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Effects of endodontic root canal irrigants on tooth dentin revealed by infrared spectroscopy: a systematic literature review

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A R T I C L E I N F O

Keywords:
Endodontic irrigation
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A B S T R A C T

Background: Root canal irrigation endodontic solutions have effects on the chemistry of dentin. Infrared spectroscopy is a non-destructive chemical characterization method where the strength of absorption often correlates with mineral or organic composition.
Objectives: To survey effects of commonly used irrigation solutions on the composition of root dentin as detected by widely-available Fourier transform infrared spectroscopy (FTIR) methods.
Methods: Electronic databases were searched for articles published between 1983 to 2023. After risk of bias assessments (OHAT), studies were grouped according to effects per irrigation solution. Inclusion criteria comprised in vitro studies that used extracted human or bovine teeth, treated by irrigation solutions characterized using FTIR spectroscopy and presenting spectral data. Publications that did not present spectra were excluded.
Results: A wide range of concentrations, durations, and treatment protocols have been tested but only 30 out of 3452 studies met our inclusion criteria. Different FTIR methods were used with Attenuated Total Reflection (ATR) variant being the most common (21 studies). Investigated solutions included sodium hypochlorite (NaOCl), ethylenediaminetetraacetic-acid (EDTA), 1-hydroxyethylidene-1–1-diphosphonic-acid (HEDP), peracetic-acid (PAA), glycolic-acid (GA), and citric-acid (CA) though most focused on NaOCl and EDTA. All solutions had detectable effects in the FTIR signature of dentin. NaOCl mainly affects the organics, revealing reduced amide/phosphate ratios with increasing concentrations. EDTA mainly effects the inorganic component, with the effects increasing with time and concentration, yet glycolic acid has stronger effects than EDTA on dentin. Beyond the type of irrigant and dentin exposure durations, concentration and protocol of application had strong effects. There is a lack of studies comparing similar irrigants under conditions that mimic clinical scenarios analyzing bulk sample because FTIR of powder dentin differs from FTIR of bulk dentin.
Significance: The ideal root-canal irrigant should combine local disinfection properties with minimal compositional effects on healthy dentin. FTIR methods appear reliable to identify important changes in root dentin chemical composition. Such information can help understand when endodontic irrigation might lead to root degradation or possibly contribute to long term failures such as vertical fractures. Awareness of chemical damage from irrigation procedures may help clinicians select procedures that reduce deleterious effects on the root canal structures.

1. Introduction

Bacterial invasion into the root canal system is the primary cause of pulp infection for which the recommended remedy is root canal treatment. The goal is to remove pathogens and ensure canal disinfection for continued tooth function [1,2]. The use of antiseptic irrigation solutions during this procedure is meant to eradicate microorganisms and remove tissue debris. This is considered a requirement for successful and lasting treatment outcomes [3,4]. However, endodontic irrigation solutions also affect the chemical composition of the tooth substance, dentin, leading to a competition between disinfection efficiency and possible chemical degradation of the natural substrate. Common irrigants directly interact with the dentin bio-composite ingredients: collagen fibers or apatite nanocrystals. In particular, chemical treatment may

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alter the composition of dentin, which in the natural state comprises approximately 70 % mineral (nm sized nanocrystals of carbonated apatite), 20 % organic matrix (mainly nano fibers of collagen type I) and 10 % fluids. Changes by dissolution or oxidation are likely to degrade the material capacity to withstand mastication forces [5–7]. Chemical measurement methods such as infrared spectroscopy have thus been used, to try and predict treatment-induced chemical changes to dentin, in researcher efforts to understand and decrease any adverse effects of the medicaments.

Beyond chemistry, the porous micro-architecture of dentin [8], characterized by densely packed micron-sized dentinal tubules, presents challenges during endodontic treatment, because bacteria can penetrate deep into these channels. It is thus desirable for effective irrigation solutions with antimicrobial activity to flow along tubules [9–13] and act there. In fact, the success of endodontic treatment relies on effective disinfection, and therefore treatment procedures often enhance the chemical effects by mechanical agitation and instrumentation [14–16]. Studies in this field have thus focused on finding a balance between effective disinfection with removal of biofilm and organic pathogenic products, while minimizing chemical degradation of healthy dentin. Yet, there is still significant ambiguity and uncertainty regarding the precise effects of different irrigant concentrations and protocols of use, and the corresponding effects on dentin composite composition and properties.

Fourier-Transform Infrared Spectroscopy (FTIR) is a chemical characterization method, and is often used to measure chemical compositional changes in dentin. FTIR reveals distinct signatures of both organic and inorganic dentin components, so that when measurements are performed within a defined aperture of illumination and the sample surface is scanned, FTIR can be used for chemical mapping [17–19]. The technique involves irradiating the sample with a broad range of infrared wavelengths. Molecular vibrations that correspond to characteristic absorbance peaks are then correlated with distinct chemical functional groups [18]. The main used FTIR configurations [19] include transmission, reflection and Attenuated Total Reflection Fourier transform infrared spectroscopy (ATR-FTIR), each with specific requirements and limitations for sample preparation and measurement. The gold standard, classical FTIR method, uses a transmission geometry through the sample, to directly establish the absorption bands of different wavenumbers [20]. However, due to the high density and absorption by dentin mineral, sample thickness cannot exceed several micrometers (<10 μm) so that practically, only powdered diluted samples can be measured. This practically precludes any spatial mapping of slices but can be used to characterize fine powders that are prepared within controlled IR transparent pellets. The reflection and ATR-FTIR modes allow measurements of the surfaces of bulk samples, however they do not directly yield absorption signatures. Both approaches require data transformation and composition/optical property assumptions, to be compared to classical FTIR methods [20,21], assuming some a-priori knowledge about the sample composition and refractive indices. The reflectance mode FTIR yields complex spectra that are essentially reflected from the sample surface, requiring samples that are smooth and reliably reflective. ATR-FTIR involves collection of spectra while maintaining tight contact of a focus tip, pressing against the sample surface. The ATR-FTIR signal depends on the pressure used for sample contact as well as the surface topography, and furthermore, strong peaks are known to distort the signal and shift peak positions non-linearly [21]. An example of an ATR-FTIR dentin spectra is shown in Fig. 1, revealing peaks comparable to classical FTIR bands, assigned, as is common in the literature[22,23].

Conflicting reports about the effects of irrigation solutions on the chemical composition of root dentin may be due to different sample preparation, measurement approaches or due to technical aspects of the FTIR analyses techniques. This review summarizes existing literature reports on the effects of irrigation solutions on dentin, based on observations by the FTIR methodology. There remain questions regarding how and if clinically used endodontic irrigation solutions impact the chemical composition of dentin.

2. Materials and methods

2.1. Protocol and registration

This systematic review is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) [24]. It was registered in the Open Science Framework (OSF) under the registration DOI (https://doi.org/10.17605/OSF.IO/4QN9X) entitled 'Infrared spectroscopy insights into the effects of endodontic root canal irrigants on tooth dentin: a systematic review'.

2.2. Eligibility criteria

Studies included in this review used extracted human or bovine teeth and reported changes in chemical composition of dentin following

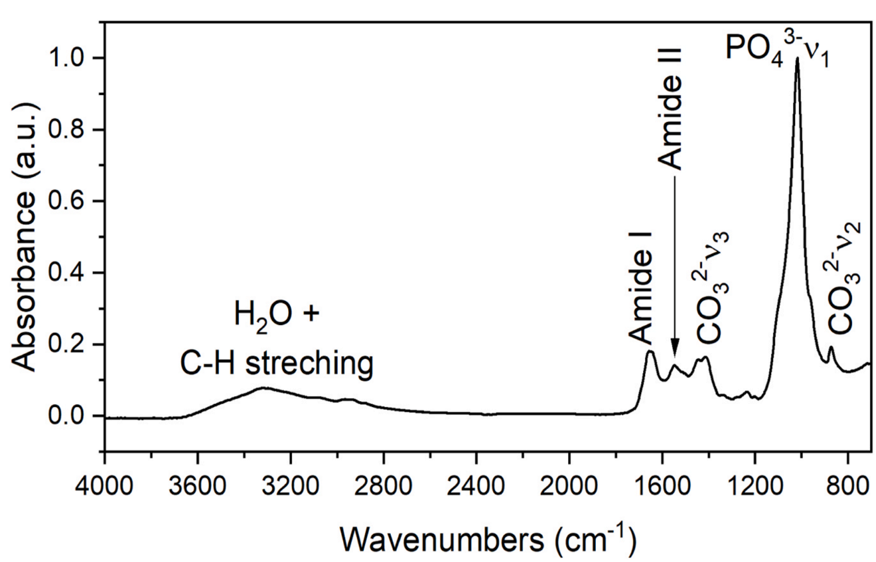

Fig. 1. Typical ATR-FTIR spectrum of bulk bovine dentin measured using Nicolet™ iN10 MX collected with a 100 μm aperture, contact pressure of 15 psi and a 4 cm^{-1} spectral resolution. The spectra shown is the result of averaging 128 scans.

exposure to one or more irrigation solutions. All relied on use of FTIR measurements employing any of several setups/geometries including transmission, specular reflectance, diffuse reflectance, photoacoustic and attenuated total reflectance (ATR).

2.3. Exclusion criteria

Clinical, in situ, in vivo, and animal studies were excluded as well as studies in languages other than English. Publications that lacked visual representation/figures showing example FTIR spectra of dentin as observed by the authors were also excluded.

2.4. Outcomes

Studies addressed the effects of commonly used endodontic irrigation solutions, including sodium hypochlorite (NaOCl), ethylenediaminetetraacetic-acid (EDTA), etidronic acid (HEDP), peracetic-acid (PAA), glycolic-acid (GA) and citric-acid (CA). Both qualitative (e.g., visual differences in spectra) and quantitative (e.g., intensity and band ratios) results were reported.

2.5. Information sources

Publications were searched on PubMed, Google Scholar, and Research Gate. Reference lists of identified full texts were screened and cross-referenced. The search period spanned 40 years, from 1 January 1983 to 1 January 2023. Neither authors nor journals were blinded to the evaluator.

2.6. Search strategy

The following keywords were used: "Endodontic irrigation protocol"; "chelating agent"; "chemical composition of dentin/e"; "collagen changes in dentin/e"; "canal wall erosion"; "minerals in dentin/e"; "EDTA" and "dentin/e" and "structure"; "NaOCl" and "dentin/e" and "structure"; "sodium hypochlorite" and "dentin/e" and "structure"; "chelating agent" and "collagen"; "chelating agent" and "mineral";" FTIR" and "irrigation"; "FTIR" and "dentin/e"; "ATR" and "dentin/e"; "photoacoustic FTIR" and dentin/e"; "infra-red" and "irrigation";" FTIR" and "sodium hypochlorite".

2.7. Data management

For data extraction, a Microsoft Excel sheet was used to collect, sort and compare the data across all studies.

2.8. Selection process

Titles and abstracts were screened by two authors (HE, FL), who compared their findings. In case of disagreement, titles were included to obtain full texts. Full texts were evaluated independently after removal of duplicates. Relevant as well as uncertain titles were included to obtain full texts. Full texts were assessed independently after the removal of duplicates. Papers that did not include explicit examples of the data obtained and quantified were excluded.

2.9. Data collection and analysis

The following items were summarized to facilitate comparison: author, publication year, journal, irrigation solutions tested, duration of application solution, concentration, exposure time, the volume of solution, wash-out solutions, type of teeth, type of analysis tests and

Modified CONSORT checklist of items for reporting in vitro studies		
Section	**Checklist item**	**Applicable / not applicable**
Abstract	Structured summary of trial design, methods, results, and conclusions	
Introduction		
Background and objectives	Scientific background and explanation of rationale along with the specific objectives and/or hypotheses.	
Methods		
Treatment and approach	The description of the intervention for every group, encompassing the methodology and timing of administration , with sufficient detail to enable replication.	
Findings and outcomes	Clearly defined, predetermined primary and secondary outcome measures, detailing the methodology and conditions of their assessment.	
Sample size calculation	The methodology employed to calculate the sample size.	
Randomization process	The technique utilized to generate the random allocation sequence.	
Statistical methods	The data analysis method and statistical tests utilized for group comparisons.	
Results		
Outcomes	Results for each primary and secondary outcome measured within each group.	
Discussion		
Discussion of the findings	Analyzing the findings and outcomes involves providing explanations, predictions, or causal factors behind the observed results.	
Constraints or shortcomings	Trial limitations encompass factors such as potential sources of bias, imprecision in measurements, and other factors that might affect the reliability or generalizability of the findings.	
Additional details		
Financial support or funding sources	Sources of funding and other support, such as irrigation solutions manufactures, and clarification on the role of funders.	

Fig. 2. Evaluation of risk of bias in the included in vitro studies using a modified CONSORT checklist.

properties analyzed, tooth pre-treatment during analysis, storage media, moisture and storage condition. Results were compared between studies and common and conflicting findings were identified. A modified CONSORT checklist of items was used to evaluate the risk of bias in the in vitro studies included (Fig. 2). Comparative analysis made it possible to identify both shared and contrasting findings.

2.10. Risk of bias assessment

The assessment of potential bias in the included articles was conducted individually by two authors (HE and FL). Any disparities in evaluations were resolved through consensus-seeking discussions, with recourse to the judgment of a third author, PZ, when necessary. Given the absence of established protocols for appraising bias risk in in vitro investigations, we employed an adapted version of the OHAT (Office of Health Assessment and Translation) Risk of Bias Tool. This adaptation was based on prior systematic reviews [25–27]. The assessment encompassed a range of aspects, including the scrutiny of experimental conditions, blinding of the researchers to the study groups during experimentation, the integrity of outcome data with respect to absence of attrition or exclusions from analytical consideration, the robustness of exposure characterization, the reliability of outcome assessment, and comprehensive reporting of all measured outcomes. Additionally, the examination extended to identifying any potential threats to internal validity, such as the appropriateness of statistical methodologies employed, adherence of researchers to the prescribed study protocol, the sufficiency of replicates within each study group, and the disclosure of overall bias tendencies. This evaluation procedure facilitated classification of studies into distinct tiers based on their collective bias profile.

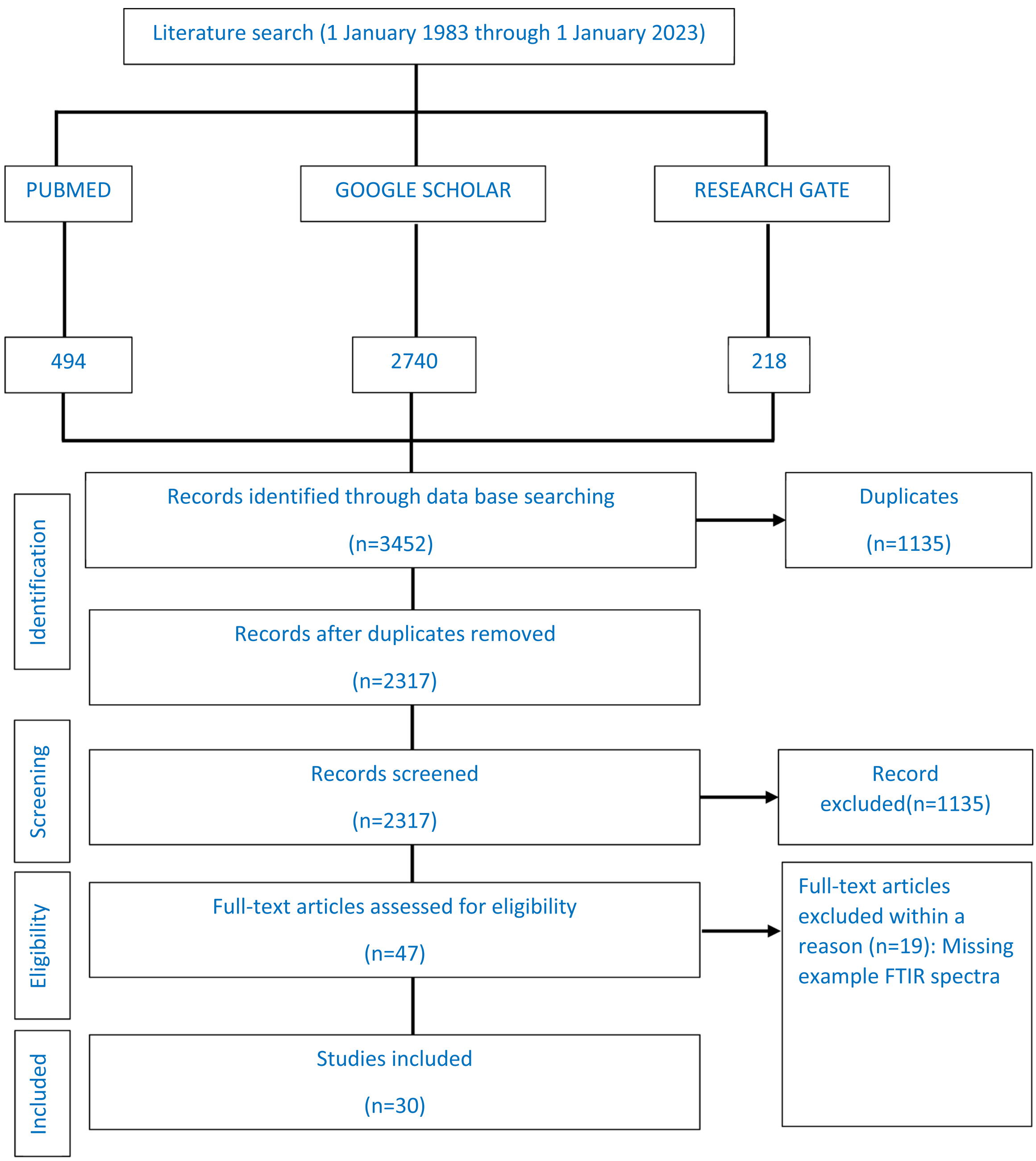

Fig. 3. PRISMA flow diagram showing the selection of articles included in the review. Electronic databases were searched for articles.

Table 1
Studies included in the present review investigating the effects of one or more irrigation solutions using FTIR methods.

No.	Author / year	Journal	Sample type	Irrigation solution	Duration	Outcome measure	Main findings
1	Sakae et al. 1988	Journal of Dental Research	Radicular dentin powder	10 % NaClO	30 min	Band intensities	Band related to carbonate ions was weakened after treatment
2	Verdelis et al. 1999	Endod Dent Traumatol	Radicular dentin sections	15 % neutral EDTA RCPrep	40 s	Orthophosphate to amide I ratio	Greater decalcification of dentin surfaces following treatment with neutral EDTA , the effect was reduced at apical regions.
3	Di Renzo et al. 2001	Biomaterials	Coronal dentin sections	CA Maleic acid Nitric acid Phosphoric acid	0, 10 and 30 s 1, 2, 3, 5, 11 and 15 min	Intensities of peaks	The spectra of citric, maleic and nitric acid-treated samples indicate similar reductions of the mineral phase. Both maleic and phosphoric acids remove similar amounts of mineral. Less mineral removed from the outer surface in samples treated with CA.
4	Di Renzo et al. 2001	Biomaterials	Coronal dentin sections	NaOCl solution (12 % w/v) maleic acid	NaOCl: 0, 0.5, 1, 2, 6, 18, 30 and 48 h sample etached with maleic acid for 2 min then NaOCl for for 0, 10 and 30 s, 1, 2, 5 and 15 min	Intensities of peaks Comparing the spectrum	NaOCl induce a slow and heterogeneous removal of organic phase. A combined sequential 2-min treatment of dentin with both maleic acid and NaOCl indicates that this treatment can produce a surface region which is neither significantly demineralized nor deproteinated.
5	Driscoll et al. 2002	Journal of Materials Science: Materials in Medicine	Radicular dentin powder	0 %, 0.5 %, 3 %, 5 % NaOCl	30 min	Band intensities	Reduction in the intensity of the C-H stretching bands (2960-2850), and amide band (about 1655, 1525, 1228). Reduction in the organic phase of dentin by NaOCl treatment. The spectrum of treated powder exhibited bands characteristic of corbonate containing apatite with predominant carbonate substitution
6	Mountouris et al. 2004	Journal of Adhesive Dentistry	Coronal dentin sections	5 % NaOCl	0, 5, 10, 20, 30, 40, 60 and 120 S	Mineral (v_4P-O stretching vibrations at 1035 cm^{-1}) to matrix (C═O stretching vibrations of amide 1 at 1655 cm^{-1})	NaOCl treatment reduced organic matrix (amide I, II, III peaks), but did not affect carbonates and phosphates
7	Hu et al. 2010	Journal of Endodontics	Radicular dentin slabs	0.5 % NaOCl 1 % NaOCl 2.25 % NaOCl. 0.9 % NaCl	1, 5, or 10 min	Collagen and apatite ratio (the ratio of absorbance of amide I peak to phosphate ν_3 peak) Carbonate/apatite ratio (the ratio of absorbance of carbonate v_2 peak to phosphate v_3 peak)	NaOCl decreased amide/phosphate ratio significantly compared with the control group. Samples treated with 0.5 % NaOCl have significantly higher amide/phosphate ratio than samples treated with 1 % and the 2.25 % NaOCl. Different exposure times (1, 5, or 10 min) of NaOCl within the same concentration did not influence the amide/phosphate ratio. NaOCl treatment did not affect the carbonate/phosphate ratio
8	Zhang et al. 2010	Journal of Endodontics	Radicular dentin powder	1.3 % NaOCl 5.25 % NaOCl 17 % EDTA	10, 20, 30, 60, 120, 180, or 240 min for NaOCl 2 min for EDTA	Apatite/collagen ratio (the amide I peak (1640) and the $v_3PO_4^{-3}$ peak (1020 cm^{-1})	Using 5.25 % NaOCl for more than 1-hour resulted in significant collagen degradation. Conversely, changes were insignificant when 1.3 % NaOCl was used as the initial irrigant
9	Zhang et al. 2010	Dental materials	Radicular dentin powder	1.3 % NaOCl 5.25 % NaOCl 17 % EDTA	10, 20, 30, 60, 120, 180, or 240 min for NaOCl 2 min for EDTA	Apatite/collagen ratio Comparing the spectrum	5.25 % NaOCl results in less intact collagen in the subsurface of the mineralized dentin powder compared with 1.3 % NaOCl, regardless of rinsing with 17 % EDTA Erosion was seen only in samples irrigated with 5.25 % NaOCl followed by 17 % EDTA.
10	Thanatvarakorn et al. 2014	Journal of Dentistry	mid-coronal dentin discs	6 % NaOCl 50 ppm HOCl	15 and 30 s	collagen to apatite ratio (the ratio of C═O stretching vibrations of amide I at 1643 cm^{-1} to ν_3 P–O stretching vibrations at 1026 cm^{-1})	Both NaOCl and HOCl significantly reduced the amide/phosphate ratio as compared with the control group regardless of the exposure time.
11	Yassen et al. 2015	Restor Dent Endod.	Coronal dentin sections	1.5 % NaOCl 17 % EDTA Ca(OH)2 TAP DTAP	1.5 % NaOCl for 5 min EDTA for 10 min intra-canal medicaments for 4 weeks	phosphate/amide I ratios ratio of integrated areas of the phosphate ν1 and ν3 peaks to the amide I peak	Dentin discs treated with different intracanal medicaments and dentin discs treated with NaOCl + EDTA showed significant reduction in phosphate/amide I ratio compared

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Table 1 (*continued*)

No.	Author / year	Journal	Sample type	Irrigation solution	Duration	Outcome measure	Main findings
				sterile water for control			to control dentin. Dentin discs treated with TAP had significantly lower phosphate/ amide I ratio compared to all other groups.
12	TARTARI et al. 2016	J Appl Oral Sci.	Coronal dentin sections	1 % NaOCl 2.5 % NaOCl 5 % NaOCl 0.9 % saline solution	0, 0.5, 1, 2, 3, 5, 8 and 10 min	Absorption bands amide III/ phosphate ratio carbonate/ phosphate ratio	The increase in NaOCl concentration and contact time intensified the dissolution of organic matter and dentin collagen with reduction in the amide III/phosphate ratio. Significant differences between all groups were observed in the dissolution of organic matter at 10 min and in the amide III/ phosphate ratio between the saline solution and 5 % NaOCl at 5 min. The carbonate/phosphate ratio decreased significantly in samples treated with 1 % NaOCl, 2.5 % NaOCl, 5 % NaOCl after 0,5 min of immersion, but more alterations did not occur in the subsequent periods. Intergroup differences were not observed.
13	Li-sha Gu et al. 2017	Acta Biomaterialia	Coronal dentin sections	2 %, 4 %, 6 %, 8 % NaOCl	30, 60, 120, 180 and 240 min	The apatite/collagen ratio by dividing the corresponding absorbance peak of the $\nu 3$ phosphate bending mode of apatite at 1020 cm^{-1} with the corresponding amide I absorbance peak of type I collagen at 1635 cm^{-1}.	A slight shift of the broad collagen peak toward a lower wavenumber, with concomitant reduction in the intensity of collagen amide peaks. Time- and concentration-resolved examination of collagen degradation kinetics revealed continuing increase in the apatite/collagen ratio (i.e. ratio of the phosphate $\nu_3 PO_4^3$ peak at 1020 cm^{-1} and the collagen amide I peak at 1635 cm^{-1}) during the 4 h of NaOCl exposure, irrespective of the NaOCl concentration. NaOCl readily infiltrates the collagen water compartments, oxidizes the organic matrix and denatures the collagen components of mineralized dentin. The deleterious effects of collagen degradation from mineralized dentin and the consequential drop in dentin flexural strength are both concentration-dependent and time-dependent.
14	WANG et al. 2017	J Huazhong Univ Sci Technol [Med Sci]	Coronal dentin sections	1 %, 5 %, 10 % NaOCl Distilled water (control group)	Total of 60 min	Mineral/matrix ratio (the ratio of the integrated areas of $v_1 v_3 PO_4^{-3}$ contour to amide I peak, M/M) Carbonate/mineral (the ratio of the integrated areas of $v_2 CO_3{}^{-2}$ contour to $v_1 v_3 PO_4{}^{-3}$ contour, C/ M)	NaOCl induces morphological changes in dentin. morphology changes were unnoticeable within 10 min in 1 % NaOCl group. No significant difference in carbonate/mineral ratio (C/M) between samples treated with NaOCl and distilled water Mineral/matrix ratio (M/M) increased with increasing the concentration and exposure time of NaOCl. Mineral/matrix ratio (M/M) unchanged after 1 % NaOCl treatment.
15	Tartari et al. 2018	Int Endod J.	Coronal dentin sections	0.9 % saline 2.5 %NaOCl 5 % NaOCl 9 % HEDP 18 % HEDP 5 % EDTANa4 10 % EDTANa4 17 %EDTAHNa3 0.5 % PAA 2.0 % PAA	0, 0.5, 1, 2, 3, 5, 8 and 10 min	amide III/phosphate ratio Carbonate/phosphate ratio	NaOCl reduced amide III/phosphate ratio significantly due to collagen degradation Increasing the concentration and immersion time for the same type of the decalcifying agent results in greater removal of phosphate, exposure of collagen matrix and consequently increases in amide III/ phosphate ratio. Significant differences were found only between

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Table 1 (*continued*)

No.	Author / year	Journal	Sample type	Irrigation solution	Duration	Outcome measure	Main findings
							the two concentrations of PAA. PAA results in a higher increase in amide III/phosphate ratio, followed by EDTAHNa 3, EDTANa4 and HEDP and this order was kept in the combinations with NaOCl. NaOCl needs 0.5 s to deproteinate the collagen matrix exposed after phosphate removal by EDTAHNa 3 and PAA. All decalcifying agents removed dentin carbonate more quickly than phosphate. The dentin amide III/phosphate and carbonate/phosphate ratios were determined by the last irrigant utilized.
16	Ramírez-Bommer et al. 2018	Int Endod J	Coronal dentin powder	2.5 % NaOCl 17 % EDTA	2.5 % NaOCl for 2-10 min 17 % EDTA for 5-1440 min	collagen/phosphate peak height ratio	NaOCl reduced the surface collagen fraction by ~40 % within 2 min of exposure and plateaued at ~60 % between 6–10 min. The average of collagen loss depth was 16 ± 13 µm at 10 min of exposure. Using EDTA for 10 min results in caused ~60 % loss of surface phosphate. The average of phosphate loss was 19 ± 12 µm and 89 ± 43 µm after 10 and 1440 min EDTA immersion, respectively. Immersion in a series of NaOCl/EDTA, results in a 62 ± 28 µm thick phosphate-depleted surface. Sequential immersion in NaOCl/EDTA/NaOCl resulted in about 85 µm of collagen loss.
17	Gandolfi et al. 2018	Materials	Coronal dentin sections	1 %EDTA 10 % EDTA 17 % EDTA 10 %CA Distilled water	1 min	Apatite/amide II ratio Carbonate/Phosphate ratio Shifts of the collagen bands (amide II, III)	10 % CA solution and 10 % EDTA showed the highest demineralizing effect. 17 % EDTA has the lowest demineralizing effect. No significant collagen alterations were seen upon using 1 % EDTA. However, subtle changes were noticed following other treatments Collagen rearrangement was seen in all treatments except for 1 % EDTA.
18	Browne et al. 2019	International Endodontic Journal.	Radicular dentin sections (Roots irrigated)	5 % NaOCl 17 % EDTA Saline	Total of 25 min	amide I/phosphate ratio amide II/phosphate ratio	Significant reduction in collagen bands near the canal lumen after NaOCl irrigation using surface EDTA-treated samples. Irrigation solutions cause significant alterations in the dentinal collagen in the mature roots. The effects in immature roots were significantly greater compared with mature roots with or without periodontal involvement.
19	Morgan et al. 2019	International Endodontic Journal.	Radicular dentin sections (Roots irrigated)	5 % NaOCl Deionised water	25 min	Spectra visual comparison amide I / phosphate ratio amide II / phosphate ratio amide III / phosphate ratio	NaOCl induces changes in the chemistry and structure of collagen in dentin. FTIR spectra of dentin close to root canals exposed to NaOCl exhibit degradation and conformational changes of the collagen. The depth of effect of NaOCl reaches at least 0.5 mm from the canal wall.
20	Naseri et al. 2019	Journal of Endodontics	Coronal dentin sections	Normal saline 2.5 % NaOCl 17 %EDTA Calcium Hydroxide (CH) Nano–calcium Hydroxide (NCH)	NaOCl for 5 min EDTA for 1 min	phosphate/amide I ratio	After 1 week, the mean phosphate/amide I ratio increased significantly in the CH and NCH groups compared to the NaOCl and intact control groups, but did not alter significantly during the later observation period.

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Table 1 (*continued*)

No.	Author / year	Journal	Sample type	Irrigation solution	Duration	Outcome measure	Main findings
21	Di Foggia et al. 2019	Journal of Inorganic Biochemistry	Dentin sections	17 %EDTA	2 h	amide II/amide I ratio amide III/1450 ratio phosphate / amide I ratio carbonate/phosphate ratio	After EDTA treatment the IR bands related to the mineral phase vanished, whereas those assigned to collagen changed significantly in intensity and wavenumber location in compared to sound dentin. The intensity of the amide I, II, and III bands increased significantly after demineralization. The profile of amide I band changed, with shifting in the wavenumber from 1641 to 1628 cm^{-1}.
22	Barón et al. 2020	Australian Endodontic Journal	Radicular dentin sections	5.25 % NaOCl 17 % EDTA	5.25 % NaOCl for 1, 5, 20 min 17 % EDTA 1 min	Carbonate/mineral ratio amide I/mineral ratio amide III/CH_2 ratio	5.25 % NaOCl induced a significant reduction in the carbonate/mineral ratio in the coronal third after 20 min, in the middle third after 1 min, in the apical third after 5 min, and significantly decreasing again after 20 min 5.25 % NaOCl induced a significant reduction in the amide I/mineral ratio after 1 min in the three root thirds 17 % EDTA for 1 min does not induce significant alteration in carbonate/mineral ratio. After exposure to 17 % EDTA for 1 min, amide I/ mineral ratio only increased in the apical third, significant increase was seen in amide III/CH_2 ratio along the root canal. Applying 5.25 % NaOCl for 20 min, 17 % EDTA and 5.25 % NaOCl, both for 1 min result in a significant increase in the carbonate/ mineral ratio in the coronal third, significant decrease in the amide I/mineral ratio in the coronal and apical thirds, and significant decrease in the amide III/ CH_2 ratio in the middle third.
23	Ng et al. 2020	Australian Endodontic Journal	Radicular dentin sections	formal-saline 5.25 % NaOCl 17 % EDTA Ca $(OH)_2$	NaOCl & EDTA 10, 20, 50, 80 or 110 min Ca $(OH)_2$ for 1, 2, 4, 12 weeks	Spectra visual comparison The collagen and phosphate peak heights at 1640 and 1000 cm^{-1} were obtained by subtracting background absorbance at 1730 and 1180 cm^{-1}, respectively	Samples treated with NaOCl or Ca $(OH)_2$ solutions showed reduction in the the organic (N-H, N-H, C=0) peak components. Dentin samples treated alternately to 5.25 % NaOCl and 17 % EDTA showed considerable reduction of the hydroxyapatite peak and an appreciable reduction in the organic components: N-H (1) and N-H (2) bands.
24	Ratha et al. 2020	Dental materials	Coronal dentin sections	0.9 % NaCl 6 % NaOCl 3 % NaOCl 12.5 % EDTA 17 % EDTA 18 % HEDP	15 min	Spectra visual comparison	Using NaOCl alone result in a sharp peak at 1000 cm^{-1} suggesting the deposition of hydroxyapatite on the surface of the sample. In comparison, NaOCl/EDTA showed a stretch at 1000 and 2300 cm^{-1}, suggesting the loss of inorganic component. The combination of NaOCl and HEDP resulted in partially degraded yet mineralized collagen fibers with a little change to the subsurface matrix. And resulted in a homogenous spreading of the organic and inorganic components on the surface of the sample. The combination of NaOCl and EDTA dissolved the hydroxyapaptite encapsulation, exposing collagen fiber bundles.
25	Bello et al. 2020	Mater Sci Eng C Mater Biol Appl	Dentin powder	Distilled water 17 % EDTA 5 % GA	1 min	Apatite/collagen ratio	All tested solutions reduced apatite/ collagen ratios compared to the no-treatment and DW group.

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Table 1 (*continued*)

No.	Author / year	Journal	Sample type	Irrigation solution	Duration	Outcome measure	Main findings
				10 % GA 17 % GA			lowest values of apatite/collagen ratio were shown with higher GA concentration EDTA, CA, and GA 5 % have similar effect on apatite/collagen ratio. GA affects both organic and inorganic dentin components. The effect of GA is dose-dependent.
26	Barcellos et al. 2020	Scientific Reports	Dentin powder	Saline 2.5 % NaOCl 17 % EDTA 17 % GA	1 min	Apatite/collagen ratio	All irrigation solutions significantly reduced apatite/collagen ratios. The lowest apatite/collagen ratio was found in samples treated with GA at pH 5.0 while EDTA and GA at pH 1.2 demonstrated statistically similar results
27	Bosaid et al. 2020	International Endodontic Journal	Radicular dentin sections	Distilled water 1.5 % NaOCl 3 % EDTA 10 % EDTA 17 % EDTA 10 % CA	NaOCl for 5 min EDTA and CA for 5 or 10 min	Spectra visual comparison Comparison of absorbance of Collagen (1640 cm^{-1}), phosphate (1010 cm^{-1}) and carbonate peak levels (871 cm^{-1})	1.5 % NaOCl results in significant reduction in the collagen peak levels (61 % reduction) compared with the negative control group. Samples treated with EDTA or 10 % CA showed significantly higher collagen peak levels than samples treated with the NaOCl group All concentrations and time exposures of EDTA showed similar effects on the collagen, phosphate, and carbonate peak levels. 10 % CA reduces the phosphate peak levels compared to the control and other EDTA groups No significant difference in carbonate peak levels between samples treated with EDTA or CA and the control group.
28	Kusumasari et al. 2020	Journal of Dentistry	Dentin at the level of cemento-enamel junction	Papacarie, Carisolv, and 6 % NaOCl	15 s	The ratio of the amide I band stretching around 1643 cm^{-1} to $\nu_3PO_4^{3-}$ vibrations around 1026 cm^{-1}. Given that the amide band is representative for collagen and the phosphate band for hydroxyapatite	A significant decrease in the amide/phosphate ratio for all the deproteinizing agents. The spectra acquired after the application of Papacarie, Carisolv, and NaOCl revealed a significant reduction in the amide I peak at 1643/ cm^{-1} on both enamel and dentin, compared to the control group.
29	Kusumasari et al. 2021	Journal of Dentistry	Coronal dentin sections	Papacarie, Carisolv and 6 % NaOCl	15 s	The ratio of an amide I band stretching (1643 cm^{-1}) to ν_3 PO_4^{3} vibrations (1026 cm^{-1}).	All agents significantly decreased the amide/phosphate ratios.
30	Padmakumar et al. 2022	journal of functional biomaterials	Radicular dentin sections	5.25 % NaOCl Ozonated olive oil Silver citrate Distilled water	Not mentioned	Collagen, phosphate, and carbonate peak levels	A decrease in the collagen level was observed in the NaOCl group compared to the experimental and negative control groups, although the results were not statistically significant. Intergroup comparisons between NaOCl and silver citrate as well as ozonated olive oil revealed significant reductions in the carbonate and phosphate peak values in the NaOCl group.

3. Results

3.1. Searched and included studies

Out of a total of 3452 identified studies, only 47 publications reported the effects of irrigants based on FTIR methods. The full texts of those papers were evaluated, but 17 reports that did not provide at least one IR spectra or did not investigate the effects of irrigation solution were discarded. Thus, 30 studies were included in the final cohort (Fig. 3). Out of those, 22 studies reported the number of teeth used (787 teeth (26 bovines and 707 human), while 8 studies did not, instead, disclosing the number of tooth sections (in the form of discs) that were used from the teeth analyzed. Regarding the FTIR technique used, 21 studies used the ATR-FTIR method [28–48], two studies used photo-acoustic fourier transform infrared spectroscopy [49,50], two studies used transmission [51,52], a single study used micro-MIR-FTIR [53], a single study in reflection mode[54], and three studies did not mention the method used to obtain the spectra [55–57].

All of the included studies reported the effects of one or more irrigation solutions on the FTIR signature of dentin (Table 1). A meta-analysis was not conducted because of the wide variation in study methodologies and heterogeneity of the results.

3.2. Reported IR peak assignments – risk of bias

The outcomes of risk of bias analysis are summarized in Fig. 4 and

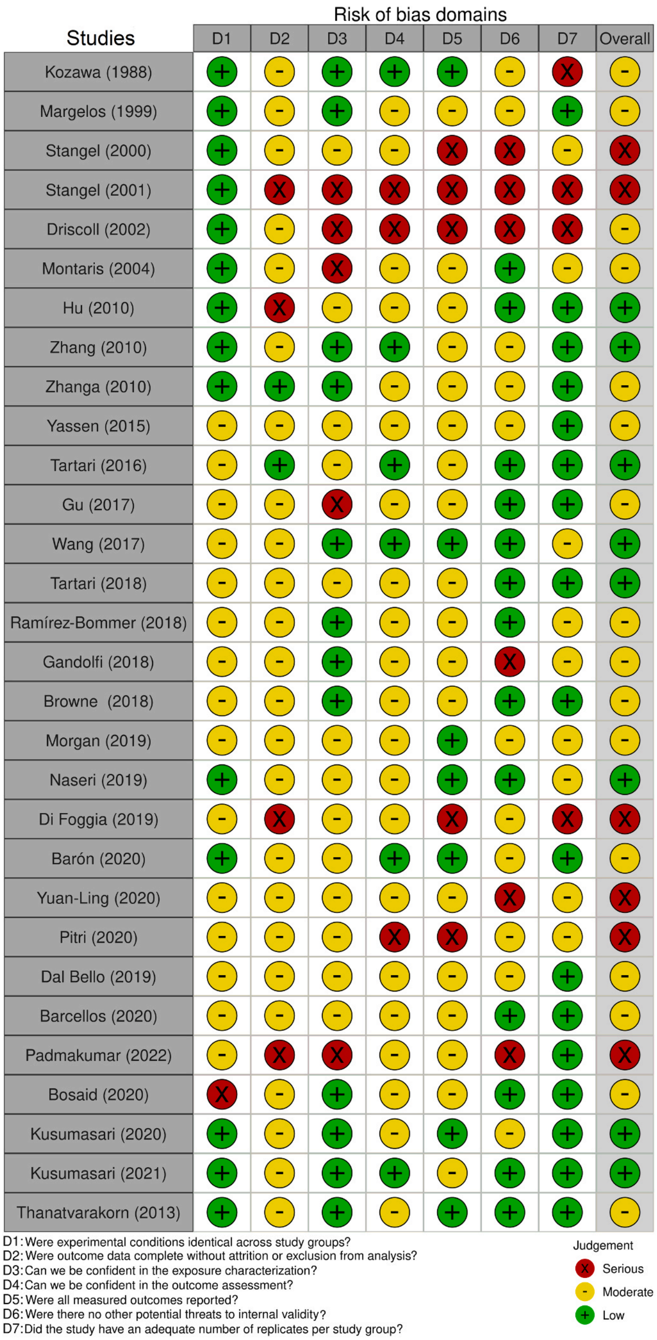

Fig. 4. 'Traffic light' plot of OHAT risk of bias analyses of cell studies displaying the judgment of each domain.

further elucidation regarding the rationale behind the assigned ratings for each category of bias can be accessed in Supplementary Table S3. 6 studies showed high, 16 moderate and 8 low risk of bias.

3.3. *Reported IR peak assignments*

In all studies included, the effects of irrigation were assessed by comparing the IR signal intensity (peak emergence or disappearance) as well as ratios between peaks, based on widely established assignments of what structural element of dentin creates these peaks (similar to bone). Table 2 lists some common FTIR assignments to peaks used in the articles included in this review.

3.4. *Sample preparation*

7 studies analyzed powdered dentin [28,32,39,40,51,52,57] whereas 23 studies examined bulk dentin in slices [29–31,33,34–38,41,42–50,53,54–56].

3.5. *The effects of irrigants on tooth substrate*

The porous nature of dentin dominated by deep penetrating tubules renders different components of the dentin nanocomposite susceptible to chemical degradation in different ways. To this end, the use of powdered samples versus bulk samples is of particular consideration and discussed below. In the following, the effects of treatment solutions on dentin, as determined by FTIR, are grouped according to effects on the organic or inorganic components of the tissue.

3.5.1. *The irrigants examined*

Multiple irrigation solutions were tested alone or combined with additional treatment steps (e.g. application of intracanal medicaments) as follows: A total of 25 studies investigated the effects of NaOCl in concentrations varying between 0.5 - 12 % [28–32,35,36,37,40,41–49,51,52,54–57]. Many of those are included in the16 studies that investigated the effects of 1 - 17 % EDTA [28,30–34,39,40,42,45,46,48,53,55–57]. Four studies investigated the effects of CA [31,33,39,50]. Only 2 studies investigated the effects of HEDP [45,56] and 2 other studies investigated the effects of GA [39,57]. One single study investigated the effects of PAA [45], and another study investigated the effects of maleic, nitric and phosphoric acids [50]. 1 study investigated silver citrate (BioAKT, New Tech Solutions s.r.l., Brescia, Italy)[41].

To explore the effects on dentin, the studies reported treatment that was simulated in different ways. In 17 studies, dentin specimens were soaked in the irrigation solution [28–34,40,41,43–45,49,50,51,53,55], whereas 10 studies applied the irrigation solution on sectioned dentin with the use of a syringe [36–39,42,48,54,56,57], and only 3 studies irrigated entire roots imitating routine clinical treatment approaches [41,46,47].

Some additional uncertainty arises from the observation that different concentrations of the irrigation solutions were used, as listed in

Table 2
Common assignments to peaks in IR spectra of dental tissues. Amide peaks correspond to the organic component of dentin (mainly collagen). Phosphate and carbonate peaks correspond essentially to the inorganic component of dentin (mineral).

Assignment	Absorption peaks and/or bands (cm^{-1})	Based on studies:
Amide A	3600	[47,56]
Amide I	1650	[28–32,34,36–40,42,43,44,46,47–57]
Amide II	1550	[29,33–35,46,47,52,54,56]
Amide III	1240	[29,30,33,34,35,43,45,47,52,54,56]
Carbonate $CO_3^{-2}\nu_2$	870	[29–31,34,41,43–45,51]
Carbonate $\nu_3CO_3^{2}$	1410	[33,52,54]
Phosphate PO_4 ν_4	554	[33]
Phosphate PO_4 ν_2	1000	[28–32,34,35–50,53,54–57]
CH_2	1452	[30,52]

Table 3
Irrigation solutions used and the concentrations used in each of the studies.

Type of irrigation solution	Concentration	Studies
NaOCl	12 %	[49]
	10 %	[44,51]
	8 %	[35]
	6 %	[35–38,56]
	5.25 %	[30,32,40,41,55]
	5 %	[43–47,52,54]
	4 %	[35]
	3 %	[52,56]
	2.5 %	[28,43],[45,48,57]
	2.25 %	[29]
	2 %	[35]
	1.5 %	[31,42]
	1.3 %	[32,40]
	1 %	[29,43,44]
	0.5 %	[29,52]
EDTA	17 %	[28,30–34,39,40,42,46,48,55–57]
	15 %	[45,53]
	12.5 %	[56]
	10 %	[31,33,45]
	5 %	[45]
	3 %	[31]
	1 %	[33]
HEDP	9 %	[45]
	18 %	[45]
PAA	0.5 %	[45]
	2 %	[45]
GA	5 %	[39]
	10 %	[39]
	17 %	[39,57]
CA	10 %	[31,33,39]
Silver citrate (BioAKT)	4.8 %	[41]

Table 3. For most, the concentration was given with but was missing for maleic acid [50], nitric acid, and phosphoric acid [50].

3.5.2. Effects of irrigation on the organic component of dentin

Changes in the intensities or intensity ratios of IR spectra is an indicator of an effect on specific dentin components (e.g., collagen amide). This typically entails a comparison between treated and untreated dentin domains. The studies analyzed the results of FTIR in multiple ways. Some compare the intensities of specific peaks in the spectrum, while other report the ratios of different components, and some studies only report descriptive changes in the spectra. Common to all of these approaches is the observation of some FTIR determined chemical changes in dentin.

Some FTIR signatures were revealed when using increasing NaOCl concentrations ranging from 0.5 % to 12 %. Certain absorption bands and shifts of the amide I peak were inconsistent. Two studies reported shifting in the collagen peak positions to lower wavenumbers with increased exposure time [49], [35]. Concentrations of 0.5 %, 1 % and 2.25 % NaOCl decrease absorption bands at 1242, 1550, and 1643 cm^{-1}[29,51]. Higher concentrations (10 % NaOCl) weakened the intensity bands at 1650 cm^{-1}, which is assigned to the amide I peak [51]. Exposure to 12 % NaOCl solution results in shifting of the amide I peak from 1655 cm^{-1} to 1640 cm^{-1}[49]. Some contradiction was observed in reports of no visible changes in peaks assigned to collagen within 10 min of exposure to 1 % NaOCl [44]. Use of a slightly higher concentration (1.5 % NaOCl) for five minutes results in a significant reduction in the collagen peak (~60 % reduction) [31]. 5 % NaOCl solution applied by a rubbing action reduced the signatures of the organic matrix (amide I, II, III peaks)[54].

There is also some uncertainty regarding the effects of NaOCl on the mineral to matrix ratios. Tartari et al. [43] reported that 1 % NaOCl reduces the amide III/phosphate ratio whereas Wang et al., 2017 [44] reported that 1 % NaOCl does not change this ratio. Use of 1.3 % NaOCl for periods up to 4 h did not result in significant changes in the apatite/collagen ratio [32].

When it comes to higher concentrations, it was shown for powdered dentin that within the first 2 min of exposure to 2.5 % NaOCl, about 40 % of the collagen signal was reduced in the following 6–10 min [28]. 2.5 % and 5 % NaOCl significantly reduced the amide III/phosphate ratio [43], and the effects of 5 % NaOCl extended to at least 0.5 mm outward from the treated canal wall [47]. Additionally, 5.25 % NaOCl significantly reduced the amide I/mineral and amide III/CH_2 ratios [30]. There is agreement in three studies that using higher NaOCl concentrations induces more detectable collagen degradation resulting in less intact collagen [32,35,40].

Gu et al. [35] found that the effect of NaOCl is time and concentration dependent and Di Renzo et al. [49] reported that 2 min of exposure to 12 % NaOCl are enough to eliminate organic signatures (mainly type I collagen) [49]. The study by Hu et al. showed that the reduction in the amide/phosphate ratio is mainly related to the irrigant concentration [29].

Two studies investigate the effects of EDTA on the organic components of dentin and found that using lower concentrations, e.g., 1 % EDTA, does not induce any significant alteration in collagen [33]. Unlike EDTA however, GA induces significant changes in the amide I, II, and III peaks [39].

3.5.3. Effects of irrigation on the inorganic component of dentin

The main NaOCl effects are on the organic components of dentin. Nevertheless, there are reports that NaOCl also affects the inorganic component. Two studies found that low concentrations of NaOCl (0.5 %, 1 %, 2.25 %) and various exposure times did not affect the carbonate/phosphate ratios [29,44]. An additional study reported that applying 1.5 % NaOCl for 5 min did not affect the mineral content [31]. However, other studies found that carbonate/phosphate ratios were affected by exposure to different concentrations of NaOCl (1 %, 2.5 %, 5 %) [43]. Barón et al. for example [30] found that 5.25 % NaOCl has a time-dependent effect that varies with the portion of root analyzed. They reported that applying 5.25 % NaOCl for 1, 5 and 20 min induced a significant reduction in the carbonate/mineral ratio only in the coronal third, after 20 min of exposure. In the middle third, the reduction was significant after 1 min and, in the apical third, after 5 min. Higher NaOCl concentrations (10 %) result in weakening of the carbonate-visible IR bands with corresponding effects on the carbonate/phosphate ratios [51].

EDTA chelates calcium and thus erodes away the inorganic component of dentin. 1 % EDTA causes a reduction in the intensity of bands assigned to B-type carbonated apatite. Curiously, there are reports of a shift in the $\nu_3 PO_4{}^3$ band from 1006 to 1000 cm^{-1} without a significant decrease in the apatite/amide II ratio or carbonate/phosphate ratio [33]. Bosaid et al. [31] found that the effect of EDTA on the inorganic content is not concentration and time-dependent, as tested for 3 %, 10 %, and 17 % irrigation solutions of EDTA. They also found no reduction in the carbonate peak levels. In contrast to these findings, Tartari et al. [45] found that increasing the immersion period and concentration results in significant phosphate removal, with increasing collagen matrix exposure, and consequently, an increase in amide III/phosphate ratios. This disparity in results may be related to the difference in methodology of application and time intervals used. Whereas Bosaid et al. [31] used time intervals of 5 and 10 min intervals, Tartari et al.[45] used time intervals of 0, 0.5, 1, 2, 3, 5, 8 and 10 min

Use of 10 % EDTA results in a decrease in the relative intensity of the apatite bands and in the apatite/amide II ratios. It also causes a shifting in the collagen amide II and III bands as well as the COO− stretching band in the spectrum, without any significant differences noted in the carbonate/phosphate ratio [33].

Using higher concentrations of 17 % EDTA, Ramírez-Bommer et al. powdered dentin and reported an attenuation of both phosphate (1010 cm^{-1}) and carbonate (871 cm^{-1}) peaks with a depth of the effect (phosphate peak intensity loss) being time-dependent; after 10 min of exposure, the average distance of phosphate loss was 19 ± 12 µm and after 1440 min the distance increased to 89 ± 43 μm [28]. Gandolfi et al. [33] found that 17 % EDTA does not reduce the apatite/amide II and carbonate/phosphate ratio significantly. These conflicting results may be due to sample preparation, since Ramírez-Bommer et al. [28] used dentin powder while Gandolfi et al. [33] used sliced coronal dentin discs that were exposed for 1 min only.

Three studies investigated the effects of citric acid on the chemical composition of dentin. Bosaid et al.[31] reported that 10 % CA does not reduce the carbonate peak levels significantly compared to the control group. Samples treated with 10 % CA showed significantly lower inorganic content than those in the control and EDTA groups [31,33]. They found that 10 % CA reduced the apatite/amide II ratio significantly and caused shifts in the collagen amide III and COO− stretching bands, with no significant differences observed in the carbonate/phosphate ratio [33]. Comparing CA with maleic and phosphoric acids in terms of mineral removal showed less mineral removal from the exposed surface treated with CA as compared with maleic and phosphoric acids [50].

Recent experiments target use of GA as a chelating agent reported that the effects are concentration-dependent [39] and that GA use significantly reduces FTIR signatures of apatite/collagen ratios [39,57]. 10 % and 17 % GA further induce major changes in the phosphate peak [39]. 17 % GA at pH 5.0 results in a significantly lower apatite/collagen ratio than GA with a pH of 1.2 [57].

3.5.4. *Effects of combining irrigation solutions*

Standard treatment procedures advocate combining NaOCl and EDTA to remove debris in treated canals, broadly known as the smear layer. Spectra of dentin discs treated by 5.25 % NaOCl followed with 17 % EDTA revealed considerable reduction of the mineral signature at ~1000 cm^{-1} (phosphate peak) and an appreciable decrease in the organic components [55]. An apatite/collagen ratio higher than that of untreated mineralized dentin indicates a reduction of the organic component following irrigation of the dentin samples. In contrast, an apatite/collagen ratio lower than that of untreated mineralized dentin indicates the presence of a collagen matrix in which the apatite phase was partially depleted[40]. The apatite/collagen ratio was analyzed for samples initially treated with two different NaOCl concentrations (1.3 % and 5.25 %), followed by a 2-minute treatment with 17 % EDTA as the final irrigant, exposed to varying time intervals (10, 20, 30, 60, 120, 180, or 240 min). For the 5.25 % NaOCl group, the lowest apatite/collagen ratio (5.62 ± 0.16) was observed after a 20-minute exposure, while the highest ratio (17.07 ± 2.23) was found after 240 min. In the case of the 1.3 % NaOCl group, the lowest apatite/collagen ratio (3.87 ± 0.32) occurred after a 20-minute exposure, whereas the highest ratio (5.86 ± 0.49) was observed after 180 min. Importantly, the apatite/collagen ratios for all samples treated with either 1.3 % or 5.25 % NaOCl were consistently higher than the corresponding values for the control group treated with 17 % EDTA alone [40]. The application of a low 1.5 % NaOCl concentration for 5 min, followed by a 10-minute treatment with 17 % EDTA, significantly reduced the phosphate/amide I ratio [42].

3.5.5. *Powdered dentin versus dentin as bulk*

Irrigants perform differently when acting on large dentin slabs versus powdered tissue[54]. Ramírez-Bommer et al. [28] describes the effects of sequential exposure of different sizes of dentin powders to NaOCl, EDTA and NaOCl on collagen and phosphate. The results, showed that a single treatment of NaOCl caused significant collagen loss (60 %) in dentin particles sized 75–106 μm, while larger dentin particles (greater than 500 μm) were minimally affected. This showcases the strong effect of powdering. Subsequent treatment with EDTA resulted in 57 ± 3 % (500–1000 μm) to 86 ± 3 % (75–106 μm) phosphate reaction and apatite dissolution, with the extent of reaction inversely related to particle size. After further treatment with NaOCl, collagen loss in smaller particles was similar to the first treatment and the collagen/phosphate peak ratios decreased after completion of the full treatment regime for all particle sizes. Zhang et al.[32] immersed dentin powders in 1.3 % or 5.25 % NaOCl for 10, 20, 30, 60, 120, 180, or 240 min then rinsed with 17 % EDTA for 2 min and found that the apatite/collagen ratios for all 1.3 % NaOCl time period subgroups were not significantly different from the untreated dentin control. However, the use of 5.25 % NaOCl as the initial irrigant for more than 60 min resulted in significant increases ($P < .05$) in the apatite/collagen ratios. Combining 3 % NaOCl with 17 % EDTA resulted in stretching of calcium phosphate peaks at 1000 and 2300 cm^{-1}, indicating the loss of inorganic substance [56]. An alternative treatment using a higher concentration of NaOCl (5.25 %) and 17 % EDTA causes a significant drop in the hydroxyapatite peak (1000 cm^{-1}) as well as a noticeable decrease in the organic N-H bands [55].

Barón et al. [30] analyzed 500– 600 μm thick slices by ATR-FTIR and found that samples treated with 5.25 % NaOCl for 20 min followed by 17 % EDTA for 1 min and final flush with 5.25 % NaOCl for 1 min showed increased carbonate/ mineral ratios in the tooth coronal root third. They also noted that the amide I/mineral ratio decreased significantly in the coronal and apical thirds, whereas the amide III/ CH_2 ratios significantly decreased in the middle third.

4. Discussion

Successful endodontic treatment requires using chemical irrigation solutions to disinfect the root canal system because mechanical instrumentation alone is unable to reach and clear all infected areas within the root canal system [58]. Chemical quantification methods of mineral and protein such as FTIR, have served to reveal and even quantify changes induced by treatment. Clinically used irrigation solutions, such as NaOCl, EDTA and HEDP expose dentin to reactive chemistries that potentially modify the protein and/or the mineral. Concerns have thus been raised about potential alterations to the integrity of the physical, mechanical, and chemical characteristics of natural dentin [28,52,59–63]. Understanding the effects of irrigation solutions and usage protocols on the chemistry and hence the long term composition of dentin is important to be able to predict how and when the performance and properties of dentin might change, possibly degrade. Such information may help tailoring the treatment needs and may be useful to explain structural or time-evolving failures. Under ideal conditions, possible changes in composition of organic and inorganic components due to root canal treatment should be known and considered as part of the treatment planning. However, measurement of changes in composition specifically by sensitive chemical mapping methods such as FTIR require paying attention to critical differences in the methods used as well as details in the sample preparation/treatment approaches used.

The works surveyed in this review included 30 studies in which IR data were presented and used to assess the effects of irrigation solutions on the chemical composition of dentin. Differences in measurement and evaluation procedures as well as differences in experimental steps yield a range of contradictory conclusions. Here we focus on studies where FTIR was used as a major analytic method and we note that many results mostly verified previous observations of the effects of irrigation solution on dentin.

FTIR is a nondestructive widely used method, that has been adopted to identify chemical groups in many solid materials, with exciting structural insights in both bones and teeth [23]. In FTIR, samples are irradiated with a range of infrared wavelengths between 12800–100 cm^{-1} [64]. As the photons impinge on the sample, they are selectively absorbed by the different chemical components inducing transitions from lower-energy to higher-energy/excited states [18]. Excitation generates molecular bond vibrations (i.e., stretching, bending, twisting, rocking and wagging) with different intensity responses at different frequencies in the infrared part of the light spectrum [17]. Typical IR absorbance peaks are proportional to the concentration of each chemical group and correspond to well-documented molecular absorption vibration, thus specific fingerprints may be associated with distinct chemical functional groups (e.g. C–H, O–H PO_4 etc.)[18]. FTIR uses an interferometer to measure the absorption determined by a Fourier Transform of the collected residual light, normalized with respect to some reference. In the case of dentin and similar to bone [23], this allows identifying organic and mineral components such as phosphate, carbonate, and amide I, II, and III bands [65] and it is common to compare the changes in the ratios of carbonate/mineral, amide I/mineral, and amide III/CH_2 [6,44].

FTIR spectra in teeth were reported for a large range of IR configurations including transmission, specular reflectance, diffuse reflectance, photoacoustic, and attenuated total reflectance (ATR) infrared configurations [19]. Usually, spectroscopy requires minimal preparation of the samples, and yields rapid analysis of dry samples with measurements performed more or less non-destructivity [44,45]. Measurements in transmission are the classical and more straightforward forms of FTIR experiments of bony samples made of mineralized collagen fibers[23, 66]. The measurements require samples with a thickness of ~5 µm, usually achieved by pulverization and dilution [65]. FTIR can also be performed on thicker sections using reflection configurations. The widely used attenuated total reflection (ATR) method yields transmission-like FTIR spectra based on a signal that arises from the outermost two micrometers of the sample [67]. This method is the most used because of the minimal sample preparation requirements. FTIR-ATR is useful for solid sample preparations. Yet the method requires good contact of the imaging lens with the sample surface which may not be totally flat, resulting in only partial acquisition. Importantly, the bands obtained with the ATR method are shifted with respect to the bands obtained by transmission [68].

Unlike transmission or ATR, reflection FTIR records the signal arising near the illuminated sample surface. Though this is an advantage in certain situations, reflection spectra results can only be compared with transmission spectra following a Kramers Kronig Transformation [69] which incorporates some sample-specific information. Both methods, ATR and reflection yield 2D maps providing important hints regarding the distribution of dentin chemical groups. All FTIR data analysis follows a baseline correction to reliably calculate absorbance [70]. There are multiple baseline correction procedures available, but in general a measure of the area under the peak is obtained by combining points above the lowest absorbance peak, ideally selected in reproducibly non-absorbing regions of the spectra. The greatest height or integrated area between the peaks is commonly used to calculate the absorbance and ratios of vibrational bands [18]. These differ between different FTIR methods and hence comparisons of results across methods is difficult.

4.1. The rationale of using FTIR

The amide I, II, and III bands in the dentin FTIR spectrum represent characteristic vibrations of peptide bonds. The complexity of the amide I and II bands arises from coupling between similar carbonyl stretching modes or variations in the hydrogen bonding of collagen peptide bonds [1]. These spectral features directly reflect the molecular conformation of intact type I collagen, with the amide I band (C═O stretching vibration at 1600–1700 cm^{-1}) [5]. Phosphate vibrations in the range of 900 to 1200 cm^{-1} typically indicate apatite-related components, while the intense $\nu_3PO_4^{3}$ peak represents the P–O stretching vibration of apatite, often utilized for normalization when assessing the removal of the organic phase [6].

The amide I band observed in the FTIR spectrum of dentine characterized by its broadness. At 1690 cm^{-1} , the signal originates from carbonyl groups situated within collagen fibrils, unaffected by hydrogen bonding. Within the collagen triple helix, a notable interaction occurs between the carbonyl group of an amino acid, typically proline, and the N-H group of glycine, forming an intra-molecular hydrogen bond crucial for helix stability. Carbonyl groups weakly bonded within this helical structure contribute to the absorbance observed at 1660 cm^{-1} . Additionally, the amide I band exhibits a contribution at 1630 cm^{-1} , attributed to carbonyl groups positioned outward from the triple helix, capable of forming strong inter-molecular hydrogen bonds. These distinct contributions render the amide I band particularly sensitive to conformational changes in collagen, highlighting its usefulness for dentin composition characterization [47,71,72].

Tartari et al. (2018) [45] justified the application of, with a specific focus on the amide III/phosphate and carbonate/phosphate ratios, as reliable indicators for assessing changes in dentin composition induced by various decalcifying agents. This choice aimed to provide a comprehensive understanding of the chemical alterations brought about by different endodontic irrigants [45]. Similarly, Baron et al. (2020) [57], highlighted that ATR-FTIR offers minimal preparation, enabling swift analysis of thick samples in their natural state, while maintaining samples intact due to the non-destructive chemical mapping characteristics [44,45]. This approach facilitates examination of changes in carbonate/mineral, amide I/mineral, and amide III/CH_2 ratios throughout the same sample taken from root dentin. In Di Renzo's study [49], the critical selection of FTIR modes elucidated chemical changes such as the hydroxyl stretching vibration at 3572 cm^{-1} and the shift of the amide I band. These chosen FTIR modes provided insights into collagen structure transformation, highlighting the water scissoring mode and the presence of surface hydrogen-bonded water. FTIR analysis, utilized by Gu et al., 2017 [35], Di Foggia et al., 2019 [34], and Browne et al. (2020) [46], played a pivotal role in comprehending molecular changes of collagen within the dentin biocomposite. The focus on amide bands revealed alterations in the protein structures, hydrogen bonding arrangements, and secondary collagen structure distribution. ATR-FTIR spectroscopy, employed by Di Foggia et al., 2019 [34], added insights into demineralization extent as well as to collagen conformation changes, showcasing the utility of FTIR modes in examining changes in the structure on a molecular level and supporting discussions on the impact of endodontic irrigants [34,35,46]. Browne et al. (2020) [46] observed that, for representing the organic component, the amide I and amide II bands were chosen over the amide III and amide A bands. The decision was based on the amide band excessive breadth and the relatively low intensity of the amide III band prior to treatment with NaOCl. The amide I vibrational mode is associated with a combination of CO stretch, CN stretch, C═CN deformation, and other minor contributions, directly correlating with the conformation of the collagen backbone. The amide II vibrational mode is linked to an NH in-plane bend, CN stretch, C═C stretch, and other minor contributions, offering information on nuanced structural changes in the collagen secondary structure [46]. The phosphate band often serves as a constant, and the spectra are typically baseline corrected and normalized to it, as demonstrated by multiple studies [28,47,49,50,52] based on the resistance of mineral to NaOCl treatment. The amide/phosphate ratios, a widely recognized structural parameter, were employed for assessing the removal of the organic phase [73]. The alterations in amide and phosphate bands are key indicators of collagen and mineral alterations facilitating understanding of the chemical modifications occurring during treatments. The collective findings underscore the indispensable role of FTIR spectroscopy, for enhancing our understanding of dental tissue compositional changes and relations to irrigation and treatment efficacy.

Due to the strong connection between systematic review quality and the quality of its incorporated studies, it is advisable to approach the review statements with caution. We evaluated bias risk for all included studies, using a modified version of the OHAT Risk of Bias Tool. Our assessment covered various aspects, including investigating experimental conditions, ensuring researchers were unaware of study groups during experiments, checking outcome data integrity without loss or exclusions, confirming robust exposure characterization, dependable outcome assessment, and comprehensive reporting of measured outcomes.

Moreover, our evaluation also looked into potential threats to the study validity. This included assessing the suitability of statistical methods, researcher adherence to the study protocol, adequate replication within study groups, and transparency regarding bias tendencies. This approach allowed us to categorize studies based on their collective bias profile.

As shown in Fig. 4, the outcome showed 6 studies with a high risk of bias, 16 with unclear bias risk, and 8 with low bias risk.

4.2. Agreement across studies

The included studies reported several common recurring findings. Generally, the use of higher concentrations of NaOCl reduces the organic component of dentin [28–32,35,40,43,47,49,51,52]. EDTA affects the carbonate peak [28,31,33] without significantly reducing the carbonate/phosphate or carbonate/mineral ratios [30,33]. Increasing concentrations of GA (5 %, 10 %, and 17 %) results in a significant reduction in the apatite/collagen ratios [39,57].

4.3. Heterogeneity and ambiguity in the current state of research

Differing results were reported regarding the effects of NaOCl on the carbonate/phosphate ratios. One possibility for the conflicting results is the variation in sample preparation methods. The way dentin samples are prepared can influence their structural integrity and the chemical signals reported by different FTIR methods. Differences in sample size, the portion of the root analyzed, and the size and number of dentinal tubules at different microstructures (e.g. due to different positions along the root), all may contribute to the divergent findings. It is important to note that the heterogeneity of the local dentin structure (e.g. changes in density) could lead to variations in how the dentin interacts with NaOCl, potentially affecting the carbonate/phosphate ratio. Furthermore, the concentration and exposure time of NaOCl may play a significant role in the observed effects on the carbonate/phosphate ratio. For example, Tartari et al., 2016 [43] found that the carbonate/phosphate ratio decreased significantly after a short exposure time (0.5 min) to various concentrations of NaOCl, but no further changes were observed in subsequent periods. This suggests that an initial exposure to NaOCl might cause a rapid alteration in the carbonate/phosphate ratio, but this effect may plateau with longer exposure times. However, our review surveys different studies using various concentrations and this variation may contribute to the literature heterogeneity. Contrasting findings have specifically been reported in studies examining various concentrations of NaOCl (0.5 %, 1 %, 2.25 %) where different exposure times found different alterations in the carbonate/phosphate ratios [29,44]. One study specifically indicated that the application of 1.5 % NaOCl for 5 min did not result in changes to the mineral content [58]. The required time needed to induce a significant difference in the amide III/phosphate ratio also appears to be a subject of controversy. Some studies suggest that short exposure times are sufficient [29,30], while others indicate that longer exposure times are necessary to observe significant changes [43].

Studies such as Zhang et al., 2010 [32] and Morgan et al., 2019 [47] have investigated the effects of NaOCl on collagen degradation and the depth of effect within the root canal system. However, more research is needed to establish a consensus on the appropriate exposure time for reduction in organic content at increasing distances from the root canal wall.

A different controversy relates to the question whether the effect of EDTA depends on the concentration and exposure time or not. Tartari et al.[45] found that increasing the immersion time and concentration results in more phosphate removal, collagen matrix exposure, and consequently the increase in amide III/phosphate ratio, while Bosaid et al. [31] found that the effect of EDTA on the inorganic content is not concentration and time-dependent. These conflicting findings may be attributed to important details of the methodology, such as the sample origin, preparation or specific time intervals used during the experiments. Bosaid et al. [31] employed time intervals of 5 and 10 min, while Tartari et al.[45] utilized a broader and higher resolution range of time intervals, including 0, 0.5, 1, 2, 3, 5, 8, and 10 min. It is possible that shorter time intervals may not provide enough opportunity for EDTA to interact adequately with the target substances, leading to limited impact on phosphate removal and exposure of the collagen matrix. Conversely, longer exposure times might enhance interaction, leading to more noticeable alterations in the measured parameters. To reach a consensus on the effects of EDTA, further studies with standardized protocols and well-defined time intervals are required.

4.4. Chemical mechanistic insights

In their investigation, Tartari et al. (2018) [45] explored the effects of various decalcifying agents on dentin, with a specific focus on the amide III/phosphate and carbonate/phosphate ratios as indicators of changes in dentin composition. The study highlighted the crucial role of the last irrigation solution in defining dentin surface characteristics for subsequent interactions during root filling or potential recontamination [45]. Zhang (2010) [40] delved into the influence of NaOCl on dentin powder, concluding that EDTA, as a final active irrigant, did not significantly contribute to mineralized collagen degradation. This suggests that there is a delicate balance between NaOCl removal of the organic phase and EDTA dissolution of collagen-depleted apatites [40].

Application of 5.25 % NaOCl, results in significant alterations in the carbonate/mineral ratio as the contour for $\nu_3PO_4^{-3}$ becomes sharper after irrigation with different exposure times required for different root dentin portions. This Baron et al. (2020) [30] study emphasized the biologically important presence of labile phosphate and carbonate ions in the hydrated layer of crystalline apatites [74,75], shedding light on the NaOCl specificity in removing organic material [30], and magnesium and carbonate ions [76]. Sakae's investigation [51] using infrared analysis identified loss of magnesium and carbonate ions and indicated a decrease in the 1650 cm^{-1} band intensity following NaOCl treatment, aligning with earlier findings by Emerson and Fischer [77].

Higher concentrations of NaOCl led to a more severe removal of the organic phase, resulting in notable collagen degradation, especially with 8 % NaOCl over a 4-hour period compared to a less concentrated solution (2 % NaOCl) [35]. This indicates a diffusion-controlled "top-down" removal of the organic phase from mineralized dentin by NaOCl, dependent on both time and concentration [35]. While peritubular dentin is noncollagenous [78], its richness in glutamic acid-containing proteins makes it susceptible to deproteinization. In contrast to uniform dentin demineralization, the collagen-sparse subsurface zone exhibited nonuniform deproteinization channels [40,49], facilitating subsequent penetration of EDTA and apatite dissolution.

The interpretation of FTIR results typically requires imaging by other methods (e.g TEM), and such work has made it possible for various authors to provide degradation insights. The application of EDTA to NaOCl-treated dentin dissolved the collagen-depleted mineral ghost layer, exposing the underlying dentin irreversibly damaged by NaOCl, traditionally interpreted as EDTA-induced canal wall erosion [79,80]. The collagen-depleted apatite crystallites within the mineral ghost layer may gradually dissolve due to acids produced by acidogenic microbes retained in the access cavity and root canal system [35]. This could create inconspicuous pathways for bacteria, fluids, and endotoxin diffusion, potentially leading to microleakage to the periapex, a significant factor in root canal treatment failure [81]. Non-mineralized collagen matrices exhibit a size-exclusion effect, restricting access to molecules larger than 40 kDa to the internal aqueous environment of collagen fibrils [82]. As collagen fibrils are encapsulated by apatite crystallites in mineralized dentin, the size-exclusion dimensions for penetrating mineralized collagen should be smaller than those for non-mineralized collagen. The high alkalinity of NaOCl enables it to destroy highly cross-linked collagen molecules once it infiltrates the water compartments of mineralized collagen, creating additional spaces for NaOCl to interact with the fibrils [35].

In the aqueous environment, NaOCl ionizes to produce Na^+ and the hypochlorite ion (OCl-), which establishes an equilibrium with hypochlorous acid (HOCl) [83]. The pH value of NaOCl reflects the chlorine forms in solution, with higher values indicating the predominance of OCl- ions and lower values depicting a higher concentration of hypochlorous acid [84]. The OCl- ion is associated with increased proteolytic activity, leading to enhanced destruction of the collagen component of the mineralized dentin matrix [85]. Regarding its effects on collagen, NaOCl is known to severely fragment long peptide chains and chlorinate protein terminal groups, with resulting N-chloroamines breaking down into various species [86,87].

Gu et al., 2017 [35] reported monitoring pH changes during the reaction of NaOCl and mineralized dentin. Despite the highly alkaline nature of 2–8 % NaOCl (pH 11.29–11.80), its pH rapidly decreased to 6.4-6.7 after mixing with mineralized dentin for 30 min. This could be attributed, in part, to the buffering capacity of dentin on the pH of NaOCl solutions [88]. These findings align with the observations of Norman [89] and Kantouch and Abdel-Fattah [90], who noted increased acidity in the reaction mixture of NaOCl and amino acids as oxidation progressed. The oxidation reaction of NaOCl with collagen was confirmed by the formation of cysteic acid (CysO3), the oxidation product of cyst(e)ine in dentin matrices, based on amino acid analysis results [91]. Additionally, the hydrolysates of mineralized dentin treated with NaOCl exhibited significantly less glycine compared to demineralized dentin hydrolyzed in 6 N HCl. This reduction is likely due to the rapid oxidation of glycine by OCl- in the presence of excess chlorine to complete the reaction [35]. In summary, the reaction between glycine and an excess of OCl- likely involves the intermediate formation of hydrocyanic acid, which is subsequently hydrolyzed into formic acid and ammonia [35]. These products may undergo complete oxidation, liberating carbon dioxide and gaseous nitrogen [89,90]. Di Foggia's ATR-FTIR spectroscopy study [34] demonstrated that EDTA treatment effectively demineralized dentin, reaching a depth of at least 2 μm. The disappearance of bands associated with B-type carbonated apatite and a significant decrease in the apatite/collagen ratio were observed. Changes in the relative intensity of IR amide bands suggested a loss of interactions between collagen, particularly its C=O groups, and Ca^{2+} ions during demineralization, as indicated by previous studies [92, 93]. These interactions play a crucial role in the formation of the triple-helix of collagen with characteristic hydrogen bonding pattern. The increase in the amide II/amide I ratio after EDTA treatment indicated a modified hydrogen bonding arrangement post-demineralization [34].

Conversely, the amide III/I1450 ratio in demineralized dentin, lower than 1, suggested an impact on the integrity of the triple-helix structure. These findings were corroborated by amide I fitting data [34]. In sound dentin, the predominant collagen conformation was the expected triple-helix. Demineralization therefore leads to a significant alteration in secondary structure distribution, with β-sheet becoming the prevailing conformation, and an increase in unordered conformation at the expense of triple-helix, α-helix, and β-turns [34].

The use of NaOCl/EDTA resulted in the removal of the smear layer, opened dentinal tubules, and exposed collagen fibrils, indicating penetration into mineralized collagen matrices, forming a "ghost" mineral layer [35]. The study by Rath et al. (2020) [56] revealing that NaOCl/HEDP treatment exhibited minimal changes, suggesting a homogenous arrangement of organic and inorganic components. Conversely, NaOCl/EDTA treatment resulted in the depletion of inorganic components, indicating weakened C-H bonding and potential weak bonding of collagen fibers [56]. Additionally, Barcellos et al. (2020) reported significant alterations in FTIR spectra for GA at pH 5.0, indicating potential reactions with mineral and the formation of salts, impacting the apatite/collagen ratio [94]. Similarly, Dal Bello et al. (2020) [39] found reduced apatite/collagen ratios with chelating agents, including GA, EDTA, and CA, suggesting modifications in both the organic and inorganic components of dentin. Barcellos et al.'s (2020) [94] emphasis on pH-dependent reactions of GA solutions and Dal Bello et al.'s (2020) [39] exploration of concentration-dependent effects of chelating. In the acid treatment experiment conducted by Di Renzo [50], an intriguing observation was the decrease in signal intensity with longer exposure times to phosphoric, maleic citric, and nitric acids. This reduction could stem from either a partial collapse of the collagen layer or a morphological change in the exposed collagen due to prolonged acid treatment [95–98]. Both effects would lead to a decrease in surface area and, consequently, a reduction in the generated photoacoustic signal. Changes to the collagen structure could significantly influence both the morphology and chemistry of the dentin surface though some alterations might be too subtle for direct observation in the spectra [50].

It is known that some acids may be less efficient in demineralization, as certain acids can form precipitates on the dentin surface during demineralization, hindering further etching [99]. The experimental procedure that Di Renzo used involving sequential measurements, likely prevented the formation of a persistent insoluble boundary layer of these species. The repeated interruptions in the experiment, necessary for monitoring etching over time, facilitated the removal of this layer during each rinse. Consequently, each interruption in the acid exposure contributed to minimizing the differences in etching effects between the acids. Hu et al.'s study [29] also provided insights into NaOCl treatment, indicating concentration-dependent collagen depletion and emphasizing the impact of NaOCl concentration on the amide/phosphate ratio [29]. Tartari et al. [43] corroborated these findings, noting that 5 % NaOCl induces more severe removal of the organic phase compared to lower concentrations. Interestingly, Wang et al. [44] found that 1 % NaOCl within a short time (10 min) does not cause structural variations in dentin, suggesting a concentration-time relationship for its effects.

4.5. Dentin powder implications and clinical relevance

An important aspect is the state of the samples used, whether bulk, or powdered. Testing the effects of irrigation using dentin powder [28,32, 39,40,51,52,57] might help understand certain chemical irrigation effects but does not represent the clinical situation. This is because when powdered, the tissue layout and tubular porosity are destroyed. Powdering increases the surface area and erases the spatially varying information of the chemical characteristics and micro structure. The importance of preparing samples as slices is to maintain the natural structure and prevent alterations in tissue composition, avoiding potential issues arising from water loss during grinding processes [43,100]. Yet many studies rely on powder sample preparation for reasons related to the FTIR measurement method: standardized pellets are simply easier to make and measure, where the mortar-crushed dentin particles minimize surface changes and subsequent FTIR spectra alterations during storage[28]. In typical experimental methods, dentin powder is commonly used to emulate bulk tissue. This powder, characterized by its extensive surface area, tends to lack carbonates due to thermal degradation during the grinding process and undergoes alterations in organic components such as collagen fragmentation and denaturation[54]. For specific applications, treatment of dentin powder with deproteinizing agents is employed to enhance analytical precision[101], however, the clinical relevance of such results remains highly questionable.

4.6. FTIR peaks shift and reductions in intensity after the application of irrigants

Shifts in FTIR peaks, particularly the amide I band, after exposure to decalcifying agents, leading to a discussion on the challenges posed by overlapping bands with water and the unreliability of the amide I band for dentin composition analysis [45]. The use of ratios, such as amide III/phosphate and carbonate/phosphate, are more reliable indicators for assessing changes induced by different irrigants [45]. Shifts and intensity reductions in FTIR peaks are due to changes in collagen structures, deproteination process and hydrogen bonding arrangements induced by endodontic irrigants [34,46,50]. Overall, these findings

Table 4
Explanations and key insights into molecular transformations reported across the included studies.

No.	Author / year	Sample type	Irrigation solution	Duration	Peak shift/reduction observed	Suggested explanation
1	Sakae et al. 1988	Radicular dentin powder	10 % NaClO	30 min	Decrease in the relative intensity of the 1650 cm^{-1}	The weakening of the band at 1650 cm^{-1} suggested that the band originates from certain organic components.
2	Verdelis et al. 1999	Radicular dentin sections	15 % neutral EDTA RCPrep	40 s	reduction in the PO_4/amide I ratio of the micro-MIR FTIR spectra	Neutral EDTA is an efficient agent in removing water-soluble non-collagenous proteins (NCPs) of dentin associated with the calcification process, such as phosphophoryns. It is reasonable to expect that calcium bonded to the extracted fractions of NCPs is also removed.
3	Di Renzo et al. 2001	Coronal dentin sections	Citric acid Maleic acid Nitric acid Phosphoric acid	0, 10 and 30 s 1, 2, 3, 5, 11 and 15 min	An overall increase in signal intensity with etching time can be observed over the entire spectral domain. Reduction of the relative intensities of peaks at 1100 and 1445 cm^{-1}. Newly formed peak at 1724 cm^{-1}.	The overall increase is the result of an increase in the sample surface/ volume ratio. As the acid removes HAP, collagen fibers are exposed. A much higher sample surface area results, and thus, the signal increases. The signal intensity begins to decrease at longer exposure times. This reduction may be caused by a partial collapse of the collagen, or by a morphological change in the exposed collagen. Changes caused to the structure of collagen may have an important impact on both the morphology and the chemistry of the dentin surface, possibly effecting the formation and durability of bonds with the restorative material
4	Di Renzo et al. 2001	Coronal dentin sections	NaOCl solution (12 % w/v) maleic acid	NaOCl: 0, 0.5, 1, 2, 6, 18, 30 and 48 h sample etached with maleic acid for 2 min then NaOCl for for 0, 10 and 30 s, 1, 2, 5 and 15 min	Spectra indicate a clear but not total removal of organic matter over the time of exposure. The presence of the HAP phosphate band becomes more apparent with time, as does the carbonate apatite band (1445 cm^{-1}). The peak at 1655 cm^{-1}, typically assigned to the amide I vibration, gradually shifts to 1640 cm^{-1}.	The reduction and apparent 15 cm^{-1} shift of the peak from 1655 cm^{-1} in the untreated dentin to 1640 cm^{-1} in the heavily deproteinated sample is the result of a decrease in intensity of the 1655 cm^{-1} amide I band of collagen which, after reduction, reveals a water scissoring mode (1640 cm^{-1})
5	Driscoll et al. 2002	Radicular dentin powder	0 %, 0.5 %, 3 %, 5 % NaOCl	30 min	Reduction in the intensity of the C-H stretching band (2960-2850 cm^{-1}) and amide bands (around 1655, 1525, 1228 cm^{-1})	FTIR spectra showed that there was substantial loss of the organic phase from dentin powder exposed to 0.5 % NaOCl for 30 min. Detection of changes in dentin mineral with NaOCl treatment was precluded by the overlap of organic bands with apatite v_3 carbonate bands.
6	Mountouris et al. 2004	Coronal dentin sections	5 % NaOCl	0, 5, 10, 20, 30, 40, 60 and 120 S	A progressive reduction in the amide I, II, and III peaks relative to phosphate was observed as function of treatment time. The carbonate peaks were not affected. The mineral to matrix ratio presented a statistically significant difference among all treatment.	It is proposed that the shear forces applied on the dentin surface during the rubbing action intensify the proteolytic effect of NaOCI by enhancing dissolution of the gelatin-converted part of the smear layer that serves as a binder of the ruptured mineral particles, with exposed collagen appearing on the intact subsurface dentin. Consequently, dentin surfaces conditioned only with NaOCI, may provide a mineralized substrate rich in hydroxyl, carbonate, and phosphate groups available for bonding. Rubbing the smear-layer covered dentin with 5 % NaOCI for 40 s results in a mineralized surface with exposed tubule orifices and inter-tubular dentin.
7	Hu et al. 2010	Radicular dentin slabs	0.5 % NaOCl 1 % NaOCl 2.25 % NaOCl. 0.9 % NaCl	1, 5, or 10 min	These spectra suggested a clear weakening of the peaks at 1242, 1550, and 1643/cm^{-1} after NaOCl treatment. However, the 1643/cm^{-1} peak existed as a weak band after NaOCl treatment. The presence of the phosphate peak became more apparent with time. The amide/phosphate ratio decreased after NaOCl treatment	NaOCl removed the organic components but did not influence the inorganic phase of human dentin. This is because the collagen exposed on the dentin surfaces can be quickly attacked and removed by the NaOCl solutions.

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Table 4 (*continued*)

No.	Author / year	Sample type	Irrigation solution	Duration	Peak shift/reduction observed	Suggested explanation
8	Zhang et al. 2010	Radicular dentin powder	1.3 % NaOCl 5.25 % NaOCl 17 % EDTA	10, 20, 30, 60, 120, 180, or 240 min for NaOCl 2 min for EDTA	Concentration-dependent and time-dependent collagen depletion by NaOCl was revealed by ATR FTIR.	5.25 % NaOCl repression line suggests that the kinetics of collagen degradation was more rapid and severe when it was used as the initial irrigant.
9	Zhang et al. 2010	Radicular dentin powder	1.3 % NaOCl 5.25 % NaOCl 17 % EDTA	10, 20, 30, 60, 120, 180, or 240 min for NaOCl 2 min for EDTA	Lower apatite/collagen ratios of EDTA-treated dentin versus untreated mineralized dentin control Dentin powder treated with either 5.25 % or 1.3 % NaOCl only, apatite–collagen ratios from all time periods were significantly higher than untreated mineralized dentin.	EDTA removes the smear layer, demineralizes the intact dentin and creates a superficial apatite-sparse, collagen-rich matrix on the surface of the radicular dentin. Both NaOCl concentrations were capable of removing the organic phase from the “superficial subsurface” of mineralized dentin.
10	Thanatvarakorn et al. 2014	mid-coronal dentin discs	6 % NaOCl 50 ppm HOCl	15 and 30 s	Reduction in the amide I peak at 1643/ cm^{-1}, compared to control. The amide/ phosphate ratio significantly decreased after NaOCl or HOCl pretreatment, regardless of the pretreatment time.	By 15- or 30-s pretreatment of NaOCl or HOCl solution the organic phase on the smear layer-covered dentin surface was removed. Micromorphological findings revealed elimination of hybridized smear layers in both pretreatment groups. The distribution of nanoleakage patterns varied in no-pretreated, NaOCl- or HOCl-ntreated dentin. NaOCl dissociates into Na^{+} and OCl and establishes HOCl in water. HOCl is a powerful oxidizer and deproteinizer.
11	Yassen et al. 2015	Coronal dentin sections	1.5 % NaOCl 17 % EDTA Ca(OH)2 TAP DTAP sterile water	1.5 % NaOCl for 5 min EDTA for 10 min intra-canal medicaments for 4 weeks	Untreated dentin had significantly higher mean phosphate/amide I ratios compared to dentin treated with NaOCl + EDTA.	Proposed to relate to the strong acidic nature of TAP (pH = 2.9) as well as the chelating effects of both EDTA and minocycline present in TAP
12	TARTARI et al. 2016	Coronal dentin sections	1 % NaOCl 2.5 % NaOCl 5 % NaOCl 0.9 % saline solution	0, 0.5, 1, 2, 3, 5, 8 and 10 min	The saline solution did not alter amide III/ phosphate ratios during the periods analyzed. The collagen was deproteinated by NaOCl solutions, resulting in decreases in the amide III/phosphate ratios. All irrigants caused a decrease in carbonate/phosphate ratio However, only the NaOCl solutions produced significant intragroup changes.	NaOCl can dissolve the organic matter and deproteinate the collagen of dentin in high quantities; otherwise, it can cause a small reduction in the carbonate in the inorganic phase of the dentin. This reduction may be related to the fact that the collagen present on the dentin surfaces is quickly hydrolyzed whereas deeper and unexposed collagen is encapsulated by hydroxyapatite, is possibly less vulnerable to the destructive effects of NaOCl, showing little changes over time
13	Li-sha Gu et al. 2017	Coronal dentin sections	2 %, 4 %, 6 %, 8 % NaOCl	30, 60, 120, 180 and 240 min	Superimpositions of the infrared spectra of NaOCl-treated dentin collected at different time periods (30–240 min) indicated that there was a slight shift of the broad collagen peak toward a lower wavenumber, with concomitant reduction in the intensity of collagen amide peaks	Due to its high alkalinity, NaOCl is capable of destroying apatite-encapsulated, highly cross-linked collagen molecules once it infiltrates the water compartments of mineralized collagen. the reaction between glycine and excess of OCl probably results in the intermediate formation of hydrocyanic acid, which is then hydrolyzed to formic acid and ammonia. An increase in the apatite/collagen ratio of NaOCl-treated dentin occurred as early as 30 min after the application of NaOCl. All NaOCl concentrations are capable of removing the organic phase from the “superficial subsurface”. As more encapsulated collagen molecules are dissolved with the use of a higher concentration of NaOCl, more unbound apatite crystallites remain within the collagen-depleted “ghost mineralized dentin matrix”.
14	WANG et al. 2017	Coronal dentin sections	1 %, 5 %, 10 % NaOCl Distilled water (control group)	Total of 60 min	The contour of $\nu_1\nu_3PO_4^{3}$ remained almost unchanged after treatment in all groups. The amide peaks were stable after treatment in the DW group, while they gradually decreased with treatment time in the NaOCl groups	The organic content changed after NaOCl treatment. The organic content was undermined and eliminated time-dependently by NaOCl. They demonstrated a concentration-dependent effect of NaOCl on dentinal organic content.

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Table 4 (*continued*)

No.	Author / year	Sample type	Irrigation solution	Duration	Peak shift/reduction observed	Suggested explanation
						The loss of networks and the presence of abundant granules along the tubular walls after NaOCl treatment revealed that the meshed collagen was degraded and deproteinized hydroxyapatite remained. Erosion at 60 min was deeper and wider than at 10 min. This indicated that NaOCl posed a time-dependent effect on the dentinal organic content. Erosion-like traces on the intertubular dentin in 5 % and 10 % NaOCl groups was deeper and wider than those in 1 % NaOCl group at the same time point.
15	Tartari et al. 2018	Coronal dentin sections	0.9 % saline 2.5 %NaOCl 5 % NaOCl 9 % HEDP 18 % HEDP 5 % EDTANa4 10 % EDTANa4 17 %EDTAHNa3 0.5 % PAA 2.0 % PAA	0, 0.5, 1, 2, 3, 5, 8 and 10 min	The higher the concentration and immersion time the greater the removal of phosphate, exposure of collagen matrix and consequently the increase in amide III/phosphate ratio. Significant differences in the carbonate/phosphate were observed after 5, 5, 2, 10, 5, 8 and 1 min of sample immersion in 9 % HEBP, 18 % HEBP, 5 % EDTANa4, 10 % EDTANa4, 17 % EDTA, 0.5 % PAA and 2 % PAA solutions, respectively. The spectra obtained from samples submitted to PAA and EDTAHNa3 revealed a shift in the position of the band assigned to amide I after immersion in the solutions. The first 5 min of immersion in NaOCl caused a slow, but significant reduction in amide III/phosphate ratios. Various decalcifying agents decreased the bands associated with phosphate and carbonate apatite at different rates. The HEDP at concentrations of 9 % and 18 % did not alter the amide III/ phosphate ratio of the dentin and the EDTANa4 at 5 % and 10 % caused minor reductions in the phosphate groups	The shift in the position of the band assigned to amide I after immersion in the solutions related with the overlapping between the amide I band and a band of water and shows that this band is not reliable for use in the analysis of dentin composition. The evaluated decalcifying agents demineralized dentin and created surfaces rich in collagen but apatite-sparse, which were expressed as increases in the amide III/phosphate ratio. The analysis of the alterations in the amide III/phosphate ratio produced by the combinations of solutions showed that NaOCl promoted the deproteination of dentin collagen matrix. NaOCl slowly denatured the collagen fibrils encapsulated by apatites on mineralized dentine. This is because they are less vulnerable to the effects of NaOCl, which has to penetrate between crystals to act. After the removal of the exposed collagen, the deproteination process reverted to that observed for encapsulated collagen on mineralized dentin, being much slower and showing little changes over time.
16	Ramírez-Bommer et al. 2018	Coronal dentin powder	2.5 % NaOCl 17 % EDTA	2.5 % NaOCl for 2-10 min 17 % EDTA for 5-1440 min	The height of 1640 cm^{-1} collagen peak decreased by ~ 40 % within the first 2 min of treatment with 2.5 % NaOCl and declined slowly thereafter. The peak height for the PO_4^{-3} (at 1010 cm^{-1}) remained relatively stable. Treatment using 17 % EDTA revealed a decrease in the phosphate (1010 cm^{-1}) and carbonate (871 cm^{-1}) peaks over time and a concomitant increase in the collagen peaks (1 640 cm^{-1}, 1530 cm^{-1}, and 1240 cm^{-1}).	NaOCl reaction with dentin may degrade collagen, rendering it water soluble and removable by the washing. Surface hydroxyapatites could subsequently be released but may either re-precipitate as new water-insoluble particles or back onto the dentin surface due to their limited water solubility. A combination of such processes may explain the decrease in surface collagen/ hydroxyapatite ratio. If the hypochlorite had penetrated dentinal tubules, then the level of collagen disruption might have been expected to occur at much greater depths. The apparent lack of bulk reaction for the largest particles could be a consequence of rapid apatite re-precipitation blocking dentin tubules or neutralization of hypochlorite by the presence of the hydroxyapatite. This could either prevent hypochlorite reaction with deeper collagen and/or prevent deeper damaged collagen from being washed away. Pre-treatment with NaOCl enables the EDTA to penetrate deeper into dentin; a second treatment with NaOCl resulted in further loss of collagen and the phosphate/collagen ratio returned to

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Table 4 (*continued*)

No.	Author / year	Sample type	Irrigation solution	Duration	Peak shift/reduction observed	Suggested explanation
						that of untreated dentine for all particle sizes.
17	Gandolfi et al. 2018	Coronal dentin sections	1 %EDTA 10 % EDTA 17 % EDTA 10 %citric acid Distilled water	1 min	Following treatment with 1 % EDTA the bands assigned to B-type carbonated apatite decreased in intensity with respect to those of collagen, without disappearing and the amide bands did not undergo any shift upon treatment; on the contrary, the $\nu_3PO_4^{-3}$ band shifted from 1006 to 1000 cm^{-1}. Following the treatment with 10 % EDTA the spectra showed decrease in the relative intensity of the apatite bands. 10 % EDTA induced a shift in the collagen amide II and III bands, as well as in the COO- stretching band at about 1338 cm^{-1}. the shift in the $\nu_3PO_4^{-3}$ band was lower than upon treatment with 1 % EDTA. Treatment with 17 % EDTA result in insignificant reduction of the apatite/amide II ratio, in agreement with the EDX results. The amide II and III bands shifted upon treatment, as well as the COO- stretching mode and the $v_3PO_4^{-3}$ band. No significant variations in the carbonate/phosphate ratio were observed. Treatment with citric acid result in significant decreased in apatite/amide II ratios, and certain shifts in the collagen amide III and COO- stretching bands were detected.	The amide bands, due to the repeating peptide unit of proteins, reveal the secondary structure and conformational transitions of proteins; the observed changes suggest that the collagen network underwent a certain conformational rearrangement upon demineralization, although to a lower extent than that previously observed by treatment with 17 % EDTA for a longer period. The shifts of the COO- collagen vibration may be ascribed to changes in the interactions between the collagen and apatite phases.
18	Browne et al. 2019	Radicular dentin sections (Roots irrigated)	5 % NaOCl 17 % EDTA Saline	Total of 25 min	The spectra for the saline-irrigated samples before and after 17 % EDTA surface treatment demonstrated no obvious difference between the outer and inner aspects. The spectra for the NaOCl-irrigated samples before surface treatment with EDTA demonstrated a similar spectral appearance to the saline-irrigated samples with no obvious difference between the outer and inner sampling sites. However, following EDTA surface treatment, the absorbance intensities for the amide I and amide II bands were visibly lower at the inner compared to the outer sampling site.	The reduction of collagen is linked to amino acid degradation and hydrolysis by NaOCl. The collagen peak reduction confirmed previous findings. There was a significant difference in the mean relative ratio for NaOCl+EDTA compared to that for NaOCl alone. This does not indicate greater penetration of the dentin matrix by NaOCl but a greater reduction in the collagen component in the NaOCl & EDTA group
19	Morgan et al. 2019	Radicular dentin sections (Roots irrigated)	5 % NaOCl Deionised water	25 min	Relative increase in absorbance at 1630 cm^{-1}, compared to that at 1660 cm^{-1}, indicating greater contribution from the carbonyl groups at 1630 cm^{-1} after NaOCl exposure. Loss of or denaturation of collagen from dentin treated with NaOCl was confirmed through a decrease in the intensity of the absorbance peaks of amide A, I, II and III, compared to the phosphate peak. The amide I/phosphate absorbance ratio and the amide II/phosphate absorbance ratio decreasedfollowing exposure to NaOCl, confirming the widely reported finding of loss of dentinal collagen. The effect was most apparent with the amide I band, whereas the amide II band formed a 'shoulder' on the carbonate peak, potentially allowing some convolution of its peak, whilst the intensity of the amide III band was initially much lower.	Reduction in the collagen content of the dentin has been linked to hydrolysis and amino acid degradation by NaOCl which occurs following chloramination of the amine functionality, resulting in the formation of organic chloramines. Breakdown of the collagen structure may result in smaller, more soluble products that are dissolved in the irrigant and removed from the dentin surface. Another mechanism for loss of organic content is amino acid neutralization, where peptide linkages are broken and the resulting free carboxylic acid group combines with the Na^+ of the NaOCl to form a soluble sodium salt. Not all of the bands associated with collagen were removed during exposure to NaOCl. The band maxima for the exposed and unexposed dentin were also similar. If denaturation of collagen is judged simply by measuring the frequencies of the band maxima, the broad peak width may cause confusion.

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Table 4 (*continued*)

No.	Author / year	Sample type	Irrigation solution	Duration	Peak shift/reduction observed	Suggested explanation
						If however, the ratio of the responses at 1660 and 1630 cm^{-1} are compared then an increase in the contribution due to the 1630 cm^{-1} carbonyl stretching frequency may be apparent. The ratio 1630/1660 for the unexposed collagen gel sample was 0.70, whilst that for the collagen gel exposed to NaOCl for 30 s was 0.91, suggesting a greater contribution to the spectrum from carbonyl groups that are not held within the triple helix. Exposing the collagen gel to NaOCl for a further 30 s gave a spectrum with a 1630/1660 ratio of 0.99, highlighting an even greater contribution from the carbonyl group(s) at 1630 cm-1. The rate of change decreased over the second 30-s period suggesting that the majority of the triple helix had degraded in the first 30-s exposure. Calculation of the ratio of the absorbances at 1630/1660 revealed a small increase in the relative contribution of the 1630 cm^{-1} carbonyl stretch. a relative increase in absorbance at 1630 cm^{-1}, compared to that at 1660 cm^{-1}, consistent with the breaking of the intra-molecular hydrogen bonds of the collagen and the unwinding of the triple helix. In doing so, the collagen would be more susceptible to chemical attack by NaOCl and subsequent dissolution of the smaller, more soluble breakdown products. A 20-min exposure of the dentin discs was selected because this was considered more clinically relevant. During this time, it is proposed that the triple helix should have broken open and the dissolution of the collagen in the NaOCl be complete. Therefore, the loss of the carbonyl contribution at 1630 cm^{-1} would have 'caught up' with the loss at the 1660 cm^{-1}, making the observed difference smaller.
20	Naseri et al. 2019	Coronal dentin sections	Normal saline 2.5 % NaOCl 17 %EDTA Calcium Hydroxide (CH) Nano–calcium Hydroxide (NCH)	NaOCl for 5 min EDTA for 1 min	The mean phosphate/amide I ratios of the CH and NCH groups were significantl higher after both 1 and 4 weeks compared with the NaOCl and control groups, which indicated deproteinization	There is a strong denaturing effect on the organic matrix of dentin. The denaturing effect of CH and NCH can occur very early even after 1 week.
21	Di Foggia et al. 2019	Dentin sections	17 %EDTA	2 h	Upon EDTA treatment, the IR bands assigned to the mineral phase disappeared while those of collagen underwent significan changes in intensity and wavenumber position with respect to sound dentin. The amide I, II and III bands significantly increased in intensity upon demineralization. At the same time, the amide I band noticeably changed its profile, with a wavenumber shift of its maximum from 1641 to 1628 cm^{-1}. Collagen conformational rearrangements were confirmed by the amide II/amide I and amide III/I1450 ratios: upon EDTA treatment, the former increased from 0.79 ± 0.02 to 0.90 ± 0.02, the latter assumed a value of 0.81 ± 0.04 The bands assignable to B-type carbonated apatite completely	The changes observed in the relative intensity of the IR amide bands may be related to the loss of interactions between collagen (in particular through its CO groups) and Ca^{2+} ions upon demineralization. These interactions appeared of key importance in order that collagen assumes its triple-helix structure as well as its characteristic hydrogen bonding pattern. Actually, the increase of the amide II/amide I ratio upon treatment with EDTA may be interpreted as a sign of a changed hydrogen bonding arrangement after demineralization. On the other hand, the value of the amide III/I1450 ratio in demineralized dentin (lower than 1) suggests that the integrity of the triple-helix is affected to a certain extent. These findings were confirmed by the amide I fitting data. In sound dentin the main collagen conformation

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Table 4 (*continued*)

No.	Author / year	Sample type	Irrigation solution	Duration	Peak shift/reduction observed	Suggested explanation
					disappeared and the apatite/collagen ratio significantly decreased.	was triple-helix, as expected. Demineralization significantly altered the secondary structure distribution: after treatment with EDTA, the prevailing secondary structure became β-sheet and the content of unordered conformation increased as well at the expenses of triple-helix, α-helix and β-turns.
22	Barón et al. 2020	Radicular dentin sections	5.25 % NaOCl 17 % EDTA	5.25 % NaOCl for 1, 5, 20 min 17 % EDTA 1 min	5.25 % NaOCl induced a reduction in the carbonate/mineral ratio that was statistically significant for the coronal third only after 20 min of treatment. In the middle third, the decrease was significant after 1 min of irrigation and, in the apical third, after 5 min, while significantly decreasing again after 20 min. amide I/mineral ratio also significantly decreased after 5.25 % NaOCl treatment for 1 min in the three root thirds. 17 % EDTA produced no significant alteration of the carbonate/mineral ratio. The amide I/ mineral ratio only increased in the apical third, while amide III/CH_2 ratio significantly increased after its application along the root canal. 5.25 % NaOCl for 20 min followed by 17 % EDTA for 1 min and, afterwards, a final flush with 5.25 % NaOCl for 1 min significantly increased carbonate/ mineral ratio in the coronal third. The amide I/mineral ratio significantly decreased in the coronal and apical thirds, and amide III/ CH_2 ratio significantly decreased in the middle third.	NaOCl is mainly responsible for the removal of organic material, but also eliminates magnesium and carbonate ions. NaOCl was able to penetrate and damage the apatite-encapsulated, highly cross-linked collagen matrix. The proteolytic action of NaOCl has been attributed to the low molecular size of the hypochlorite anion that penetrates and oxidizes the collagen, reducing the content of alanine and glycine and increasing the intensity of ammonia. The links of amide I are more sensitive to NaOCl action than those of amide III. The reaction of 17 % EDTA with calcium ions in hydroxyapatite crystals promotes their elimination from dentin Neutral EDTA is well known as an efficient agent in removing water-soluble non-collagenous proteins (NCPs) of dentine associated with the calcification process.
23	Ng et al. 2020	Radicular dentin sections	formal-saline 5.25 % NaOCl 17 % EDTA Ca (OH)2	NaOCl & EDTA 10, 20, 50, 80 or 110 min Ca (OH)2 for 1, 2, 4, 12 weeks	5.25 % NaOCl and 17 % EDTA revealed considerable reduction of the hydroxyapatite peak at 1000 cm^{-1} and an appreciable decrease in the organic components: N-H(1) and N-H(2) bands, which mirrored the FTIR spectra for samples reacted to 5.25 % NaOCl alone. FTIR spectra (normalised at 1000 cm^{-1}) showed that the 'C═O′ and 'N-H′ peaks at 1640 and 1540 cm^{-1}, respectively progressively diminished with reaction time with Ca $(OH)_2$, regardless of pH.	Complete denaturation of the exposed collagen, which may in turn influence its state of hydration.
24	Ratha et al. 2020	Coronal dentin sections	0.9 % NaCl 6 % NaOCl 3 % NaOCl 12.5 % EDTA 17 % EDTA 18 % HEDP	15 min	All the groups showed elevated amide A peaks, confirming the presence of collagen on the dentinal surface. Dentin treated with NaOCl alone showed a sharp peak at 1000 cm^{-1}indicating the deposition of hydroxyapatite. By contrast, NaOCl/EDTA showed a stretch at 1000 and 2300 cm^{-1}, indicating the loss of inorganic substance. The FTIR spectra for the NaOCl/HEDP treated dentin resembled that of the control group, indicating a homogenous arrangement of organic and inorganic components. NaOCl/EDTA-treated dentin showed no distinct peaks at 1000 cm^{-1}, indicating the depletion of inorganic components from the surface. Such data can be related with significantly less phosphorus content in NaOCl/EDTA group. Importantly, the stretch at amide III peak in this group indicates	NaOCl causes proteolysis of the organic content of the superficial smear layer and the subsurface dentin. This is characterized by the loss of banding pattern of the collagen. As the organic layers dissolve, there is an increase in the concentration of inorganic crystallites at the surface, blocking deeper dentinal penetration of NaOCl, restricting its actions against the underlying organic matrix. Demineralization of this dentin surface by EDTA results in a surface characterized by loosely arranged, "naked" collagen fibrils, extending up to 880 nm into the dentin, as revealed by the TEM images. The control group showed a higher atomic percentage of C, N and Ca, P indicating the presence of both organic and inorganic components respectively. No significant difference in C and P content were observed between the

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Table 4 (*continued*)

No.	Author / year	Sample type	Irrigation solution	Duration	Peak shift/reduction observed	Suggested explanation
					that the–C-H bonding is medium to weak in nature.	NaOCl/HEDP and control group suggesting the homogenous presence of organic and inorganic components of the dentin at the exposed surface. The NaOCl/EDTA protocol showed higher atomic wt% of C and N. HEDP is a weak chelator. The NaOCl/HEDP-treated dentin showed elevated peaks at amide I and the stretch at amide III that indicate potential weak bonding of C N (collagen fibres and polypeptide cross links) The NaOCl/HEDP protocol is characterized by simultaneous dissolution of organic fibres, demineralization and collagen destruction. The protocol resulted in a significant increase in the amide content, compared to the control and NaOCl/EDTA protocol. NaOCl/EDTA-treated dentin showed an increased exposure of unprotected surface collagen bundles. The dentin surface treated with the NaOCl/EDTA protocol contains "naked" collagen fibres that are devoid of mineral encapsulation. Conversely, the NaOCl/HEDP protocol resulted in surface erosion, an increase in surface roughness and disorientation of the organic matrix at the interface. Yet, the collagen fibrils were still encapsulated by minerals and there was no significant compositional change in the dentin treated with this protocol.
25	Bello et al. 2020	Dentin powder	Distilled water 17 % EDTA 5 % GA 10 % GA 17 % GA	1 min	The apatite/collagen ratio was reduced with chelating agent applications because all groups showed differences from the no-treatment and DW groups. The lowest values were shown with increased GA concentration, while EDTA, CA, and GA 5 % demonstrated similar results. The main alterations generated for GA 10 % and 17 % were verified in the phosphate peak. However, considerable alterations in the amide I, II, and III peaks indicated that the action of this acid occurs in organic and inorganic dentin components. Moreover, the changes were dose dependent because GA 5 % did not create substantial alterations. GA 10 % and 17 % showed considerable alterations in the apatite/collagen ratio and EDTA showed drastic reductions in the apatite/collagen ratio	The apatite/collagen ratio alterations showed by chelating agents, mainly by GA 17 %. These alterations are intended because they facilitate the biomechanical preparation under clinical conditions.
26	Barcellos et al. 2020	Dentin powder	Saline 2.5 % NaOCl 17 % EDTA 17 % GA	1 min	The apatite/collagen proportion significantly decreased after irrigation with all experimental solutions, as the results obtained were statistically lower than the control group (no irrigation). The lowest values were obtained for GA at pH 5.0 while EDTA and GA at pH 1.2 demonstrated statistically similar results.	2.5 % NaOCl resulted in less collagen breakdown, not enough to significantly reduce the flexural strength when compared to the control group. When comparing the FTIR spectra among the groups, the most significant changes are observed for the GA pH 5. Aminomethyl propanol was used to obtain a GA solution with pH 5.0, this substance may have reacted with mineral and formed salts, reflecting the significant changes in the apatite/collagen ratio found in this study. Similar spectra are noted for EDTA and GA pH 1.2, demonstrating a potential chelating binding of calcium.

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Table 4 (*continued*)

No.	Author / year	Sample type	Irrigation solution	Duration	Peak shift/reduction observed	Suggested explanation
27	Bosaid et al. 2020	Radicular dentin sections	Distilled water 1.5 % NaOCl 3 % EDTA 10 % EDTA 17 % EDTA 10 % CA	NaOCl for 5 min EDTA and CA for 5 or 10 min	FTIR analysis revealed that 1.5 % NaOCl significantl decreased the collagen peak levels (61 % reduction) compared with the negative control. The use of EDTA or 10 % CA resulted in significantly greater collagen peak levels compared with the NaOCl group. 10 % CA for 5 or 10 min decreased the phosphate peak levels compared to the control and other EDTA groups. The use of EDTA at all concentrations or 10 % CA decreased the carbonate peak levels, compared to the control group	1.5 % NaOCl did not change the inorganic components of root canal dentin compared with the control. High concentrations of NaOCl (5 % or 9 %) could significantly reduce the carbon and nitrogen levels of dentin 1.5 % NaOCl had no effect on the inorganic part of the root canal dentin, it had a detrimental effect on the dentin collagen content. The chelating solutions appear to neutralize the effect of the remaining NaOCl on dentin through a chemical interaction between chelating solutions and NaOCl which may prevent further reduction in collagen degradation. NaOCl alone decreased the collagen levels.
28	Kusumasari et al. 2020	Dentin at the level of cemento-enamel junction	Papacarie, Carisolv, and 6 % NaOCl	15 s	The spectra acquired after the application of Papacarie, Carisolv, and NaOCl revealed a significant reduction in the amide I peak at 1643/ cm^{-1} on both enamel and dentin, compared to the control group.	15 s-NaOCl treatment could partially eliminate the hybridized smear layer and the longer application of NaOCl for 30 s could completely eliminate it. A plausible reason for these is the presence of residual free-radicals generated by the oxidizing effect of NaOCl, which leads to premature chain termination and incomplete polymerization
29	Kusumasari et al. 2021	Coronal dentin sections	Papacarie, Carisolv and 6 % NaOCl	15 s	Papacarie and 6 % NaOCl showed a significant reduction in the amide/ phosphate ratio for both dentin substrates. Carisolv showed a decrease in the amide I peak at 1643/cm^{-1} for normal and caries-affected dentin, compared to the control group.	ATR-FTIR confirmed the reduction in amide/phosphate ratio which indicated the elimination of protein
30	Padmakumar et al. 2022	Radicular dentin sections	5.25 % NaOCl Ozonated olive oil Silver citrate Distilled water	Not mentioned	Decrease in the collagen peak was observed in the NaOCl group compared to the experimental and negative control groups. Significant reduction observed in the carbonate and phosphate peak values in the NaOCl group	NaOCl significantly reduced the organic components of the dentin such as carbonate and phosphate compared to novel irrigating solutions such as silver citrate and ozonated olive oil. NaOCl spreads on the intrafibrillar water volume of apatite-encapsulated collagen matrix owing to its low molecular weight. Collagen from the "superficial subsurface" undergoes oxidative chemical destruction when it comes into contact with sodium hypochlorite . The silver citrate group displayed a slightly larger quantity of calcium dissolution, which is consistent with a capacity to decalcify hard dental tissues by the chelation of Ca^{2+} ions

underscore the importance of understanding the molecular changes reflected in FTIR peaks for a comprehensive assessment of the impact of endodontic irrigants on dentin properties. The changes in FTIR peaks and their explanations provided in Table 4 summarize the molecular transformations reported across different studies.

4.7. Concentrations of irrigants and dentin damage

In general, as the concentration and contact time of the irrigation solution with dentin increase, the resulting effect tends to become more pronounced. The 2.0 % PAA is a strong demineralizing agent, causing substantial phosphate removal and exposing the collagen matrix [45]. Various concentrations of NaOCl, including 1 %, 2.5 %, and 5 %, result in a significant reduction in the carbonate/phosphate ratio within 0.5 min of immersion [43]. Additionally, collagen degradation has been highlighted as concentration- and time-dependent, with even low concentrations such as 1.5 % NaOCl significantly impacting collagen levels and consequently affecting dentin mechanical properties [35]. Treatment with NaOCl/EDTA associated with potential damage, while NaOCl/HEDP treatment resulted in minimal alterations, indicating a potentially more favorable outcome [56]. It has been emphasized that increased NaOCl concentration may lead to potential damage, highlighting the importance of considering concentrations in the selection of irrigants [29]. This insight aids in the discussion on concentrations that may potentially damage dentin, providing guidance for clinical considerations in the selection of irrigants.

Despite an extensive coverage in the literature, there is no complete agreement between the studies regarding the effects of the concentration and the duration of the exposure to each irrigant. Thus, FTIR studies have not yet delivered clear guidelines or recommendations for clinically effective irrigation solutions or protocols.

4.8. Recommendations for future studies

Based on the literature, it may be concluded that:

1. Further investigations are needed to identify the effects and importance of irrigation on bone-derived structural parameters in dentin including collagen cross-linking and changes in the crystallinity index.
2. FTIR studies have not fully revealed the depth of effects of irrigation solutions that are used in the canal space and how far they penetrate outwards from the canal wall, extending into the dentin root bulk.
3. Further investigation is needed to find out the relation between concentration of different irrigation solutions, exposure time and the amount of loss of both organic and inorganic components of dentin.

5. Conclusion

FTIR methods reveal, for both the researcher and the clinician, chemical and nanostructural changes that are induced by use of irrigation solutions during root canal treatment. Studies based on powdered samples augment studies using mapping across treated root sections. Based on the studies included in this review, NaOCl mainly affects the organic component of dentin with minor, inconclusive effects on the inorganic component of dentin. Higher concentrations result in more loss and depletion of the organic components of dentin and the effects of NaOCl appear to extend several hundreds of microns in the dentin wall. EDTA has a minor effect on the organic component of dentin and its effects on the mineral component are time and concentration dependent. GA significantly reduces apatite/collagen ratios and induces major changes in the phosphate peak. Lower concentrations of irrigation solutions are recommended to reduce erosion and removal of structurally-important domains in root dentin. Future research should ideally better link physical observations, such as alteration in the chemical composition of dentin and changes in structural and mechanical properties, etc., with bacteriological assessments of the biofilm, of infection spread and of clinical outcomes (e.g., tooth survival).

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Compliance with ethical standards

The authors declare that they have no conflict of interest.
Informed consent was not required.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.dental.2024.05.014.

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