range 9–13 years old Gender ratio: 22 males/14 females Comorbidities: None	Number of treated teeth: 24 Tooth type(s): 11 (*n*=8); 12 (*n*=5); 21 (*n*=5); 22 (*n*=6) Root development stage: Incomplete root development and open apicis (*n*=24)	Pulp tests CPT: MD HPT: MD EPT: MD Percussion examination: MD Palpation examination: MD Periapical radiolucent lesion: MD Diagnosis: PN (*n*=24)	Number of visits: 2 First visit: Instrumentation: Minimal (*n*=24); irrigant: 10 mL of 2.6% of NaOCl (*n*=24); irrigant activation: MD; type of medication: TAP (*n*=24); temporary sealer: Zinc oxide cement (*n*=24) Second visit: Irrigant: 10 mL 2.6% of NaOCl; 10 mL sterile saline (*n*=24); irrigant activation: MD; scaffold used: Blood clot (*n*=12); blood clot + injectable hydrogel scaffold with bFGF (*n*=12); bioactive material: MTA (*n*=24); final restorative material: Composite resin (*n*=24)	Follow-up: 3, 6, 12,18 months (*n*=20) Clinical success: Yes (*n*=9) blood clot; (*n*=8) blood clot + injectable hydrogel scaffold with bFGF; no (*n*=1) blood clot, (*n*=2) of blood clot + injectable hydrogel scaffold with bFGF Response to vitality tests: MD Periapical healing: MD Apex closure: Yes (*n*=20) Root lengthening: Yes (*n*=20) Root thickening: Yes (*n*=20) Coronal discoloration: MD
Nazzal *et al.*, 2020[57] Prospective cohort study Eur Arch Paediatr Dent No funding	Sample size: *n*=15 Mean age: 8.3 years old range 7–10 years old Gender ratio: 12 males/3 females Comorbidities: No relevant medical history (ASA I and ASA II)	Number of treated teeth: 12 Tooth type(s): 11 (*n*=8); 21 (*n*=3); 22 (*n*=1) Root development stage: Cvek's stage II (*n*=1); Cvek's stage III (*n*=8); Cvek's stage IV (*n*=3)	Pulp tests CPT: Negative (*n*=10); positive (*n*=2) HPT: MD EPT: Negative (*n*=9); positive (*n*=3) Percussion examination: Sensibility (*n*=12) Palpation examination: Sensibility (*n*=12) Periapical radiolucent lesion(s): Yes (*n*=8); no (*n*=3); unclear (*n*=1) Diagnosis: PN following trauma (*n*=12)	Number of visits: 2 First visit: Instrumentation: Minimal; Irrigant: 0.5% of NaOCl (*n*=12); Irrigant activation: MD; Type of medication: DAP (*n*=12); Temporary sealer: Glass ionomer cement (*n*=12) Second visit: Irrigant: Sterile saline (*n*=12); irrigant activation: MD; scaffold used: Blood clot (*n*=12); bioactive material: N/D; final restorative material: Pure Portland cement, glass ionomer cement, composite resin (*n*=12)	Follow-up: 3, 9, 12, 24, 48 months (average recall time: 43.42 months, range 27–59 months) (*n*=12) Clinical success: Yes (*n*=12) Response to vitality tests: *n*=9 of no to CPT; *n*=1 of positive to CPT; *n*=2 of unclear to CPT; *n*=8 of no to EPT; *n*=4 of positive to EPT Periapical healing: Yes (*n*=12) Apex closure: Yes (*n*=12) Root lengthening: No (*n*=12) Root canal thickening: Yes (*n*=12) Coronal discoloration: Yes (*n*=4)
Priya *et al.*, 2016[58] Case report J Endod No funding	Sample size: *n*=1 Mean age: 11 years old Gender ratio: 1 male Comorbidities: MD	Number of treated teeth: 1 Tooth type(s): 21 Root development stage: Cvek's stage V (*n*=1)	Pulp tests CPT: MD HPT: Negative (*n*=1) EPT: MD Percussion examination: MD Palpation examination: MD Periapical radiolucent lesion(s): Yes (*n*=1) Diagnosis: Avulsion with uncomplicated fracture (*n*=1)	Number of visits: 2 First visit: Instrumentation: MD; irrigant: 5.25% NaOCl; sterile saline (*n*=1); irrigant activation: MD; type of medication: MD; temporary sealer: MD Second visit: Irrigant: MD; irrigant activation: MD; type of medication: DAP (*n*=1); scaffold used: PRP (*n*=1); bioactive material: N/D; final restorative material: Glass ionomer cement, composite resin (*n*=1)	Follow-up: 6, 9, 12 months (*n*=1) Clinical success: Yes Response to vitality tests: Yes to CPT, HPT (*n*=1) Periapical healing: Yes (*n*=1) Apex closure: MD Root lengthening: MD Root canal thickening: MD Coronal discoloration: MD
Ravikumar *et al.*, 2021[59] Case report J Pharm Bioallied Sci No funding	Sample size: *n*=1 Mean age: 17 years old Gender ratio: 1 male Comorbidities: MD	Number of treated teeth: 1 Tooth type(s): 22 (*n*=1) Root development stage: Open apex (*n*=1)	Pulp tests CPT: MD HPT: MD EPT: MD Percussion examination: Sensibility (*n*=1) Palpation examination: MD Periapical radiolucent lesion(s): Yes (*n*=1) Diagnosis: Ellis class III fracture (*n*=1)	Number of visits: 2 First visit: Instrumentation: MD; irrigant: 20 mL of 5.25% NaOCl; sterile saline (*n*=1); irrigant activation: MD; type of medication: Ca(OH)2 (*n*=1); temporary sealer: Cotton pellet and IRM (*n*=1) Second visit: Irrigant: 17% EDTA, sterile saline (*n*=1); irrigant activation: MD; scaffold used: Blood clot, absorbable collagen matrix (*n*=1); bioactive material: Parrot MTA and glass ionomer (*n*=1); final restorative material: Composite resin (*n*=1)	Follow-up: 3, 6, 12, 18 months (*n*=1) Clinical success: Yes (*n*=1) Response to vitality tests: MD Periapical healing: Yes (*n*=1) Apex closure: Yes (*n*=1) Root lengthening: MD Root canal thickening: Yes (*n*=1) Coronal discoloration: MD

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Table 1: Contd...

Studies	Population	Teeth characteristics	Signs and symptoms	Treatment of root(s) with ret	Posttreatment outcomes
Ray *et al*., 2016[60] Case report Dent Traumatol No funding	Sample size: *n*=1 Mean age: 11 years old Gender ratio: 1 male Comorbidities: MD	Number of treated teeth: 1 Tooth type(s): 21 Root development stage: Immature teeth with incomplete root development and open apex (*n*=1)	Pulp tests CPT: Negative (*n*=1) HPT: MD EPT: Negative (*n*=1) Percussion examination: No sensibility (*n*=1) Palpation examination: Slight sensibility (*n*=1) Periapical radiolucent lesion(s): Yes (*n*=1) Diagnosis: PN, ERR, AP (*n*=1)	Number of visits: 2 First visit: Instrumentation: N/A; Irrigant: 0.5% NaOCl; 17% EDTA (*n*=1); irrigant activation: MD; type of medication: DAP (*n*=1); temporary sealer: Cotton pellet and glass ionomer (*n*=1) Second visit: Irrigant: 0.5% NaOCl; 17% EDTA (*n*=1); irrigant activation: MD; scaffold used: PRF; bioactive material: MTA, glass ionomer base (*n*=1); final restorative material: Composite resin (*n*=1)	Follow-up: 24; 36 months (*n*=1) Clinical success: No (*n*=1) Response to vitality tests: Negative CPT, positive to EPT (*n*=1) Periapical healing: MD Apex closure: MD Root lengthening: Yes (*n*=1) Root canal thickening: MD Coronal discolouration: Yes (*n*=1)
Rizk *et al*., 2020[61] Saudi Dent J Double-blinded RCT No funding	Sample size: *n*=25 EG: *n*=13 CG: *n*=12 Mean age: 9±1 years old; range 8–14 years old Gender ratio: 13 males/12 females Comorbidities: None	Number of treated teeth: 25 Tooth type(s): Permanent necrotic central incisors (*n*=25) Root development stage: Incomplete root development with open apex (≥1.0 mm) (*n*=25)	Pulp tests CPT: MD HPT: MD EPT: MD Percussion examination: MD Periapical radiolucent lesion(s): MD Diagnosis: Enamel-pulp-dentine fracture (*n*=18); enamel-dentin fracture (*n*=7)	Number of visits: 2 (*n*=25) First visit: Instrumentation: Endodontic files (*n*=25); Irrigant: 20 mL of 2% NaOCl; 20 mL of 17% EDTA (*n*=25); Irrigant activation: MD; type of medication: Equal proportion of TAP (*n*=25); temporary sealer: Dry steryle cotton pellet and IRM (*n*=25) Second visit: Irrigant: 20 ML of sterile saline; 20 mL of 17% EDTA (*n*=25); irrigant activation: MD; scaffold used: PRP (*n*=13); PRF (*n*=12); bioactive material: MTA (*n*=25); final restorative material: Glass ionomer; composite (*n*=25)	Follow-up: 3, 6, 9, 12 months (*n*=25) Clinical success: Yes (*n*=25) Response to vitality tests: No response to CPT, HPT, EPT (*n*=25) Periapical healing: MD Apex closure: Yes (*n*=25) Root lengthening: Yes (*n*=25) Root canal thickening: Yes (higher in PRP group) Coronal discoloration: Yes (higher in PRF group)
Timmermann, 2017[63] Case report J Endod. No funding	Sample size: *n*=1 Mean age: 16 years old Gender ratio: 1 male Comorbidities: MD	Number of treated teeth: 1 Tooth type(s): 22 Root development stage: Immature root with open apex (*n*=1)	Pulp tests CPT: MD HPT: MD EPT: MD Percussion examination: MD Palpation examination: MD Periapical radiolucent lesion(s): Yes (*n*=1) Diagnosis: AP and dens invaginatus (*n*=1)	Number of visits: 2 First visit: Instrumentation: Lightly instrumented by hand by using a 60/0.04 ProFile rotary instrument (*n*=1); irrigant: 20 mL of 1% NaOCl (*n*=1); irrigant activation: Passive ultrasonic activation for 20 s 3 times (*n*=1); type of medication: Ca(OH)2 (*n*=1); temporary sealer: IRM and reinforced glass ionomer cement (*n*=1) Second visit: Irrigant: 20 mL 1% NaOCl and 20 mL 15% EDTA (*n*=1); irrigant activation: MD; scaffold used: Blood clot (*n*=1); bioactive material: MTA (*n*=1); final restorative material: Polycarboxylate cement base, resin composite (*n*=1)	Follow-up: 12, 24, 36 months (*n*=1) Clinical success: Yes (*n*=1) Response to vitality tests: No sensibility (*n*=1) Periapical healing: Yes (*n*=1) Apex closure: Yes (*n*=1) Root lengthening: MD Root canal thickening: Yes (*n*=1) Coronal discoloration: MD
Timmerman and Parashos, 2018[62] Case report J Endod No funding	Sample size: *n*=1 Mean age: 12 years old Gender ratio: 1 female Comorbidities: MD	Number of treated teeth: 1 Tooth type(s): 15 Root development stage: Immature root with open apex (*n*=1)	Pulp tests CPT: MD HPT: MD EPT: Negative (*n*=1) Percussion examination: MD Palpation examination: MD Periapical radiolucent lesion(s): MD Diagnosis: AP, irreversible pulpitis, dens evaginatus (*n*=1)	Number of visits: 1 First visit: Instrumentation: Minimal (*n*=1); irrigant: 20 mL of 1% NaOCl; 20 mL of 15% EDTA (*n*=1); irrigant activation: MD; scaffold used: Blood clot, matrix (*n*=1); bioactive material: White MTA (*n*=1); final restorative material: Polycarboxylate cement base, resin composite onlay (*n*=1)	Follow-up: N/D Clinical success: Yes (*n*=1) Response to vitality tests: Responsive to EPT (*n*=1) Periapical healing: Yes (*n*=1) Apex closure: Yes (*n*=1) Root lengthening: MD Root canal thickening: Yes (*n*=1) Coronal discoloration: Yes (*n*=1) (after 3 months)

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Table 1: Contd...

Studies	Population	Teeth characteristics	Signs and symptoms	Treatment of root(s) with ret	Posttreatment outcomes
Topçuoğlu and Topçuoğlu, 2016[64] Case series J Endod No funding	Sample size: *n*=3 Mean age: 8.33 years old; range 8–9 years old Gender ratio: 1 male/2 female Comorbidities: MD	Number of treated teeth: 3 Tooth type(s): 46 (*n*=2); 36 (*n*=1) Root development stage: Immature roots with open apices (*n*=3)	Pulp tests CPT: Negative (*n*=3) HPT: MD EPT: Negative (*n*=3) Percussion examination: No sensibility (*n*=3) Palpation examination: No sensibility (*n*=3) Periapical radiolucent lesion(s): No (*n*=3) Diagnosis: PN (*n*=3)	Number of visits: 1 First visit: Instrumentation: None (*n*=3); irrigant: 20 mL of 2.5% NaOCl; 10 mL of sterile saline; 10 mL of 17% EDTA (*n*=3); irrigant activation: MD; scaffold used: PRP (*n*=3); bioactive material: Biodentine (*n*=3); final restorative material: Composite resin (*n*=3)	Follow-up: 3, 6, 9, 12, 18 months (*n*=3) Clinical success: Yes (*n*=3) Response to vitality tests: Not responsive to CPT, EPT, percussion, or palpation (*n*=3) Periapical healing: N/D Apex closure: Yes (*n*=3) Root lengthening: MD Root canal thickening: Yes (*n*=3) Coronal discoloration: MD
Ulusoy *et al.*, 2019[65] Prospective cohort study J Endod No funding	Sample size: *n*=77 Mean age: 9.36 years old; range 8–11 years old Gender ratio: 44 males/33 females Comorbidities: None	Number of treated teeth: 73 Tooth type(s): Maxillary incisors (*n*=73) Root development stage: Incomplete root development and open apex (>1 mm) (*n*=73)	Pulp tests CPT: Negative (*n*=73) HPT: MD EPT: Negative (*n*=73) Percussion examination: MD Palpation examination: MD Periapical radiolucent lesion(s): N/D Diagnosis: PN (*n*=73)	Number of visits: 2 First visit: Instrumentation: None (*n*=73); irrigant: 20 mL of 1.25% NaOCl (*n*=73); irrigant activation: MD; type of medication: TAP (*n*=73); temporary sealer: Glass ionomer cement (*n*=73) Second visit: Irrigant: 2% CHX; 10 mL of sterile saline; 1 mL of 17% EDTA (*n*=73); irrigant activation: MD; scaffold used: PRP (*n*=18); PRF (*n*=17); platelet pellet (*n*=17); blood clot (*n*=21); bioactive material: White MTA (*n*=73); final restorative material: Glass ionomer (*n*=73)	Follow-up: from 10 to 49 months (*n*=73) Clinical success: Yes (*n*=71); no (*n*=1 from blood clot group; *n*=1 from PRF group) Response to vitality tests: Yes (*n*=63); no (*n*=10) CPT, EPT Periapical healing: MD Apex closure: Yes (*n*=12 PRP group; *n*=12 PRF group; *n*=14 platelet pellet group; *n*=16 blood clot group); ongoing (*n*=3); no (*n*=16); MD (*n*=1) Root lengthening: Yes (*n*=73) Root canal thickening: Yes (*n*=73) Coronal discoloration: MD
Yoshpe *et al.*, 2021[66] Case series Eur Arch Paediatr Dent No funding	Sample size: *n*=6 Mean age: 8.25 years old; range 7–11 years old Gender ratio: 1 male/5 females Comorbidities: MD	Number of treated teeth: 6 Tooth type(s): 46 (*n*=3); 36 (*n*=2); 26 (*n*=1) Root development stage: Immature roots with open apicis (*n*=6)	Pulp tests CPT: Negative (*n*=6) HPT: MD EPT: Negative (*n*=6) Percussion examination: No sensibility (*n*=6) Palpation examination: Slight sensibility (*n*=6) Periapical radiolucent lesion(s): Yes (*n*=6) Diagnosis: PN, CAA and ST (*n*=1); PN and AP (*n*=1); AP and previously initiated endodontic treatment (*n*=3); AP and ERR (*n*=1)	Number of visits: 2 First visit: Instrumentation: N/D; irrigant: 20 mL of 1.5% NaOCl (*n*=6); irrigant activation: MD; type of medication: TAP (*n*=3); "Ledermix" + TAP (*n*=2); Ca(OH)2 + TAP (*n*=1); Temporary sealer: Interim restorative material (*n*=6) Second visit: Irrigant: 20 mL of 17% EDTA (*n*=6); Irrigant activation: MD; scaffold used: PRF and resorbable collagen plug (*n*=6); Bioactive material: White MTA (*n*=6); final restorative material: Glass ionomer (*n*=6)	Follow-up: 16, 23, 24, 30 months (*n*=6) Clinical success: Yes (*n*=6) Response to vitality tests: No (EPT, sensitivity) (*n*=6) Periapical healing: Yes (*n*=6) Apex closure: Yes (*n*=6) Root lengthening: Yes (*n*=6) Root canal thickening: Yes (*n*=6) Coronal discoloration: No (*n*=6)

Study characteristics: Author, year of publication, study design, journal, reference number, and funding. Population: Sample size (*n*); mean age (years old) and age range (years old); gender ratio (male/female); comorbidities. Teeth characteristics: Treated teeth (*n*); tooth type(s) (ISO classification); root development stage (description and Cveck's classification). Pretreatment signs and symptoms: Pulp tests (cold, heat, electric pulp tests); percussion and palpation examination (sensibility/slight sensibility/no sensibility); periapical radiolucent lesion(s) (yes/no) and diagnosis. Protocol of RET of root(s): Number of visits; instrumentation, type of irrigant; irrigant activation; type of medication (DAP/TAP/$Ca(OH)_2$); temporary sealer; and second visit: Type of irrigant; irrigant activation; scaffold used; final restorative material. Posttreatment outcomes: Follow-up (months or mean months); clinical success (yes/no); response to vitality tests (yes/no); root lengthening (yes/no; mm if any); root thickening (yes/no; mm if any); coronal discoloration (yes/no and number). #: Tooth number, RET: Regenerative endodontic treatment, MD: Missing data, N/A: Not available, N/D: Not defined, CPT: Cold pulp test, HPT: Heat pulp test, EPT: Electric pulp test, IRR: Internal root resorption, ERR: External root resorption, NaOCl: Sodium hypochlorite, CHX: Chlorhexidine, EDTA: Ethylenediaminetetraacetic acid MTA: Mineral trioxide aggregate, $Ca(OH)_2$: Calcium hydroxide, TAP: Triple antibiotic paste, DAP: Double antibiotic paste, PR: Platelet-rich fibrin, PRP: Platelet-rich plasma, b-FGF: Fibroblast growth factor-basic, PN: Pulp necrosis, AP: Apical periodontitis, CAA: Chronic apical abscess, ST: Sinus tract, IRM: Intermediate restorative material, ASA: American Society of Anesthesiologists, RCT: Randomized controlled trial, EG: Examination group, CG: Control group, PRF: Platelet-rich fibrin

Seven studies[42,43,45,51,54,56,61] reported the use of triple antibiotic paste on 67 teeth in total; one study[60] mentioned double antibiotic paste as a medicament on one tooth; calcium hydroxide was used on five teeth;[50,63]

calcium hydroxide and chlorhexidine gel were used on one tooth;[44] and double antibiotic paste or triple antibiotic paste or calcium hydroxide on 19 teeth was mentioned to be used by one study.[39]

The temporary sealer used was intermediate restorative material on 58 teeth, as reported by five studies;[39,43,50,54,61] intermediate restorative material and glass ionomer cement on six teeth, as cited by two studies;[51,63] zinc-oxide cement and composite resin on one tooth;[44] zinc oxide cement on 24 teeth;[56] Cavit G on one tooth as reported by one study;[45] and glass ionomer cement on two teeth, as reported by one study.[42]

As a scaffold, a blood clot was used by eight studies[39,42-44,51,55,56,63] on 50 teeth; blood clot and the collocate barrier were employed on one tooth;[45] blood clot and PRP were used on one tooth;[54] blood clot and injectable hydrogel scaffold with bFGF on 12 teeth in one study;[56] blood clot and absorbable adhesion barrier on four teeth;[50] PRF was used on 13 teeth, as reported by two studies;[60,61] and PRP on 13 teeth.[42,61]

The final restorative material employed was composite resin on 36 teeth;[42,43,56,60] bonded resin on 20 teeth;[39,54] glass ionomer cement and composite resin on 27 teeth;[51,61] zinc oxide cement and composite resin on 1 tooth;[44] cavity-G and resin on 1 tooth;[45] glass ionomer cement on 8 teeth;[50,55] polycarboxylate cement base and resin composite on 1 tooth;[63] and on 3 teeth[51] metal crown restorations.

Thirteen studies[39,42-45,50,51,54-56,60,61,63] reported to have done follow-up recalls, with a range between 1 and 48 months, carried out on 86 teeth in total.

Clinical success was stated in 77 teeth, as reported by 10 studies[39,42-45,51,55,56,61,63] failure was declared in 12 teeth by 5 studies.[39,50,54,56,60]

Response to vitality test was as follows: no sensibility to cold and electric pulp test and percussion and palpation was found in three teeth as reported by three studies;[44,45,63] negative response to cold, heat, and electric pulp test in 26 teeth was mentioned by two studies,[42,61] whereas a positive one was found in 1 tooth;[42] no response to cold, but a positive one to electric pulp test was found in 1 tooth as reported by one study.[60]

Periapical healing was assessed by eight studies:[42,43,45,50,51,54,55,63] an amount of 19 teeth[42,43,45,51,55,63] showed healing, whereas 5[50,54] showed none.

Nine studies[43-45,50,51,55,56,61,63] mentioned apex closure evaluation, as follows: 64 teeth showed apex closure, as reported by nine studies;[43-45,50,51,55,56,61,63] 2 teeth[51] showed not yet completed apex closure; and 1[51] showed no closure.

Posttreatment root lengthening was assessed by 10 studies,[39,43-45,50,51,55,56,60,61] finding an increased length in 78 teeth. Root thickening was evaluated by 11 studies,[39,42-45,50,51,55,56,61,63] finding an increased thickness in 84 teeth.

Coronal discoloration was reported in four studies,[39,51,60,61] of which only for three,[39,51,60] it was possible to deduce the number of teeth involved, which was four.

The success rate according to the AAE criteria reported in the studies using MTA as a bioactive material is depicted in Figure 2.

White mineral trioxide aggregate

The use of white MTA as a bioactive material to perform pulp regeneration has been reported in 11 studies.[37,38,40,46,48,49,52,53,62,65,66]

The total sample of the 11 studies[37,38,40,46,48,49,52,53,62,65,66] examined included 143 subjects, with a mean of 9.28 years old and an age range between 7 and 13.

For nine studies,[37,38,46,49,52,53,62,65,66] it was possible to obtain the male-to-female ratio, which consisted of 58 males and 49 females (male:female = 1.18:1).

Four studies[37,40,53,65] reported no presence of comorbidities.

A total of 148 teeth[37,38,40,46,48,49,52,53,62,65,66] were treated: 5[38,46,49,53] were maxillary right central incisors; 3[38,52,53] maxillary left central incisors; 97[37,65] maxillary incisors; 13[48] central incisors; 1[49] maxillary lateral right incisor; 13[40] permanent anterior teeth; 1[37] a maxillary first premolar; 3[37] mandibular first premolars; 1[62] a maxillary second premolar; 5[37,48] mandibular second premolars; 5[66] mandibular first molars; and 1[66] maxillary first molar.

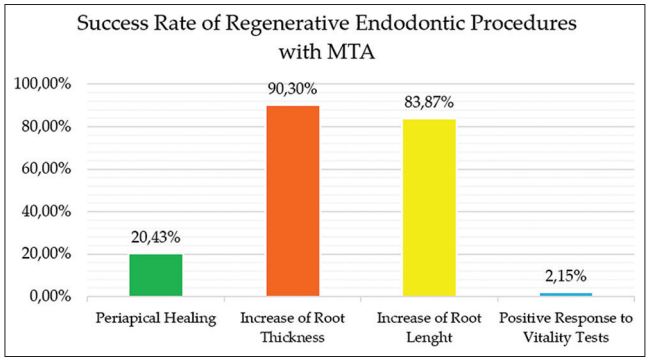

Figure 2: American Association of Endodontists criteria of success rate (periapical healing, increased root thickness and length, and positive response to vitality tests) of regenerative endodontic procedures with mineral trioxide aggregate. MTA: Mineral trioxide aggregate

Incomplete root development and open apices status were reported by eight studies[37,38,40,46,48,53,62,65,66] in 144 teeth; immature roots development in three teeth was mentioned by one study;[49] and Cvek's development Stage II was stated to be present in one tooth, as mentioned by one study.[52]

Seven studies[37,38,49,52,53,65,66] reported a negative response to the cold pulp test for 117 treated teeth. The electric pulp test was negative for 95 teeth, as mentioned by 7 studies,[48,49,52,53,62,65,66] and positive for 5, as reported by one.[48] Percussion examination registered sensibility in 29[37,52,53] teeth, whereas 12[38,46,49,66] proved no sensibility, and 6[66] had a slight sensibility. Palpation examination registered sensibility in 29[37,52,53] teeth, whereas 6[38,46,49] showed no sensibility. Four studies[37,48,49,66] reported periapical radiolucid lesions for 47 teeth, and 10[37,38] had none.

The diagnosis was pulp necrosis in 120 teeth;[37,38,40,48,65] apical periodontitis in 12;[46,48,66] chronic periapical abscess in 5;[48] internal root resorption with apical periodontitis in 1;[48] pulp necrosis with apical periodontitis in 4;[49,66] complicate crown fracture with sinus tract and internal root resorption in 1;[52] intrusion associated with pulp necrosis and acute apical abscess in 1;[53] pulp necrosis, acute apical abscess, and not complicated enamel fracture in 1;[53] irreversible pulpitis, apical periodontitis, and dens evaginatus in 1;[62] and pulp necrosis, chronic apical abscess, and sinus tract in 1.[66]

Pulp regeneration treatments were made by three studies in a single-visit approach,[38,46,62] seven in a two-visit,[37,40,48,49,52,65,66] and one in a three-visit.[53]

No instrumentation was performed on 79 teeth,[38,49,52,65] minimal instrumentation was carried out on one,[62] and a hand file was made on one[53] tooth.

As irrigant, NaOCl was used on 108 teeth in different volumes and concentrations, as reported by four studies;[40,48,65,66] NaOCl and EDTA were used on eight teeth in different volumes and concentrations, as mentioned by five studies;[46,49,52,53,62] the use of NaOCl and chlorhexidine on 30 teeth was cited by one study;[37] and chlorhexidine gluconate and physiological solution were reported by one study[38] on two teeth.

The medication type was double antibiotic paste on 13 teeth;[40] triple antibiotic paste on 125 teeth in four studies;[37,48,65,66] calcium hydroxide on 1;[52] 2% of chlorhexidine gel on 1;[53] triamcinolone acetonide and demeclocycline paste ("Ledermix") with triple antibiotic paste in 2;[66] and calcium hydroxide and triple antibiotic paste in 1.[66]

The temporary sealer used was as follows: glass ionomer cement on 76 teeth, as mentioned by two studies;[49,65] intermediate restorative material on 19 teeth, as reported by two studies;[40,66] Cavit temporary restorative material on two teeth as cited by one;[38] reinforced zinc-oxide eugenol cement on 30 teeth, as evidenced by one study;[37] Cavit and glass ionomer cement on 16 teeth, as mentioned by one;[48] Cavit G on one tooth, reported by one;[52] and resin-modified glass ionomer on two teeth,[62] detected by one study.

As a scaffold, a blood clot was used by six studies[37,38,40,48,53,65] on 69 teeth in total; PRP was mentioned by two studies[37,65] on 33 teeth; PRF was used on 20 teeth, as reported by two studies;[49,65] platelet pellet on 17 teeth has been used as stated by one study;[65] blood clot with absorbable adhesion barrier was used on one tooth, as reported in one study;[46] blood clot with collagen sponge on one tooth, as cited by one study;[52] and PRF with resorbable collagen plug was used on six teeth as reported by one study.[66]

The final restorative material used hereafter: glass ionomer cement on 95 teeth, as reported by three studies;[48,65,66] glass ionomer cement and composite resin on 32 teeth, as mentioned by three studies;[37,46,52] bonded resin on five teeth, as explicated by two studies;[38,49] composite resin on 13 teeth, reported by one study;[40] resin-modified glass ionomer on two teeth, mentioned by one study;[53] and polycarboxylate cement base and resin composite restoration on one tooth, as reported by one study.[62]

Ten studies[37,38,40,46,48,49,52,53,65,66] reported a follow-up, with a range between 3 and 144 months, carried out on a total of 138 teeth.

Clinical success was reported for 141 teeth,[37,38,40,48,49,52,62,66] and failure was declared for six teeth, as mentioned by four studies.[40,46,53,65] For one study,[38] the clinical success or failure was not declared, as the follow-up was lost.

The posttreatment response to the vitality test and sensibility to percussion and palpation was mentioned by six studies,[37,49,53,62,65,66] as follows: 82 teeth[37,65] were responsive to cold pulp test and electric pulp test, 12[37,65] were not responsive; 9[49,66] were not responsive to cold pulp test or palpation/percussion; 1[62] was responsive to electric pulp test; and 2[53] teeth were not responsive to palpation/percussion.

Periapical healing was assessed by seven studies:[37,48,49,52,62,66] 52 teeth[37,48,52,62,66] healed, and 3[49] healed periodically or periapical lesions decreased in size.

Apex closure evaluations were reported by 10 studies,[37,38,46,48,49,52,53,62,65,66] which referred to achieving a successful closure for 114 teeth,[37,38,48,49,52,53,62,65,66] an ongoing closure for 3 teeth,[65] and no closure for 17 teeth.[46,65]

Root lengthening was mentioned by 8 studies: [37,38,40,48,49,52,65,66] 131[37,38,40,48,65,66] teeth increased their root length, whereas 4[49,52] showed none. Root thickening was assessed by eight studies[38,46,48,49,52,62,65,66] as follows: 92 teeth[38,46,48,52,62,65,66] showed an increased thickness, and 3[49] did not exhibit any.

Seven studies[40,46,48,52,53,65,66] evaluated posttreatment coronal discoloration, which was present in 23 teeth,[40,46,48,52,62] whereas in 8,[53,66] no discoloration was registered.

The success rate according to the AAE criteria reported in the studies using white MTA as a bioactive material is shown in Figure 3.

Gray mineral trioxide aggregate

Gray MTA was used in one study.[47] The sample consisted of a 17-year-old male subject with noncontributory comorbidities.[47]

A total of 2 teeth[47] were treated, one maxillary right central incisor and one maxillary left central incisor; immature roots with open apices were reported in both.

Cold and electric pulp tests were negative; no sensibility to percussion and slight sensibility to palpation exams were reported for the two teeth.[47] The study[47] reported the presence of periapical radiolucid lesions, and the diagnosis was pulp necrosis.

The pulp regeneration treatment was carried out with a single-visit approach for the two teeth.[47] For both teeth,[47] the treatment was instrumentation with the use of K files; 20 mL of 5.25% NaOCl, physiological saline, and 17% EDTA for irrigation; the irrigant activation was performed through photoactivated disinfection with diode laser; the scaffold used was PRF, and the final restorative material was coltosol and composite resin.

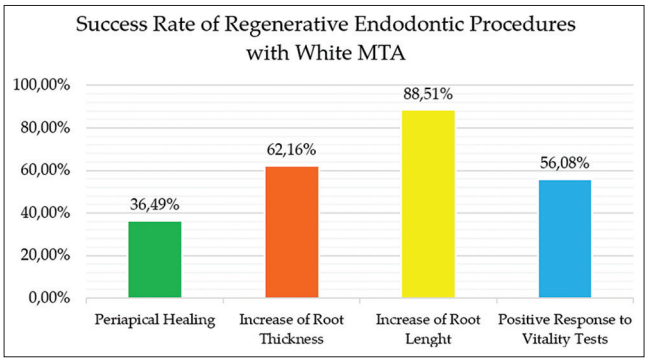

Figure 3: American Association of Endodontists criteria of success rate (periapical healing, increased root thickness and length, and positive response to vitality tests) of regenerative endodontic procedures with white-Mineral trioxide aggregate. MTA: Mineral trioxide aggregate

The study[47] had follow-ups for two teeth at 6 and 10 months, with one clinical success and one failure. Neither tooth[47] was responsive to electric, palpation, or percussion tests. Periapical healing was found for one tooth,[47] but none for the other. Apex closure and increased root lengthening and thickening occurred in both teeth.[47]

Biodentine

Biodentine for pulp regeneration treatment was used in a total of two studies.[40,64]

The total sample size was 27,[40,64] with a mean age of 8.66 years old and an age range between 8 and 9.89. No comorbidities were reported in one study.[40]

A total of 16 teeth were treated, of which 13 were permanent anterior teeth,[40] two were mandibular right first molars, and one was mandibular left first molar.[64] Both studies[40,64] reported immature roots with open apices.

One study[64] reported a negative response to cold and electric pulp tests, no sensibility to palpation, and no periapical radiolucent lesions for the three treated teeth.

The diagnosis[40,64] was pulp necrosis for all 16 teeth treated with Biodentine.

One study[40] performed two visits to achieve pulp regeneration, whereas the other[64] implemented a one-visit approach.

No instrumentation was carried out for three teeth,[64] and both studies[40,64] used NaOCl as an irrigant but in different concentrations and volumes.

A double antibiotic paste consisting of equal amounts of metronidazole and ciprofloxacin was used in 13 treated teeth, followed by a dry cotton pellet and intermediate restorative material as a temporary sealer. In contrast, 20 mL of 17% EDTA was used as an irrigant during the second visit, as reported by one study.[40]

One study[40] employed blood clots as a scaffold; PRP was used in the other.[64]

Both studies[40,64] have chosen composite resin as a final restorative material and conducted follow-up visits at 3, 6, 9, and 12 months, with clinical success in 16 teeth.[40,64]

One study[40] assessed an increase in root lengthening in 13 teeth, with a mean value of 0.7 ± 0.32 mm and one case of coronal discoloration was found.

For the three elements treated in one study,[64] no response to vitality tests, percussion, or palpation was found. An increase in root canal thickening was reported in 3 teeth.[64]

Repair high plasticity mineral trioxide aggregate

Repair high plasticity (HP) MTA was used by one study[41] to treat one tooth.

The sample size reported by the study[41] was one 6-year-old male subject. Pulp regeneration treatment was carried out on one tooth, a maxillary left central incisor, with incomplete root formation, as mentioned by the study.[41]

The treated teeth responded negatively to cold pulp tests, but they showed sensibility to palpation and percussion and the presence of a radiolucent periapical lesion.[41] The diagnosis detected in the study[41] was of pulp necrosis and sinus tract.

A two-visit approach was performed as follows: the irrigant was NaOCl and EDTA; the medication was calcium hydroxide, and Coltosol and resin composite were employed as temporary sealers. The scaffold was a blood clot, and the final restorative material was glass ionomer cement and resin composite.[41]

The follow-ups ranged from 6 to 36 months,[41] and clinical success was achieved. The element responded negatively to the cold pulp test and showed increased root lengthening and thickening.[41] No coronal discoloration was recorded.[41]

Overall results

In the population investigated, which amounted to 312 subjects,[37-66] the absence of comorbidities was reported by 10 studies in 183 subjects.[37,40,43,45,53,55-57,61,65]

A total of 273 teeth have been treated with pulp regeneration treatment, as mentioned by 29 studies;[37-58,60-66] 181 were central incisors;[37,38,41-47,49-53,55-58,60,61,65] 15 lateral incisors;[48,50,56,57,63] 45 anterior teeth;[39,40] 4 first premolars;[37] 8-second premolars;[37,48,50,62] and 20 first molars.[43,51,54,64,66]

The root development was: 213 teeth incomplete root development and open apices;[37,40,46,48,52,54-56,60-62,64] 13 teeth immature roots;[49-51,63] 3 teeth incomplete root formation;[41,44,45] 4 teeth belonged to Cvek's Stage II;[49,57,60] 13 to Cvek's Stage III;[57,60] 5 to Cvek's Stage IV;[57,60] 1 to Cvek's Stage V;[58] and19 from a Stage I to IV.[39]

The presence or absence of radiolucent periapical lesions was assessed by 20 studies,[37-39,41-43,45,47-51,54,55,57,58,60,63,64,66] which reported its presence in 86 teeth[37,39,41-43,45,47-51,54,55,57,58,60,63,66] and none in 27 teeth.[37-39,57,64]

Instrumentation was minimal or none on 146 teeth[38,39,42,49,51,52,56,57,62-65] a k-file or a hand-file was performed on 30 teeth.[44,47,53,54,61]

As irrigant NaOCl in different volumes and concentrations was used on 148 teeth, as mentioned by 13 studies;[40,42,48,50,51,54,56-58,62,64-66] NaOCl and EDTA in different volumes and concentrations were employed on 47 teeth;[41,43,46,47,49,52,53,55,60,61,63] chlorhexidine and sterile saline were used on three teeth;[38,44] NaOCl and chlorhexidine in different volumes and concentrations were used on 31 teeth, as reported by two studies[37,45] and different amount, concentrations, volumes, and combinations of NaOCl, chlorhexidine, or EDTA was used on 19 teeth, as reported by one study.[39]

Irrigant activation was reported by three studies[47,55,63] on four teeth, with a photoactivated activation with a diode laser on two teeth and an ultrasonic activation on two teeth.

The type of medication was double antibiotic paste on 40 teeth, as mentioned by four studies;[40,57,58,60] triple antibiotic paste on 192 teeth, as reported by eleven studies;[37,42,43,45,48,51,54,56,61,65,66] calcium hydroxide on seven teeth;[41,50,52,63] calcium hydroxide and chlorhexidine gel on one tooth;[44] 2% of chlorhexidine gel in one tooth;[53] calcium hydroxide and triple antibiotic paste in 1 tooth;[66] triamcinolone acetonide; and demeclocycline paste ("Ledermix") with triple antibiotic paste in two teeth.[66]

As a scaffold, blood clot was employed on 145 teeth;[37-44,48,51,53,55-57,63,65] PRP was used on 50 teeth;[37,42,58,61,64,65] PRF on 35 teeth;[47,49,60,61,65] platelet pellet on 17 teeth;[46] blood clot and collacote barrier on 1 tooth;[45] blood clot and PRP on 1 tooth;[54] blood clot and injectable hydrogel scaffold with bFGF in 12 teeth;[56] blood clot and absorbable adhesion barrier in 4 teeth;[50] blood clot and collagen sponge on 1 tooth;[52] and PRF and resorbable collagen plug on 6 teeth.[66]

MTA was used in 93 teeth in 14 studies;[39,42-45,48,50,51,54-56,60,61,63] white MTA for 148 in 11 studies;[37,38,40,46,48,49,52,53,62,65,66] gray MTA for 2;[47] biodentine for 16 in 2 studies;[40,64] and repair HP MTA for one tooth in 1 study.[41]

Clinical success evaluation was: clinical success in 249 teeth[37-45,47-49,51,52,55-58,61-64,66] and failure in 19 teeth.[39,40,46,47,50,53,54,56,60,65]

The posttreatment response to vitality tests can be grouped as follows: negative response for a total of 45 teeth[37,40,42,44,45,47,49,57,61,63-66] (which did not respond to cold, heat, or electric tests); a positive response in 90 teeth[37,42,57,58,62,65] (which responded to cold, or heat, or electric tests); and an unclear response on 4 teeth.[57]

Periapical healing was stated to be present in 84 teeth;[37,42,43,45,47,48,51,52,55,57,58,62,63,66] complete healing or a decrease in lesion size was present in 3 teeth;[49] and no healing was found in a total of 6 teeth.[47,50,54]

Apex closure was mentioned as follows: 222 tee th[37,38,43-45,47-49,51-53,55-57,61-63,65,66] showed a complete apex closure; ongoing apex closure was stated in 3 teeth;[51,65] and no closure was found in 18 teeth.[46,51,65]

An increase in root length was found in 223 tee th;[37-41,43-45,47,48,50,51,55,56,60,61,65,66] no increase in 16 teeth.[49,52,57]

An increase in root thickening was found in 194 tee th,[38,39,41,43-48,50-52,55-57,60-62,64-66] whereas none was found in 8 teeth.[53,66]

Coronal discoloration was found in 31 teeth[39,40,46,48,51,52,57,60,62] and none in 9.[41,53,66]

Success rates according to the AAE criteria in the studies included in the present systematic review are shown in Figure 4.

Quality assessment

The RoB of the included nonrandomized studies is listed in Table 2.

The RoB of the included randomized studies is listed in Table 3.

The RoB of the included case report is listed in Table 4.

The RoB of the included case series is listed in Table 5.

DISCUSSION

The present systematic review aimed to evaluate the success rate of pulp regeneration treatment according to the AAE criteria in pediatric subjects (between 6 and 17 years of age) in permanent teeth using different types of bioactive material.

Pulp regeneration is a biological procedure intended to replace damaged dental structures, including dentin and roots and the cells of the pulp–dentin complex.[18] To perform pulp regeneration treatment, it is important to determine the case selection properly,[24] especially in patients with systemic diseases such as hemophilia or von Willebrand disease, which can affect blood clotting, or patient taking drugs such as anticoagulants due to uncontrolled bleeding, or subjects with systemic disease inhibiting healing,[21] impaired immune diseases, uncontrolled diabetes or hypertension, recent myocardial infarction, coronary diseases, and prolonged use of hormone drugs.[21,24]

In the population examined in the present systematic review, which amounted to 312 subjects, no comorbidities were reported in 183 subjects.

Age represents another aspect to highlight in pulpal regeneration because some evidence[24,28,33,67] showed that younger subjects had better results than the older ones, which might be because of a better healing capacity and a higher proliferation and differentiation of mesenchymal stem cells (MSCs).

For this reason, the age range in this systematic review has been set up between 6 and 17 years of age, also considering that regenerative endodontics is not recommended for deciduous teeth[21,28] and since the youngest age at which a permanent element erupts is 6 years of age,[29,30]

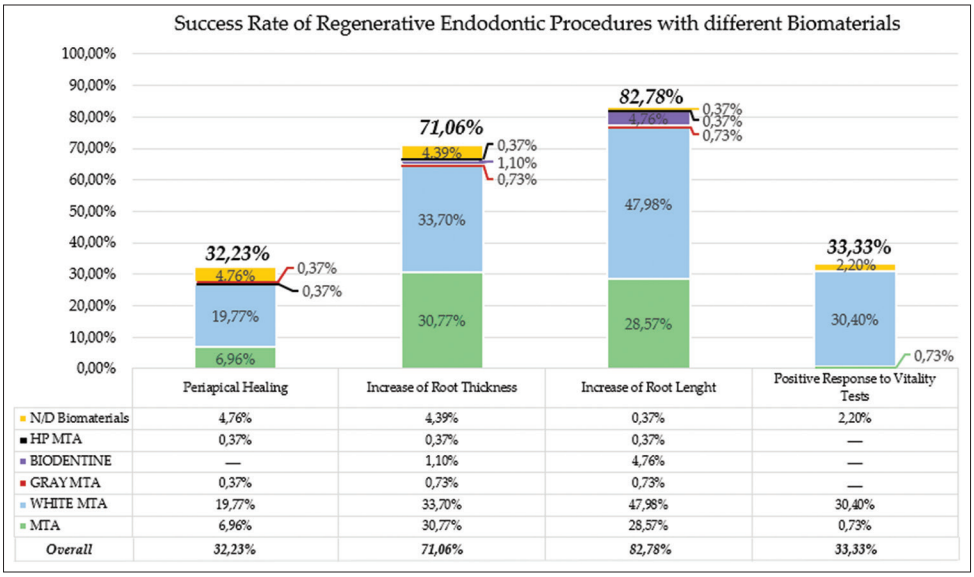

Figure 4: Percentage of American Association of Endodontists success rate (periapical healing, increased root thickness, and length and positive response to vitality tests) of regenerative endodontic procedures with different biomaterials. MTA: Mineral trioxide aggregate, HP MTA: High plasticity MTA

Table 2: Risk of bias of included studies sorted alphabetically

Study	Bias due to confounding	Bias in the selection of participants	Bias in the classification of intervention	Bias due to deviations from intended intervention	Bias due to missing data	Bias in measurement of outcomes	Bias in selection of the reported result	Overall quality judgments
Alagl *et al.*, 2017[37]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Alobaid *et al.*, 2014[39]	Low risk	Low risk	Moderate risk	Moderate risk	Moderate risk	Serious risk	Low risk	Serious risk
Caleza-Jiménez *et al.*, 2022[43]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Kahler *et al.*, 2014[48]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk
Nazzal *et al.*, 2020[57]	Low risk	Low risk	Moderate risk	Low risk	Low risk	Low risk	Low risk	Moderate risk
Ulusoy *et al.*, 2019[65]	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk	Low risk

According to the domains of the ROBINS-I assessment tool, the options were - Y: Yes, PY: Probably yes, PN: Probably no, N: No, NI: No information. ROBINS-I: Risk of bias in non-randomized studies of interventions

Table 3: Risk of bias of included studies sorted alphabetically

	Aly *et al.*, 2019[40]	Nagy *et al.*, 2013[56]	Rizk *et al.*, 2020[61]
Randomization process (item 1)			
1.1	Yes	NI	Yes
1.2	Yes	PN	PY
1.3	No	NI	NI
Effect of assignment to intervention (item 2.a)			
2.1	No	PN	NI
2.2	PY	PY	Yes
2.3	No	PN	PN
2.4	PN	PY	NA
2.5	PY	NA	Yes
2.6	Yes	PY	Yes
2.7	NA	NA	NA
Effect of adhering to intervention (item 2.b)			
2.1	No	PN	NI
2.2	PY	PY	Yes
2.3	No	PN	No
2.4	No	PN	No
2.5	No	PN	No
2.6	PY	PY	Yes
Missing outcome data (item 3)			
3.1	Yes	Yes	Yes
3.2	NA	NA	NA
3.3	NA	NA	NA
3.4	PY	No	No
Measurement of the outcome (item 4)			
4.1	No	PY	PN
4.2	No	No	PN
4.3	Yes	NI	No
4.4	PN	PN	NA
4.5	No	PN	NA
Selection of the reported studies (item 5)			
5.1	Yes	Yes	No
5.2	PN	PN	No
5.3	No	No	PN
Overall risk of bias	High risk	High risk	Some concern

According to the domains of the RoB-2 assessment tool, the options were: Yes, PY, PN, no, NI. PY: Probably yes, PN: Probably no, NI: No information, RoB-2: Risk of Bias-2

it represents then the minimum subjects' age of choice. In addition to that, although pulp regeneration was carried out in subjects older than 17 years old,[31] there is evidence of a greater risk of complications or failure of the procedure,[18,32,33] along with the knowledge that regenerative potential is limited in adult permanent teeth and decreases with aging,[34,35,68] which led to determine the cutoff of 17 as the maximum age.[21] Since pulp regeneration treatment is most frequently carried out in pediatric subjects,[24] it is important to establish proper management and select patients to ensure compliance is achieved[69] so that multiple visits and follow-ups can be sustained.[24]

In the case selection, the evaluation of the root development and the apical diameter size should be considered.[22,24] Root development was classified into five stages by Cvek's classification:[1] regenerative endodontic therapy is recommended for immature permanent teeth at Stage 1 (less than half of root formation and open apex),

Table 4: Risk of bias of included studies sorted alphabetically

Study	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8
Alsofi *et al*., 2019[38]	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes
Alencar *et al*., 2022[41]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Arango-Gómez *et al*., 2019[42]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
De Jesus *et al*., 2013[44]	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes
Dhiman *et al*., 2018[45]	Yes	Unclear	Yes	Yes	Yes	Yes	NA	Yes
D' Mello *et al*., 2017[46]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Johns *et al*., 2014[47]	Yes	NA	Yes	Yes	Yes	Yes	NA	Yes
Loroño *et al*., 2021[52]	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes
Maniglia-Ferreira *et al*., 2020[53]	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes
Martin *et al*., 2013[54]	Yes	N	Yes	Yes	Yes	Yes	Yes	Yes
McCabe *et al*., 2015[55]	Yes	NA	Yes	Yes	Yes	Yes	NA	Yes
Priya *et al*., 2016[58]	Yes	Yes	Yes	Yes	Yes	Yes	NA	Yes
Ravikumar *et al*., 2021[59]	Yes	Yes	Yes	Unclear	Yes	Yes	NA	Yes
Ray *et al*., 2015[60]	Yes	Yes	Yes	Yes	Unclear	Yes	Yes	Yes
Timmerman *et al*., 2017[63]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Timmerman *et al*., 2018[62]	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Yes

According to the domains of the JBI critical appraisal checklist for case reports, the options were: Yes, no, unclear, NA. JBI: Joanna Briggs Institute, NA: Not applicable

Table 5: Risk of bias of included studies sorted alphabetically

Study	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10
Kandemir *et al*., 2020[49]	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	NA
Lin *et al*., 2018[50]	Yes	Yes	Unclear	Unclear	Yes	Yes	Yes	Yes	Yes	NA
López *et al*., 2017[51]	Yes	Unclear	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	NA
Topçuoğlu *et al*., 2016[64]	Yes	Yes	Yes	Unclear	Yes	Yes	Yes	Yes	Yes	NA
Yoshpe *et al*., 2021[66]	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	NA

According to the domains of the JBI critical appraisal checklist for case series, the options were: Yes, no, unclear, NA. JBI: Joanna Briggs Institute, NA: Not applicable

Stage 2 (half of root formation and open apex), and Stage 3 (two-third of root development and open apex)[15,24] because of the short root, thin canal walls, and wide-open apex which can be more prone to the canal walls thickening and continue root development.[15,70] Cvek's Stage 4 (almost completed root formation and open apex)[1] can be treated either with pulp regeneration or apexification.[15,24] In contrast, for Stage 5 (completed root development and closed apical foramen),[1] conventional root therapy is recommended.[15,70]

Apical diameter size has been a vexed question in literature,[15,70] as some studies[28,71] report better results in teeth that preoperatively had wide-open apex (>1 mm) in terms of root thickness, length, and apical narrowing due to an easier migration of MSCs into the root canal. Nevertheless, it also succeeded in smaller diameters,[72] showing periapical healing, continued root development, and the highest clinical success in apical diameters between 0.5 and 1 mm.[73] The root development stage found in the current study was as follows: 213 teeth with incomplete root development and open apices, 13 teeth with immature roots, 3 teeth with incomplete root formation, 4 teeth belonged to Cvek's Stage II, 13 to Cvek's Stage III, 5 to Cvek's Stage IV, 1 to Cvek's Stage 5, and 19 belonged variously from a Stages I to IV.

Teeth with incomplete development and open apices, immature roots, and teeth belonging to Stages I to IV showed comparable results at follow-up in this study in terms of treatment success, absence of complications, and continuing root development. In addition, the tooth belonging to Stage V of Cvek's classification achieved successful regenerative treatment without postoperative complications and a positive response to the vitality test.

According to clinical guidelines on regenerative endodontics, minimal or no instrumentation is required,[22,25] as mechanical preparation could undermine the fragile root walls of immature teeth, increasing the risk of fractures.[3,74] In the present study, minimal or no instrumentation was done on 146 teeth, whereas a k-file or a hand-file one on 30.

Minimal or no instrumentation may not ensure microorganism removal from the endodontic system,[75] which can lead to a pulp regeneration failure.[76] Since the main purpose of endodontics is to remove bacterial biofilm,[77] as a consequence, it is essential to improve irrigation procedures to overcome the instrumentation limitations.[78]

The AAE[22] recommends using NaOCl as an irrigant in lower concentrations (1.5% NaOCl), followed by EDTA 17%, to perform a regenerative procedure.

Although success in regenerative endodontics was found using irrigant concentrations which deviate

from the guidelines,[79] it was also reported that NaOCl had concentration-dependent effects on stem cells, reducing their survival,[80] differentiation potential, and growth-factor release from dentin, which are important for promoting pulp regeneration.[81] Concerning this, it is advisable to maintain NaOCl concentrations between 1.5% and 3%, as suggested by the AAE and the European Society of Endodontology (ESE).[22,25] In the studies included in this systematic review, NaOCl was used on 148 teeth in different concentrations and volumes. In comparison, NaOCl followed by EDTA 17% was employed on 31 teeth, and chlorhexidine as an irrigant was reported in 3 teeth, of which 2 reported successful regenerative treatment in terms of apex closure and root lengthening and thickening and no complications at follow-up. However, chlorhexidine is not advisable in pulp regeneration, as there is evidence of its cytotoxic effect on stem cells[82] and its incapacity of tissue dissolution.[83]

It was demonstrated that the regenerative procedure was more prone to fail if the necrotic tissue was not properly removed, as it is a potential substrate for infection.[21,32] However, it may be possible that not all the infected and necrotic tissue is accessible by irrigation alone, as the endodontic system includes lateral canals, loops, isthmuses, and ramifications. Thus, remnant tissue and biofilms in such anatomies could be an infection source.[84] From this perspective, irrigant activation methods could be used to increase the efficacy of disinfection in root canals.[21] Irrigant activation can consist of several methods, such as subsonic, sonic, and ultrasonic activation and preheating of the irrigant.[85]

In the case of teeth with open apex, it is not advisable to perform irrigation actively due to the risk of irrigant extrusion.[86,87] For this reason, the application of negative apical pressure has been proposed as a safer method for immature or open apices.[85] Even though applying negative pressure can minimize extrusion through the apical foramen,[24] this technique is not as powerful as the others in accessing the lateral anatomies.[85] In the case of immature teeth with open apices and periapical lesions, such as those found in this study in 86 teeth, one possibility could be the application of the CAB technique, which consists of the application of a collagen apical barrier, to ensure irrigant activation without risks of extrusion.[85] It has also been demonstrated that including the diode laser in the AAE regenerative guidelines improved disinfection and periapical healing.[88] On this basis, irrigant activation through different methods could be widely investigated to perform pulp regeneration. In the present study, irrigant activation was mentioned on four teeth in total, which in particular employed a photoactivated activation with a diode laser on two teeth and an ultrasonic activation on two teeth as well.

In pulp regeneration treatment, different types of intracanal medicament were proposed, such as triple antibiotic paste (TAP), double antibiotic paste (DAP), and $Ca(OH)_2$.[23]

The use of topical antibiotics to sterilize root canals was proposed by Grossman[89] for the first time, and triple antibiotic paste has also been reported.[90] Combining agents such as triple or double antibiotic paste could nevertheless bring side effects.[15] The antimicrobial combination can indeed increase the risk of antibiotic resistance[91] or allergic reaction, as the root canal could act like a systemic sensitization pathway.[92] The use of triple antibiotic paste has been recommended by AAE.[22] Antimicrobial combinations could synergistically affect polymicrobial infections.[22,93] TAP comprises a 1:1:1 mix of ciprofloxacin, metronidazole, and minocycline.[22] Ciprofloxacin stops DNA gyrase synthesis, metronidazole inhibits DNA synthesis, and minocycline suppresses protein synthesis of microbes, which means that these medicaments are effective when microbes are in an active state of replication and not in a stationary state, so residual bacteria could actually persist in the canal space after the medication.[90] However, the efficacy of TAP in disinfecting necrotic root canals has been proven.[94,95] The AAE guidelines[22] recommend using TAP in a final concentration between 0.1 and 1.0 mg/ml, as there is evidence that higher concentration could prejudice stem cells from the apical papilla (SCAPs). From this perspective, it has been demonstrated *in vitro* that amoxicillin and clavulanic acid can kill 100% of microorganisms isolated from infected root canals, as these two active drugs inhibit the bacterial cell walls, not DNA synthesis or protein synthesis. Since human cells are devoid of cell walls, the medicament would be active against the bacterial cells alone.[15,96] Amoxicillin and clavulanic acid might represent an open scenario for further *in vivo* studies to avert any possible damage toward stem cells. Another consideration is that TAP can lead to tooth discoloration because of minocycline.[22]

The AAE[22] suggests using a DAP (ciprofloxacin and metronidazole) or replacing minocycline with clindamycin, amoxicillin, or cefaclor. When TAP is used, a dentin bonding agent should seal the chamber before the medicament, which should be placed below the cementoenamel junction, to minimize the possibility of crown staining.[22]

Calcium hydroxide is another intracanal medicament used in pulp regeneration.[22,25] It has advantages over antibiotic paste-based medicaments, such as no discoloration, lower cytotoxicity to stem cells, and growth factors release.[15] Based on this evidence, the ESE suggests $Ca(OH)_2$ as the prior intracanal medicaments in pulp regeneration.[25] However, it has also been reported that there is less efficacy of $Ca(OH)_2$ than TAP in antibacterial capacity.[24,97] A meta-analysis[98] showed that antibiotic paste-based medicaments promoted

higher root thickening, whereas $Ca(OH)_2$ an increased apex closure.

In the present study, the double antibiotic paste was employed on 40 teeth, triple antibiotic paste on 192, calcium hydroxide on 7, calcium hydroxide, and chlorhexidine gel on one.

A similar success rate of treatment using TAP or DAP has been found in this study. However, TAP was associated with a higher discoloration rate, which is likely to be related to minocycline content. However, teeth treated with DAP also showed discoloration in some cases, probably due to the type of bioactive material used. $Ca(OH)_2$, used in 7 teeth, showed 4 cases of pulpal regeneration treatment failure and 2 cases of discoloration of the tooth.

Moreover, triamcinolone acetonide and demeclocycline paste ("Ledermix") with triple antibiotic paste were mentioned in two teeth. However, there is evidence that Ledermix can lead to discoloration, which appears to be higher in immature teeth.[99]

During the regenerative endodontics procedure, the induction of intracanal bleeding determines a blood clot formation, which acts as a scaffold promoting growth factors and stem cell migration from the apical region to the canal lumen to ensure tissue regeneration.[24] By the introduction of scaffolds, which in the base of the origin can be natural (e.g., blood clot, PRP, and PRF) or artificial (e.g., polymers),[100] vital tissue formation is promoted, ensuring the mineral deposition through which dentin is strengthened, and roots of immature teeth grow.[32,101] PRP and PRF represent a valid alternative to blood clots.[22] Platelet-rich plasma is a source of growth factors as PDGF, TGF-B, IGF, and VEGF, released by the degranulation of alpha granules and stimulating bone and soft-tissue healing.[102] However, some disadvantages include blood collection from young patients, the equipment to prepare PRP, and the cost of treatment.[102] PRF contains a great number of cytokines, glycoproteins, and glycans, which encourage healing processes and guide angiogenesis.[103] Evidence on the outcomes of pulp regeneration on various scaffolds is contradictory.[21] According to Lolato *et al*.,[104] PRP- or PRF-induced continued root development in pulp regeneration treatments is better than blood clots. This statement might be explained considering that blood clots have fewer cytokines than PRP and PRF, provide slower healing than PRP and PRF, and have fewer growth factors.[100]

It has also been reported that PRP and PRF were more successful than bleeding induction for apical closure, periapical healing, and root lengthening.[105] Prasad *et al*.[106] assessed the different outcomes between PRF or PRP in pulp regeneration. The two scaffolds provided dental wall thickening, root lengthening, and apical closure in upper incisors, but only PRF-induced apical bridge formation. Such a conclusion might be explained because PRF has a higher cytokine concentration than blood clots and PRP. It induces faster and stronger healing than PRP and determines the proliferation and differentiation of bone marrow stem cells (BMSCs). PRP also inhibits the differentiation of BMSCs, and in PRF, the fibrin matrix is stronger and more stable than the PRP one.[100]

However, it has also been mentioned that PRP was better than PRF or blood clots in periapical healing, whereas no significant differences in root lengthening or thickening were found.[107] It was also reported that there was no significant improvement in outcomes when comparing blood clots alone to the blood clots with PRF during regenerative treatments.[108]

Blood clots, autologous platelet concentrates, and synthetic materials can be used as scaffolds in pulp regeneration treatment. However, the most commonly used ones are blood clots and platelet concentrates.[24]

In the studies included in the systematic review, the blood clot was the most frequent scaffold employed, which was used on 145 teeth; PRP was used on 50 teeth, PRF on 35 platelet pellets on 17, and blood clot and PRP on one tooth. Most studies reported increased root thickening, lengthening, apex closure, and periapical healing. However, in the included studies that made a comparison between the different scaffold types in relation to pulpal regeneration treatment outcomes, it was found that for one study, the rate of apex closure was nearly similar between the blood clot group, and PRP, PRF, or platelet pellet and no significant differences among groups in increase in root width and length were found,[65] whereas in another study[61] comparing PRP and PRF, a slightly higher increase in thickness and length was found in the PRP group.

Various exogenous scaffolds, such as collagen[109] and hydrogel[56] have been clinically used in pulp regeneration procedures.

Collagen as a scaffold stimulates dentin cells in the extracellular matrix, has great alkaline phosphatase activity, empowers soft and hard tissue formation, and creates a network for osteoinductive factors.[110,111] Collagen can be used in different forms, such as sponges, gels, and sheets.[111] Furthermore, the AAE guidelines[22] include using the resorbable matrix to place over the blood clot. In this study, blood clot and injectable hydrogel scaffold with bFGF were used in 12 teeth; blood clot and collagen sponge on 1; PRF and resorbable collagen plug on 6; blood clot and absorbable adhesion barrier in 4, showing heterogeneous results in terms of periapical healing, apex closure, root thickening, or lengthening.

The AAE and the ESE guidelines suggest using bioactive materials, including MTA or bioceramics sealers such as biodentine.[22,25] MTA is used widely during regenerative endodontics procedures due to its several properties, such as biocompatibility, bioactivity, and scaffold protection.[112,113] MTA is available in two forms: white MTA or gray MTA.[114,115] The main difference between the two variations is the concentration of Al_2O_3, MgO, and FeO.[116,117] The presence of iron, aluminum, magnesium, or bismuth oxides can lead to coronal discoloration.[116,118] Thus, new materials such as Biodentine have been introduced to minimize the risk of discoloration.[118,119] Biodentine is a calcium silicate-based material with similar properties to MTA.[120] Biodentine induces pulpal healing and mineralization through growth factors, the release of silicon ions, and odontoblast stimulation.[121,122] MTA HP has been introduced to overcome the drawbacks of MTA, which replaced the bismuth oxide with calcium tungsten, powering the physiochemical, antibacterial, and biological properties.[123]

Coronal discoloration was reported in 27 teeth treated with MTA (in particular, 4 of the MTA group and 23 of the white MTA one), in one tooth treated with Biodentine, whereas no discoloration was found in 8 teeth treated with white MTA and in the case treated with repair HP MTA. However, as mentioned above, the discoloration can also be related to the use of TAP.[22] Therefore, it is impossible to define whether the intracanal medicament or the bioactive material could have determined the discoloration. However, it is likely that in cases where DAP was employed as an intracanal medicament, discoloration could have been driven by the bioactive material or from blood contamination if the blood clot was not separated from barrier materials.[25] However, this study finds a higher discoloration rate when MTA is the bioactive material used. However, the paucity of the Biodentine or repair HP MTA samples should be considered. Further studies may highlight the correlation between biomaterial used and discoloration rate.

As mentioned above, regenerative endodontics is carried out most frequently in pediatric subjects, and it is important to manage younger patients properly because they are more likely to lose multiple visits and follow-ups.[24]

In the present systematic review, follow-ups were carried out between 1 and 144 months.

However, it was also mentioned in some of the included studies[37,40,50,56] that follow-up needed to be recovered. From this perspective, the literature suggests that teledentistry offers numerous applications, particularly in the care of pediatric patients.[124] Considering that periodic follow-ups are recurrent in pulp regeneration treatment, one perspective to ensure long-term evaluation for the pediatric population is teledentistry.[125]

In the present study, the primary goal of pulp regeneration treatment, according to the AAE criteria, which consists of symptom elimination and periapical healing, has been accomplished by 87 teeth. In comparison, none was found in 6 teeth. As the literature mentions,[15,21,24] the primary goal will likely be achieved. In contrast, the secondary goal, an increase in root thickness and length, was sometimes found, and the rate of changes was highly variable among the studies.[48,126,127] This study found root lengthening in 223 teeth, none in 16, root thickening in 194, and no increase in 8.

The third goal refers to obtaining a positive response to vitality tests, indicating a well-organized, innerved, and vascularized pulp.[22] However, it has been reported that a positive response to vitality test after pulp regeneration amounted to 50%–60% of the published cases.[128,129] However, it has been suggested that a positive response to pulp sensibility tests after pulp regeneration should not be an indicator of the success of pulp regeneration, as histologic and immunohistochemical findings showed that after regenerative endodontics treatment, the vital tissue was cementum-like, bone-like, and nerve fibers and not a well-organized pulp.[130] Hence, a positive response to the vitality test could occur because of the presence of vascularized and innervated vital tissue.[24]

The present study found a negative response to vitality tests for a total of 45 teeth, a positive one for 90 teeth, and an unclear response for four teeth. However, it should be noticed that despite the fact that 45 teeth showed a negative response to the vitality test and 4 showed an unclear response, the majority of the teeth showed successful pulpal regeneration treatment during follow-ups in relation to radiographic signs of periapical healing, root lengthening or thickening, and apex closure.

Moreover, it should also be mentioned that cold, heat, and electric tests are not vitality tests but sensitive tests, and they are nonobjective, as they refer to vitality through sensory responses. They could have a margin of error,[131] giving false negatives and false positives.[132] In this perspective, objective tests such as dual-wavelength spectrophotometry, laser Doppler flowmetry, and pulse oximetry have been proposed to overcome the nonpredictability of conventional vitality tests.[133] However, dual-wavelength spectrophotometry detects the presence of hemoglobin and not blood circulation, and it has been examined only in laboratories. At the same time, laser Doppler flowmetry is not a reproducible method due to its costs and sensitivity to movements.[134] Among them, pulse oximetry is the most reliable diagnostic device that can be used in dentistry. It is based on measuring the oxygen saturation level of arterial blood through a probe with two lights emitting diodes.[132] One light transmits red (wavelength of 640 nm) and the other one infrared (wavelength of 940 nm).[133] The association between the pulsatile change in the

absorption of red light and infrared light is measured through a pulse oximeter to quantify the oxygen saturation levels of arterial blood.[132] This method may be useful in measuring the oxygen saturation after regenerative endodontics, as some studies have been focusing on the evaluation of pulp oximetry tests in teeth in different conditions such as traumatic cases, vital, nonvital, and teeth with open apices.[135,136] In addition, the accuracy of pulp oximetry was higher compared to the conventional pulp tests,[136] and its sensitivity was reported to be 100% when compared to cold tests or electric tests, respectively, 81% and 71%.[137] Further studies can assess the usefulness of the pulp oximetry test after pulp regeneration treatment, especially whether a negative or unclear response to the conventional pulp test is found.

Based on the results achieved in the present systematic review, to precisely interpret the findings, some limitations of the study should be deemed.

First of all, to analyze the different biomaterials used for the pulpal regeneration procedure, it must be taken into account that the materials most used were MTA and white MTA. At the same time, as mentioned above, the paucity of the sample of other bioceramic cements such as Biodentine or HP MTA may lead to a less extensive evaluation that could be deepened in further studies.[138] Furthermore, the presence of heterogeneous data, especially regarding tooth types, number of visits, various follow-up timing, and some missing data, precluded the possibility of conducting a meta-analysis.

A limitation to be also considered is that the studies included in this systematic review had different designs and therefore the quality assessment for RoB was assessed with different tools.

In relation to the tools used and the studies evaluated, studies with severe and even critical risks were found.

Further and more extensive studies may be necessary concerning the different biomaterials used in pulpal regeneration and success rate regarding periapical healing, root thickening and lengthening, and response to vitality tests.

CONCLUSIONS

The present systematic review aimed to assess the success rate of pulp regeneration treatment in permanent teeth of pediatric subjects (age range between 6 and 17 years of age) according to the AAE criteria.

The material associated with a higher success rate of periapical healing, increase of root thickness and lengthening, and positive response to vitality tests was white MTA.

Despite the limitations of this study concerning the small sample size of some biomaterials (Biodentine or other bioceramic sealers) and some missing data, to provide an overall view of pulpal regeneration treatment, it should be considered that overall success – taking into consideration the essential primary goal, that is periapical healing and the secondary goal which is root lengthening and thickening – was reported for 249 out 273 teeth, for a global amount of 91.20%. These data support the strong potential that regenerative endodontics has in continuing root development in permanent immature teeth. Therefore, further studies are needed to support the AAE guidelines and provide additional clinical recommendations for implementing the procedure.

In this perspective, further studies are needed for a more extensive evaluation of different biomaterials and the success rate in regenerative endodontics involving periapical healing, increased root thickness and length, and a positive response to vitality tests.

Author contributions

Conceptualization, A.I. and M.P.; Methodology, D.A.; Validation, F.G.; Investigation, M.P. and G.D.B.; Data curation, A.I.; Writing-original draft preparation, A.I. and M.P.; Writing-review and editing, F.G. and G.D.B.; and Supervision, D.A. All authors have read and agreed to the published version of the manuscript.

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Conflicts of interest

There are no conflicts of interest.

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