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 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. Among these, 32 were in vitro, three animal studies, and one with both models. The EDTA concentrations ranged from 3%-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 (VEGF) 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; further research to evaluate its influence on tissue regeneration is necessary due to low methodological quality of the animal studies.

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Introduction

Treatment of immature necrotic teeth is challenging, due to incomplete root formation and open apex of these teeth (Law 2013, Albuquerque et al. 2014). 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 (Verma et al. 2016, Deniz Sungur et al. 2019). 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 (Bucchi et al. 2017, Bracks et al. 2019) 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 (Gonçalves et al. 2016, Bucchi et al. 2017, Bracks et al. 2019), 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 (Galler et al. 2015, Gonçalves et al. 2016, Bracks et al. 2019). 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), while others have shown some negative effects from this conditioning on cell behaviour, such as impaired stem cell survival and migration (Deniz Sungur et al. 2019, Aksel et al. 2020), 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 immunolabeling/expression; and on blood clot characterization, tissue inflammation, tissue in-growth, 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 1.

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. A 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 (Yaylali et al. 2015, Dos Reis-Prado et al. 2021). 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 2-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, Nagata et al. 2014, Yassen et al. 2014, Yassen et al. 2015, Nerness et al. 2016, Saghiri et al. 2016, Ustun et al. 2018, Widbiller et al. 2018, Buldur et al. 2019, Bosaid et al. 2020, Ivica et al. 2020, Kandemir et al. 2020, Shawli et al. 2020) were outside of the proposed theme, and 6 had no control group (Graham et al. 2006, Tomson et al. 2007, Galler et al. 2011, Trevino et al. 2011, Hristov et al. 2018, Ferreira et al. 2020) (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/among reviewers according to the benchmark scale of Landis & Koch (1977). No 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 (Galler et al. 2015, Gonçalves et al. 2016, Sadaghiani et al. 2016, Zeng et al. 2016, Duncan et al. 2017, Widbiller et al. 2017, Chae et al. 2018, Ranc et al. 2018, Deniz Sungur et al. 2019, Bracks et al. 2019, Cameron et al. 2019, Ivica et al. 2019, Liu et al. 2019, Aksel et al. 2020, Atesci et al. 2020, Li et al. 2020, Kucukkaya Eren et al. 2021). Table 2 summarizes information from the in vitro studies that evaluated cell behaviour (Ring et al. 2008, Huang et al. 2011, Martin et al. 2014, Pang et al. 2014, Kim et al. 2015, Park et al. 2015, Galler et al. 2016, Gonçalves et al. 2016, Kawamura et al. 2016, Mollashahi et al. 2016, Sadaghiani et al. 2016, Shrestha et al. 2016, Alghilan et al. 2017, Chae et al. 2018, Hashimoto et al. 2018, Prompreecha et al. 2018, Scott et al. 2018, Widbiller et al. 2019, Ivica et al. 2019, Liu et al. 2019, Deniz Sungur et al. 2019, Tunç et al. 2019, Aksel et al. 2020, Atesci et al. 2020, Li et al. 2020, Kucukkaya Eren et al. 2021), and Table 3 shows studies that evaluated tissue regeneration in immature teeth models (Yamauchi et al. 2011, El Ashry et al. 2016, Kawamura et al. 2016, Taweewattanapaisan et al. 2019). 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 (Galler et al. 2015, Duncan et al. 2017, Widbiller et al. 2017), while most studies used dentine discs and different stem cells. The roots of permanent human teeth with stem cells (Ring et al. 2008, Huang et al. 2011, Martin et al. 2014, Prompreecha et al. 2018, Atesci et al. 2020, Li et al. 2020) or alone (Zeng et al. 2016, Widbiller et al. 2017, Chae et al. 2018, Ranc et al. 2018, Cameron et al. 2019) were also used as an experimental model. Among these studies, EDTA concentrations ranged from 3%-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 (Yamauchi et al. 2011, El Ashry et al. 2016, Kawamura et al. 2016, Bracks et al. 2019). Two studies only used 17% EDTA as the irrigating solution (Yamauchi et al. 2011, Bracks et al. 2019), while 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 (Yamauchi et al.

2011, El Ashry et al. 2016) and mice posterior teeth (Bracks et al. 2019), while 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

Among the 17 studies that evaluated the growth factors' release/expression (Table 1), transforming growth factor (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% (Galler et al. 2015, Gonçalves et al. 2016, Sadaghiani et al. 2016, Duncan et al. 2017, Widbiller et al. 2017), 12% (Liu et al. 2019), and for the most part of studies, 17% (Chae et al. 2018, Ranc et al. 2018, Bracks et al. 2019, Cameron et al. 2019, Ivica et al. 2019, Atesci et al. 2020). 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 vascular endothelial growth factor (VEGF), bone morphogenetic protein 2 (BMP-2), and fibroblast growth factor (FGF)-2 (Atesci et al. 2020). Conversely, 7 studies found no significant differences in TGF-β release after dentine conditioning with EDTA, compared to their control groups without EDTA (Sadaghiani et al. 2016, Zeng et al. 2016, Cameron et al. 2019, Deniz Sungur et al. 2019, Aksel et al. 2020, Atesci et al. 2020, Kucukkaya Eren et al. 2021).

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 4 studies (Sadaghiani et al. 2016, Bracks et al. 2019, Atesci et al. 2020, Li et al. 2020), FGF in 2 studies (Zeng et al. 2016, Atesci et al. 2020), and BMP-2 in 1 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, 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) (Ducan et al. 2017). On the other side, 10% EDTA did not influence the expression of fibroblast growth factor members (FGF), 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

Among the 19 studies that evaluated cell viability (Table 2), most studies (n=10) showed no influence from use of 17% EDTA-treated dentine (Ring et al. 2008, Galler et al. 2016, Kim et al. 2015, Park et al. 2015, Alghilan et al. 2017, Chae et al. 2018, Deniz Sungur et al. 2019, Liu et al. 2019, Widbiller et at. 2019, Li et al. 2020). On the other hand, 6 studies showed a reduction in cell viability, whereas the other 6 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, 9 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 5 studies. Similarly, among 13 studies that evaluated cell attachment, 8 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), while the others reported no influence from EDTA.

For cell differentiation or mineralization protein assay, all 7 articles (Pang et al. 2013, Martin et al. 2014, Park et al. 2015, Galler et al. 2016, Kawamura et al. 2016, Sadaghiani et al. 2016, Hashimoto et al. 2018) 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 minutes 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, while 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 (Yamauchi et al. 2011, El Ashry et al. 2016) 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, while another reported no EDTA influence.

One record stated that irrigation with 17% EDTA improves mineralized tissue formation (Yamauchi et al. 2011), while 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 among 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 2 and Figure 2. Regarding the critical appraisal of in vitro studies (Figure 2A), a high risk of bias was observed

only 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 among 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 was 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 (Trevino et al. 2011, Martin et al. 2014), 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 (Galler et al. 2011, Chae et al. 2018, Aksel et al. 2020).

The expression of signaling 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 (Cameron et al. 2019, Atesci et al. 2020) 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). Among the studies that investigated VEGF release, a molecule that supports angiogenic activity (Wibdiller et al. 2017), most reports found no influence on irrigation with EDTA (Bracks et al. 2019, Atesci et al. 2020, 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 other hand, 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 among 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 (Oyarzun et al. 2002, Hashimoto et al. 2018). 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 (Galler et al. 2015, 2016, Gonçalves et al. 2016, Chae et al. 2018, 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 (Galler et al. 2016, AAE 2018). 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 among 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 have the limitation of transferability findings to the clinical practice, thus clinical studies and patient-

centered 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/among 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 1-10 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.

References

Aksel H, Albanyan H, Bosaid F, Azim AA (2020) Dentin conditioning protocol for regenerative endodontic procedures. Journal of Endodontics **46**, 1099-104.

Albuquerque MTP, Valera MC, Nakashima M, Nör JE, Bottino MC (2014) Tissue-engineering-based strategies for regenerative endodontics. Journal of Dental Research **93**, 1222–31.

Alghilan MA, Windsor LJ, Palasuk J, Yassen GH (2017) Attachment and proliferation of dental pulp stem cells on dentine treated with different regenerative endodontic protocols. International Endodontic Journal **50**, 667-75.

Alobaid AS, Cortes LM, Lo J, Nguyen TT, et al. **(**2014) Radiographic and clinical outcomes of the treatment of immature permanent teeth by revascularization or apexification: a pilot retrospective cohort study. Journal of Endodontics **40**, 1063-70.

American Association of Endodontists (AAE) (2018) AAE Clinical Considerations for a Regenerative Procedure [Online]. Chicago: American Association of Endodontists. Available at: https://www.aae.org/specialty/wp-content/uploads/sites/2/2018/06/ConsiderationsForRegEndo_AsOfApril2018.pdf

Arslan H, Capar ID, Saygili G, et al. (2014) Efficacy of various irrigation protocols on the removal of triple antibiotic paste. International Endodontic Journal **47**, 594-9.

Atesci AA, Avci CB, Tuglu MI, Ozates Ay NP, Eronat AC (2020) Effect of different dentin conditioning agents on growth factor release, mesenchymal stem cell attachment and morphology. Journal of Endodontics **46**, 200-8.

Benetti F, Gomes-Filho JE, Ferreira LL, et al. (2018) Concentration-dependent effect of bleaching agents on the immunolabelling of interleukin-6, interleukin-17 and CD5-positive cells in the dental pulp. International Endodontic Journal **51**, 789-99.

Bosaid F, Aksel H, Makowka S, Azim AA (2020) Surface and structural changes in root dentine by various chelating solutions used in regenerative endodontics. International Endodontic Journal **53**, 1438-45.

Bracks IV, Espaladori MC, Barros P, et al. (2019) Effect of ethylenediaminetetraacetic acid irrigation on immune-inflammatory response in teeth submitted to regenerative endodontic therapy. International Endodontic Journal **52**, 1457-65.

Bucchi C, Valdivia-Gandur IM, Sánchez-Bizjak R, Tallón-Walton V, Manzanares-Céspedes C (2017) Regenerative endodontic therapy: a systematic review of clinical protocols. International Journal of Clinical and Experimental Medicine **10**, 2006-15.

Buldur B, Oznurhan F, Kaptan A (2019) The effect of different chelating agents on the push-out bond strength of Proroot MTA and Endosequence root repair material. European Oral Research **53**, 88-93.

Cameron R, Claudia E, Ping W, Erin S, Ruparel NB (2019) Effect of a residual biofilm on release of transforming growth factor B1 from dentin. Journal of Endodontics **45**, 1119-25.

Chae Y, Yang M, Kim J (2018) Release of TGF-B1 into root canals with various final irrigants in regenerative endodontics: an in vitro analysis. International Endodontic Journal **12**, 1389-97.

Conde MC, Chisini LA, Demarco FF, Nör JE, Casagrande L, Tarquinio SB (2016) Stem cell-based pulp tissue engineering: variables enrolled in translation from the bench to the bedside, a systematic review of literature. International Endodontic Journal **49**, 543-50.

Deniz Sungur D, Aksel H, Ozturk S, Yılmaz Z, Ulubayram K (2019) Effect of dentine conditioning with phytic acid or etidronic acid on growth factor release, dental pulp stem cell migration and viability. International Endodontic Journal **52**, 838-46.

Dos Reis-Prado AH, Abreu LG, Tavares WLF, et al. (2021) Comparison between immediate and delayed post space preparations: a systematic review and meta-analysis. Clinical Oral Investigations **25**, 417-40.

du Sert NP, Ahluwalia A, Alam S, et al. (2020) Reporting animal research: Explanation and elaboration for the ARRIVE guidelines 2.0. PLoS Biology **18**, e3000411.

Duncan HF, Smith AJ, Fleming GJ, et al. (2017) Release of bio-active dentine extracellular matrix components by histone deacetylase inhibitors (HDACi). International Endodontic Journal **50**, 24-38.

El Ashry SH, Abu-Seida AM, Bayoumi AA, Hashem AA (2016) Regenerative potential of immature permanent non-vital teeth following different dentin surface treatments. Experimental and Toxicologic Pathology **68**, 181-90.

Ferreira LN, Puppin-Rontani RM, Pascon FM (2020) Effect of intracanal medicaments and irrigants on the release of transforming growth factor beta 1 and vascular endothelial growth factor from cervical root dentin. Journal of Endodontics **11**, S0099-2399(20)30580-X.

Galler KM, Buchalla W, Hiller KA, et al. (2015) Influence of root canal disinfectants on growth factor release from dentin. Journal of Endodontics **41**,363-8.

Galler KM, D'Souza RN, Federlin M, et al. (2011) Dentin conditioning codetermines cell fate in regenerative endodontics. Journal of Endodontics **37**, 1536-41.

Galler KM, Widbiller M, Buchalla W, et al. (2016) EDTA conditioning of dentine promotes adhesion, migration and differentiation of dental pulp stem cells. International Endodontic Journal **49**, 581-90.

Gonçalves LF, Fernandes AP, Cosme-Silva L, et al. (2016) Effect of EDTA on TGF-β1 released from the dentin matrix and its influence on dental pulp stem cell migration. Brazilian Oral Research **30**, e131.

Gonçalves SB, Dong Z, Bramante CM, Holland GR, Smith AJ, Nör JE (2007) Tooth slice-based models for the study of human dental pulp angiogenesis. Journal of Endodontics **33**, 811-4.

Graham L, Cooper PR, Cassidy N, Nor JE, Sloan AJ, Smith AJ (2006) The effect of calcium hydroxide on solubilisation of bio-active dentine matrix components. Biomaterials **27**, 2865-73.

Hargreaves KM, Diogenes A, Teixeira FB (2013) Treatment options: biological basis of regenerative endodontic procedures. Journal of Endodontics **39,** S30–43.

Hashimoto K, Kawashima N, Ichinose S, Nara K, Noda S, Okiji T (2018) EDTA treatment for sodium hypochlorite–treated dentin recovers disturbed attachment and induces differentiation of mouse dental papilla cells. Journal of Endodontics **44**, 256–62.

Herrera DR, Martinho FC, de-Jesus-Soares A, et al. (2016) Clinical efficacy of EDTA ultrasonic activation in the reduction of endotoxins and cultivable bacteria. International Endodontic Journal **50**, 933-40.

Hooijmans CR, Rovers MM, de Vries RBM, Leenaars M, Ritskes-Hoitinga M, Langendam MW (2014) SYRCLE's risk of bias tool for animal studies. BMC Medical Research Methodology **26**, 14-43.

Hristov K, Gateva NH, Stanimirov P, Ishkitiev N, Tsikandelova R, Mihaylova Z. Influence of citric acid on the vitality of stem cells from apical papilla. Acta Medica Bulgarica **45**, 31-35

Huang X, Zhang J, Huang C, Wang Y, Pei D (2011) Effect of intracanal dentine wettability on human dental pulp cell attachment. International Endodontic Journal **45**, 346-53.

Ivica A, Deari S, Patcas R, Weber FE, Zehnder M (2020) Transforming growth factor beta 1 distribution and content in the root dentin of young mature and immature human premolars. Journal of Endodontics **46**, 641-7.

Ivica A, Zehnder M, Mateos JM, Ghayor C, Weber FE (2019) Biomimetic conditioning of human dentin using citric acid. Journal of Endodontics **45**, 45-50.

Kandemir Demirci G, Güneri P, Çalışkan MK (2020) Regenerative endodontic therapy with platelet rich fibrin: case series. Journal of Clinical Pediatric Dentistry **44**, 15-9.

Kawamura R, Hayashi Y, Murakami H, Nakashima M (2016) EDTA soluble chemical components and the conditioned medium from mobilized dental pulp stem cells contain an inductive microenvironment, promoting cell proliferation, migration, and odontoblastic differentiation. Stem Cell Research & Therapy **7**, 77.

Kim KW, Yassen GH, Ehrlich Y, Spolnik K, Platt JA, Windsor LJ (2015) The effects of radicular dentine treated with double antibiotic paste and ethylenediaminetetraacetic acid on the attachment and proliferation of dental pulp stem cells. Dental Traumatolology **31**, 374-9.

Kim SG, Malek M, Sigurdsson A, Lin LM, Kahler B (2018) Regenerative endodontics: a comprehensive review. International Endodontic Journal **51**, 1367-88.

Kucukkaya Eren S, Bahador Zırh E, Zeybek ND, et al. (2021) Effect of benzalkonium chloride addition to EDTA on attachment and proliferation of dental pulp stem cells on dentin and on transforming growth factor-β1 release. Odontology **109**, 313-20.

Landis JR, Koch GG (1977) The measurement of observer agreement for categorical data. Biometrics **33**, 159-74.

Law AS (2013) Considerations for regeneration procedures. Journal of Endodontics **39**, 44-56.

Li Y, Ge H, Wu L, et al. (2020) Pretreatment of root canal with photodynamic therapy facilitates adhesion, viability and differentiation of stem cells of the apical papilla. Photochemistry and Photobiology **96**, 890-6.

Liao J, Al Shahrani M, Al-Habib M, Tanaka T, Huang GT-J (2011) Cells isolated from inflamed periapical tissue express mesenchymal stem cell markers and are highly osteogenic. Journal of Endodontics **37**, 1217-24.

Liu L, Leng S, Yue J, et al. (2019) EDTA enhances stromal cell-derived factor 1α-induced migration of dental pulp cells by up-regulating chemokine receptor 4 expression. Journal of Endodontics **45**, 599-605.

Maciel KF, Neves de Brito LC, Tavares WLF, et al. (2012) Cytokine expression in response to root canal infection in gnotobiotic mice. International Endodontic Journal **45**, 354-62.

Martin DE, De Almeida JFA, Henry MA, et al. (2014) Concentration-dependent effect of sodium hypochlorite on stem cells of apical papilla survival and differentiation. Journal of Endodontics **40**, 51-5.

Mollashahi NF, Saberi E, Karkehabadi H (2016) Evaluation of cytotoxic effects of various endodontic irrigation solutions on the survival of stem cell of human apical papilla. Iranian Endodontic Journal **11**, 293-7.

Nagata JY, Soares AJ, Souza-Filho FJ, et al. (2014) Microbial evaluation of traumatized teeth treated with triple antibiotic paste or calcium hydroxide with 2% chlorhexidine gel in pulp revascularization. Journal of Endodontics **40**, 778-83.

Nerness AZ, Ehrlich Y, Spolnik K, Platt JA, Yassen GH (2016) Effect of triple antibiotic paste with or without ethylenediaminetetraacetic acid on surface loss and surface roughness of radicular dentine. Odontology **104**, 170-5.

Oyarzún A, Cordero AM, Whittle M (2002) Immunohistochemical evaluation of the effects of sodium hypochlorite on dentin collagen and glycosaminoglycans. Journal of Endodontics **28**, 152-6.

Page MJ, McKenzie JE, Bossuyt PM, et al. (2021) Updating guidance for reporting systematic reviews: development of the PRISMA 2020 statement. Journal of

Clinical Epidemiology **134**, 103-12.

Pang N-S, Lee SJ, Kim E, et al. (2014) Effect of EDTA on attachment and differentiation of dental pulp stem cells. Journal of Endodontics **40**, 811-7.

Park M, Pang N-S, Young Jung Il (2015) Effect of dentin treatment on proliferation and differentiation of human dental pulp stem cells. Restorative Dentistry & Endodontics **40**, 290–8.

Prompreecha S, Sastraruji T, Louwakul P, Srisuwan T (2018) Dynamic irrigation promotes apical papilla cell attachment in an ex vivo immature root canal model. Journal of Endodontics **44**, 744-50.

Ranc V, Žižka R, Chaloupková Z, Ševčík J, Zbořil R (2018) Imaging of growth factors on a human tooth root canal by surface-enhanced Raman spectroscopy. Analytical and Bioanalytical Chemistry **410**, 7113-20.

Ring KC, Murray PE, Namerow KN, Kuttler S, Garcia-Godoy F (2008) The comparison of the effect of endodontic irrigation on cell adherence to root canal dentin. Journal of Endodontics **34**, 1474-9.

Sadaghiani L, Gleeson HB, Youde S, Waddington RJ, Lynch CD, Sloan AJ (2016) Growth factor liberation and DPSC response following dentine conditioning. Journal of Dental Research **95**, 1298-307.

Saghiri MA, Asatourian A, Garcia-Godoy F, Sheibani N (2016) Effect of biomaterials on angiogenesis during vital pulp therapy. Dental Materials Journal **35**, 701-9.

Sakai VT, Cordeiro MM, Dong Z, Zhang Z, Zeitlin BD, Nör JE (2011) Tooth slice/scaffold model of dental pulp tissue engineering. Advances in Dental Research **23**, 325-32.

Scott MB 2nd, Zilinski GS, Kirkpatrick TC, Himel VT, Sabey KA, Lallier TE (2018) The effects of irrigants on the survival of human stem cells of the apical papilla, including endocyn. Journal of Endodontics **44**, 263-8.

Shamszadeh S, Asgary S, Nosrat A (2019) Regenerative endodontics: a scientometric and bibliometric analysis. Journal of Endodontics **45**, 272-80.

Shrestha S, Torneck CD, Kishen A (2016) Dentin conditioning with bioactive molecule releasing nanoparticle system enhances adherence, viability, and differentiation of stem cells from apical papilla. Journal of Endodontics **42**, 717-23.

Taweewattanapaisan P, Jantarat J, Ounjai P, Janebodin K (2019) The effects of EDTA on blood clot in regenerative endodontic procedures. Journal of Endodontics **45**, 281-6.

Tomson PL, Grover LM, Lumley PJ, Sloan AJ, Smith AJ, Cooper PR (2007) Dissolution of bio-active dentine matrix components by mineral trioxide aggregate. Journal of Dentistry **35**, 636-42.

Trevino EG, Patwardhan AN, Henry MA, et al. (2011) Effect of irrigants on the survival of human stem cells of the apical papilla in a platelet-rich plasma scaffold in human root tips. Journal of Endodontics **37**, 1109-15.

Tunç H, Islam A, Kabadayı H, Vatansever HS, Çetiner S, Yilmaz HG (2019) Evaluation of low-level diode laser irradiation and various irrigant solutions on the biological response of stem cells from exfoliated deciduous teeth. Journal of Photochemistry and Photobiology B: Biology **191**, 156-63.

Ulusoy AT, Turedi I, Cimen M, Cehreli ZC (2019) Evaluation of blood clot, platelet-rich plasma, platelet-rich fibrin, and platelet pellet as scaffolds in regenerative endodontic treatment: A prospective randomized trial. Journal of Endodontics **45**, 560-6.

Ustun Y, Düzgün S, Aslan T, Aktı A (2018) The efficiency of different irrigation solutions and techniques for the removal of triple antibiotic paste from simulated immature root canals. Nigerian Journal of Clinical Practice **21**, 287-92.

Verma P, Nosrat A, Kim JR, et al. (2016) Effect of residual bacteria on the outcome of pulp regeneration in vivo. Journal of Dental Research **96**, 100-6.

Widbiller M, Althumairy RI, Diogenes A (2019) Direct and indirect effect of chlorhexidine on survival of stem cells from the apical papilla and its neutralization. Journal of Endodontics **45**, 156-60.

Widbiller M, Driesen RB, Eidt A, et al. (2018) Cell homing for pulp tissue engineering with endogenous dentin matrix proteins. Journal of Endodontics **44**, 956-62.

Widbiller M, Eidt A, Hiller K-A, et al. (2017) Ultrasonic activation of irrigants increases growth factor release from human dentine. Clinical Oral Investigations **21**, 879–88.

Yamauchi N, Yamauchi S, Nagaoka H, et al. (2011) Tissue engineering strategies for immature teeth with apical periodontiti. Journal of Endodontics **37**, 390-7.

Yassen GH, Al-Angari SS, Platt JA (2014) The use of traditional and novel techniques to determine the hardness and indentation properties of immature radicular dentin treated with antibiotic medicaments followed by ethylenediaminetetraacetic acid. European Journal of Dentistry **8**, 521-7.

Yassen GH, Sabrah AH, Eckert GJ, Platt JA (2015) Effect of different endodontic regeneration protocols on wettability, roughness, and chemical composition of surface dentin. Journal of Endodontics **41**, 956-60.

Yaylali IE, Kececi AD, Kaya BU (2015) Ultrasonically activated irrigation to remove calcium hydroxide from apical third of human root canal system: A systematic review of in vitro studies. Journal of Endodontics **41**, 1589-99.

Zeng Q, Nguyen S, Zhang H, et al. (2016) Release of growth factors into root canal by irrigations in regenerative endodontics. Journal of Endodontics **42**, 1760-6.

Figure legends

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

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).

Table 1. Effects of EDTA on growth factors release/expression

Author	Experi-mental 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 § #	There was no significant difference in EDTA without adMSC regarding TGF-β1, VEGF and FGF-2,

				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	but EDTA increase BMP-2 release. EDTA with adMSC increased release of all growth factors
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 no 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	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

			placed in 1 mL HBSS for 24 h, TGF-β1 quantification		released more TGF-β1 compared to NaOCl. EDTA did not influence TGF-β1 release in the presence of biofilm compared to control
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 to 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 to PBS.
Liu et al. 2019	Pulp tissue from human premolars or third molars	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:	Cell culture, cells treated with EDTA in different time points and concentrations, cells maintained in fresh medium for 6, 12 and 24 h,	TGF-β1, ELISA (pg/mL). NC: ≅ 400 =, G1: ≅ 500 #, G2: < 500 #, G3: < 500 #	EDTA increased TGF-β1 release.

	and DPCs (n = 5)	12% EDTA + fresh medium for 24 h.	analyses		
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^{-1}). NC: 43 = G1: 53 # G2: 231 # G3: 516 # G4: 240 # G5: 53	EDTA increased TGF- β1 compared to saline solution
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 to 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 to 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^{-1}), TGF- β1. G1: > 100000 # G2: < 20000 = G3: > 10000 = G4: < 30000 = G5: ≅ 30000; 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: >	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.

				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: > 15000 # G2: ≅ 2500 # G3: < 2500 = G4: < 2500 # G5: > 5000; IGFBP-2. G1: > 1000 # G2: < 250 # G3: > 1000 # G4: > 2500 # G5: > 250; IGFBP-3. G1: > 60000 # 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 decreased GDF-15, FGF-4, EGFR-1, NGFR.
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	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

		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			
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-labeled 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 among 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 to control; but increased TGF-b1, and did not influence BMP-2 and VEGF in ELISA analysis.

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

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

n.a.: not applicable, G: group, n: number of specimens, d: days, w: week, min: minutes, h: hour, mm: millimeter, mL: milliliter, nm: nanometre, mg: milligram, pg: picogram, ng: nanogram, µL: microliter, °C: degree Celsius, MC: minimal concentration or zero, NM: not mentioned, DMC: dentine matrix component, semi-qRT-PCR: semi-quantitative reverse transcriptase-PCR, DMPs: dentine matrix protein extracts, BC: blood clot, DPCs: dental pulp cells, DPSCs: dental pulp stem cells, EDTA: ethylenediaminetetraacetic acid, NB: nanobubble water, NaOCl: sodium hypochlorite, PBS: sterile phosphate-buffered saline, FBS: fetal bovine sérum, PC: positive control, NC: negative control, adMSCs: adipose-derived mesenchymal stem cells, DW: distilled water, CA: citric acid, IP6: phytic acid; PHA: phosphoric acid, BMP-2: bone morphogenetic protein-2, FGF-: fibroblast growth factor, TAP: triple antibiotic paste (ciprofloxacin, metronidazole and minocycline), CHX: chlorhexidine, CH: calcium hydroxide, BAC: benzalkonium chloride, USA: ultrasonic activation, PDT: photodynamic therapy, SCAPs: stem cells of the apical papilla, a-MEM:alpha- minimum essential medium, RT-PCR: real- time polymerase chain reaction, PDGF: platelet-derived growth factor, IGF: Insulin-like growth factor, NGF: nerve growth factor, IL-: interleukin,

HBSS: Hank´s balanced salt solution, MSCs: mesenchymal stem cells, HEDP: etidronic acid, ELISA: enzyme linked immunosorbent assay, HDACis: histone deacetylase inhibitors, TSA: trichostatin A, VPA: valproic acid, SAHA: suberoylanilide, PBS: sterile phosphate-buffered saline, g: grass, BMP-7: bone morphogenetic protein-7, FGF-: fibroblast growth factor-, BDNF: brian-derived neurotrophic factor, GDF-15: growth/differentiation factor 15, GDNF: glial cell-line-derived neurotrophic factor, TGF-: transforming growth factor, nAb TGF-β1: TGF-β1 neutralizing antibody, VEGF-: vascular endothelial growth factor, SCF-R: mast/stem cell growth factor receptor kit, IGFBP: Insulin-like growth factor-binding protein, EGFR: epidermal growth factor receptor, NGFR: tumour necrosis factor receptor, HGF: hepatocyte growth factor, PIGF: placenta growth factor, US: ultrassonic ativation, PD: predentine layer, GdnHCl: guanidine hydrochloride, HCl: hydrochloric acid, MDPSCs: mobilized dental pulp stem cells, , CM: conditioned medium, PA: polyacrylic acid, DME: dentine matrix extracts, TCD: trisodium citrat, CB: citrate buffer, CAPB: citric acid phosphate buffer, TP: trisodium phosphate, M: molar, WMTA: white mineral trioxide aggregate, GMTA: grey mineral trioxide aggregate, NCPs: non-collagenous proteins, GAGs: glycosaminoglycans, ADM: adrenomedullin, BCA: benzalkonium chloride, NCP: non-collagenous protein, MDPC-23: mouse odontoblast-like cells, OD-21: undifferentiated pulp cells, 3T3: mouse fibroblast cells.

Table 2. Effects of EDTA on stem cells behaviour (in vitro analyses)

Author	Experi-mental 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:	Discs preparation, first conditioning protocols, DPSCs seeded on dentine discs, incubation for 3 and 7 d, cell	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: ≅	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 to EDTA groups	n.a.	Final rinse with EDTA decreased cell viability compared to others. The activation EDTA protocols increased cell viability/ migration, and influenced cell morphology

		EDTA with NBs, G8: EDTA + 5 min USA, G9: EDTA with NBs + USA, G10: PBS, control: DPSC, PC: DPSC in 10% FBS, NC: DPSCs	viability/morphology assays, second conditioning protocols, incubation for 7 d, final analyses	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)				
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 to the other groups
Li et al. 2020	Human mandibular single-root premola	G1: 10 min 1.5% NaOCl (20 mL), G2: NaOCl + 5 min 17% EDTA (10 mL), G3: NaOCl + PDT, G4: NaOCl + EDTA +	Preparation of root segment, irrigating protocols (PDT groups: irradiation for	Luminescence analysis (n° 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

	rs and SCAPs (n = 6)	PDT	120 s), final rinse with sterile saline, SCAP with hydroxyapatite-based scaffolds seeded into root canals, incubation at 37°C for 7 d, analyses					
Ivica et al. 2019	Human dentine discs/ MSCs (n = 4: viability / migration, n = 3: attach-ment)	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: ≅ $1x10^5$ # G2: ≅ $5x10^4$ # G3: > $5x10^4$. 48 h: G1: ≅ $6x10^5$ # G2: ≅ $2x10^5$ = G3: >	n.a	n.a.	EDTA had higher cell viability and migration than PBS, and higher cell attachment at 24 h

					$2x10^5$			
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	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 to other EDTA concentration groups and control; no difference in the other exposure times and the control. EDTA significantly enhanced cell migration.

			#G4: ≅ 90)					
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 to G1; 5 d: major cell number in the groups.	n.a.	EDTA decreased cell viability than DW at 3 d, while 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%	SHEDs seeded, irrigants and laser application,	MTT assay (%). 5 min: (G1 ≅ 60, G2: ≅ 60, G3: ≅ 55, G4: >60, G5: >60, G6:	n.a.	n.a.	n.a.	EDTA decreased cell viability compared with control

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	incubation for 5, 10 and 15 min, analyses	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)

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: ≅ 10^4 # G1: < 10^2 # G4: ≅ 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 among 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%	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: ≅ $1x10^{-3}$ = G1: ≅ $2x10^{-3}$ # G2: ≅ $4x10^{-3}$ # G3: > $4x10^{-3}$; Control: ≅ $6x10^{-4}$ = G4: ≅ $6x10^{-4}$ # G6: > $6x10^{-4}$ # G12: ≅ $7x10^{-4}$; DMP. Control: 0 = G1: < $2x10^{-3}$ # G2: ≅ $2x10^{-3}$ # G3: ≅ $4x10^{-3}$; Control: ≅ $2.5x10^{-6}$ = G4: < $5x10^{-6}$ # G6: > $1x10^{-5}$ # G12: ≅ $1.5x10^{-5}$; DSPP. control: 0 = G1: < $2x10^{-3}$ # G2: > $2x10^{-3}$ # G3: ≅ $4x10^{-3}$; control: < $1x10^{-7}$ = G4: ≅ $5x10^{-7}$ = G6: < $5x10^{-7}$ = G12: > $1x10^{-6}$	10 min EDTA after 1.5% NaOCl increased cell viability, attachment, and differentiation with cells ≅ odontoblast-like

		NaOCl +10 min 3% EDTA, G17: 6% NaOCl +10 min 17% EDTA						
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-dinamic 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 to NSS in the dynamic irrigation; however, no difference was observed between EDTA and NSS in non-dinamic 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=,	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

			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 #)				
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, 7d incubation, EDTA and PBS irrigation, DPSC on dentine, incubation for 24h and 3 d,	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 to the others, without difference with untreated control. Moreover, EDTA showed higher cell attachment than CH and untreated control

			analyses					
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 to G1, G3: < expression compared to 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 to conditioned slices without PD; however, EDTA does not impact SHED migration
Kawamura et al. 2016	C2C12 and HUVEC (n = 4, cell attachment; n = 3, cell differentiation)	For in vitro analyses, G5: nonextracted, 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	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;	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:	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, Immunocytochemi	EDTA extracts with CM promoted cell proliferation, migration, and odontoblastic differentiation; however, EDTA extracts showed no increase cell adhesion and in endothelial differentiation

			the extracts for 28 d and 14 d, analyses	48 h. G5: ≅ 0.4 = G6: ≅ 0.4 # G7: ≅ 0.8 # G8: ≅ 1.0	≅ 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			stry of VE-cadherin (fluorescence) G5 and PC: not mentioned, G6: in increase, G7 and G8: induced differentiation	
Mollashahi et al. 2016	Stem cells from immature third molars (n =	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 =	n.a.	n.a.	n.a.	EDTA and the other groups had higher cytotoxicity than sterile saline and untreated control

	n.a.)		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				
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 =,	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 14d.

							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 =)	
Shrestha et al.	Human dentine	G1: control (no treatment), G2: 10 min	Dentine discs preparation,	n.a.	Attachment, fluorescence. G1:	Fluorescence microscope. G1: fibroblast-like morphology, G2	n.a.	EDTA alone promoted similar cell adherence and

2016	discs and SCAPs (n = 6)	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	irrigating protocols, rinse with DW, nanoparticle conditioning, SCAPs cultured on discs for 24 h, staining with calcein-AM, analyses		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	and G5: rounded cells without cytoplasmic extensions and less ctytoplasmic F-actin compared with the other groups, G3: similar morphology of G1 but more flattened, G4: few cells with cytoplasmic extensions		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
Park et al. 2015	Human dentine discs	G1: 30 min 5.25% NaOCl, G2: 30 min 5.25% NaOCl +7 d 1	Flow cytometric analysis,	MTT assay (%). Control: 100 # G1: ≅ 2 = G2: ≅ 17 =	Attachment, RT-PCR. FN-1. G2: 1 #, G3: ≅ 1.7 =,	SEM analysis. 7 d. G1: DPSCs not attached to the dentine, G2/G3/G4/G5: elongated cells	RT-PCR. DMP-1. Control: 1 # G2: ≅	EDTA did not influence cell viability, but additional treatment with EDTA after

	and DPSCs (n = 250)	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	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	G3: ≅ 22 = G4: 20 = G5: ≅ 22	G4: ≅ 2 =, G5: ≅ 2 =; SPP-1. G2: 1 #, G3: ≅ 1.4 =, G4: approx. 1.3 =, G5: ≅ 1.8	with longer cytoplasmic processes, G5: dentine overlapped by proliferated cell layers	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	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	Preparation of root segments, scaffold preparation, irrigation protocols, SCAPs with hyaluronic acid–based scaffold seeded into the canals, samples	Luminescence ($x10^3$). 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

		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	cultured for 7 d, analyses					
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 to 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/osteo-blastic differentiation
Huang et al.	Human single-	Control: 1 min 5 mL DW, G1: 1 min 5.25%	Root slices preparation,	n.a.	Attachment, fluorescence	Fluorescence microscope. Control and G1: round shape	n.a.	EDTA and MTAD significantly increased

2011	rooted premolars and HDPSCs (n = 5)	NaOCl, G2: 1 min 17% EDTA, G3: 1 min MTAD	irrigating protocols (5 mL), DW rinse, cells onto the root canal, incubation for 72 h, samples dyed, analysis		microscope (cell/field). Control: ≅ 1.6 = G1: ≅ 1.6 # G2: ≅ 6 # G3: 11.4	cells, G2 and G3: spindle-shaped cells		HDPC attachment with spindle-shaped cells
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

The simbol * indicates additional group per analysis; = indicates no significant differences between/among groups; # indicates significant differences between/among groups; ≅ indicates “approximately”; > indicates “greater than”; < indicates “less than”.

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

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 ingrowth	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.

El Ashry et al. 2016	Premo-lars 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, disinfect-tion 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:	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:	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 #,	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 =,	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 #,	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.

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#	1.7 #, PC: 0.9 #, NC: NM	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 =,	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 =,	PC: 100 #, NC: 0 #

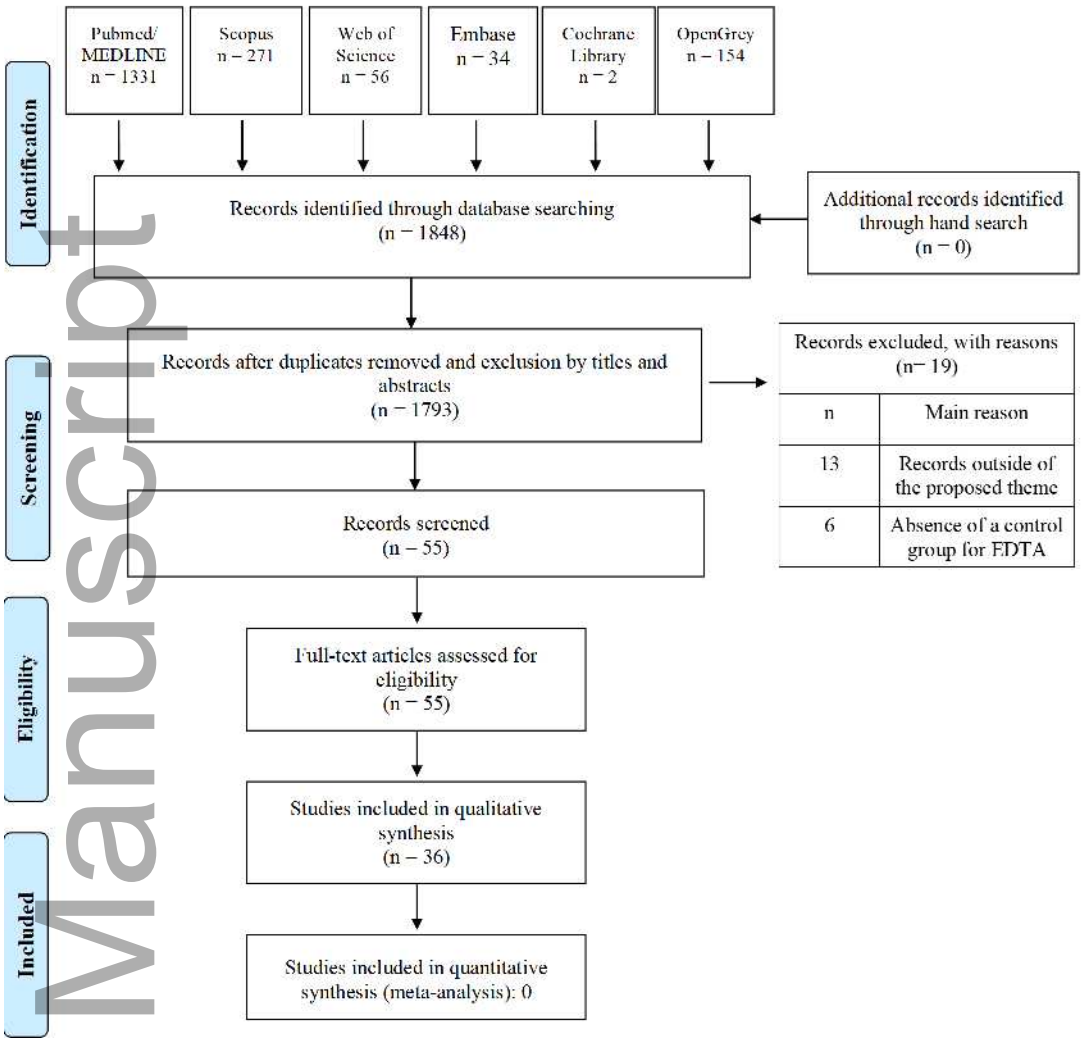
				PC: 0 #, NC: 15.8 =)	G4: 58.3 =, G5: 50 =, G6: 58.3 =, PC: 0 =, NC: 58.3 =		
Kawamura et al. 2016	Porcine roots, immu-nodefi-ciency mice and MDPSC (n = 4), In vivo	P1. Regenera-tive / angiogenesis area, cell differentia-tion. G1: nonextrac-ted tooth, G2: HCl-extracted tooth, G3: GdnHCl-extracted tooth, G4: EDTA-extracted tooth. P2. ENM+	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	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-		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 =	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

		cells, PLAP-1, and angiogenic potential. G5: nonextrac-ted, G6: EDTA extracts, G7: CM, G8: CM + EDTA extracts, G9: GdnHCl, G10: CM + GdnHCl, NC: autoclaved teeth	teeth with collagen (P1) and with CM or not (P2), teeth sealed, subcuta-neous implanta-tion, analyses at 28 d		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			autocl.: MC.			periodontal ligament cells. EDTA allowed higher ODD and endothelial differentiation when associated with CM.
Yamauchi et al. 2011	Double -rooted canine	G1: BC, G2: BC + CCS, G3:	PA lesion induction, 2.5%	n.a.	n.a.	RGe (%). G1: 64.2 =, G2: 87.5 #, G3:	HE. G2 and G4: most	HMM (%). DAMT. G1: ≅ 5=, G2: > 10=,	Periapi-cal radiolu-cencies	n.a.	EDTA did not influence the root

premo-lars (n = 12), In vivo	BC + 2 min 17% EDTA, G4: BC + CCS + EDTA, PC: infected only, NC: untreated	NaOCl irrigation, TAP, IRM for 2 w; 2.5% NaOCl and saline irrigations, protocols, BC inducted, sealing, 3,5 months, analysis	54.2 =, G4: 83.3 #	specimens had apical closure, some showed no complete apical closure and forming bony islands	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 #	impro-vement. RGe (%). G1: 56.52 =, G2: 79.2 #, G3: 58.3 =, G4: 87.5#		thickening and periapical radiolucencies ; EDTA allowed higher apical closure and mineralization .

The symbol # indicates significant differences between/among groups; ≅ indicates "approximately"; > indicates "greater than"; < indicates "less than".

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



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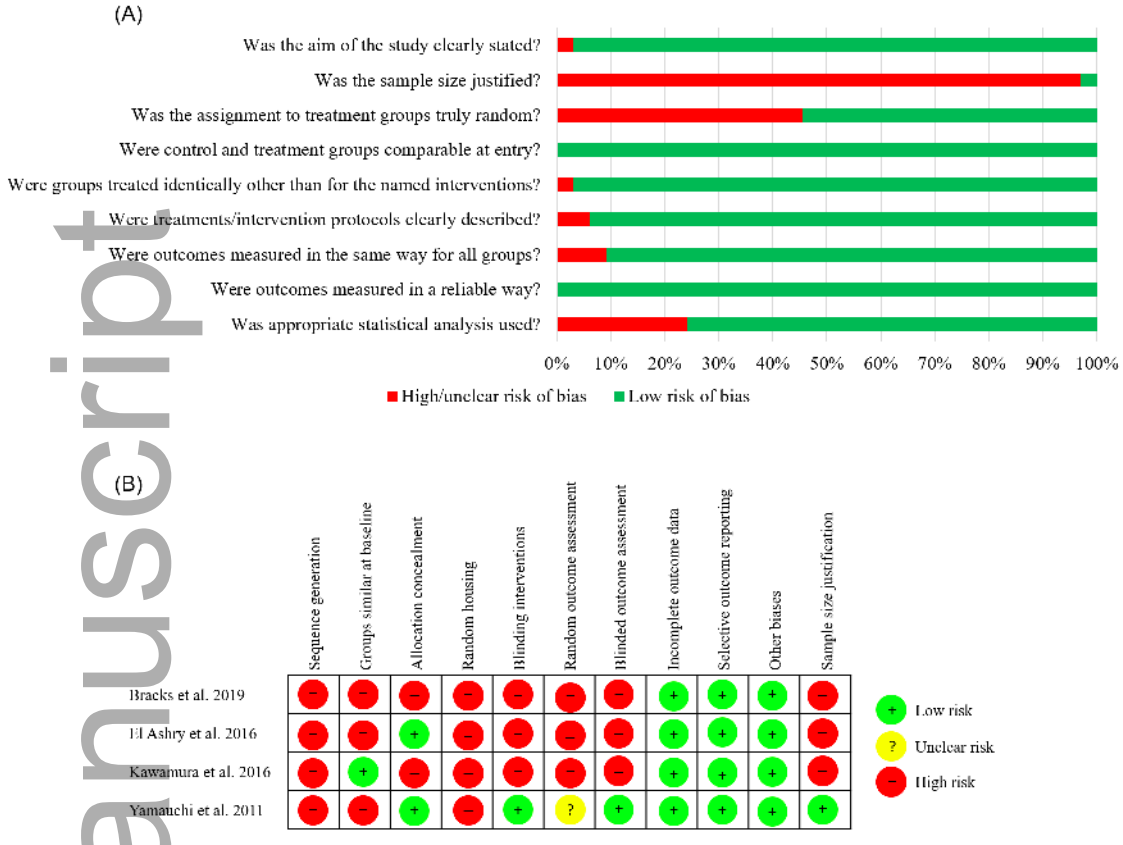

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