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REVIEW ARTICLE

Influence of ethylenediaminetetraacetic acid on regenerative endodontics: A systematic review

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Abstract

Background: The effects of ethylenediaminetetraacetic acid (EDTA) on regenerative endodontic procedures (REPs) are controversial, because, despite releasing growth factors from dentine, some studies show negative effects on cell behaviour.

Objectives: The aim of the study was to investigate the influence of the use of EDTA in REP on the growth factors' release, cell behaviour and tissue regeneration.

Methods: A systematic search was conducted (PubMed/Medline, Scopus, Cochrane Library, Web of Science, Embase, OpenGrey and reference lists) up to February 2021. Only *in vivo* and *in vitro* studies evaluating the effects of EDTA on the biological factors of dentine, pulp/periapical tissues and cell behaviour were eligible. Studies without a control group or available full text were excluded. The growth factors' release was the primary outcome. Risk of bias in the *in vitro* and *in vivo* studies was performed according to Joanna Briggs Institute's Checklist and SYRCLE's RoB tool, respectively.

Results: Of the 1848 articles retrieved, 36 were selected. Amongst these, 32 were *in vitro*, three animal studies and one with both models. The EDTA concentrations ranged from 3% to 15%, at different times. Regarding growth factors' release (17 studies), 15 studies found significant transforming growth factor (TGF)-β release after dentine conditioning with EDTA, and most found no influence on vascular endothelial growth factor release. Regarding cell behaviour (26 studies), eight studies showed no influence of EDTA-treated dentine on cell viability, whereas, five, nine and six studies showed higher cell migration, adhesion and differentiation respectively. No influence of EDTA conditioning was observed in animal studies. *In vitro* studies had a low risk of bias, whereas animal studies had high risk of bias. Meta-analysis was unfeasible.

Discussion: This review found that EDTA increased TGF-β release and improved cell activity. However, well-designed histological analyses using immature teeth models are needed.

Conclusions: High-quality *in vitro* evidence suggests that EDTA-treated dentine positively influences TGF-β release, cell migration, attachment and differentiation;

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further research to evaluate its influence on tissue regeneration is necessary due to low methodological quality of the animal studies.

KEYWORDS

EDTA, growth factor(s), guided tissue regeneration, stem cells, systematic review, tissue engineering

INTRODUCTION

Treatment of immature necrotic teeth is challenging, due to incomplete root formation and open apex of these teeth (Albuquerque et al., 2014; Law, 2013). Regenerative endodontic procedures (REP) are a promising biological approach (Albuquerque et al., 2014; Verma et al., 2016) with long-term success rates ranging between 95% and 100% (Alobaid et al., 2014). REP may be helpful in restoring the physiologically functional dentition through the repair of damaged dental structures (Law, 2013). In addition, sensory and defence mechanisms of the pulp-dentine complex may also be repaired after REP (Hargreaves et al., 2013).

REP relies on the use of scaffolds, stem cells' availability and growth factors' release (Hashimoto et al., 2018). The dentine matrix plays an important role in REP, since it acts as a reservoir of various growth factors. Root canal disinfection and an appropriate conditioned dentine for stem cell adhesion and differentiation are crucial for achieving promising results (Deniz Sungur et al., 2019; Verma et al., 2016). Although there is no standard irrigation protocol for REP (Kim et al., 2018; Shamszadeh et al., 2019), sodium hypochlorite (NaOCl) remains the most common irrigant used (Bracks et al., 2019; Bucchi et al., 2017) due to its well-established properties, such as antimicrobial effect and solvent potential of the tissues (Galler et al., 2011). In addition, the complementary use of ethylenediaminetetraacetic acid (EDTA) may help to reduce the amount of endotoxins of the contaminated root canal (Herrera et al., 2016), but mainly, conditioning dentine with EDTA after NaOCl irrigation and before induced bleeding in REP is important for exposing bioactive molecules entrapped in the dentine matrix (Bracks et al., 2019; Bucchi et al., 2017; Gonçalves et al., 2016), such as transforming growth factor (TGF)-β and angiogenic factors as vascular endothelial growth factor (VEGF), which play a key role in pulp-dentine complex regeneration.

REP outcomes are strongly related to cellular responses (Galler et al., 2015). TGF-β1 is one of the most important stimulating molecules for the recruitment, proliferation and differentiation of stem cells (Bracks et al., 2019; Galler et al., 2015; Gonçalves et al., 2016). Besides enhancing odontoblastic differentiation and reparative dentinogenesis (Kucukkaya Eren et al., 2021), TGF-β1 also shows substantial anti-inflammatory effects by regulating pro-inflammatory cytokines (Bracks et al., 2019). Moreover, the vascular formation after intracanal bleeding plays a critical role in cellular activity (Galler et al., 2015). Thus, bioactive molecules, such as VEGF secreted by EDTA-treated dentine and delivered from periapical blood, also present potent angiogenic effects (Zeng et al., 2016) in promoting stem cell differentiation into endothelial cells (Gonçalves et al., 2007; Sakai et al., 2011).

In addition to promoting the expression of bioactive molecules from treated dentine, the use of EDTA after NaOCl is also recommended in REP to reduce cytotoxicity (Chae et al., 2018; Kim et al., 2018), thus optimizing cell activity and tissue regeneration (Conde et al., 2016). However, some studies showed no influence of EDTA-treated dentine on cell viability (Chae et al., 2018; Li et al., 2020), whilst others have shown some negative effects from this conditioning on cell behaviour, such as impaired stem cell survival and migration (Aksel et al., 2020; Deniz Sungur et al., 2019), which might negatively influence tissue regeneration. To our knowledge, no previous systematic review has been conducted to evaluate whether EDTA yields a more favourable environment for tissue regeneration after REP. Thus, this systematic review evaluated the influence of EDTA conditioning on factors associated with REP, through the assessment of *in vivo* and *in vitro* studies. The release of growth factors from EDTA-treated dentine was the primary outcome evaluated. The effects of EDTA-treated dentine on cell behaviour and on different parameters of tissue regeneration were also assessed.

METHODS

Protocol

Reporting this systematic review was carried out according to the checklist of the Preferred Report Items for Systematic Reviews and Meta-analyses (PRISMA; Page et al., 2021). A research protocol was registered at the International Prospective Register of Systematic Review (PROSPERO) database (CRD42020205417).

Eligibility criteria

The inclusion criteria were: (1) *in vivo* studies that evaluated the effects of conditioned dentine with EDTA on the biological factors of dentine or pulp/periapical tissues in REP, and (2) *in vitro* studies that determined the effects of EDTA-treated dentine or EDTA dilutions on cell viability, migration, attachment, morphology and on biological factors from dentine. Exclusion criteria were: (1) studies that examined the effects of EDTA conditioning on dentine, cells or pulp/periapical tissues without a control group or another irrigant without the EDTA-treated group, and (2) studies for which the full text was unavailable. There were no restrictions on the language and date of publication.

The population, intervention, comparison and outcome (PICO) approach was used to address the following question: 'Does EDTA conditioning during REP influence growth factors' release, cells' behaviour, or tissue regeneration?' The study population is composed of dentine, cells or pulp/periapical tissues of humans or animals who/that had been submitted to irrigation/conditioning with EDTA. The intervention was irrigation/conditioning with EDTA; the comparison was irrigation/conditioning with other solutions. The primary outcome assessed was the effect of EDTA on the release of growth factors after EDTA-treated dentine. The secondary outcomes were the effects of EDTA-treated dentine or contact of cells with dilutions of EDTA on cell viability, migration, attachment, morphology and protein immunolabelling/expression; and on blood clot characterization, tissue inflammation, tissue ingrowth, root length/root thickness, apical diameter, mineralization and root/bone resorption in immature teeth (incomplete root formation and open apex) or extracted teeth with simulated open apex.

Search strategy and information sources

Electronic searches were conducted in the PubMed/MEDLINE, Scopus, Cochrane Library, Web of Science and Embase databases up to February 2021. Grey literature was consulted through OpenGrey, and manual searches were carried out in the reference list of the selected articles. The search strategy used a combination of keywords and Medical Subject Heading (MeSH) terms associated with the Boolean operators 'AND' and 'OR' as shown in Supplementary File S1.

Study selection

Study selection was carried out independently by two authors (A.H.R.P. and R.R.F.) in a two-step process. The records were organized alphabetically by title and duplicates could be identified and removed manually. In Step 1, the authors appraised titles and abstracts of the studies retrieved from the search. In Step 2, full text of the remaining records was obtained for further evaluation by the authors. Only studies that fulfilled the eligibility criteria were included. Disagreements were solved through discussion, and when necessary, a third reviewer (F.B.) was consulted. Cohen's kappa coefficient for inter-investigator agreement during studies' selection was assessed (Landis & Koch, 1977).

Data collection and analyses

Two authors (A.H.R.P. and R.R.F.) undertook data collection independently and in duplicate for all studies using a piloted data extraction form in an Excel spreadsheet. The following data were retrieved: first author's last name, year of publication, study design, experimental model, sample size, groups, experimental protocol and analyses. Unavailable data were classified as not applicable. The third author (F.B.) revised the data. In cases of missing data, the authors were contacted twice by email.

Risk-of-bias assessment

Two investigators (A.H.R.P. and S.C.O.) independently assessed the selected studies' methodological quality according to their levels of evidence, as proposed by a modified version of the Joanna Briggs Institute's (JBI) Critical Evaluation Checklist for Experimental Studies (Dos Reis-Prado et al., 2021; Yaylali et al., 2015). The assessed items were as follows: clearly stated aim, sample size justification (the authors either described the sample size and power calculation methods or justified the sample size used in the study), sample randomization, blind treatment allocation, possibility of comparison between control and treatment groups, baseline equivalence of control and treatment groups, measurement standardization, reliable measurement method, and appropriate statistical analysis. Each item was assessed on a two-point scale: 0, *not reported or reported inappropriately*, and 1, *reported and appropriate*. A 10-criteria tool, 'Systematic Review Centre for Laboratory animal Experimentation' (SYRCLE's RoB tool), was used for risk-of-bias assessment of the animal studies (Hooijmans et al., 2014). The assessed items included: adequate generation of allocation sequence, similarity of groups at baseline or adjustment of confounder due to differences between groups, adequate allocation concealment, random housing, blinded intervention/outcome assessment, sample randomization for outcome assessment, incomplete outcome data, selective outcome reporting, presence of other biases and sample size justification to further characterize reporting in the selected animal studies. A judgment of 'no' indicated a high risk of bias, 'yes' represented a low risk of bias, and 'unclear' indicated either lack of information or uncertainty. Discrepancies were resolved with a third examiner (L.G.A.).

RESULTS

Study selection

Figure 1 displays a flowchart of the selection process of the studies. A total of 1848 articles were identified after searching the databases. After the first screening (Step 1), 55 articles were selected and submitted to a full-text review (Step 2). Then, 19 studies were excluded, in which 13 (Arslan et al., 2014; Bosaid et al., 2020; Buldur et al., 2019; Ivica et al., 2020; Kandemir Demirci et al., 2020; Nagata et al., 2014; Nerness et al., 2016; Saghiri et al., 2016; Shawli et al., 2020; Ustun et al., 2018; Widbiller et al., 2018; Yassen et al., 2014, 2015) were outside of the proposed theme, and 6 had no control group (Ferreira et al., 2020; Galler et al., 2011; Graham et al., 2006; Hristov et al., 2018; Tomson et al., 2007; Trevino et al., 2011; Figure 1). A total of 36 studies were included in the qualitative analysis and are presented in Tables 1, 2 and 3.

The assessed Cohen's kappa coefficient for inter-investigator agreement during the studies' selection was 0.843 for PubMed, 0.970 for Scopus, 1.000 for the Cochrane Library and OpenGrey, 0.925 for the Web of Science and 0.876 for the Embase. These values indicated an almost perfect agreement between/amongst reviewers according to the benchmark scale of Landis and Koch (1977). No

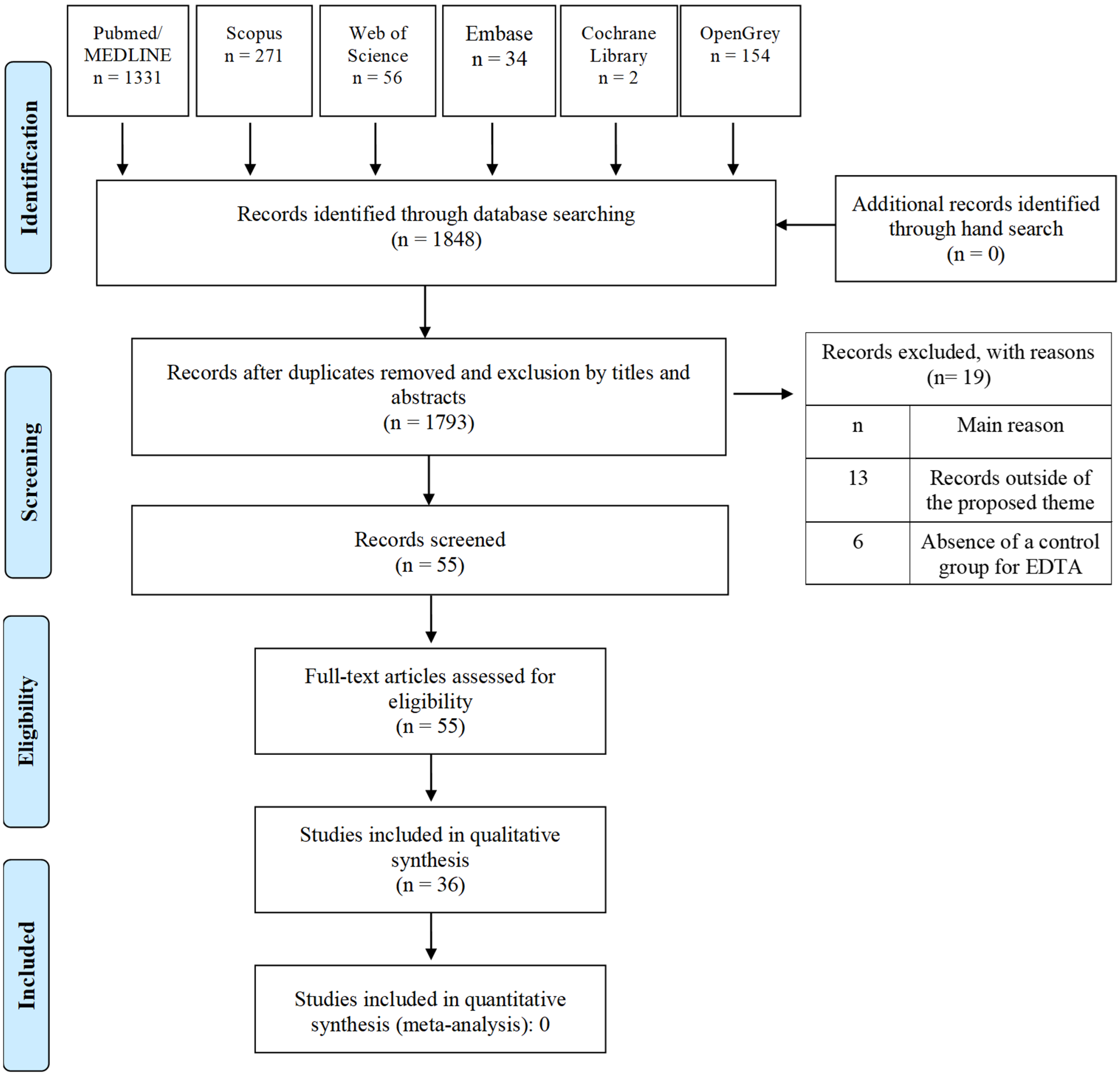

FIGURE 1 Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart

TABLE 1 Effects of EDTA on growth factors release/expression

Author	Experimental model (n)	Groups	Experimental protocol	Growth factor release	Outcomes
Kucukkaya Eren et al. (2021)	Human dentine discs and DPSCs (n = 9)	PC: cells, NC: no treatment, G1: 1 min EDTA +cells, G2: 1 min EDTA, G3: 5 min EDTA +cells, G4: 5 min EDTA, G5: 10 min EDTA +cells, G6: 10 min EDTA, G7: 1 min EDTA +BAC + cells, G8: 1 min EDTA +BAC, G9: 5 min EDTA +BAC + cells, G10: 5 min EDTA +BAC, G11: 10 min EDTA +BAC + cells, G12: 10 min EDTA+BAC, G13: cells without disk	Dentine discs preparation, conditioning protocols with or without 17% EDTA or 0.008% BAC, DPSCs seeded on dentine discs, analyses at 24 and 72 h	TGF-β1, ELISA (pg/ml). PC: >600 = NC: 600 = G1: >700 = G2: ≅600 = G3: >700 = G4: 600 = G5: ≅700 = G6: >600 = G7: >700 = G8: >600 = G9: ≅700 = G10: ≅600 = G11: >600 = G12: >500 # G13: ≅1300	EDTA solutions had no significant effect on the TGF-β1 release
Aksel et al. (2020)	Human dentine discs (n = 3)	Second protocols of the study. G1-G5: groups used for analyses not considered in this table; G6: optimized EDTA, G7: EDTA with NBs, G8: EDTA +5 min USA, G9: EDTA with NBs +USA, G10: PBS	Discs preparation, conditioning protocols, discs placed in 12-well plates with PBS for 24 h, analysis	TGF-β, ELISA (pg/ml). G6: ≅780 = G7: ≅780 = G8: 872 = G9: 799 = G10: ≅780	EDTA did not increase TGF-β release
Atesci et al. (2020)	Human roots/ dentine discs and adMSCs (n = 4)	NC: DW, G1: 17% EDTA, G2: 10% CA, G3: 1% IP6, G4: 37% PHA, PC: DW +adMSCs, G5: 17% EDTA +adMSCs, G6: 10% CA +adMSCs, G7: 1% IP6 + adMSCs, G8: 37% PHA +adMSCs	Root fragments disinfection, irrigation protocols for 5 min (G1, G2, G3, G5, G6, G7) or 30 s (G4, G8), final irrigation with PBS, cell seeding in half of the group, incubation for 3 d, analysis	ELISA (pg/mg). TGF-β1. NC: ≅100 § = G1: >200 § # G2: ≅500 § # G3: ≅300 § = G4: ≅370; PC: ≅370 # G5: ≅700 = G6: >600 = G7: ≅580 # G8: ≅980; VEGF. NC: ≅0 § = G1: ≅1 § = G2: ≅1 § = G3: ≅1 § = G4: ≅1; PC: ≅8 # G5: ≅24 = G6: ≅33 = G7: ≅20 # G8: ≅. 57; BMP−2. NC: ≅60 § # G1: ≅240 § = G2: ≅. 250 § = G3: ≅250 § = G4: ≅230; PC: ≅150 # G5: ≅400 = G6: ≅400 = G7: ≅450 = G8: ≅520; FGF−2. NC: ≅1 § = G1: <10 § = G2: ≅5 § = G3: ≅10 § = G4: <10; PC: <10 # G5: >80 # G6: ≅40 = G7: >20 # G8: ≅99	There was no significant difference in EDTA without adMSC regarding TGF-β1, VEGF and FGF−2, but EDTA increase BMP−2 release. EDTA with adMSC increased release of all growth factors

(Continues)

TABLE 1 (Continued)

Author	Experimental model (n)	Groups	Experimental protocol	Growth factor release	Outcomes
Li et al. (2020)	Human mandibular single-root premolars and SCAPs ($n = 6$)	G1: 10 min 1.5% NaOCl (20 ml), G2: NaOCl +5 min 17% EDTA (10 ml), G3: NaOCl +PDT, G4: NaOCl +EDTA + PDT	Preparation of root segment, irrigating protocols (PDT groups—irradiation for 120 s), final rinse with sterile saline, SCAP with hydroxyapatite-based scaffolds seeded into root canals, incubation for 7 d, analyses	PDGF, qRT-PCR. G1: 1 =, G2: <1 =, G3: >3 #, G4: ≅2.5 #; VEGF, qRT-PCR. G1: ≅1 =, G2: >2 =, G3: ≅7 #, G4 ≅ 7 #	EDTA did not influence PDGF and VEGF expression, with or without PDT
Bracks et al. (2019)	Maxillary first molars from Balb/c mice ($n = 18$)	NC: empty, G1: BC, G2: 17% EDTA +BC	Endodontic access, pulpectomy, groups allocation (G2 - irrigation with 1 min 17% EDTA), dried with sterile paper points, intracanal bleeding with #15 K-file 0.5 mm beyond the apical foramen, coronal seal, euthanasia at 7, 14 or 21 d	TGF-β, RT-PCR. (7 d. NC: ≅0 =, G1: ≅50 =, G2: <300 #; 14 d. NC: ≅0 =, G1: >0 =, G2: >0 =; 21 d. NC: ≅0 #, G1: >100 =, G2: >100 =). VEGF, RT-PCR. (7 d. NC: ≅9 =, G1: ≅6 =, G2: ≅2 =; 14 d. NC: ≅2 =, G1: ≅2 =, G2: ≅13 =; 21 d. NC: ≅2 =, G1: >2 =, G2: ≅4 =). IGF, RT-PCR. (7 d. NC: ≅0 =, G1: ≅0 =, G2: 40 #; 14 d. NC: >0 =, G1: >0 =, G2: >10 #; 21 d. NC: >0 =, G1: ≅0 =, G2: <10 #). NGF, RT-PCR. (7 d. NC: ≅0 =, G1: ≅0 =, G2: >0 =; 14 d. NC: ≅0 =, G1: ≅0 =, G2: >500 #; 21 d. NC: ≅0 =, G1: ≅0 =, G2: >0 =)	EDTA increased TGF-β at 7 d, IGF at all periods and NGF at 14 d. EDTA not influenced in the VEGF expression
Cameron et al. (2019)	Human teeth ($n = 6$)	Sterile versus infected root canals. Control: untreated, G1: 1,5% NaOCl, G2: 17% EDTA, G3: 1.5% NaOCl +17% EDTA	Sterile (control) and infected root canals with polymicrobial biofilm, disinfection protocols (10 ml of each solution for 10 min), root segments placed in 1 ml HBSS for 24 h, TGF-β1 quantification	TGF-β1, ELISA (ng/gm). Sterile root canal, control: ≅2.5 # G1: 0 # G2: ≅8.5 = G3: ≅7.5; Infected root canals, control: <2.5 # G1: 0 # G2: ≅1 = G3: ≅3.5	EDTA increased TGF-β1 release from dentine under sterile conditions. EDTA released more TGF-β1 compared with NaOCl. EDTA did not influence TGF-β1 release in the presence of biofilm compared with control

Author	Experimental model (*n*)	Groups	Experimental protocol	Growth factor release	Outcomes
Deniz Sungur et al. (2019)	Human dentine discs ($n = 3$)	G1: 17% EDTA, G2: 1% IP6, G3: 9% HEDP, control: DW	Preparation of dentine discs, immersion in the solutions for 5 min, immersion in PBS, incubation at 37 °C up to 28 d, analysis	TGF-β, ELISA (nm). 4h. G1: >25 = G2: ≅25 = G3: <25 = control: >25; 1 d. G1: ≅75 = G2: >50 = G3: <75 = control <75; 3 d. G1: <100 = G2: 75 = G3: 100 = control: ≅100; 5 d. G1: 125 = G2: 100 = G3: >125 = control: ≅125; 7 d. G1: <150 = G2: >125 = G3: >150 = control: 150; 14 d. G1: ≅175 = G2: >150 = G3: 200 = control: 175; 28 d. G1: <200 = G2: 175 = G3: 225 = control: >200	EDTA did not influence TGF-β release compared with other groups
Ivica et al. (2019)	Human dentine discs and human bone marrow–MSCs ($n = 3$)	G1: 10% CA, G2: 17% EDTA; G3: PBS	Preparation of dentine discs, 300 µl of conditioning agents (CA, EDTA and PBS) for 10 min, analysis	TGF-β1, Slot Blot Protein Immunoassay (ng). G1: 382 ± 30 # G2: 66 ± 3 # G3: no staining	EDTA increased TGF-β1 release compared with PBS
Liu et al. (2019)	Pulp tissue from human premolars or third molars and DPCs ($n = 5$)	For TGF-β1 release, NC: no EDTA stimulation, G1: 5 min 12% EDTA+fresh medium for 6 h, G2: 5 min 12% EDTA +fresh medium for 12h, G3: 12% EDTA +fresh medium for 24 h	Cell culture, cells treated with EDTA in different time points and concentrations, cells maintained in fresh medium for 6, 12 and 24 h, analyses	TGF-β1, ELISA (pg/ml). NC: ≅400 =, G1: ≅500 #, G2: <500 #, G3: <500 #	EDTA increased TGF-β1 release
Chae et al. (2018)	Root of permanent human teeth ($n = 3$)	G1: 5 min saline, G2: 17% EDTA, G3: 10% CA, G4: 10% PHA, G5: 37% PHA, NC: internal surface coated with nail varnish	Preparation of root segments, irrigation with 1.5% NaOCl, randomization, irrigation protocols, dried with paper points, samples placed into 1 ml a-MEM for 24 h, analysis	TGF-β1, ELISA (pg/ml). NC: 43 = G1: 53 # G2: 231 # G3: 516 # G4: 240 # G5: 53	EDTA increased TGF-β1 compared with saline solution

(Continues)

TABLE 1 (Continued)

Author	Experimental model (n)	Groups	Experimental protocol	Growth factor release	Outcomes
Ranc et al. (2018)	Immature single-rooted human premolars ($n = 5$)	NC: no irrigation, PC: 0.9% saline, G1: 5 min 17% EDTA, G2: 20 min 17% EDTA	Samples preparation, randomization, irrigating protocols, rinse with 0.9% saline (PC, G1 and G2), tagging of antibodies and preparation of samples, wash in water, analyses	TGF-β1, Raman intensity. NC: decrease of 70%, G1 increase of 10% compared with the G2, G2 increased 80% compared PC bFGF and BMP−2, Raman intensity. NC: decrease of 50% for bFGF and 82% for BMP−2, G1: NM, G2 increased >300% compared with PC	EDTA increased TGF-β1 expression, and 20 min EDTA increased bFGF and BMP-2
Duncan et al. (2017)	Human dentine discs ($n = 3$)	G1: 10% EDTA, G2: TSA, G3: VPA, G4: SAHA, G5: PBS	HDACis preparation, isolation of DMCs, DMCs extraction from 5 g of powdered dentine using 25 ml of each solution, constant agitation for 14 d, daily refreshing of extractant, analyses	Proteomic assay (pg/ml), TGF-β1. G1: >100 000 # G2: <20 000 = G3: >10 000 = G4: <30 000 = G5: ≅30 000; GDF−15. G1: >25 = G2: <50 = G3: >50 # G4: >200 # G5: 150; BMP−7. G1: >1000 # G2: 0 = G3: 0 = G4: 0 = G5: 0; FGF−2. G1: >55 = G2: >45 # G3: >15 = G4: >40 = G5: 50; FGF−4. G1: 0 # G2: 160 # G3: 30 # G4: ≅120 = G5: 80; FGF−7. G1: ≅70 = G2: ≅30 = G3: <20 = G4: >60 = G5: <90; BDNF. G1: ≅21 # G2: ≅32 = G3: 30 = G4: >30 # G5: 0; GDNF. G1: 35 # G2: ≅20 = G3: ≅18 = G4: ≅24 # G5: ≅36; PDGF-AA. G1: >3000 # G2: 0 = G3: 0 = G4: 0 = G5: 0; VEGF-A. G1: 250 #, G2: >200 =, G3: ≅150 #, G4: >200 =, G5: ≅175 #; SCF-R. G1: >500 # G2: >1000 = G3: >1000 # G4: ≅2.000 # G5: >500; IGFBP−1. G1: >15 000 # G2: ≅2500 # G3: <2500 = G4: <2500 # G5: >5000; IGFBP−2. G1: >1000 # G2: <250 # G3: >1000 # G4: >2500 # G5: >250; IGFBP−3. G1: >60 000 # G2: >0 = G3: >0 = G4: >5000 = G5: 0; IGF−1. G1: 3500 = G2: >3500 = G3: 4000 = G4: ≅3250 # G5: 0; Insulin. G1: >1300 = G2: >1400 = G3: 1400 = G4: >1400 = G5: >1600; EGFR−1. G1: >500 # G2: ≅1000 # G3: >1500 # G4: >2000 # G5: >1500; NGFR. G1. 0 # G2: >200 # G3: >125 = G4: >125 # G5: >300; HGF. G1: 65 # G2: ≅20 # G3: 5 # G4: ≅30 = G5: ≅35; PIGF. G1: 50 # G2: 15 = G3: 10 = G4: >15 = G5: >15	EDTA effectively released TGF-β1, BMP−7, BDNF, PDGF-AA, VEGF-A, IGFBP−1 and −3, IGF−1, HGF and PIGF. EDTA not influenced FGF−2 and −7, GDNF, SCF-R, IGFBP−2, insulin. EDTA decreased GDF−15, FGF−4, EGFR−1, NGFR

TABLE 1 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Growth factor release	Outcomes
Widbiller et al. (2017)	Human dentine discs and straight roots from first/second molars (*n* = 9—dentine discs, *n* = 12—root canal)	Dentine discs. One step, G1: 10 min PBS, G2: 10 min PBS+US, G3: 1 min 10% EDTA, G4: 1 min EDTA +US, G5: 3 min EDTA, G6: 3 min EDTA +US, G7: 10 min EDTA; Two step, G1: 10 min PBS +1 min PBS +US, G2: 1 min EDTA +1 min PBS +US, G3: 10 min EDTA +3 min PBS, G4: 10 min EDTA +3 min PBS +US, G5: 10 min EDTA +5 min PBS, G6: 10 min EDTA +5 min PBS+US. Root canal model. G1: 10 min PBS +US, G2: 10 min EDTA +5 min PBS +US, G3: 3 min EDTA +US	Dentine discs preparation, irrigation protocols, analysis. Root canal model preparation, irrigation protocols, ultrasonic activation, frozen immediately, analysis	TGF-β1, ELISA (pg/ml). Dentine discs. One step. G1: <50 = G2: <50 # G3: 197 # G4: 313 = G5: 535 = G6: ≅700 = G7: 908; Two step. G1: <50 # G2: 286 = G3: >300 # G4: 735 # G5: >300 # G6: 1334. Root canal model. G1: ≅500 # G2: 1023 # G3: 3445	EDTA increased TGF-β1 release, mainly after US activation
Gonçalves et al. (2016)	Human dentine samples (*n* = 10)	G1: PBS with PD, G2: PBS without PD, G3: 1 min 10% EDTA with PD, G4: 1 min 10% EDTA without PD, G5: 5 d 2.5% NaOCl with PD, G6: 5 d 2.5% NaOCl without PD	Tooth slice preparation with or without PD, slices conditioned with irrigating protocols, transferred to 24-well plates incubation at for 3 d, analysis	TGF-β1, ELISA (pg/ml). G1: MC =, G2: MC =, G3: 1.262.175 #, G4: 1.197.095 #; G5: MC =, G6: MC =	EDTA significantly released TGF-β1 from dentine matrix
Sadaghiani et al. (2016)	Dentine powder, human dentine slices and DPSCs (*n* = 6 or 15)	G1: 10% EDTA, G2: 37% PHA, G3: 10% CA, G4: 25% PA, NC: PBS, PC: CH, *control: no DME or denatured EDTA-extracted DMEs for DME stimulation	Extraction of DME or dentine immersion in the conditioning solutions for 5 or 10 min, frozen, growth factor quantification, washed with DW, immunogold localization, DPSC seeded onto conditioned dentine, cell morphology examined after 1 and 8 d, and analysis	SEM, Immunogold-labelled particles (number of particles). (TGF-β1, 5 min. NC: ≅10 =, PC: ≅55 #, G1: >10 =, G2: >15 #, G3: >10 =; 10 min. NC: >10 =, PC: >40 #, G1: ≅15 =, G2: 20 #, G3: 20 #; VEGF, 5 min. NC: MC =, PC: 70 #, G1: ≅70 #, G2: >30 #, G3: >60 #; 10 min. NC: 0 =, PC: >80, G1: ≅25 #, G2: ≅20 =, G3: >10 =; BMP2, 5 min. NC: 0 =, PC: >60 #, G1: >75 #, G2: 20 =, G3: 65 #; 10 min. NC: MC =, PC: ≅70 #, G1: ≅30 #, G2: >10 =, G3: ≅20 =); ELISA, Concentrations in the conditioned dentine (pg/ml). (TGF-β1, 5 min. NC: 0 =, PC: 0 =, G1: >650 #, G2: 0 =, G3: 0 =; 10 min. NC: 0 =, PC: 0 =, G1: >600 #, G2: 0 =, G3: 0 =; VEGF. G3 significantly released VEGF after 5 min amongst the groups; BMP2, 5 min. NC: 0 =, PC: <200 =, G1: 0 =, G2: <200 =, G3: ≅600 #; 10 min. NC: 0 =, PC: 0 =, G1: 0 =, G2: <200 =, G3: 800 #)	EDTA did not influence TGF-b1 and increased BMP−2 and VEGF in SEM analysis, compared with control; but increased TGF-b1, and did not influence BMP−2 and VEGF in ELISA analysis

(Continues)

TABLE 1 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Growth factor release	Outcomes
Zeng et al. (2016)	Human permanent teeth (*n* = 12)	G1: 1.5% NaOCl +17% EDTA for 5 min, G2: 2.5% NaOCl +17% EDTA for 5 min, G3: 5 min 17% EDTA, G4: 5 min DW	Preparation of root segments, growth factor array, irrigating protocols, segments placed into 1 ml a-MEM for 4 h, 1 or 3 d, analyses	ELISA (ng/ml). TGF-β1. 4 h. G1: ≅16 =, G2: ≅30 =, G3: 4 =, G4: 0.78 =; 1 d. G1: 69.04 #, G2: 59.26 #, G3: 6.92 =, G4: 0.78 =; 3 d. G1: 15.16 =, G2: 13.04 =, G3: 16.25 =, G4: 0.78 =; bFGF 4 h. G1: 0 =, G2: 0.43 =, G3: 0, G4: 0; 1 d. G1: 0 = G2: MC=, G3: 0=, G4: 0 =; 3 d. G1: 0=, G2: MC =, G3: 0=, G4: 0=	EDTA alone did not influence released of TGF-β1 or bFGF
Galler et al. (2015)	Human dentine discs (*n* = 3)	TGF-β−1 release, G1: 10% EDTA (0.268 mol/L, pH 6), G2: 10% EDTA (0.268 mol/L, pH 7), G3: 17% EDTA (0.456 mol/L, pH 7), G4: 10% CA (0.476 mol/L, pH 2), G5: CB (CA 0.476 mol/L + TCD 1.55 mol/L) pH 5, G6: CAPB (CA 0.476 mol/L + TCD 0.68 mol/L + TP 1.09 mol/L)	Dentine discs preparation, treated with solutions at different concentrations or pH for 5, 10 and 20 min, sample collection, analysis	TGF-β1, ELISA (pg/ml). 5 min. G1: >200 = G2: >300 # G3: <200 # G4: MC =G5: MC =G6: MC; 10 min. G1: >300 = G2: >400 = G3: 400 # G4: 57 # G5: MC =G6: MC; 20 min. G1: 449 # G2: 923 = G3: 827 # G4: 57 # G5: MC =G6: MC	EDTA increased the released of TGF-b1

Note: The symbol * indicates additional group per analysis; indicates no significant differences between/amongst groups; # indicates significant differences between/amongst groups; ≅ indicates 'approximately'; > indicates 'greater than'; < indicates 'less than'.

°C, degree Celsius; 3T3, mouse fibroblast cells; ADM, adrenomedullin; adMSCs, adipose-derived mesenchymal stem cells; a-MEM, alpha-minimum essential medium; BAC, benzalkonium chloride; BC, blood clot; BCA, benzalkonium chloride; BDNF, brain-derived neurotrophic factor; BMP-2, bone morphogenetic protein-2; BMP-7, bone morphogenetic protein-7; CA, citric acid; CAPB, citric acid phosphate buffer; CB, citrate buffer; CH, calcium hydroxide; CHX, chlorhexidine; CM, conditioned medium; d, days; DMC, dentine matrix component; DME, dentine matrix extracts; DMPs, dentine matrix protein extracts; DPCs, dental pulp cells; DPSCs, dental pulp stem cells; DW, distilled water; EDTA, ethylenediaminetetraacetic acid; EGFR, epidermal growth factor receptor; ELISA, enzyme linked immunosorbent assay; FBS, foetal bovine sérum; FGF-, fibroblast growth factor; FGF-, fibroblast growth factor-; g, grass; G, group; GAGs, glycosaminoglycans; GDF-15, growth/differentiation factor 15; GDNF, glial cell-line-derived neurotrophic factor; GdnHCl, guanidine hydrochloride; GMTA, grey mineral trioxide aggregate; h, hour; HBSS, Hank´s balanced salt solution; HCl, hydrochloric acid; HDACis, histone deacetylase inhibitors; HEDP, etidronic acid; HGF, hepatocyte growth factor; IGF, Insulin-like growth factor; IGFBP, Insulin-like growth factor-binding protein; IL-, interleukin; IP6, phytic acid; M, molar; MC, minimal concentration or zero; MDPC-23, mouse odontoblast-like cells; MDPSCs, mobilized dental pulp stem cells; mg, milligram; min, minutes; ml, millilitre; mm, millimetre; MSCs, mesenchymal stem cells; *n*, number of specimens; nAb TGF-β1, TGF-β1 neutralizing antibody; NaOCl, sodium hypochlorite; NB, nanobubble water; NC, negative control; NCP, non-collagenous protein; NCPs, non-collagenous proteins; ng, nanogram; NGF, nerve growth factor; NGFR, tumour necrosis factor receptor; nm, nanometre; NM, not mentioned; OD-21, undifferentiated pulp cells; PA, polyacrylic acid; PBS, sterile phosphate-buffered saline; PBS, sterile phosphate-buffered saline; PC, positive control; PD, predentine layer; PDGF, platelet-derived growth factor; PDT, photodynamic therapy; pg, picogram; PHA, phosphoric acid; PIGF, placenta growth factor; RT-PCR, real-time polymerase chain reaction; SAHA, suberoylanilide hydroxamic acid; SCAPs, stem cells of the apical papilla; SCF-R, mast/stem cell growth factor receptor kit; semi-qRT-PCR, semi-quantitative reverse transcriptase-PCR; TAP, triple antibiotic paste (ciprofloxacin, metronidazole and minocycline); TCD, trisodium citrat; TGF-, transforming growth factor; TP, trisodium phosphate; TSA, trichostatin A; US, ultrasonic activation; USA, ultrasonic activation; VEGF-, vascular endothelial growth factor; VPA, valproic acid; w, week; WMTA, white mineral trioxide aggregate; µl, microlitre.

TABLE 2 Effects of EDTA on stem cells behaviour (*in vitro* analyses)

Author	Experimental model (n)	Groups	Experimental protocol	Cell Viability	Cell Migration/ attachment	Cell morphology	Cell differentiation/ mineralization	Outcomes
Kucukkaya Eren et al. (2021)	Human dentine discs and DPSCs (n = 9)	Control: no treatment, G1: 1 min EDTA, G2: 5 min EDTA, G3: 10 min EDTA, G4: 1 min EDTA +BAC, G5: 5 min EDTA +BAC, G6: 10 min EDTA +BAC	Dentine discs preparation, conditioning protocols with or without 17% EDTA or 0.008% BAC, DPSCs seeded on dentine discs, analyses at 24 and 72 h	WST−1 assay (%). 72 h: G1: ≅31% = G2: ≅29% = G3: ≅29% = G4: ≅31% = G5: ≅30% = G6: ≅31% # control: ≅7%	Attachment, LDH assay (%). 24 h: G1: ≅43% = G2: ≅38% = G3: ≅34% = G4: ≅45% = G5: ≅35% = G6: ≅38% # control: ≅24%	SEM. 72 h: control: mostly spherical and smaller cells; EDTA groups: mostly spindle-shaped cells with elongated cytoplasmic processes	n.a.	EDTA groups increased cell proliferation and attachment, influencing cell morphology
Aksel et al. (2020)	Human dentine discs and DPSC (n = 3)	First protocol, G1: 5 min NaOCl +3 min PBS, G2: G1 + 5 min EDTA, G3: G2 + 3 min PBS, G4: NaOCl +5 min EDTA, G5: G4 + 3 min PBS. Second protocol using G3, G6: optimized EDTA protocol, G7: EDTA with NBs, G8: EDTA +5 min USA, G9: EDTA with NBs +USA, G10: PBS, control: DPSC, PC: DPSC in 10% FBS, NC: DPSCs	Discs preparation, first conditioning protocols, DPSCs seeded on dentine discs, incubation for 3 and 7 d, cell viability/morphology assays, second conditioning protocols, incubation for 7 d, final analyses	WST−1 analysis. First protocol (3 d: G1: ≅0.04 # G2: ≅0.01 # G3: ≅0.11 # G4: >0.00 # G5: ≅0.07 # control: ≅0.15 #; 7 d: G1: ≅0.14 # G2: ≅0.03 # G3: ≅0.24 # G4: ≅0.03 # G5: ≅0.015 # control: ≅0.33); Second protocol (7 d: G6: ≅0.23 = G7: ≅0.21 # G8: ≅0.27 # G9: ≅0.24 # G10: 0.18 # control: 0.27)	Transwell migration assay. 7 d: G6: ≅0.14 # G7: ≅0.17 # G8: ≅0.18 = G9: ≅0.18 # G10: ≅0.13 # NC: ≅0.11 # PC: ≅0.17	CLSM. First protocol: G3 and G5 had elongated, fibroblastic-like with flattened cell morphology on the dentine surface; Second protocol: PBS group showed round cell morphology compared with EDTA groups	n.a.	Final rinse with EDTA decreased cell viability compared with others. The activation EDTA protocols increased cell viability/ migration, and influenced cell morphology

(Continues)

TABLE 2 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Cell Viability	Cell Migration/ attachment	Cell morphology	Cell differentiation/ mineralization	Outcomes
Atesci et al. (2020)	Human dentine discs and adMSCs ($n = 4$)	Control: DW, G1: 17% EDTA, G2: 10% CA, G3: 1% IP6, G4: 37% PHA	Root fragments disinfection, irrigation protocols for 5 min (G1, G2, G3) or 30 s (G4), final irrigation with PBS, cell seeding, cultured in GM for 3 d, analysis	n.a.	Attachment, CLSM. 3 d: control: reduced cell attachment, G1: abundance of cells, G4: well attached	CLSM. 3 d: control: round-shaped cells; G1: oblong, flattened, and round-shaped cells; G2: flattened cells with extending cytoplasmic processes, G3: flattened cells and round shape; G4: round and oblong-like cells and flattened and well-attached cells with extending cytoplasmic processes	n.a.	EDTA group had abundance of cells, mostly round-shaped compared with the other groups
Li et al. (2020)	Human mandibular single-root premolars and SCAPs ($n = 6$)	G1: 10 min 1.5% NaOCl (20 ml), G2: NaOCl +5 min 17% EDTA (10 ml), G3: NaOCl +PDT, G4: NaOCl +EDTA + PDT	Preparation of root segment, irrigating protocols (PDT groups: irradiation for 120 s), final rinse with sterile saline, SCAP with hydroxyapatite-based scaffolds seeded into root canals, incubation at 37°C for 7 d, analyses	Luminescence analysis (no of cells). 7 d: G1: ≅37 = G2: ≅50 # G3: ≅62 = G4: ≅70	n.a.	SEM. 7 d: G1/G2: cells with small cytoplasmic processes, G3/G4: spindle-shaped cells with elongated cytoplasmic processes	n.a.	EDTA did not influenced in the viability or morphology of the cells
Ivica et al. (2019)	Human dentine discs/ MSCs ($n = 4$: viability/ migration, $n = 3$: attachment)	G1: 10% CA, G2: 17% EDTA; G3: PBS	Preparation of dentine discs, 300 µl of conditioning agents for 10 min, cells seeded on conditioned dentine discs, analyses after 24 and 48 h	Automatic Cell Counter (cells /dentine area). 48 h: G1: ≅6000 # G2: ≅2000 = G3: ≅2000	Transwell migration assay (no cells). 24 h: G1: ≅5000 # G2: ≅2000 # G3: ≅100. Attachment, automatic cell counter (cells/ dentine area). 24 h: G1: $≅1 \times 10^5$ # G2: $≅5 \times 10^4$ # G3: $>5 \times 10^4$. 48 h: G1: $≅6 \times 10^5$ # G2: $≅2 \times 10^5$ = G3: $>2 \times 10^5$	n.a	n.a.	EDTA had higher cell viability and migration than PBS, and higher cell attachment at 24 h

TABLE 2 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Cell Viability	Cell Migration/ attachment	Cell morphology	Cell differentiation/ mineralization	Outcomes
Liu et al. (2019)	Pulp tissue from human premolars or third molars and DPCs ($n = 5$)	Cell viability. According to exposure time: G1: 1 min, G2: 3 min, or G3: 5 min 17% EDTA, control: α-tubulin; and concentration: G1: 3%, G2: 6%, G3: 12%, or G4: 17% EDTA, control: α-tubulin. Cell migration. G1: SDF-1α, G2: EDTA+SDF-1α, G3: EDTA+SDF-1α+siCXCR4, NC: medium without SDF-1α	Cell culture, cells treated with EDTA in different time points and concentrations, cells maintained in fresh medium for 24, 48 and 72 h, analyses	CCK−8 assay (%). According to time of exposure. 24 h: Control: 100 = G1: ≅100 = G2: >100 # G3: ≅90; 48 h: Control: 100 = G1: <100 = G2: <100 # G3: <90; 72 h: Control: 100 = G1: ≅100 = G2: <100 # G3: ≅90); According to concentration. 24 h: Control: 100 = G1: >100 = G2: >100 = G3: >100 # G4: ≅90; 48 h: Control: 100 = G1: ≅100 = G2: >100 = G3: <100 # G4: <90; 72 h: Control: 100 = G1: <100 =G2: >100= G3: ≅100 #G4: ≅90)	Transwell migration assay (cells per field): NC: ≅80 # G1: ≅125 # G2: ≅160 # G3: ≅90	n.a.	n.a.	17% EDTA for 5 min decreased DPCs viability compared with other EDTA concentration groups and control; no difference in the other exposure times and the control. EDTA significantly enhanced cell migration
Deniz Sungur et al. (2019)	Dentine discs from human third molars and DPSCs ($n = 9$)	G1: 17% EDTA, G2: 1% IP6, G3: 9% HEDP, G4: DW, PC: 20% FBS, NC: 1% FBS	Dentine discs preparation, *proliferation/ morphology*: disinfection, conditioning (5 min), DPSCs seeded on discs, incubation in GM for 1, 3, 5 d, analysis; *migration*: chambers with culture media, discs in lower chamber, cells incubated for 1 and 3 d, analysis	MTT assay. 1 d: G1: ≅0.4 =, G2: <0.4 =, G3: ≅0.3 #, G4: <0.4 =; 3 d: G1: <0.6 # G2: ≅0.4 # G3: <0.4 # G4: >0.6; 5 d: G1: ≅0.8 =, G2 < 0.6 #, G3: 0.4 #, G4: ≅0.8 =	Transwell migration assay. 1 d: G1: ≅0.7 =, G2 > 0.6 #, G3: >0.8 ##, G4: <0.6 =, NC: ≅0.6 =, PC: >0.6 =; 3 d: G1: <0.6 =, G2: <0.6 #, G3: ≅0.6 #, G4: <0.6 =, NC: 0.4 =, PC: <0.6 =.	SEM. 1 d: G1: contracted cells with a spherical morphology, G2/ G3: polygonal morphology and more stretched out onto the dentine, G4: flattened cells compared with G1; 5 d: major cell number in the groups	n.a.	EDTA decreased cell viability than DW at 3 d, whilst not influenced at 1 and 5 d. EDTA did not influence cell migration, but influenced morphology cell (contracted cells).
Tunç et al. (2019)	SHEDs (n = n.a.)	G1: 5% EDTA, G2: 8.5% EDTA, G3: 17% EDTA, G4: 1% NaOCl, G5: 2.5% NaOCl, G6: 5% NaOCl, G7: 5 μg/ ml OW, G8: 10 μg/ ml OW, G9: 20 μg/ ml OW, G10: 0.5 j/ cm^2 GaAlAs, G11: 1 j/cm^2 GaAlAs, G12: 1.5 j/ cm^2 GaAlAs, Control: no exposure	SHEDs seeded, irrigants and laser application, incubation for 5, 10 and 15 min, analyses	MTT assay (%). 5 min: (G1 ≅ 60, G2: ≅60, G3: ≅55, G4: >60, G5: >60, G6: 55.3) # (G7: ≅100, G8: ≅100, G9: >100, G10: ≅100, G11: ≅100, G12: >100, cont.: 100); 10 min: (G1: >60, G2: >60, G3: 57.2, G4: >60, G5: ≅60, G6: ≅60) # (G7: >100, G8: >100, G9: 103.7, G10: >100, G11: >100, G12: >100, cont.: 100); 15 min: (G1: ≅25, G2: ≅25, G3: 21.7, G4: ≅30, G5: ≅30, G6: ≅30) # (G7: >100, G8: 100.7, G9: 100, G10: >100, G11: >100, G12: 99.9, cont.: 100)	n.a.	n.a.	n.a.	EDTA decreased cell viability compared with control

(Continues)

TABLE 2 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Cell Viability	Cell Migration/ attachment	Cell morphology	Cell differentiation/ mineralization	Outcomes
Widbiller et al. (2019)	Human dentine discs and SCAPs (n = n.a.)	Control: saline, G1: 2% CHX, G2: 17% EDTA, G3: L-α-lecithin, G4: CHX +L-α-lecithin, G5: CHX +EDTA, G6: CHX +L-α-lecithin +EDTA	Dentine discs preparation, rehydration, conditioning 5 min (mixed or not mixed solutions), SCAPs cultured for 5 d, analysis	Luminescence (%). No mixed solutions: Control: $>10^4$ # G1: $<10^2$ # G2: $>10^4$ = G3 $> 10^4$; Mixed solutions: control: $\cong 10^4$ # G1: $<10^2$ # G4: $\cong 10^4$ # G5: $>10^3$ # G6: $<10^4$ =	n.a.	n.a.	n.a.	There was no difference between EDTA and control in cytotoxicity
Chae et al. (2018)	Root human teeth and SCAPs ($n = 3$)	G1: saline, G2: 17% EDTA, G3: 10% CA, G4: 10% PHA, G5: 37% PHA, PC: non-treated dentine	Discs irrigated 1.5% NaOCl, irrigating, surfaces dried, transwell system, SCAPs loaded for 2 in the lower chamber, discs in the upper chamber, cells cultured for 24 h, analysis	MTS assay (nm). PC: ≅2.4 =, G1: ≅2.3 =, G2: ≅2.7 = #, G3: ≅2.5 = #, G4: ≅2.4 =, G5: ≅2.0 #	n.a.	n.a.	n.a.	There was no difference amongst saline, PC and EDTA in viability
Hashimoto et al. (2018)	Dentine discs from bovine teeth and MDP cells ($n = 9$)	G1: PBS, G2: PBS +10 min 3% EDTA, G3: PBS +10 min 17% EDTA, G4: 1.5% NaOCl, G5: 1.5% NaOCl +1 min 3% EDTA, G6: 1.5% NaOCl +5 min 3% EDTA, G7: 1.5% NaOCl +10 min 3% EDTA, G8: 1.5% NaOCl +20 min 3% EDTA, G9: 1.5% NaOCl +60 min 3% EDTA, G10: 1.5% NaOCl +1 min 17% EDTA, G11: 1.5% NaOCl +5 min 17% EDTA, G12: 1.5% NaOCl +10 min 17% EDTA, G13: 1.5% NaOCl +20 min 17% EDTA, G14: 1.5% NaOCl +60 min 17% EDTA, G15: 6% NaOCl, G16: 6% NaOCl +10 min 3% EDTA, G17: 6% NaOCl +10 min 17% EDTA	Preparation of dentine discs, irrigating protocols, washes with PBS, MDP cells seeded on dentine discs for 24 and 48 h, analyses	CCK−8 assay (cells/ field). G4: ≅0.003 = G5: ≅0.002 = G6: 0.003 # G7: ≅0.010 = G8: ≅0.007 = G9: ≅0.010 # G10: ≅0.002 = G11: ≅0.004 # G12: ≅0.008 = G13: ≅0.007 = G14: ≅0.008	Attachment, cell density (cell/ field). G1: 158 =, G2: ≅260 #, G3: ≅350 ##, G4: 24.5*, G7: ≅60, G12: ≅62, G15: 1.5$, G16: 1.5$, = G17: 1.5$ SEM. G1: cytoplasmic process to the smear layer, G3 and G12: cytoplasmic process to the dentine matrix, G4: few cells; TEM analysis. G7 and G12: cytoplasmic process to the dentine matrix, presence of collagen fibre	Fluorescence photomicrograph. G1/G2/G3: spindle and fibroblastic appearances, G4: round cells, G5 and G6: absence of viable cells, G7: most fibroblastic appearance, G12: most round cells, G15/G16/G17: reduced cells with fibroblastic appearances	RT-PCR. ALP. Control: $\cong 1 \times 10^{-3}$ = G1: $\cong 2 \times 10^{-3}$ # G2: $\cong 4 \times 10^{-3}$ # G3: $>4 \times 10^{-3}$; Control: $\cong 6 \times 10^{-4}$ = G4: $\cong 6 \times 10^{-4}$ # G6: $>6 \times 10^{-4}$ # G12: $\cong 7 \times 10^{-4}$; DMP. Control: 0 = G1: $<2 \times 10^{-3}$ # G2: $\cong 2 \times 10^{-3}$ # G3: $\cong 4 \times 10^{-3}$; Control: $\cong 2.5 \times 10^{-6}$ = G4: $<5 \times 10^{-6}$ # G6: $>1 \times 10^{-5}$ # G12: $\cong 1.5 \times 10^{-5}$; DSPP. control: 0 = G1: $<2 \times 10^{-3}$ # G2: $>2 \times 10^{-3}$ # G3: $\cong 4 \times 10^{-3}$; control: $<1 \times 10^{-7}$ = G4: $\cong 5 \times 10^{-7}$ = G6: $<5 \times 10^{-7}$ = G12: $>1 \times 10^{-6}$	10 min EDTA after 1.5% NaOCl increased cell viability, attachment, and differentiation with cells ≅odontoblast-like

TABLE 2 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Cell Viability	Cell Migration/ attachment	Cell morphology	Cell differentiation/ mineralization	Outcomes
Prompreecha et al. (2018)	Plastic tooth models, human dentin discs and APCs ($n = 9$)	NI (G1: 16 min NSS, G2: 15 min EDTA +1 min NSS, G3: 5 min CHX +10 min EDTA +1 min NSS), NI+EA (G4: 16 min NSS, G5: 15 min EDTA +1 min NSS, G6: 5 min CHX +10 min EDTA +1 min NSS), NI+PUI (G7: 16 min NSS, G8: 15 min EDTA +1 min NSS, G9: 5 min CHX +10 min EDTA +1 min NSS), control (non-dynamic irrigation, G10: 16 min NSS, G11: 15 min EDTA+1 min NSS, G12: 5min CHX +10 min EDTA+1 min NSS)	Preparation of immature root canal models (plastic tooth +dentine discs), smear layer removal, samples with CH paste for 1w, samples inserted into the models, irrigating protocols for 16 min, analyses	n.a.	Attachment, immuno-fluorescent assay (positive cells/field) G1: ≅230 # G2: ≅180 = G3: ≅160. G4: ≅240 # G5: ≅170 = G6: ≅150. G7: ≅230 = G8: ≅160 = G9: ≅175. G10: ≅80 = G11: ≅85 # G12: ≅25	n.a.	n.a.	EDTA groups had less cell attachment compared with NSS in the dynamic irrigation; however, no difference was observed between EDTA and NSS in non-dynamic irrigation
Scott et al. (2018)	SCAPs, PDL fibroblasts and UMR−106 cells ($n = 8$)	G1: DW, G2: 10% Endocyn, G3: 6% NaOCl, G4: 17% EDTA, G5: 2% CHX	Cell lines isolated and cultured for 24 h to 48-well plates, exposure to the irrigants, treatment with calcein AM for 1 h, rinse with PBS, analyses	Autofluorescence (% cell). PDL survival (10 min. G1: ≅105 =, G2: ≅110 =, G3: ≅18 #, G4: ≅10 #, G5: ≅7 #; 1 h. G1: ≅100 =, G2: ≅90 #, G3: ≅7#, G4: ≅16#, G5: 20#; 24 h. G1: 100 =, G2: 80#, G3: ≅5*, G4: ≅5*, G5: ≅5*); UMR survival (10 min. G1: ≅85 =, G2: ≅100 =, G3: 0#, G4: ≅0#, G5: ≅0#; 1 h. G1: 100=, G2: ≅90=, G3: ≅5 #, G4: ≅5#, G5: ≅5 #; 24h. G1: ≅90 =, G2: ≅100=, G3: 0#, G4 < 0#, G5: ≅3#); SCAP survival (10min. G1: >100=, G2: ≅80 =, G3: 20 #, G4: ≅10 #, G5: ≅10 #; 1h. G1: >100 =, G2: 20 #, G3: ≅5 #, G4: ≅18 #, G5: 20 #; 24h. G1: 100 =, G2: 0 #, G3: >0 #, G4: ≅5 #, G5: ≅5 #)	n.a.	n.a.	n.a.	EDTA and the other irrigants showed more cytotoxicity to PDL, UMR−106 and SCAP cells than DW and Endocyn

(Continues)

TABLE 2 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Cell Viability	Cell Migration/attachment	Cell morphology	Cell differentiation/mineralization	Outcomes
Alghilan et al. (2017)	Human dentine samples and DPSCs (*n* = 10)	G1: TAP, G2: DTAP, G3: DAP, G4: CH, PC: 10 min 17% EDTA, NC: untreated	Dentine samples preparation, intracanal dressing, control groups treated with 200 µl of water, 7 d incubation, EDTA and PBS irrigation, DPSC on dentine, incubation for 24 h and 3 d, analyses	WST−1 assay (%). NC: ≅116 =, PC: ≅95 =, G1: ≅10 #, G2: ≅90 =, G3: ≅20 #, G4: ≅36 *	Attachment, LDH assay (%). PC: ≅22 =, G1: 40 =, G4: ≅28, G3: 15 #, G4: 10 *, NC: 10 *	n.a.	n.a.	EDTA and DTAP had more DPSC proliferation compared with the others, without difference with untreated control. Moreover, EDTA showed higher cell attachment than CH and untreated control
Galler et al. (2016)	Human dentine discs and DPSCs (*n* = 9, cell viability/migration; *n* = 3, cell differentiation)	Cell viability. G1: Polystyrene, G2: DW, G3: EDTA, G4: 5.25% NaOCl; Cell migration. PC: αMEM +20% FBS, NC: αMEM +1% FBS, G1: 10 min 10% EDTA, G2: 10 min 5.25% NaOCl, G3: DW; Cell differentiation. G1: DW, G2: EDTA +saline, G3: polystyrene	Cell migration: cell seeding onto dentine discs, chambers with αMEM, discs in lower chambers, solutions, cells removed from the membrane after 24 and 48 h, analysis. Cell viability: discs conditioned, cells seeded, assays at 24 and 48 h. Cell differentiation: cells seeded onto discs, immersion in the solutions for 7 d, analysis	MTT assay (%). 24 h. G1: ≅140 # G2: 100 = G3: ≅95 # G4: ≅10. 48 h. G1: ≅120 = G2: 100 = G3: ≅95 # G4: ≅15	Crystal violet assay (%). 24 h. PC: 100 # G1: 70 # G2: ≅30 = G3: ≅25 = NC: ≅25; 48 h. PC: 10 # G1: ≅75 # G2: ≅20 # G3: ≅30 = NC: ≅30	n.a.	RT-PCR. G1: 1 for mineralization markers, G2: > expression compared with G1, G3: < expression compared with G1, except for COL1A1 and RUNX2	EDTA did not influence viability, but increased migration and differentiation of DPSCs
Gonçalves et al. (2016)	Human tooth slices and SHEDs (*n* = 10)	G1: PBS with PD, G2: PBS without PD, G3: 1 min 10% EDTA with PD, G4: 1 min 10% EDTA without PD, G5: 5 d 2.5% NaOCl with PD, G6: 5 d 2.5% NaOCl without PD, Control 1: alpha-MEM with 20% FBS, Control 2: alpha-MEM with 10% FBS	Tooth slice with or not PD, slices conditioned with irrigating, transferred to 24-well plates, incubation at for 3 d, cells seeded onto tooth slice CM, migration assay	n.a.	Fluorescent analysis (Arbitrary units). G1: ≅4000 # G2: 3000 # G3: ≅4000 # G4: 3000 # G5: 4000 # G6: >3000 # Control 1: >4000 # Control 2: ≅3000	n.a.	n.a.	Dentine slices with PD increased cell migration compared with conditioned slices without PD; however, EDTA does not impact SHED migration

TABLE 2 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Cell Viability	Cell Migration/ attachment	Cell morphology	Cell differentiation/ mineralization	Outcomes
Kawamura et al. (2016)	C2C12 and HUVEC (n = 4, cell attachment; n = 3, cell differentiation)	For *in vitro* analyses, G5: non-extracted, G6: EDTA extracts, G7: CM, G8: CM +EDTA extracts, *PC: 1 μg/ml VEGF, FGF-β, and IGF for endothelial differentiation	For proliferation: cells cultured in the extracts for 2, 12, 24, 36, and 48 h; for adhesion: cells cultured on dentine and CM for 48 h; for differentiation: cells cultured in the extracts for 28 and 14 d, analyses	Proliferation, CCK−8 (absorbance). 2h. G5 < 0.1 = G6: <0.1 = G7: <0.1 = G8: <0.1; 12 h. G5: >0.1 = G6: >0.1 # G7: ≅0.4 = G8: >0.4; 24 h. G5: ≅0.2 = G6: >0.2 # G7: ≅0.6 # G8: >0.6; 36 h. G5: >0.3 = G6: >0.3 # G7: ≅0.7 # G8: 0,8; 48 h. G5: ≅0.4 = G6: ≅0.4 # G7: ≅0.8 # G8: ≅1.0	Chemotaxis assay (no cell). 3 h. G5: >10 = G6: >12.5 # G7: ≅22.5 # G8: >25; 6 h. G5: >12.5 # G6: ≅17.5 # G7: >27.5 # G8: ≅32.5; 9 h. G5: ≅15 = G6: >17.5 # G7: ≅30 # G8: ≅34; 12 h. G5: ≅15 = G6: >17.5 # G7: ≅30 # G8: ≅35; 15 h. G5: >15 = G6: >17.5 # G7: >30 # G8: ≅35; 18 h. G5: >15 = G6: >17.5 # G7: >30 # G8: >35; 21 h. G5: >15 = G6: >17.5 # G7: >30 # G8: >35; 24 h. G5: ≅15 = G6: ≅17 # G7: ≅30 # G8: ≅35. Adhesion, Giemsa stain (mm^2). G5: ≅35 = G6: ≅35 # G7: ≅56 # G8: ≅60 # PC: ≅80	n.a.	C2C12 odontoblastic differentiation, DSPP, enamelysin and β-Actin, RT-PCR. G5 and G7: no effect, G6 and G8: induced differentiation; HUVEC endothelial differentiation, Immunocytochemistry of VE-cadherin (fluorescence) G5 and PC: not mentioned, G6: in increase, G7 and G8: induced differentiation	EDTA extracts with CM promoted cell proliferation, migration and odontoblastic differentiation; however, EDTA extracts showed no increase cell adhesion and in endothelial differentiation
Mollashahi et al. (2016)	Stem cells from immature third molars (n = n.a.)	G1: 2% CHX, G2: 17% EDTA, G3: Qmix, G4: 5.25% NaOCl, G5: BioPure MTAD Cleanser, G6: sterile saline, Control: untreated	SCAPs cultured for 1 w, exposure to the solutions for 1.5 and 15 min, MTT assay	MTT assay (%). 1 min. G1: 60 # G2: ≅50 = G3: ≅57 = G4: ≅57 # G5: ≅48 # G6: ≅100 = Control: 100; 5 min. G1: 60 = G2: 50 = G3: ≅53 = G4: ≅53 # G5: 40 # G6: ≅100 = Control: 100; 15 min. G1: 60 # G2: ≅41 = G3: ≅43 = G4: ≅43 # G5: ≅30 # G6: ≅100 = Control: 100	n.a.	n.a.	n.a.	EDTA and the other groups had higher cytotoxicity than sterile saline and untreated control

(Continues)

TABLE 2 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Cell Viability	Cell Migration/ attachment	Cell morphology	Cell differentiation/ mineralization	Outcomes
Sadaghiani et al. (2016)	Dentine powder, human dentine slices and DPSCs (n = 3)	G1: 10% EDTA, G2: 37% PHA, G3: 10% CA, G4: 25% PA, NC: PBS, PC: CH	Extraction of dentine matrix, conditioned for 5 or 10 min, frozen, rinse with DW, immunogold localization, DPSC seeded onto conditioned dentine, analysis at 1 and 8 d	n.a.	n.a.	SEM. 2 d. Many cells on conditioned slices. Cell coverage against the open dentinal tubules with G1, G2, G3 and G4 compared with PC; 8 d. G1, G3 and PC: uniform/thick coverage by cells ≅odontoblast-like, G2: reduced cells; NC: few cells visible	RT-PCR (%). (RUNX2, 3 d. control: >1.4 =, G1: >1.2 =, G2: >1.2 =, G3: 1.2; 21 d. control: 1.2 =, G1: >1.2 =, G2: >1.2 =, G3: >0.8 =; ALP, 3 d. control: MC =, G1: MC =, G2: MC =, G3: MC =; 21 d. control: ≅0.25 =, G1: ≅0.4 #, G2: >0.4 #, G3: >0.3 =; OPN, 3 d. control: MC =, G1: MC =, G2: MC =, G3: MC =; 21 d. control: MC =, G1: >0.045 #, G2: >0.025 #, G3: 0.04 #). Conditioned dentine slices. (ALP, 5 d. NC: MC =, PC: MC =, G1: MC =, G2: MC =, G3: MC =; 14 d. NC: <0.2 =, PC: <0.2 =, G1: 0.6 #, G2: >0.1 =, G3: 0.55 #; OPN, 5 d. NC: <0.01 =, PC: >0.01 #, G1: 0.01 =, G2: <0.01 =, G3: <0.01 =; 14 d. NC: ≅0.03 =, PC: >0.02 =, G1: 0.05 #, G2: ≅0.04 =, G3: ≅0.035 =)	EDTA-treated dentine showed a thick coverage by cells with odontoblast-like appearances. In DMEs, EDTA increased ALP and OPN at 21d. In dentine slice, EDTA increased ALP and OPN at 14 d

TABLE 2 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Cell Viability	Cell Migration/ attachment	Cell morphology	Cell differentiation/ mineralization	Outcomes
Shrestha et al. (2016)	Human dentine discs and SCAPs ($n = 6$)	G1: control (no treatment), G2: 10 min 5.25% NaOCl, G3: 2 min 17% EDTA, G4: 10 min NaOCl +2 min EDTA, G5: 10 min NaOCl +1 min EDTA +1 min NaOCl	Dentine discs preparation, irrigating protocols, rinse with DW, nanoparticle conditioning, SCAPs cultured on discs for 24 h, staining with calcein-AM, analyses	n.a.	Attachment, fluorescence. G1: unidirectional cells, G2 and G5: less cells, G3: similar cell nº of G1, G4: increase in no of SCAP adherence when nanoparticle conditioning with CSnp or Dex-CSnp	Fluorescence microscope. G1: fibroblast-like morphology, G2 and G5: rounded cells without cytoplasmic extensions and less cytoplasmic F-actin compared with the other groups, G3: similar morphology of G1 but more flattened, G4: few cells with cytoplasmic extensions	n.a.	EDTA alone promoted similar cell adherence and morphology to control
Kim et al. (2015)	Human dentine discs and DPSCs ($n = 4$)	G1: 500 mg DAP (metronidazole and ciprofloxacin), G2: 500 mg DAP +10 min 17% EDTA, G3: 1 mg DAP, G4: 1 mg DAP +10 min 17% EDTA, G5: 10 min 17% EDTA, Control: no treatment	Dentine discs preparation, DAP medicated, 1w incubation, DAP-samples rinsed with DW, EDTA, DPSCs seeded on discs, LDH assay at 24 h, assay at 3 d	WST−1 assay (%). G1: 0 = G2: 20 # G3: ≅65 = G4: ≅77 = G5: ≅100 = control: 85	Attachment, LDH assay (%). G1: ≅7 # G2: ≅32 # G3: ≅10 # G4: 35 = G5: ≅25 = control: ≅35	n.a.	n.a.	10 min EDTA may have positive effects cell attachment, but not influenced the proliferation

(Continues)

TABLE 2 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Cell Viability	Cell Migration/ attachment	Cell morphology	Cell differentiation/ mineralization	Outcomes
Park et al. (2015)	Human dentine discs and DPSCs ($n = 250$)	G1: 30 min 5.25% NaOCl, G2: 30 min 5.25% NaOCl +7 d 1 mg/ml CH +PBS, G3: 30 min 5.25% NaOCl +7 d 1 mg/ ml CH +3 min 17% EDTA, G4: 30 min 5.25% NaOCl +7 d 1 mg/ml CH +3 min 17% EDTA +24 h culture media, G5: 30 min 5.25% NaOCl +7 d 1 mg/ml CH +instrumentation +3 min 17% EDTA, control: cell culture	Flow cytometric analysis, dentine slices preparation, cells seeded onto dentine, samples cultured for 7 d, viability and morphology assays, cells cultured for 4 w for cell differentiation assay	MTT assay (%). Control: 100 # G1: ≅2 = G2: ≅17 = G3: ≅22 = G4: 20 = G5: ≅22	Attachment, RT-PCR. FN−1. G2: 1 #, G3: ≅1.7 =, G4: ≅2 =, G5: ≅2 =; SPP−1. G2: 1 #, G3: ≅1.4 =, G4: approx. 1.3 =, G5: ≅1.8	SEM analysis. 7 d. G1: DPSCs not attached to the dentine, G2/G3/ G4/G5: elongated cells with longer cytoplasmic processes, G5: dentine overlapped by proliferated cell layers	RT-PCR. DMP−1. Control: 1 # G2: ≅1.2 # G3: ≅1.5 # G4: ≅2.3 = G5: ≅1.8; DSPP−1. Control: 1 = G2: ≅1.2 # G3: ≅1.4 # G4: ≅1.7 =G5: ≅2	EDTA did not influence cell viability, but additional treatment with EDTA after NaOCl and CH enhanced cell attachment and differentiation
Martin et al. (2014)	Human root canals and SCAPs ($n = 9—12$)	G1: 10 min 0,5% NaOCl +5 min saline, G2: 10 min 0,5% NaOCl +5 min EDTA, G3: 10 min 1.5% NaOCl +5 min saline, G4: 10 min 1.5% NaOCl +5 min EDTA, G5: 10 min 3% NaOCl +5 min saline, G6: 10 min 3% NaOCl +5 min EDTA, G7: 10 min 6% NaOCl +10 ml 5% ST+5 min saline, G8: 10 min 6% NaOCl +5 min EDTA, PC: EDTA, NC: saline	Preparation of root segments, scaffold preparation, irrigation protocols, SCAPs with hyaluronic acid–based scaffold seeded into the canals, samples cultured for 7 d, analyses	Luminescence ($\times 10^3$). NC: 38 =, PC: ≅52 #, G1: ≅23 =, G2: ≅38 #, G3: ≅22 =, G4: ≅42 #, G5: ≅23 =, G6: ≅39 #, G7: ≅5.5 =, G8: ≅30 #	n.a.	n.a.	RT-PCR (DSPP expression). NC: 1.0 =, PC: ≅2.2 #, G3: ≅1.2 =, G4: ≅1.8 #, G5: ≅0.5 =, G6: >1.2 #, G7: 0 =, G8: ≅0.4 #	EDTA increased SCAPs survival and DSPP expression

TABLE 1 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Cell Viability	Cell Migration/attachment	Cell morphology	Cell differentiation/mineralization	Outcomes
Pang et al. (2014)	Human dentine discs and DPSCs ($n = 20$)	Cell attachment, G1: 17% EDTA, control: no treatment; Cell differentiation/mineralization, NC: proliferation medium, ND: untreated dentine +proliferation medium, ED: proliferation medium +EDTA-treated dentine, UED: proliferation medium +upper chamber EDTA-treated dentine, PC: differentiation medium	Attachment: dentine discs preparation, cells seeded on dentine slices, cultured for 3 d, analyses; Differentiation /mineralization: cells placed on dentine for 21 d, dentine slices replenished every 3d, analyses	n.a.	Attachment, cell density. G1: ≅2.4 # control: ≅1; RT-PCR (FN−1 expression). G1: ≅3.1 # control: ≅1; SEM analysis. G1: longer cytoplasmic processes with many granules compared with control	n.a.	RT-PCR. DMP−1. NC: 1 = ND: ≅1.2 = UED: ≅1 # ED: ≅3.1 # PC: ≅5.2; DSPP. PC: 1 = ND: 1 = UED: ≅1 # ED: 3 # PC: ≅4.3; ALP. NC: 1 = ND: 1 = UED: ≅1.3 # ED: ≅1.8 # PC: ≅3.2; OCN. NC: 1 = ND: 1 = UED: ≅1 = ED: ≅1.1 # PC: ≅2.7	EDTA-treated dentine promoted cell attachment and odontoblastic/osteoblastic differentiation
Huang et al. (2011)	Human single-rooted premolars and HDPSCs ($n = 5$)	Control: 1 min 5 ml DW, G1: 1 min 5.25% NaOCl, G2: 1 min 17% EDTA, G3: 1 min MTAD	Root slices preparation, irrigating protocols (5 ml), DW rinse, cells onto the root canal, incubation for 72 h, samples dyed, analysis	n.a.	Attachment, fluorescence microscope (cell/field). Control: ≅1.6 = G1: ≅1.6 # G2: ≅6 # G3: 11.4	Fluorescence microscope. Control and G1: round shape cells, G2 and G3: spindle-shaped cells	n.a.	EDTA and MTAD significantly increased HDPC attachment with spindle-shaped cells

(Continues)

TABLE 2 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Cell Viability	Cell Migration/attachment	Cell morphology	Cell differentiation/mineralization	Outcomes
Ring et al. (2008)	Human root canals, SHEDs and L929 ($n = 30$, experimental groups; $n = 8$, control)	G1: 6% NaOCl, G2: 6% NaOCl +15 s EDTA +6% NaOCl, G3: 6% NaOCl +5 min MTAD +15 s MTAD, G4: 2% CHX +15 s EDTA +2% CHX, G5: AquatineEC +15 s EDTA +AquatineEC, G6: MCJ +15 s EDTA +MCJ, G7: saline +DPSCs, G9: saline +15 s EDTA +saline +DPSCs, G10: saline +15 s EDTA +saline +L929 cells, control: saline without DPSCs	Teeth preparation, Protaper and ProFile instrumentation, irrigation, cells seed into root canals, samples cultured for 7 d, analyses at 2, 4 and 7 d	LDH assay (560 nm). G1: ≅0.19 = G2: 0.15 = G3: ≅0.08 = G4: ≅0.08 # G5: ≅0.025 = G6: >0.025 = G7: 0.025 = G8: <0.025 = G9: <0.025	Attachment, SEM analysis (cell count). G1: ≅2.7 # G2: ≅3.2 # G3: 1.5 #, G4: ≅2.6 # G5: ≅5.7 # G6: ≅4.2 # G7: ≅5.4 = G8: ≅5 = G9: ≅5.5	SEM analysis. G1/G2/G3 and G4: round to oblong-shaped cells, G5/G6/G7/G8 and G9: oblong to flattened cells	n.a.	EDTA did not influence cytotoxicity and cell adherence

Note: The symbol * indicates additional group per analysis; = indicates no significant differences between/amongst groups; # indicates significant differences between/amongst groups; ≅ indicates 'approximately'; > indicates 'greater than'; < indicates 'less than'.

Abbreviations: °C, degree Celsius; adMSCs, adipose-derived mesenchymal stem cells; ALP, alkaline phosphatase; a-MEM, alpha-minimum essential medium; APCs, apical papilla cells; AquatineEC, aquatine endodontic cleanser; BAC, benzalkonium chloride; C2C12, mouse embryonic muscle myoblast cells; CA, citric acid; CCK-8, Cell Counting Kit-8; CH, calcium hydroxide paste; CHX, chlorhexidine; CLSM, confocal laser scanning microscope; CM, conditioned medium; COL1A1, collagen type I; d, days; DAP, double antibiotic paste (ciprofloxacin and metronidazole); DME, dentine matrix extract; DMP, dentine matrix protein extracts; DMP-1, dentine matrix acidic phosphoprotein-1; DPCs, dental pulp cells; DPSCs, dental pulp stem cells; DSPP, dentine sialophosphoprotein; DTAP, diluted triple antibiotic paste; DW, distilled water; EA, EndoActivator; EDTA, ethylenediaminetetraacetic acid; FBS, foetal bovine serum; FGF-b, fibroblast growth factors beta; FN-1, fibronectin-1; G, group; GaAlAs, gallium–aluminium–arsenide; GM, growth culture medium; h, hour; HDPC, human dental pulp cell; HDPSCs, human dental pulp stem cells; HEDP, etidronic acid; HUVEC, human umbilical vein endothelial cells; IGF, insulin-like growth factor,; IP6, phytic acid; j/cm2, joules per square centimetre; LDH, lactate dehydrogenase activity; MC, minimal concentration or zero; MCJ, morinda citrifolia juice; MDP, mouse dental papilla; mg, milligram; min, minutes; ml, millilitre; $mm^{2,}$ square millimetre; MSCs, mesenchymal stem cells; MTAD, mixture tetracycline citric acid and detergent; *n*, number of specimens; n.a., not applicable; NaOCl, sodium hypochlorite; NBs, nanobubble water; NC, negative control; NI, needle irrigation; nm, nanometre; NSS, normal saline solution; OCN, osteocalcin; OPN, Osteopontin; OW, ozonated water; PA, polyacrylic acid; PBS, sterile phosphate-buffered saline; PC, positive control; PD, predentine layer; PDL, human periodontal ligament; PDT, photodynamic therapy; PHA, phosphoric acid; PUI, passive ultrasonic irrigation; RT-PCR, real-time polymerase chain reaction; RUNX2, runt-related transcription factor 2; s, seconds; SCAPs, stem cells of the apical papilla; SDF-1α, stromal cell-derived factor 1 α; SEM, scanning electron microscope; SHEDs, stem cells from human exfoliated deciduous teeth; siCXCR4, silencing CXCR4; SPP-1, secreted phosphoprotein-1; ST, sodium thiosulfate; TAP, triple antibiotic paste (ciprofloxacin, metronidazole and minocycline); TEM, transmission electron microscopy; UMR-106, rat osteosarcoma cells; USA, ultrasonic activation; VEGF, vascular endothelial growth factor; w, week; WST-1, water soluble tetrazolium salts; α-tubulin, alpha tubulin; μg, micrograms; μl, microlitre.

TABLE 3 The effects of EDTA on blood clot, tissue healing and regeneration of immature teeth models

Author	Experimental model (*n*)	Groups	Experimental protocol	Tissue inflammation	Tissues in-growth	Increase in the root length /root thickness	Decrease in apical diameter	Mineralization/differentiation	Bone or root resorption	Blood clot characterization	Outcomes
Taweewattanapaisan et al. (2019)	Human mandibular premolars ($n = 7$), *in vitro*	G1: 5 min SS, G2: 1 min 17% EDTA +SS (E1N), G3: 5 min 17% EDTA +SS (E5N), G4: 1 min 17% EDTA (E1), G5: 5 min 17% EDTA (E5)	Roots preparation, irrigations, specimens split vertically in half, BC sample collection spread on specimens, analyses	n.a.	n.a.	n.a.	n.a.	n.a.	n.a.	SEM. G1 and G2: dense meshwork of fibrins with abundant biconcave erythrocytes, G3: meshwork of fibrins with biconcave and shrinkage erythrocytes, G4 and G5: < and shorter fibrins than G1 with inactivated platelets. Fibrin density. SEM (no./10 µm). Coronal. G1: 11.60 =, G2: 8.99 =, G3: 8.34 =, G4: 0.22 #, G5: 0.17 #; Middle. G1: 12.38 =, G2: 9.51 =, G3: 9.13 =, G4: 0.22 #, G5: 0.23 #; Apical. G1: 13.33 =, G2: 7.69 =, G3: 11.14 =, G4: 0.23 #, G5: 0.30 #	1 and 5 min EDTA alone decreased BC formation; EDTA with SS final flushing had results similar to the group that used only SS

(Continues)

TABLE 3 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Tissue inflammation	Tissues in-growth	Increase in the root length /root thickness	Decrease in apical diameter	Mineralization/ differentiation	Bone or root resorption	Blood clot characterization	Outcomes
El Ashry et al. (2016)	Premolars of mongrel dogs (*n* = 48), *in vivo*	G1: BC, G2: BC +collagen, G3: BC +2 min 17% EDTA, G4: BC +collagen +17% EDTA, G5: BC +MTAD, G6: BC +collagen + MTAD, PC: exposed teeth, NC: untouched teeth	PA lesion induction, disinfection with 2.6% NaOCl, intracanal dressing with TAP, coronal seal for 3 w, TAP removed by NaOCl, treatment protocols, evaluations at 2, 6 and 12 w	HE (scores). 2 w. G1: 2.1=, G2: 2.4=, G3: 2.3=, G4: 2.6=, G5: 1.4#, G6: 1.6#, PC: 2.7=, NC: 0#; 6 w. G1: 1.3=, G2: 1.6=, G3: 1.4=, G4: 1.7=, G5: 0.9=, G6: 1=, PC: 2.9#, NC: 0#; 12 w. G1: 0.4=, G2: 0.7=, G3: 0.6=, G4: 0.9=, G5: 0.5=, G6: 0.6=, PC: 3#, NC: 0#; (cell count) 2 w. G1: 25=, G2: 25.8=, G3: 25.5=, G4: 26=, G5: 20#, G6: 20.9#, PC: 28.4=, NC: 2.5#; 6 w. G1: 16.4=, G2: 16.9=, G3: 16.6=, G4: 17.1=, G5: 14.9=, G6: 15.6=, PC: 36.7#, NC: 2.3#; 12 w. G1: 10.2=, G2: 10.7=, G3: 10.4=, G4: 10.9=, G5: 9.2=, G6: 9.7=, PC: 38.9#, NC: 2.1#	HE (scores). 2 w. G1: 0.6 =, G2: 0.7 =, G3: 0.9 =, G4: 1 =, G5: 0.4 =, G6: 0.6 =, PC: 0.3 =, NC: NM; 6 w. G1: 1.3 =, G2: 1.4 =, G3: 1.9 #, G4: 2 #, G5: 1 =, G6: 1.1 =, PC: 0.9 =, NC: NM; 12 w. G1: 1.9 =, G2: 2 =, G3: 2.6 #, G4: 2.7 #, G5: 1.6 =, G6: 1.7 #, PC: 0.9 #, NC: NM	RGe (mm). Increase in root length (2 w. G1: 4.9 =, G2: 5.4 =, G3: 5.1 =, G4: 5.7 =, G5: 4.8 =, G6: 5.3 =, PC: 0 #, NC: 6.2 =; 6 w. G1: 13.9 =, G2: 14.6 =, G3: 14 =, G4: 14.8 =, G5: 13.7 =, G6: 14.5 =, PC: 0 #, NC: 15.5 =; 12 w. G1: 16.3 =, G2: 17.1 =, G3: 16.5 =, G4: 17.7 =, G5: 16.1 =, G6: 16.9 =, PC: 0 ± 0 #, NC: 19.7 =); Increase in root thickness (2 w. G1: 4 =, G2: 4.8 =, G3: 4.7 =, G4: 5.3 =, G5: 3.9 =, G6: 4.5 =, PC: 0 #, NC: 6.1 =; 6 w. G1: 11.4 =, G2: 12.1 =, G3: 11.6 =, G4: 12.3 =, G5: 11.2 =, G6: 11.7 =, PC: 0 #, NC: 12.4 =; 12 w. G1: 13.4 =, G2: 14.2 =, G3: 13.6 =, G4: 14.4 =, G5: 12.7 =, G6: 13.8 =, PC: 0 #, NC: 15.8 =)	RGe (mm). 2 w. G1: 2.9 =, G2: 3.6 =, G3: 3.1 =, G4: 3.8 =, G5: 2.7 =, G6: 3.5 =, PC: 0 #, NC: 6.2 #; 6 w. G1: 16.4 =, G2: 17.3 =, G3: 16.5 =, G4: 17.6 =, G5: 16.2 =, G6: 16.9 =, PC: 0 #, NC: 20.5 #; 12 w. G1: 30.2 =, G2: 31.9 =, G3: 30.8 =, G4: 32.2 =, G5: 29.8 =, G6: 31.5 =, PC: 0 #, NC: 47 #; HE (%). 2 w. G1: 0 =, G2: 0 =, G3: 0 =, G4: 0 =, G5: 0 =, G6: 0 =, PC: 0 =, NC: 0 =; 6 w. G1: 33.3 =, G2: 41.7 =, G3: 33.3 =, G4: 41.7 =, G5: 33.3 =, G6: 41.7 =, PC: 0 =, NC: 50 =; 12 w. G1: 50 =, G2: 58.3 =, G3: 50 =, G4: 58.3 =, G5: 50 =, G6: 58.3 =, PC: 0 =, NC: 58.3 =	HE (scores). 2 w. G1: 0.4 =, G2: 0.6 =, G3: 0.6 =, G4: 0.7 =, G5: 0.3 =, G6: 0.4 =, PC: 0 =, NC: NM; 6 w. G1: 0.7 =, G2: 0.9 =, G3: 0.9 =, G4: 1 =, G5: 0.6 =, G6: 0.7 =, PC: 0 =, NC: NM; 12 w. G1: 1.3 =, G2: 1.4 =, G3: 1.4 =, G4: 1.6 =, G5: 1.1 =, G6: 1.3 =, PC: 0 =, NC: NM	HE (%). 2 w. G1: 75 =, G2: 83.3 #, G3: 75 =, G4: 83.3 #, G5: 75 =, G6: 83.3 #, PC: 83.3 #, NC: 0 #; 6 w. G1: 41.6 =, G2: 58.3 #, G3: 41.6 =, G4: 58.3 #, G5: 41.6 =, G6: 58.3 #, PC: 100 #, NC: 0 #; 12 w. G1: 16.6 =, G2: 25 #, G3: 16.6 =, G4: 25 #, G5: 16.6 =, G6: 25 #, PC: 100 #, NC: 0 #	n.a.	The use of EDTA no influenced the inflammation, the root length or thickness, apical diameter, mineralization and bone resorption; EDTA allowed significant tissue in-growth in the pulp space at 6 and 12 w

TABLE 3 (Continued)

Author	Experimental model (*n*)	Groups	Experimental protocol	Tissue inflammation	Tissues in-growth	Increase in the root length /root thickness	Decrease in apical diameter	Mineralization/ differentiation	Bone or root resorption	Blood clot characterization	Outcomes
Kawamura et al. (2016)	Porcineroots, immunodeficiency mice and MDPSC ($n = 4$), *in vivo*	P1. Regenerative / angiogenesis area, cell differentiation. G1: non-extracted tooth, G2: HCl-extracted tooth, G3: GdnHCl-extracted tooth, G4: EDTA-extracted tooth. P2. ENM+cells, PLAP−1, and angiogenic potential. G5: non-extracted, G6: EDTA extracts, G7: CM, G8: CM +EDTA extracts, G9: GdnHCl, G10: CM +GdnHCl, NC: autoclaved teeth	P1 and P2. Cell culture, preparation of CM (P2), roots preparation, teeth demineralized with the solutions for 7 d, washing with PBS, MDPSCs were injected into the teeth with collagen (P1) and with CM or not (P2), teeth sealed, subcutaneous implantation, analyses at 28 d		P1. Regenerative area, (%). G1: ≅80 # G2: 70 = G3: ≅63 # G4: ≅7; RECA1 IMS (%) G1: ≅16 # G2: 10 = G3: 10 # G4: ≅3; PLAP−1 (%) G1: 10 = G2: 13 # G3: ≅17 # G4: ≅58; P2. PLAP−1 (%) G5: ≅12 # autocl.: MC =G6: MC # G7: >5 = G8: >6 # G9: MC # G10: >5 =; IHC, PLAP−1 (%) G5: ≅15#autocl.: ≅1 = G6: ≅1 # G7: ≅25 # G8: ≅16			P1. Hoechst 33342+ cells (FLC/ mm^2). G1: ≅3.000 #, G2: ≅2.500 =, G3: ≅2.000 #, G4: >1.750 #; ODD (ENM+cells). G1: >120 =, G2: ≅120 =, G3: ≅120 =, G4: ≅20 #; P2. ODD (ENM+cells). G5: ≅140 = G8: <140 # G7: >100 # G6: MC =autocl.: MC.			P1 (Cell with collagen). EDTA had less regenerative/ angiogenesis area and cells; fewer ODD and endothelial differentiation. However, it had more periodontal ligament cells. P2 (cells with CM or not). EDTA did not influence periodontal ligament cells. EDTA allowed higher ODD and endothelial differentiation when associated with CM
Yamauchi et al. (2011)	Double-rooted canine premolars ($n = 12$), *in vivo*	G1: BC, G2: BC +CCS, G3: BC +2 min 17% EDTA, G4: BC +CCS + EDTA, PC: infected only, NC: untreated	PA lesion induction, 2.5% NaOCl irrigation, TAP, IRM for 2 w; 2.5% NaOCl and saline irrigations, protocols, BC inducted, sealing, 3,5 months, analysis	n.a.	n.a.	RGe (%). G1: 64.2 =, G2: 87.5 #, G3: 54.2 =, G4: 83.3 #	HE. G2 and G4: most specimens had apical closure, some showed no complete apical closure and forming bony islands	HMM (%). DAMT. G1: ≅5=, G2: >10=, G3: >2.5 =, G4: >12.5 =; bony islands. G1: >5 =, G2: ≅14=, G3: 5 =, G4: >17=; total. G1: >10 =, G2: ≅25#, G3: >7.5=, G4: ≅32 #	Periapical radiolucencies improvement. RGe (%). G1: 56.52 =, G2: 79.2 #, G3: 58.3 =, G4: 87.5#	n.a.	EDTA did not influence the root thickening and periapical radiolucencies; EDTA allowed higher apical closure and mineralization

Note: The symbol # indicates significant differences between/amongst groups; ≅ indicates 'approximately'; > indicates 'greater than'; < indicates 'less than'.

Abbreviations: °C, degree Celsius; BC, blood clot; CCS, cross-linked type I collagen scaffold; CM, conditioned medium; d, days; DAMT, dentine-associated mineralized tissue; EDTA, ethylenediaminetetraacetic acid; ENM, enamelysin; FLC, fluorescence; G, group; GdnHCl, guanidine hydrochloride; HCl, hydrochloric acid; HE, haematoxylin and eosin; HMM, histomorphometry; IHC, immunohistochemistry; IMS, immunostaining; IRM, intermediate restorative material; MC, minimal concentration or zero; MDPSCs, mobilized dental pulp stem cells; min, minutes; mm, millimetre; MTAD, mixture tetracycline citric acid and detergent; n.a., not applicable; *n*, number of specimens; NaOCl, sodium hypochlorite; NC, negative control; NM, not mentioned; NSS, normal saline solution; ODD, odontoblastic differentiation; P1, part one; P2, part two; PA, periapical; PBS, sterile phosphate-buffered saline; PC, positive control; PLAP-1, periodontal ligament-associated protein 1; RGe, radiographic evaluation; SEM, scanning electron microscope; TAP, triple antibiotic paste (ciprofloxacin, metronidazole and minocycline); w, week; μm, micrometre.

additional records were found through manual search in the references lists.

Characteristics of the included studies

Table 1 summarizes the studies that evaluated growth factors' release/expression (Aksel et al., 2020; Atesci et al., 2020; Bracks et al., 2019; Cameron et al., 2019; Chae et al., 2018; Deniz Sungur et al., 2019; Duncan et al., 2017; Galler et al., 2015; Gonçalves et al., 2016; Ivica et al., 2019; Kucukkaya Eren et al., 2021; Li et al., 2020; Liu et al., 2019; Ranc et al., 2018; Sadaghiani et al., 2016; Widbiller et al., 2017; Zeng et al., 2016). Table 2 summarizes information from the *in vitro* studies that evaluated cell behaviour (Aksel et al., 2020; Alghilan et al., 2017; Atesci et al., 2020; Chae et al., 2018; Deniz Sungur et al., 2019; Galler et al., 2016; Gonçalves et al., 2016; Hashimoto et al., 2018; Huang et al., 2011; Ivica et al., 2019; Kawamura et al., 2016; Kim et al., 2015; Kucukkaya Eren et al., 2021; Li et al., 2020; Liu et al., 2019; Martin et al., 2014; Mollashahi et al., 2016; Pang et al., 2014; Park et al., 2015; Prompreecha et al., 2018; Ring et al., 2008; Sadaghiani et al., 2016; Scott et al., 2018; Shrestha et al., 2016; Tunç et al., 2019; Widbiller et al., 2019), and Table 3 shows studies that evaluated tissue regeneration in immature teeth models (El Ashry et al., 2016; Kawamura et al., 2016; Taweewattanapaisan et al., 2019; Yamauchi et al., 2011). According to the study design, 32 studies were only *in vitro*, three studies were *in vivo*, and one study used both models.

Of the 33 *in vitro* studies, three studies used dentine discs alone (Duncan et al., 2017; Galler et al., 2015; Widbiller et al., 2017), whilst most studies used dentine discs and different stem cells. The roots of permanent human teeth with stem cells (Atesci et al., 2020; Huang et al., 2011; Li et al., 2020; Martin et al., 2014; Prompreecha et al., 2018; Ring et al., 2008) or alone (Cameron et al., 2019; Chae et al., 2018; Ranc et al., 2018; Widbiller et al., 2017; Zeng et al., 2016) were also used as an experimental model. Amongst these studies, EDTA concentrations ranged from 3% to 15% at different exposure times. The second most frequent irrigating agent used after EDTA was 0.5%–6% NaOCl.

For the *in vivo* category, four animal studies were evaluated (Bracks et al., 2019; El Ashry et al., 2016; Kawamura et al., 2016; Yamauchi et al., 2011). Two studies only used 17% EDTA as the irrigating solution (Bracks et al., 2019; Yamauchi et al., 2011), whilst other studies also used guanidine hydrochloride (GdnHCl; Kawamura et al., 2016) and mixture tetracycline citric acid and detergent (MTAD; El Ashry et al., 2016). Regarding the regenerative protocols, three studies performed pulpectomies associated or not with an induction of periapical lesion in canine (El Ashry et al., 2016; Yamauchi et al., 2011) and mice posterior teeth (Bracks et al., 2019), whilst one used a subcutaneous tooth roots transplant into immunodeficient mice comparing an EDTA-treated tooth to an untreated control (Kawamura et al., 2016).

Growth factors release and expression

Amongst the 17 studies that evaluated the growth factors' release/expression (Table 1), TGF-β was assessed in 16 studies. EDTA effectively released TGF-β1 at 7 days (Bracks et al., 2019) or at all periods in 12 studies using concentrations of 10% (Duncan et al., 2017; Galler et al., 2015; Gonçalves et al., 2016; Sadaghiani et al., 2016; Widbiller et al., 2017), 12% (Liu et al., 2019), and for the most part of studies, 17% (Atesci et al., 2020; Bracks et al., 2019; Cameron et al., 2019; Chae et al., 2018; Ivica et al., 2019; Ranc et al., 2018). One available study reported an increase in TGF-β1 release, mainly after ultrasonic activation (Widbiller et al., 2017). Additionally, only two other studies found a significant TGF-β1 release in dentine conditioned with 17% EDTA under sterile conditions without biofilm (Cameron et al., 2019), or when a combination with adipose-derived mesenchymal stem cells (adMSCs) was applied, also increasing VEGF, bone morphogenetic protein-2 (BMP-2) and fibroblast growth factor (FGF)-2 (Atesci et al., 2020). Conversely, seven studies found no significant differences in TGF-β release after dentine conditioning with EDTA, compared with their control groups without EDTA (Aksel et al., 2020; Atesci et al., 2020; Cameron et al., 2019; Deniz Sungur et al., 2019; Kucukkaya Eren et al., 2021; Sadaghiani et al., 2016; Zeng et al., 2016).

When the expression of growth factors was evaluated with ELISA and PCR assays, 10% and 17% EDTA without any cell association or technique combination did not influence VEGF expression in four studies (Atesci et al., 2020; Bracks et al., 2019; Li et al., 2020; Sadaghiani et al., 2016), FGF in two studies (Atesci et al., 2020; Zeng et al., 2016) and BMP-2 in one study (Sadaghiani et al., 2016). On the contrary, one study that used immunogold localization visualized by scanning electron microscopy (SEM) showed that conditioning with 10% EDTA (5 and 10 min) enhanced the number of BMP-2 and VEGF particles released (Sadaghiani et al., 2016), in addition to a PCR analysis that found a great expression of nerve growth factor (NGF) at 14 days and insulin-like growth factor (IGF) at all evaluated periods after irrigation with 17% EDTA (Bracks et al., 2019).

The results of a proteomic assay showed that 10% EDTA extracted a significantly greater quantity of expressed angiogenic-associated growth factors (PDGF-AA and VEGF-A), BMP-7, brain-derived neurotrophic factor (BDNF), placenta growth factor (PIGF), hepatocyte growth factor (HGF) and some integrin growth factor-related family (IGFBP; Duncan et al., 2017). On the contrary, 10% EDTA did not influence the expression of FGF members, glial cell-line-derived neurotrophic factor (GDNF), IGFBP-2, mast/stem cell growth factor receptor (SCF-R) and insulin, in addition to decreasing the expressions of FGF-4, NGF receptor, epidermal growth factor receptor (EGFR-1) and growth/differentiation factor (GFD-15).

Assessment of cell behaviour using in vitro studies

Amongst the 19 studies that evaluated cell viability (Table 2), most studies ($n = 10$) showed no influence from the use of 17% EDTA-treated dentine (Alghilan et al., 2017; Chae et al., 2018; Deniz Sungur et al., 2019; Galler et al., 2016; Kim et al., 2015; Li et al., 2020; Liu et al., 2019; Park et al., 2015; Ring et al., 2008; Widbiller et al., 2019). On the contrary, six studies showed a reduction in cell viability, whereas the other six reported higher cell viability after using EDTA for conditioning or after performing an irrigation activation protocol.

Out of the 11 studies that evaluated cell morphology, nine found the presence of elongated- to flattened-shaped cells with fibroblastic-like appearances in EDTA-treated dentine. Regarding cell migration ($n = 7$), 10%–17% EDTA-treated dentine or extracts of different dilutions of EDTA for different exposure times significantly enhanced cell migration in five studies. Similarly, amongst 13 studies that evaluated cell attachment, eight found a higher adherence to conditioned dentine with EDTA. One study showed a reduction in cell adhesion in the dentine after EDTA conditioning (10 and 15 min), whilst the others reported no influence from EDTA.

For cell differentiation or mineralization protein assay, all seven articles (Galler et al., 2016; Hashimoto et al., 2018; Kawamura et al., 2016; Martin et al., 2014; Park et al., 2015; Pang et al., 2014; Sadaghiani et al., 2016) that performed this evaluation using real-time polymerase chain reaction (RT-PCR) found an increased odontoblastic differentiation and expression of mineralization markers, such as dentine sialophosphoprotein (DSPP) and dentine matrix acidic phosphoprotein (DMP)-1, after EDTA conditioning.

In matrix dentine extracts and dentine discs, 10% EDTA significantly increased alkaline phosphatase (ALP) and osteopontin (OPN) expression at 21 and 14 days respectively (Sadaghiani et al., 2016). One study observed that 5–10 min of 10% EDTA did not influence runt-related transcription factor 2 (RUNX2; Sadaghiani et al., 2016).

Tissue regeneration of immature teeth models

Data of tissue regeneration using immature teeth models are presented in Table 3. One animal study using 17% EDTA irrigation in REPs protocols found that this solution did not display influence on the presence of inflammatory cells (El Ashry et al., 2016). Regarding tissues' in-growth in the root canal space, one article found no statistical difference, whilst another reported a decrease in the regenerative area in the EDTA-treated group (El Ashry et al., 2016; Kawamura et al., 2016). Two studies using dogs' teeth (El Ashry et al., 2016; Yamauchi et al., 2011) observed no significant improvement in 17% EDTA irrigation in the root length/thickening and periapical radiolucencies. No influence on bone resorption was observed. One study showed higher apical diameter closure, whilst another reported no EDTA influence.

One record stated that irrigation with 17% EDTA improves mineralized tissue formation (Yamauchi et al., 2011), whilst another study found no EDTA influence (El Ashry et al., 2016). Regarding cell differentiation, one study found less odontoblastic/endothelial differentiation of dental pulp stem cells (DPSCs) in the EDTA-treated group; however, higher cell differentiation was observed in the group that used EDTA combined with Dulbecco's modified Eagle's medium (DMEM) containing mobilized DPSCs (Kawamura et al., 2016).

Synthesis of results

Meta-analysis was not performed due to wide variations amongst studies in methods for assessment, irrigation protocols, and different concentrations and time of exposure of EDTA. Included studies also failed to report the dispersion measure (standard deviation) of the measure of effect (mean difference).

Risk-of-bias assessment within in vitro and animal studies

Risk-of-bias analyses are presented in Supplementary File S2 and Figure 2. Regarding the critical appraisal of *in vitro* studies (Figure 2a), a high risk of bias was observed only

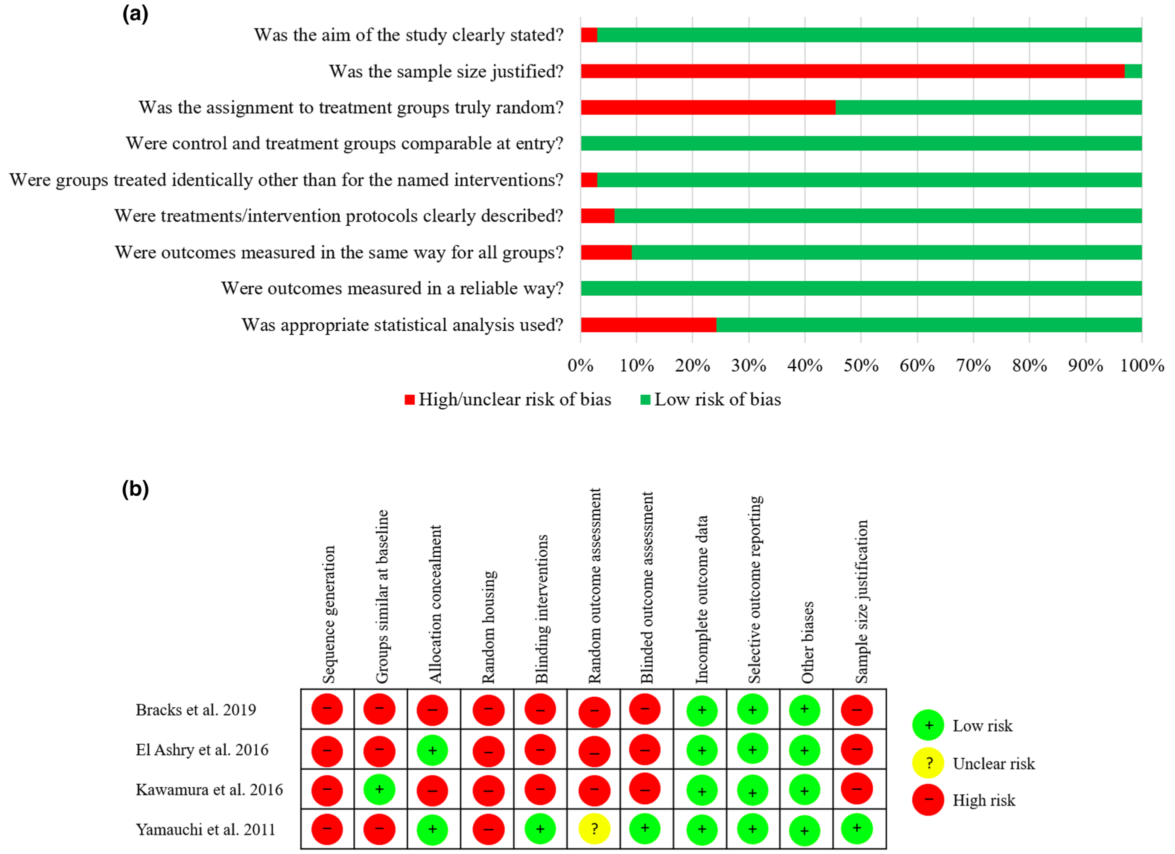

FIGURE 2 Risk-of-bias assessment of the included studies. (a) Assessment of the risk of bias in the *in vitro* studies according to the percentage of scores attributed to each evaluated study (Joanna Briggs Institute's Critical Appraisal Checklist); (b) Risk of bias of the selected animal studies (SYRCLE's RoB tool for assessing risk of bias)

for the absence of sample randomization and justification of the sample size. All studies presented a possible comparison between the control and treatment groups at entry and a reliable outcome assessment tool. A low risk of bias was found in the clearly stated aim, baseline equivalence amongst the groups, clear conditioning protocols, measurement standardization and appropriate statistical approach. Figure 2b summarizes the risk of bias of animal studies by using the SYRCLE tool. Information to judge most domains was frequently missing. A low risk of bias was observed for incomplete outcome data, selective outcome reporting and the presence of other sources of bias. Overall, the eligible animal studies showed a high risk of bias.

DISCUSSION

This systematic review primarily evaluated the effects of conditioning dentine with EDTA on the release of growth factors in REP; also analysed the influence of conditioning of dentine with EDTA on stem cell behaviour and in tissue regeneration with data from 36 included *in vitro* and *in vivo* studies. We found that EDTA-treated dentine at different concentrations effectively released TGF-β, in addition to improving cell morphology, migration, adherence and differentiation.

REP is currently considered one of the most favourable treatments for immature permanent teeth with pulp necrosis (Taweewattanapaisan et al., 2019; Ulusoy et al., 2019), promoting root development and apical closure (Deniz Sungur et al., 2019; Ulusoy et al., 2019). The European Society of Endodontology (Galler et al., 2016) and the American Association of Endodontists' clinical guidelines (2018) have recommended the use of 17% EDTA after NaOCl for REP to optimize cell viability and differentiation and to enhance the release of growth factors from the dentine matrix (Chae et al., 2018; Kim et al., 2018).

NaOCl was noted to be the most common irrigation solution after EDTA in this systematic review, probably due to its organic solvent potential and antimicrobial effectiveness (Galler et al., 2011; Gonçalves et al., 2016). The presence of a sterile environment plays a crucial role in the success of REP, since there is an impact on the chemotaxis of mesenchymal stem cells, and consequently, on the mineralized tissue neoformation (Verma et al., 2016). Nevertheless, NaOCl is known to show negative effects on stem cells' survival (Martin et al., 2014; Trevino et al., 2011), in addition to being a potential irritant for periapical tissues, especially at high concentrations (Gonçalves et al., 2016). Therefore, additional conditioning with EDTA may neutralize the cytotoxicity provoked by NaOCl, enhancing cellular spreading and the liberation of bioactive molecules from the conditioned dentine (Aksel et al., 2020; Chae et al., 2018; Galler et al., 2011).

The expression of signalling molecules following dentine demineralization might modulate cellular activity from the periapical area (Gonçalves et al., 2016; Taweewattanapaisan et al., 2019), thus playing a crucial role in the intracanal tissues' neoformation (Bracks et al., 2019). A total of 16 studies evaluated the release of endogenous growth factors from dentine using different irrigating protocols with dentine discs. The most assessed protein was TGF-β. This growth factor performs well as a substantial chemoattractant/stimulant of the activation of stem cells (Gonçalves et al., 2016). In addition, TGF-β shows the ability to induce odontoblastic differentiation and contribute to dentinogenesis (Chae et al., 2018; Kucukkaya Eren et al., 2021).

Most articles demonstrated an effective release of TGF-β1 after dentine conditioning with 10%–17% EDTA. Widbiller et al. (2017) found an increase in TGF-β1 liberation using only ultrasonic activation, which might be associated with an improved superficial erosion in the dentine, dissolution of the smear layer and other debris removal, thus exposing growth factors entrapped on the dentine surface. A significant release of TGF-β1 in EDTA-treated dentine was also reported in two studies (Atesci et al., 2020; Cameron et al., 2019) under sterile conditions or when associated with adMSCs. These findings support the importance of other pillars of tissue engineering for clinical success of REP, such as the presence of a sterile environment and stem cells in the root canal.

Angiogenesis occurs especially during the early stages of wound healing (Liao et al., 2011). Amongst the studies that investigated VEGF release, a molecule that supports angiogenic activity (Widbiller et al., 2017), most reports found no influence on irrigation with EDTA (Atesci et al., 2020; Bracks et al., 2019; Li et al., 2020). Moreover, Sadaghiani et al. (2016), using ELISA assay, found no significant effects of EDTA-treated dentine on this protein. The reason for these findings might be explained by the longer observation periods, which included the very short half-life of VEGF and its basal levels in dentine (Atesci et al., 2020). On the contrary, when adMSCs were added, EDTA-treated dentine effectively released this protein; the presence of these cells, which were not receiving sufficient oxygen, might have also induced VEGF production (Bracks et al., 2019).

Whereas TGF-β may have immunosuppressive effects against the production of pro-inflammatory cytokines (Maciel et al., 2012), the conditioning of the dentine with EDTA during REP did not influence tissue inflammation (El Ashry et al., 2016). In other analysis, one study found a high expression of IL-1 in the group of EDTA-treated dentine using real-time PCR (Bracks et al., 2019). These controversial results may have taken place due to differences in the methods of analysis, since the presence of pro-inflammatory cytokine does not exactly depend on the presence of inflammatory cells (Benetti et al., 2018). Moreover, inflamed areas are associated with an increased vascularity (Liao et al., 2011), especially during the initial phases of healing. Furthermore, the interaction between stem cells in inflamed tissues and their potential to control the inflammatory reaction that promotes tissue healing is less understood (Liao et al., 2011). Taweewattanapaisan et al. (2019) reinforced the importance of a minimal inflammatory reaction for an ideal scaffold for stem cell homing.

The conditioning of dentine with EDTA did not show superiority in the analysis of cell survival. Differences regarding stem cells' lineages, periods of analysis, solutions and methods of assessment amongst the studies may have impacted the results. Overall, most studies showed a positive influence of the conditioning of dentine with EDTA on cell migration, attachment and differentiation. For cell morphology, irrigation with EDTA was associated with the presence of an oblong and fibroblastic-like appearance with flattened morphology. EDTA treatment is capable of exposing organic components in the superficial dentine layer, such as collagen and glycosaminoglycans, which play a crucial role in cell attachment (Hashimoto et al., 2018; Oyarzún et al., 2002). Moreover, this systematic review found an increased amount of TGF-β released from EDTA-treated dentine, being that TGF-β is a potent chemoattractant that promotes cell migration into dentine and cell differentiation when in contact with dentinal tubules (Chae et al., 2018; Galler et al., 2015, 2016; Gonçalves et al., 2016; Hashimoto et al., 2018).

The few *in vivo* studies included have evaluated blood clot and tissue regeneration using, for the most part, dentine conditioning with 17% EDTA, as proposed by current clinical protocols (AAE, 2018; Galler et al., 2016). This assortment of protocols made it difficult to systematically discuss these data. In most studies, the use of EDTA positively influenced the majority of the included parameters related to tissue healing or regeneration. Additionally, the animal studies showed a high risk of bias and a low reporting quality using SYRCLE's RoB tool, in which limitations were observed mostly in the absence of randomization, intervention and blinded outcome. Randomization and blinding are essential items of the 'Animal Research: Reporting *In Vivo* Experiments' (ARRIVE) guidelines (du Sert et al., 2020), and they increase the internal validity of the study.

According to SYRCLE'S RoB tool, blinding refers to all measures used, if any, to blind caregivers/researchers and outcome assessors from knowing, which treatment was applied (Hooijmans et al., 2014). In animal studies, the investigators are usually responsible for the way the animals are housed. As housing can influence study outcomes, randomizing the housing conditions within the animal room is essential to providing a comparable between the evaluated groups (Hooijmans et al., 2014). Random allocation is crucial in cases in which blind evaluations are not possible, mainly by the professionals responsible for application of the interventions and to house the animals (du Sert et al., 2020). For instance, most selected studies that showed no blinding performance during intervention should at least provide a prior randomization of the samples. Hence, these non-reporting domains are more likely to report exaggerated effects in eligible animal studies.

These limitations of the animal studies demonstrate that important changes have to be made to the way research using animal models are performed in REP. Thus, a continuous update with additional well-designed histological analyses and clear descriptions using immature teeth and optimal conditions of EDTA treatment for REP are warranted in order to improve report quality and provide stronger evidence.

In this current systematic review, a modified version of the JBI critical appraisal tool was selected for assessing quality of the *in vitro* studies. Although most studies in the review had high-quality evidence, which may lead to more accurate conclusions, some issues, such as the absence of adequate sample randomization and no justification of sample size, were reported. A high level of methodological heterogeneity amongst the studies was also found, and therefore, all these limitations should be considered when the results of this systematic review are evaluated. In addition, the review of these *in vitro* and animal studies has the limitation of transferability findings to the clinical practice; thus, clinical studies and patient-centred outcomes after REP with EDTA irrigation should be also performed using randomized and long-term analyses.

The studies included in this review provide an understanding of influence related to the use of EDTA in the treatment outcome after REP. The present findings provide evidence that EDTA conditioning induces positive effects during REP, because they might favour tissue neoformation and accelerate the repair process, since there is increased TGF-β1 release of the dentine and improved stem cell migration and differentiation, mainly in the initial periods. Besides, the results of the risk-of-bias assessment provide important information about the methodological improvements needed for future laboratory research in the area of regenerative endodontics. Future well-designed histologic analyses and randomized clinical trials comparing effectiveness between/amongst EDTA and other irrigation agents for immature permanent teeth with pulp necrosis are needed to address these limitations and provide a strong level of evidence.

CONCLUSION

High-quality *in vitro* evidence showed significant liberation of TGF-β1 from EDTA-treated dentine and the presence of flattened fibroblastic-like cells after irrigation with different concentrations of EDTA at periods ranging from 1 to 10 min of exposure, in addition to enhanced cell migration, attachment and differentiation. However, further research to evaluate its influence on tissue regeneration is necessary due to the low methodological quality of the animal studies.

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CONFLICT OF INTEREST

The authors have explicitly stated that there are no conflicts of interest in connection with this article.

ETHICAL APPROVAL

Ethical approval was not necessary as this article is not a research study that involved any human or animal experiments.

AUTHOR CONTRIBUTIONS

Conceptualization: Francine Benetti, Antônio P. Ribeiro-Sobrinho, Marco C. Bottino; Study selection: Alexandre H. dos Reis-Prado, Rogéria R. Fagundes, Francine Benetti; Data collection: Alexandre H. dos Reis-Prado, Rogéria R. Fagundes, Francine Benetti; Quality assessment: Alexandre H. dos Reis-Prado, Sabrina de C. Oliveira, Lucas G. Abreu; Methodology: Alexandre H. dos Reis-Prado, Sabrina de C. Oliveira, Lucas G. Abreu, Francine Benetti; Project administration: Francine Benetti, Antônio P. Ribeiro-Sobrinho, Marco C. Bottino; Resources: Francine Benetti, Antônio P. Ribeiro-Sobrinho; Supervision: Lucas G. Abreu, Antônio P. Ribeiro-Sobrinho, Francine Benetti; Validation: Lucas G. Abreu, Marco C. Bottino, Antônio P. Ribeiro-Sobrinho, Francine Benetti; Visualization: Lucas G. Abreu, Marco C. Bottino, Francine Benetti; Writing (original draft): Alexandre H. dos Reis-Prado, Rogéria R. Fagundes, Sabrina de C. Oliveira; Writing (review/editing): Lucas G. Abreu, Marco C. Bottino, Antônio P. Ribeiro-Sobrinho, Francine Benetti. All authors read and approved the final manuscript.

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