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Review Article

Effects of Final Root Canal Irrigants in Conventional and Regenerative Endodontic Treatments: A Systematic Review

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Abstract
Background: The effectiveness of final root canal irrigants is crucial for successful conventional and regenerative endodontic treatments. This study aimed to systematically review the impact of final irrigants on dentin, disinfection, and regenerative potential in endodontic therapies.

Methods: MEDLINE, Cochrane Library, and Web of Science databases were electronically searched for articles on final irrigants in conventional and regenerative endodontics, evaluating effects on dentin, smear layer, fracture resistance, stem cells, growth factors, and antimicrobial properties. The risk of bias was assessed using the Systematic Review Centre for Laboratory Animal Experimentation risk of bias tool.

Results: Overall, 25 eligible studies were included in this review after screening 2842 articles. Commonly assessed irrigants included ethylenediaminetetraacetic acid (EDTA) (21 studies), sodium hypochlorite (NaOCl; 12 studies), and citric acid (6 studies). EDTA could enhance growth factors but could weaken dentin. NaOCl affected dentin despite its antimicrobial benefits. Nanoparticles, chitosan, peracetic acid (PAA), and citric acid showed potential benefits. A meta-analysis was not performed due to methodological heterogeneity.

Conclusion: In general, optimized irrigation strategies balancing disinfection, biocompatibility, and regenerative potential are necessary. More research is required to develop improved irrigation protocols.

Registration: PROSPERO CRD42023420406.

Keywords: Dentin, Endodontics, Regenerative medicine, Root canal therapy

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Background

Regenerative endodontics is defined as a set of biologically oriented procedures intended to physiologically replace injured tooth structures, encompassing both dentin and root structures, as well as cells of the pulp-dentin complex (1). Following current clinical protocols for regenerative endodontics, root canals should be irrigated with disinfectants. Additionally, bleeding from periapical tissues should be induced to form a blood clot within the canal. The stem cells, growth factors, and scaffolds in the blood clot promote new tissue development and sustained root formation. Disinfectants are essential for root canal irrigation in these protocols (2).

Periapical lesions are a common complication of root canal treatments, often caused by bacterial infections. As the root canal infection spreads to tissues surrounding the root apex, bone loss can occur around the apical foramen. Effective disinfection of the root canal system is therefore crucial for successful root canal therapy. The goal of endodontic treatment is to eliminate bacterial infections and disinfect the intricate root canal anatomy. Chemical irrigants play a vital role in this disinfection process by helping to remove microorganisms, necrotic tissue, and debris from areas inaccessible to mechanical instrumentation. Research findings emphasize the necessity of using chemical agents during root canal treatment to achieve thorough disinfection and improve treatment outcomes. Root canal irrigation with antimicrobial solutions is considered an indispensable component of the disinfection protocol in root canal therapy (3-7).

Various types of irrigation have been introduced so far.

Antiseptic irrigation and antimicrobial root canal irrigants caused a change in the microenvironment of canals (8). It is generally accepted that irrigation in regenerative treatments should begin with sodium hypochlorite (NaOCl) at a concentration of 1.5%, followed by saline or ethylenediaminetetraacetic acid (EDTA) at the initial appointment, followed by a final irrigation with 17% EDTA at the second visit (6,7). Although there are a variety of endodontic irrigants, root canal disinfection has not been accomplished yet. Techniques to increase irrigation efficiency, such as stimulating irrigant solutions with different techniques and introducing new irrigation solutions, are being investigated in this regard (9,10). Different final irrigation solutions have been evaluated to determine their efficacy in removing the smear layer; however, only a few have assessed their capacity to restrict bacterial growth inside the dentinal tubules. Achieving complete elimination of bacteria harboring in dentinal tubules may not be possible through conventional irrigation protocols and intracanal medicaments. One of the reasons behind this can be the existence of a smear layer, along with the limited antimicrobial capability of the irrigants (11). Various studies have shown the benefits and drawbacks of irrigants (12-14). It was observed that irrigation solutions can affect biological signaling molecules, such as growth factors, which are responsible for the proliferation and differentiation of cells within the dentin-pulp complex (15,16). Root canal dentin hardness and flexural strength may be affected, depending on the chemical composition and the concentration of irrigation agents. In regenerative endodontic treatments, it is crucial to disinfect the root canals using chemical irrigants since mechanical instrumentation can damage roots with weak dentin walls (16,17). The irrigation solutions may make chemically modified dentin susceptible to fractures and cracks. According to recent evidence, final rinses may impact tooth fracture resistance (18-20).

The effectiveness of the final irrigants in eliminating the smear layer, preserving dentin structure, facilitating the release of growth factors, and sustaining the viability of stem cells in the apical region of the tooth is crucial after the establishment of the root canal preparation system. This intricately influences the ultimate outcomes of regenerative endodontic treatments (2,11,21,22). Based on the above rationale and evidence, this review was conducted to comprehensively assess the outcomes of multiple techniques and solutions used in final irrigants in conventional and regenerative endodontic treatments on root canal disinfection and dentin structure using available databases.

Materials and Methods

The research protocol was registered in the International Prospective Register of Systematic Reviews database (CRD42023420406).

Search Strategy and Study Selection

Research articles published in English until February 2023 were analyzed using relevant terms in Cochrane Library, EMBASE, Web of Science, Medline/PubMed, ScienceDirect, and Scopus matching the keyword strategy (Table 1). Initially, all the searches related to our topic were performed by using certain keywords. Search terms for screening articles included "final root canal irrigants", "root canal", "dentin structure", "root canal treatment", "regenerative endodontics", "endodontics", "irrigation", "dentin", and "regenerative". The combination of these words with the operators "AND" and "OR" was examined as well. All articles related to the topic were reviewed.

Eligibility Criteria

The eligibility criteria for this systematic review were as follows:

The study was conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) statement (23).

1. Participants: Those without age or gender restrictions, permanent teeth with undeveloped, fully formed roots treated under regenerative endodontic treatments, and permanent teeth with fully formed

Table 1. Search Strategy

Database	Search Strategy	Limits/Filters
MEDLINE/PubMed	("final root canal irrigants" OR "root canal" OR "dentin structure" OR "root canal treatment" OR "regenerative endodontics" OR endodontics OR irrigation OR dentin OR regenerative) AND (English[lang])	- English language - Journal articles
Cochrane Library	("final root canal irrigants" OR "root canal" OR "dentin structure" OR "root canal treatment" OR "regenerative endodontics" OR endodontics OR irrigation OR dentin OR regenerative): ti,ab,kw	- Title, abstract, keywords
Web of Science	TOPIC: ("final root canal irrigants" OR "root canal" OR "dentin structure" OR "root canal treatment" OR "regenerative endodontics" OR endodontics OR irrigation OR dentin OR regenerative)	- Topic (Title, abstract, author keywords, keywords Plus)
EMBASE	('final root canal irrigant'/exp OR 'root canal'/exp OR 'dentin structure'/exp OR 'root canal treatment'/exp OR 'regenerative endodontics'/exp OR endodontics/exp OR irrigation/exp OR dentin/exp OR regenerative/exp) AND [english]/lim	- Exploded subject headings - English language
ScienceDirect	TITLE-ABSTR-KEY ("final root canal irrigants" OR "root canal" OR "dentin structure" OR "root canal treatment" OR "regenerative endodontics" OR endodontics OR irrigation OR dentin OR regenerative)	- Title, abstract, keywords
Scopus	TITLE-ABS-KEY ("final root canal irrigants" OR "root canal" OR "dentin structure" OR "root canal treatment" OR "regenerative endodontics" OR endodontics OR irrigation OR dentin OR regenerative) AND (LIMIT-TO (LANGUAGE, "English"))	- Title, abstract, keywords - English language

roots treated under conventional root canal therapy.
2. Intervention: Final root canal irrigation with EDTA/NaOCl/chlorhexidine (CHX)/chitosan/ethanol/peracetic acid (PAA) solutions/Qmix/MTAD.
3. Comparators: Final irrigation of the root canal with normal saline by the conventional syringe method (the control group).
4. Outcomes: Treatment success based on smear layer removal, sealer penetration into the dentin tubules, and the effect on tooth fracture resistance and stimulation of growth factor release in conventional and regenerative endodontic treatments.

Inclusion and Exclusion Criteria

If the full content of the article was unavailable, the details present in the abstract were utilized, and if the abstract was inadequate, that article was removed from the study. First, two colleagues evaluated the titles of all the collected papers and discarded any duplicates. Then, the titles and abstracts of the rest underwent thorough examination, and any study that failed to meet the requirements of this structure was eliminated from the investigation. Ultimately, the complete text of the applicable associated articles underwent analysis. Appropriate articles were selected and compared with the removal of irrelevancies. The process of choosing the articles was performed by two colleagues independently, and in cases of disagreement, the study was conducted by the third researcher.

On the other hand, the exclusion criteria for studies in this review were defined as those containing no control or test group. Studies involving patients/teeth that had undergone prior treatment were excluded from the analysis. Additionally, studies with results deemed ineligible for this review were not included in this study. The exclusion criteria encompassed completely off-topic articles, reviews, case reports, comment letters, letters to the editor, books, surveys, conference abstracts, studies solely evaluating instrumentation, investigations on irrigant flow or penetration, research on apical extrusion of debris or irrigants, and studies related to the removal of medicaments, root canal filling, subsequent procedures, or retreatment.

Data Extraction and Screening

A systematic study reporting system, PRISMA guidelines were followed (24), to present data in this research, involving the identification of the issue under investigation, data acquisition, examination, and explanation of outcomes. The eligible studies were appraised independently by two reviewers. These criteria were based on the published standards for the systematic reviews of clinical experiments as well as potential factors that could influence irrigation (25,26).

Data Extraction

Study Selection

Two reviewers independently assessed the titles and abstracts of the articles that were initially obtained. Then, a comprehensive review of the full text of possibly applicable articles was conducted by both reviewers. The final inclusion of articles was determined by consensus after conferring with a third reviewer if any discrepancies were noticed throughout the process.

Bias Assessment

The risk of bias was evaluated using the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias tool (27). In this method, studies were scored based on having or not having a risk of bias in desired areas at unclear, low, and high levels.

Results

Selection and Type of Samples

In total, 25 eligible articles (Table 2) were selected from 2,842 reviewed articles after reviewing inclusion and exclusion criteria and content (Figure 1). Among 25 articles, 13 evaluated the effect of final irrigation on growth hormone regulation (2,26,28-38). Four articles focused on the smear layer (39-42), and three studies investigated root structure (43-45). Furthermore, there were two studies on the effect of final irrigation on tooth fracture resistance variables (46,47), and one study addressed root canal cleaning (48). Further, one study focused on microorganisms' lives (49), and a dependent variable study examined dentine discoloration (50).

Contents of Articles and Study Design

Among the studies, 13 focused on the dentin section (31-36,38-40,42,49-51), and 8 studies addressed the root canal section (2,29,30,37,44-47). Dentin structure and root canal were investigated in three studies (28,41,48), and one study assessed root canal dentin (43).

In 5 studies, EDTA had a significant effect on growth hormones compared to other detergents (29,33,37,42,51). The positive effect of NaOCl on mechanical and antimicrobial properties, dentin, and root structure was observed in 4 studies (42,44,45,49). Using nanoparticles was more effective in 3 studies regarding root structure, dentin structure, and removal of the smear layer (39,40,46). In two studies, the effect of 10% citric acid compared to 17% EDTA on growth hormone release was greater (2,32), and in two studies, the opposite was lower (29,33). In a study, chitosan was better than 10% citric acid and 17% EDTA to remove the smear layer (39). The most studied irrigation was 10% or 17% EDTA (21 studies). Then, it was NaOCl, which was investigated in 12 studies at different concentrations, and citric acid was used in 6 studies. Nine studies were concentrated on the effects of final irrigation in root canal treatments. Three studies focused on irrigations in regenerative endodontic treatments.

Due to the vast variance in the assessment protocols, irrigation techniques, concentrations, and exposure times to irrigation, a meta-analysis was impossible. The

Table 2. Investigated Studies and Target Variables

Author	Root Canal Irrigant	Target Section	Variable Under Consideration	Summary of Findings
Padmakumar et al (43)	5.25% NaOCl, ozonated olive oil, and silver citrate	Root canal dentin	Mineral ions present in dentin	The organic and mineral contents of dentin exhibited fewer alterations when treated with silver citrate solution and ozonized olive oil compared to treatment with NaOCl.
Kucukkaya Eren et al (51)	17% EDTA and 17% EDTA + 0.008% benzalkonium chloride	Dentin structure	TGF-β1	Both 17% EDTA and 17% EDTA + 0.008% benzalkonium chloride demonstrated an increase in cell proliferation. They were effective in promoting growth factors and enhancing dentin structure improvement.
Elnaggar et al (44)	1.5%NaOCl, 1.5%NaOCl + PUI, 5.25%NaOCl, 5.25%NaOCl + PUI	Root canal	Mechanical properties	A rise in NaOCl concentration resulted in a highly significant reduction in mechanical properties.
Khan et al (28)	17% EDTA, 9% etidronic acid, and 1% phytic acid	Dentin structure and root canal	VEGF	The release of VEGF by 9% etidronic acid was more pronounced in dentin tubules compared to 17% EDTA and 1% phytic acid.
Hancerliogullari et al (29)	17% EDTA, 10% citric acid (break), both irrigants were tested with the irrigation activation technique	Root canal	TGF-β1, IGF-I, BMP-7, and VEGF-A	The application of 17% EDTA resulted in significantly higher IGF-I release compared to 10% citric acid, whereas no significant differences were observed for TGF-β1, BMP-7, and VEGF-A.
de Lima Dias-Junior et al (48)	17% EDTA, 2.5% NaOCl, and 70% ethanol	Dentin structure and root canal	Calcium hydroxide removal Root canal cleaning	70% ethanol improved the washing of the apical third of the root compared to 17% EDTA and 2.5% NaOCl.
de Oliveira Brandão-Neto et al (49)	2.5% NaOCl and 2% CHX	Dentin structure	Antimicrobial and toxic properties on the dentine surface	The NaOCl and CHX groups had good antimicrobial effects.
Ferreira et al (30)	2.5% NaOCl, 2% CHX, and 10% EDTA	Root canal	TGF-β1 and VEGF	The irrigants 2% CHX and 10% EDTA exhibited a significantly higher release of TGF-β1 compared to 2.5% NaOCl. No release of VEGF was detected in any groups.
Ulusoy et al (45)	17% EDTA, 9% etidronic acid, and 2% PAA or NaOCl	Root canal	Changes in root canal dentin structure	Etidronic acid alone or in combination with NaOCl was associated with structural changes in root canal dentin.
Sarkees et al (39)	17% EDTA, 2% chitosan, and 10% citrate buffer	Dentin structure	Smear layer changes	Chitosan can be a promising root cleaning solution.
Barros et al (50)	Four different NaOCl concentrations (0.5%, 1%, 2.5%, and 5.25%) and 2% CHX	Dentin structure	dentin pigmentation	NaOCl caused dentin color change and tubular obstruction.
Jowkar et al (46)	2% CHX, 17% EDTA + 2.5% NaOCl, and EDTA + 0.1% SNP	Root canal	Fracture resistance of root	The ultimate irrigation of root canals with SNP augmented the fracture resistance of the endodontically treated roots.
Aksel et al (31)	1.5% NaOCl + PBS + 17% EDTA + PBS, 17% EDTA with Nanobubble water, and 17% EDTA activated with ultrasonic	Dentin structure	TGF-β	While there was no significant difference among the groups of applied irrigants, ultrasonic activation notably increased the release of TGF-β.
Ratih et al (40)	2% chitosan nanoparticle, 17% EDTA, and 2.5% NaOCl	Dentin structure	Smear layer of dentinal tubules	A final rinse using 0.2% chitosan nanoparticles compared to 17% EDTA. A comparable result can be achieved by eliminating the smear layer.
Atesci et al (32)	17% EDTA, 10% citric acid, 1% phytic acid, 37% phosphoric acid, and distilled water	Dentin structure	TGF-β1, BMP-2, FGF-2, and VEGF	A significant difference was observed in the amount of TGF-β1 released when 10% citric acid was used, as opposed to EDTA and IP6.
Ivica et al (33)	10% Citric Acid, 17% EDTA, and phosphate-buffered saline	Dentin structure	TGF-β1	EDTA at 17% released a concentration of TGF-β1 five times higher than that released by 10% citric acid.
Deniz Sungur et al (34)	17% EDTA, 1% phytic acid, 9% etidronic acid, and distilled water	Dentin structure	TGF-β	The highest amount of TGF-β1 was observed in the 9% etidronic acid group, while the least was noted in the 1% phytic acid group, with no significant distinction between the two.
Nogo-Živanović et al (41)	17% EDTA, Qmix, and MTAD	Dentin structure and root canal	Smear layer	Qmix had a comparable ability to MTAD in eliminating the smear layer in the apical third but was superior to EDTA.
Chae et al (2)	Saline, 17% EDTA, 10% citric acid, 10% phosphoric acid, and 37% phosphoric acid	Root canal	TGF-β1	The release of TGF-β1 was highest when 10% citric acid was used, followed by 10% phosphoric acid and 17% EDTA, while 37% phosphoric acid and saline had the lowest release.
Bhandary et al (47)	17% EDTA and 8% EDTA	Root canal	Fracture resistance of endodontically treated roots	The length of time spent irrigation had an influence on the effectiveness of treatment and the strength against root fracture.
Tartari et al (42)	2.5% NaOCl, 17% $EDTAHNa_3$, 10% $EDTANa_4$, 20% $EDTANa_4$, 5% NaOCl+17% $EDTAHNa_3$, 5% NaOCl+10% $EDTANa_4$, and 5% NaOCl+20% $EDTANa_4$	Dentin structure	Smear layer	The NaOCl + 17% EDTA group experienced the removal of the smear layer in 1 minute.
Gonçalves et al (35)	EDTA 10%, NaOCl 2.5%, and phosphate-buffered saline	Dentin structure	TGF-β1	10% EDTA released a much higher amount of TGF-β1 in the dentin matrix than 2.5% NaOCl or PBS.

Table 2. Continued

Author	Root Canal Irrigant	Target Section	Variable Under Consideration	Summary of Findings
Sadaghiani et al (36)	10% EDTA, 37% phosphoric acid, 10% citric acid, 25% calcium hydroxide, buffered saline, and polyacrylic acid	Dentin structure	TGF-β1, BMP-2, and VEGF	A significant rise in TGF-β1 occurred within 5 minutes of calcium hydroxide conditioning, along with BMP-2 and VEGF within 10 minutes. In addition, 10% EDTA, 10% citric acid, and 37% phosphoric acid displayed average values for TGF-β1 release, while BMP-2 and VEGF exhibited different outcomes.
Zeng et al (37)	1.5% NaOCl + 17% EDTA, 2.5% NaOCl + 17% EDTA, 17% EDTA, and deionized water	Root canal	TGF-β1 and bFGF	TGF-β1 release was significantly higher in the groups with 1.5% NaOCl + 17% EDTA and 2.5% NaOCl + 17% EDTA than with 17% EDTA. The release of bFGF was observed at a low level in all irrigants.
Galler et al (38)	10% EDTA, 17% EDTA, 10% citric acid, citrate buffer, and citric acid phosphate buffer	Dentin structure	TGF-β1	EDTA conditioning at 10% concentration generated the greatest release of TGF-β1.

Note. PUI: Passive ultrasonic irrigation; PAA: Peracetic acid; EDTA: Ethylenediaminetetraacetic, etidronic, and peracetic acids; SNP: Silver nanoparticle; CHX: Chlorhexidine; MTAD: Mixture of doxycycline, citric acid, and a detergent; TGF-β1: Transforming growth factor beta 1; NaOCl: sodium hypochlorite; SNP: sodium nitroprusside.

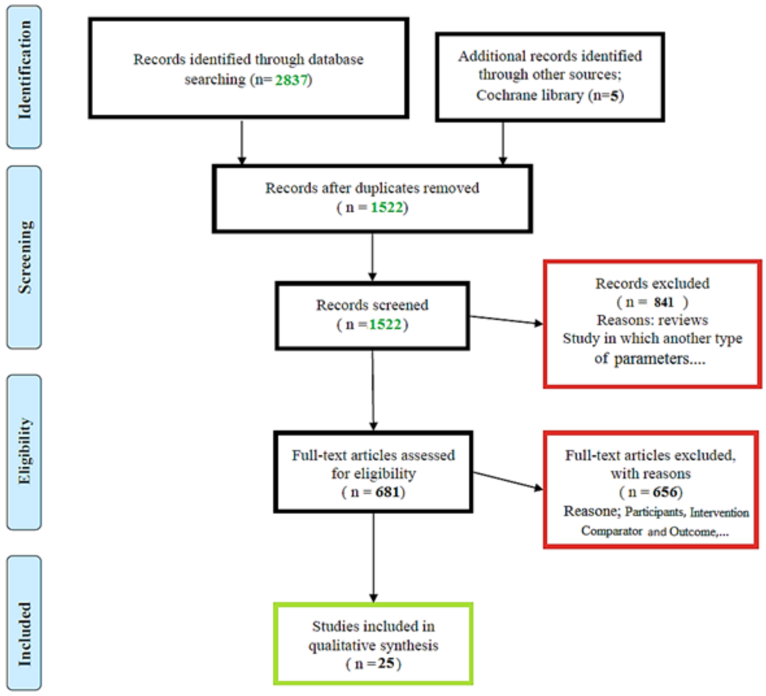

Figure 1. PRISMA Flowchart of Article Selection Process. *Note*. PRISMA: Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

investigated studies neglected to express the dispersion measure (standard deviations) of the measure of effect (mean differences).

Risk-of-Bias Assessment

It was observed that the lack of randomization of the sample and the insufficient justification for the sample size posed a significant risk of bias. All the research demonstrated a potential comparison between the control and treatment groups at the start, accompanied by a dependable result assessment tool. The risk of bias was assessed as low due to the clearly articulated objective, similarity in baseline characteristics between the groups, detailed conditioning protocols, standardization of measurements, and appropriate statistical approach. The SYRCLE tool has been used to display the risk of bias in studies in Figure 2. There was a regular absence of information to properly assess most domains. The risk of bias was diminished because of the incomplete outcome data, selective outcome reporting, and the presence of other biases. Generally, the eligible studies had a substantial risk of bias.

Discussion

This systematic review comprehensively analyzed the effects of different final irrigants utilized in endodontic treatments, balancing efficacy in disinfection, and smear layer removal and thus affecting dentin structure, biocompatibility, and regenerative potential. This study also evaluated the effects of various final irrigants used in endodontic treatments on dentin microstructure, antimicrobial efficacy, smear layer removal, stem cells, and growth factor liberation. A strength of this study was adhering to an a priori protocol, although heterogeneity

Figure 2. Assessment of Risk of Bias Using the SYRCLE Tool. *Note*. SYRCLE: Systematic Review Centre for Laboratory Animal Experimentation.

precluded meta-analysis.

Dentin Microstructure

In general, various studies revealed that final root canal irrigants used during chemomechanical debridement in different endodontic treatments can affect dentin microstructure (43,52,53). Elnaggar et al found that using 5.25% NaOCl had severe effects on the mechanical properties of root dentin (44). Based on the findings of another study, the final root canal irrigation using etidronic acid alone or together with NaOCl was associated with structural changes in the root canal dentin of extracted teeth (45).

Based on the study by Padmakumar et al that compared the peak values of carbonate and phosphate in root canal dentin in the NaOCl group with those in silver citrate and ozonized olive oil, they reported a significant decrease and observed that the amounts of carbon, oxygen, phosphorus, and calcium were significantly different among the groups. Silver citrate solution and ozonized olive oil generated fewer alterations in the organic and mineral composition of dentin than NaOCl (43). NaOCl can modify the carbon and nitrogen contents of dentin at greater concentrations between 5% and 9%, lowering the microhardness of the dentin. Numerous studies have already proven the effects of NaOCl as an irrigant on the collagen component of dentin's microhardness and organic-tissue dissolving capabilities (43,52-54). According to Gu et al (55), the loss of toughness and flexural strength in dentin specimens can occur with just a 1 μm depth of collagen degradation on the dentin surface.

Elnaggar et al (44) concluded that even with low NaOCl concentrations, the mechanical properties of dentin could be negatively impacted by the combined use of passive ultrasonic irrigation and prolonged irrigation time. Therefore, it is advisable to optimize both the concentration and activation duration to minimize the impacts on the mechanical attributes of root dentin (44,56,57).

Uzunoglu et al (58) reported the erosive consequences of 17% EDTA on dentin after being applied for 10 minutes. This may lead to a reduction in dentin microhardness and, subsequently, lower fracture strength values. Conversely, the application of 5% EDTA for 10 minutes and 17% EDTA for 1 minute exhibited a favorable impact on the fracture resistance of root canal-treated teeth. It is possible that these irrigation regimens effectively remove the smear layer without causing any erosive effect, thus explaining the phenomenon. The increased strength of the bond of the resin-based root canal sealer may be the reason behind the high fracture strength values obtained with 5% EDTA for 10 minutes and 17% EDTA for 1 minute (58,59).

Based on research, rinsing with 70% ethanol was more effective than rinsing with 2.5% NaOCl and 17% EDTA-T in terms of cleaning the root canal walls and the greater depth of clean dentin tubules. Moreover, 70% ethanol improved the removal of calcium hydroxide from the apical third of the root compared to 2.5% NaOCl or 17% EDTA-T (48). Nevertheless, NaOCl cannot completely debride dentinal tubules, which is not considered an ideal solution. Aside from not being durable, it is irritating when it comes into contact with the tissues around the mouth (12,13). Barros et al found that regardless of the concentration of NaOCl, it discolors and blocks dentinal tubules when it is in contact with CHX (50).

Using three nanoparticle solutions, another study indicated a substantial decrease in root fracture resistance after endodontic therapy (silver, zinc oxide, or titanium dioxide). It was reported that the final irrigating of root canals with nanoparticles increased root failure resistance. The lowest fracture resistance value was observed for NaOCl (46). Furthermore, a study showed that a 0.2% chitosan solution could be a viable root irrigant solution when compared to 17% EDTA and 10% trisodium citrate solutions (39).

The findings indicated that NaOCl, even at low concentrations, can negatively impact the mechanical properties of dentin, lowering dentin microhardness and fracture resistance. Alternatives such as ozonized olive oil and silver citrate appear to cause fewer alterations to dentin composition. EDTA can also have an erosive effect on dentin at higher concentrations and longer application times, compromising fracture strength. Final rinses with nanoparticles and chitosan seem to improve dentin hardness and tubule cleanliness.

The Antimicrobial Effect

de Oliveira et al concluded that, in contrast to 2% PAA, NaOCl and CHX as final rinses showed significant antimicrobial activities. Their results revealed that 2% PAA had the least detrimental effect on the cell viability of the tested agents (49). It has been recently proposed that PAA can be used in the final irrigation of root canals as an alternative to EDTA in terms of its ability to disinfect (60). The PAA utilized as the final irrigation for root canals was reported to remove smear layers as effectively as EDTA when used as a final irrigation (61). Nevertheless, there are various conflicting findings concerning the effects of PAA on root canal dentin (62). It was determined that a combination of irrigation methods was effective in this regard. Based on the findings of Ghoddusi et al, a mixture of doxycycline, citric acid, and a detergent (MTAD) was equally effective as EDTA in reducing coronal bacterial viability when used with AH Plus and gutta-percha (63). In another study, it was found that irrigation with 5.25% NaOCl/15% EDTA did not differ from irrigation with 1.3% NaOCl/Biopure MTAD in the third apical of roots infected with *Enterococcus faecalis* (64).

It has been stated that the combination of EDTA and NaOCl decreases the amount of free chlorine in the solution, thus diminishing NaOCl's antibacterial and tissue-soluble abilities (28,65).

In terms of antimicrobial effects, NaOCl, CHX, and PAA all demonstrated efficacy, but PAA probably had the least detrimental effect on cell viability. Combinations such as MTAD, followed by gutta-percha, are as effective as NaOCl/EDTA against residual bacteria. However, combining NaOCl and EDTA reduces chlorine levels and antimicrobial potential.

Smear Layer Removal

Nogo-Živanovi et al found that smear layer removal by Qmix was similar to that of MTAD but better than that of EDTA in the apical third (41). Khan et al reported that 9% etidronic acid was more effective than 17% EDTA when used in the extraction of premolar teeth (28).

The final irrigation with 0.2% chitosan nanoparticles had a similar effect on removing the smear layer as the final irrigation with 17% EDTA. In addition, 0.2% chitosan in 17% EDTA was observed to enhance root canal dentin hardness and decrease surface roughness (31).

For smear layer removal, etidronic acid may perform better than EDTA in the apical third of root canals. Chitosan nanoparticles also match EDTA's efficacy in smear layer elimination while improving hardness.

Stem Cell and Growth Factor Effects

NaOCl, EDTA, and CHX are the most commonly utilized irrigants by physicians in regenerative endodontic treatments (66). Although these solutions exhibit good antimicrobial properties, they adversely affect root dentin composition, mechanical properties, and matrix metalloproteinase activity (32,67). Regenerative endodontic procedures typically involve the use of EDTA as the final irrigant after the root canal has been disinfected with calcium hydroxide. This could be useful in the elimination of calcium hydroxide and the removal of the smear layer caused by canal instrumentation. According to earlier studies, EDTA conditioning of root canal dentin promoted the cell survival of apical papilla stem cells and facilitated the process of the attachment and development of dental pulp stem cells (2,68,69).

According to the research by Meeprasert et al (21), EDTA alone or EDTA followed by normal sterile saline could facilitate the migration of stem cells from the apical papilla through irrigating dentin. A final irrigation with 20 mL of normal sterile saline after EDTA had no effect on the differentiation of stem cells from the apical papilla while promoting their proliferation.

Vital pulp treatments are closely linked to the exposure and release of growth factors (36). Moreover, EDTA was shown to significantly affect growth hormone levels in several studies (29,33,37,42,51).

A recent study has demonstrated that 10% citric acid as a final rinse released more transforming growth factor beta 1 (TGF-β1) from root canal dentin without any added cytotoxic effects when compared to 17% EDTA. A number of studies advocate that when looking at a regenerative endodontic treatment, choosing an alternate final rinse should be taken into account in view of the broader release of growth factors other than TGF-1 and the role of these growth factors in stem cell migration, attachment, proliferation, and differentiation (2). Zeng et al reported that 1.5% NaOCl and 17% EDTA caused higher TGF-β1 release than 17% EDTA (37). Moreover, calcium hydroxide significantly increased the release of TGF-β1, bone morphogenetic protein 2 (BMP-2), and vascular endothelial growth factor (VEGF). The presence of 10% EDTA, 10% citric acid, and 37% phosphoric acid induced a moderate release of TGF-β1 (36). A different study found that 10% EDTA elicited the greatest amount of TGF-1, whereas 17% EDTA had a lower effect (38). Sadaghiani et al (36) concluded that applying citric acid to dentine for five minutes resulted in the release of BMP2 and VEGF. The release of BMP2 and VEGF into the aqueous environment was considerably more effective with citric acid compared to EDTA and phosphoric acid.

EDTA facilitates the migration, attachment, proliferation, and differentiation of stem cells from the apical papilla for regenerative endodontics. It also aids in releasing growth factors, including TGF-β1. Alternatives such as citric acid induce comparable or slightly higher release of growth factors that favor regeneration.

Future advancements could enable tailored irrigation protocols customized to the microbiome and anatomy of each root canal system. Bacterial detection methods and understanding biofilm interactions with the host may allow protocol optimization based on microbiome characterization. Research on irrigation effectiveness in different canal types could guide suitable protocols for each anatomy. However, accurately identifying canal types clinically remains challenging, though microscope observations and anatomy insights could help in this respect. While extensive research exists on debris/smear layer removal, other critical aspects such as irrigant penetration, biofilm interactions, and long-term outcomes are comparatively understudied. New studies should prioritize clinically relevant comparisons, address methodology limitations, and ensure adequate sample sizes (70,71). Overall, opportunities exist to revolutionize protocols through customization and redefinition of research priorities toward clinically impactful questions.

Limitations and Future Overlook

While this systematic review provides valuable insights into the impact of final root canal irrigants on dentin, disinfection, and regenerative potential in endodontic therapies, several limitations should be acknowledged. Firstly, the included studies exhibited methodological heterogeneity, which precluded a meta-analysis and may have affected the robustness of our findings. Additionally, the limited number of eligible studies, despite comprehensive electronic searches, suggests that the available evidence on this topic may be insufficient to draw definitive conclusions. Furthermore, the majority of studies focused on a few commonly used irrigants, such as EDTA and NaOCl, potentially overlooking the effects of other emerging irrigants or novel formulations. Moreover, while the risk of bias was assessed using the SYRCLE tool, variations in the study design and reporting quality may have influenced the overall reliability of the included evidence. Therefore, caution should be exercised when interpreting the findings of this review, and further research is warranted to address these limitations and provide more conclusive insights into the optimal use of final root canal irrigants in endodontic practice.

Conclusion

In general, while conventional irrigants such as NaOCl and EDTA demonstrated efficacy, they could negatively impact dentin structure and regeneration. Accordingly, combinations or alternatives such as nanoparticles, ozonized oils, PAA, and citric acid may provide comparable antimicrobial and cleaning effects while better supporting regenerative potential. More research is still needed to optimize irrigation strategies balancing disinfection, smear removal, biocompatibility, and pulp regeneration.

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Competing Interests

The authors do not possess any relevant financial or non-financial interests to disclose. No financial or proprietary benefit has been received by the authors in relation to any material discussed in this article. The authors disclaim any conflict of interests.

Consent for Publication

Not applicable.

Data Availability Statement

The corresponding authors, Elham Khoshbin and Roshanak Abbasi, hold ownership of the complete documentation of participants in this study, and the obtained data are available upon a reasonable request.

Ethical Approval

The research protocol was registered on the International Prospective Register of Systematic Reviews (PROSPERO) database (CRD42023420406). The study was approved by the Ethics Committee of Hamadan University of Medical Sciences (IR.UMSHA.REC.1401.179).

Informed Consent

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