



Effect of Gutta-percha Solvents on the Bond Strength of Sealers to Intraradicular Dentin: A Systematic Review

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ABSTRACT

Introduction: The aim of our systematic review was to assess the impact of gutta-percha solvents on the bond strength of endodontic sealers to intraradicular dentin using the push-out bond test. **Methods and Materials:** The literature was searched in databases (PubMed, Web of Science and Scopus) up to September 2020, using the following search terms: (bond strength AND solvent* AND sealer* AND (tooth root OR dentin OR retreatment OR root canal). No date limits were implemented, and English languages were included. The question research was constructed based on the PICO (*Population, Intervention, Comparison, and Outcome*) strategy: “Does gutta-percha solvents effect the bond strength of sealers to intraradicular dentin?”. The studies were analyzed by two reviewers and were included if they utilized extracted permanent human teeth with completely formed apices, as well as assessed the influence of gutta-percha solvents on the bond strength of sealers to intraradicular dentin using push-out bond test. Review articles, case reports and studies that included immature, bovine or artificial teeth were excluded. The risk of bias was evaluated based on the Cochrane criteria adopted to *in vitro* studies. **Results:** Thirty-two papers were analyzed, seven accomplished the eligibility criteria and were selected for our systematic review. The global risk of bias was high. Due to variations in the methodological variables, a meta-analysis could not be performed. **Conclusion:** Our systematic review highlighted the adverse effect of chloroform, which decreased the bond strength of different sealers, and the generally higher bond strength of epoxy resin-based sealers, detected with the push-out bond test. We recommend the standardization of methods in future studies to obtain a more definitive conclusion about the influence of solvents on the bond strength of sealers to intraradicular dentin.

Keywords: Dentin; Root Canal Sealer; Root Canal Therapy; Solvent; Systematic Review

Introduction

Endodontic solvents are usually applied to facilitate the removal of filling materials enhancing the penetration of rotary or manual instrumentation [1-5]. In what concerns sealers, studies showed that each chemical composition may be sensitive to a specific solvent [2]. A new strategy has been recently suggested, applying solvents after the removal of the bulk of the obturation with the purpose of eliminating filling remnants instead of only softening. A non-traditional solvent, methyl ethyl ketone, and the known gutta-percha solvent, tetra

chloro-ethylene, were greatly enhanced by ultrasonic agitation reaching or surpassing chloroform's dissolution ability, without its potential hazards [6-9]. However, their efficacy was time-dependent, which can raise concerns about the potential damage to the collagen fibrils of dentin thus influencing the bond strength of endodontic sealers [6, 7, 10]. That, in turn, could jeopardize the success of the endodontic treatment, by preventing microleakage and assuring the integrity of the root canal seal [11].

The push-out bond strength assessment is a widely accepted method to measure the adhesion quality of root filling

materials [12-15]. Nevertheless, a greater standardization in the experimental set-up and a more precise report of all research variables are required to clarify the real impact of some materials/solutions may have on the dentin adhesion [16]. It is important to understand how dentin reacts to different root canal solutions and to ensure that the filling materials achieve their intended properties, namely, promoting a hermetic seal, avoiding disruption and resisting dislocation during the occlusal forces or operative procedures they are submitted to. Although the preliminary results of *ex vivo* investigations have been quite enthusiastic [8], it would be important to detect whether a prolonged use of solvents has any potential harmful effects on dentin for its application in the clinical environment.

To date, studies have reported conflicting results about the effect of gutta-percha solvents on the push-out bond strength of sealers. Besides, at the time this review was carried out, there had been no previous systematic reviews addressing this matter. Therefore, the purpose of the present systematic review was to examine *ex vivo* studies in the literature that assessed the influence of gutta-percha solvents on the bond strength of endodontic sealers to intraradicular dentin using the push-out bond test (PBT).

Materials and Methods

Search approach and study selection

The literature review was conducted according to the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses statement) guidelines [17]. The research question was based on the PICO (Population, Intervention, Comparison, and Outcome) framework: The *Population* were human teeth, the *Intervention* was the use of gutta-percha solvents, the *Comparison* was not using gutta-percha solvents and the *Outcome* was the bond strength of sealers to dentin. Thus, the research question was set as follow: Do gutta-percha solvents influence the bond strength of sealers to intraradicular dentin?

An electronic search was performed in three databases (PubMed, Scopus and Web of Science) up to September 2020. No date limits were implemented, and English paper were included. The search strategy included the use of Medical Subject Heading MeSH terms, truncation terms and free words: (bond strength AND solvent* AND sealer* AND (tooth root OR dentin OR retreatment OR root canal). A manual search was performed in the electronic portal of the following journals: Journal of Endodontics, International Endodontic

Journal, Clinical Oral Investigations, Iranian Endodontic Journal and Australian Endodontic Journal. An additional search was done in the reference list of all papers included. After the removal of duplicates from the search results obtained, using EndNote X8 software (Thomson Reuters, New York, USA), two reviewers individually evaluated the abstracts and titles of the recognized publications and removed articles that were out of scope. Full-text articles were used to verify the relevance of the article content. All doubts were resolved with discussions between the reviewers until a unanimous decision was obtained.

Eligibility criteria

Studies were included if they conducted the tests on extracted permanent human teeth with completely formed apices and assessed the influence of endodontic solvents on the bond strength of sealers to intraradicular dentin using the PBT. Review articles, case reports and studies that included immature, bovine or artificial teeth were excluded.

Risk of bias

The risk of bias assessment was performed based on the Cochrane criteria adapted to *in vitro* studies [18, 19]. Two reviewers individually analyzed the methodological quality of each included study using the following parameters: sample size calculation, randomization (method applied to generate the random allocation sequence), allocation concealment, blinding of the observer during the experimental protocols, standardization of samples (tooth type) and standardization of the sample preparation (single operator). Similarly to another systematic review [18] performed with *in vitro* studies, if the above parameters were clearly mentioned, the risk of bias was recorded as low; if the parameters were not mentioned, it was recorded as high; if their mention was not clear, it was recorded as unclear. Discrepancies concerning the parameters mentioned were resolved by consensus between the two reviewers.

Data extraction

Two reviewers independently extracted the subsequent data from each included study and recorded them as follows: study characteristics (authors and year), root-filling materials, solvents (type, volume and exposure time), retreatment procedures and parameters of the push-out bond strength test (slice thickness, diameter of the canal and taper of the instrument used, timing of the sectioning of the root canal, plunger size, speed and direction of plunger loading and root canal segments used). Discrepancies in the data extraction were resolved by consensus

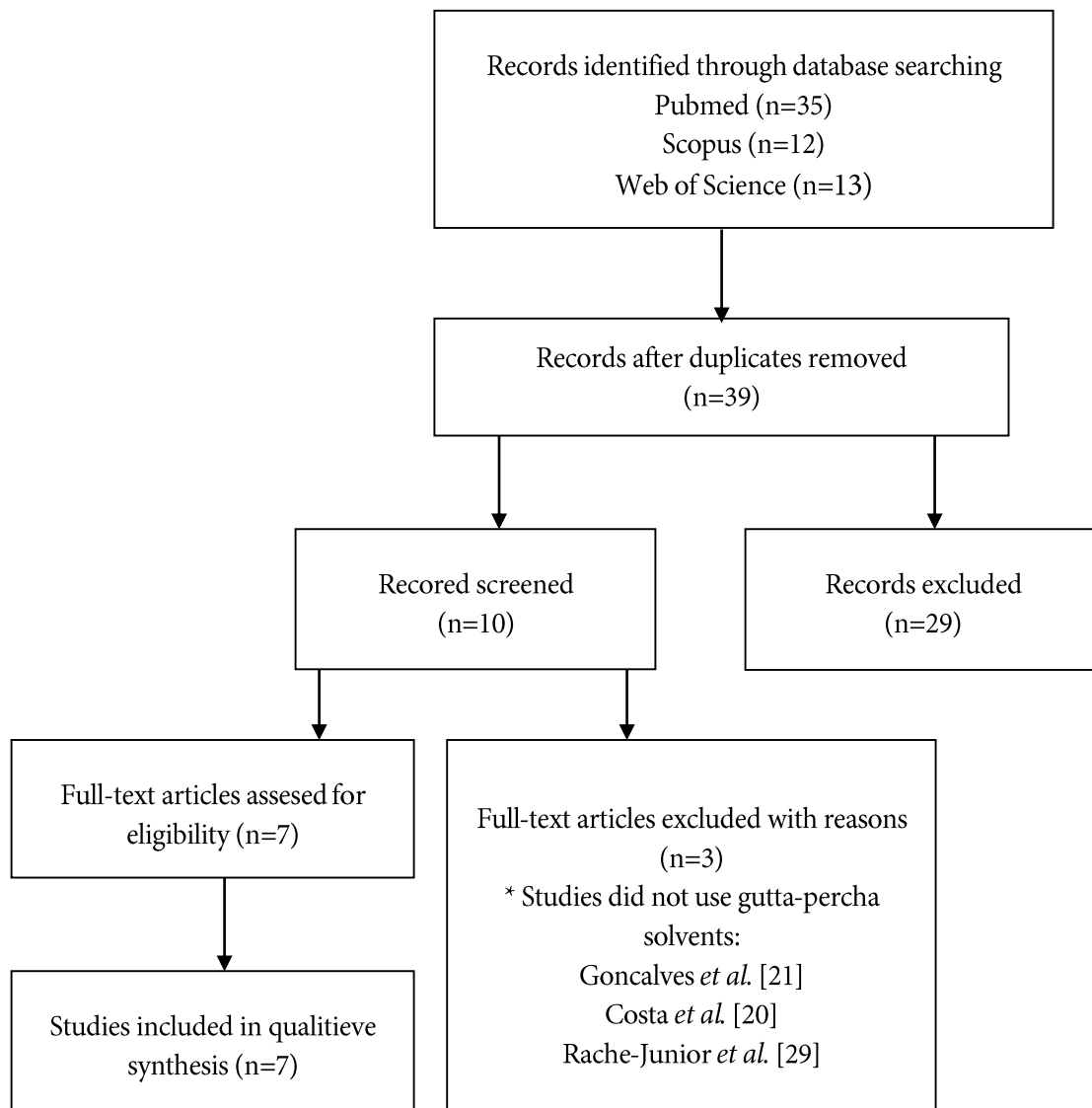


Figure 1. A flowchart of the search strategy

Results

The flowchart of the search strategy is shown in Figure 1. Two reviewers independently assessed the selected studies and examined the results. A total of 32 papers were obtained. After removing the duplicates, a data screening based on the titles and abstracts was conducted, and three studies were excluded because they did not use gutta-percha solvents [20-22]. Seven studies met the eligibility criteria and were included in this systematic review. No additional study was identified as eligible.

The results for the risk of bias of the integrated studies are presented in Table 1. The risk of bias was high in all the papers for sample size calculation, allocation concealment

and blinding because these parameters were not mentioned. All papers had an unclear risk of bias regarding the randomization parameter, as none of the studies clearly stated the method applied to generate the random allocation sequence and only stated that the samples were “randomly” divided into groups. Only one study stated that the sample was prepared by a single operator [23] and this information was not reported in the other six studies. Four studies [24-27] clearly mentioned the type of teeth used, as well as the inclusion and exclusion criteria to select the teeth. The overall risk of bias was high. A meta-analysis could not be conducted because of the considerable heterogeneity in the methodological variables. The descriptive data of all included articles are shown in Table 2.

Root filling materials

In four studies, teeth were filled with gutta-percha and sealer, using lateral compaction [27-29] or the single-cone technique [26]. One study assessed the Resilon/Epiphany obturation system using lateral compaction [23]. In two studies, teeth were filled only with sealers (silicate-based [24], epoxy or methacrylate resin-based sealers [25]). The epoxy resin-based sealer (AH Plus) was tested in four studies [25-28], MTA-based sealers in three studies [24, 26, 28], the Resilon/Epiphany SE system in two studies [23, 25], and Sealapex only in one study [26]. In Rached-Junior *et al.*'s study [29], teeth were filled with a zinc oxide eugenol-based sealer (Endofill) and refilled with an epoxy resin-based sealer (AH Plus) to assess the impact of sealers of different chemical nature.

Solvents (type, volume and exposure time)

The solvents assessed were chloroform in four studies [23, 24, 26, 28], xylol in three [25, 27, 29], orange oil and eucalyptol in three [24, 26, 27], EndoSolv E in two [24, 25] and EndoSolv R in one [23]. The volume used varied between studies from 1 drop to 5 mL.

Only one study assessed two times of exposure of the dentin (2 and 5 min) to various solvents- chloroform, orange oil and eucalyptol [26]. The use of chloroform for 5 min had a negative influence on the bond strength of all the sealers studied [AH Plus (Dentsply, Tulsa Dental, Tulsa, OK, USA), MTA Fillapex

(Angelus, Londrina, PR, Brazil) and Sealapex (Sybron-Kerr, Romulus, MI, USA)] [23-29]. On the other hand, neither orange oil nor eucalyptol influenced the bond strength of the root sealers, independently of the time [26].

Shokouhinejad *et al.* [23] tested 1 min of exposure to chloroform, refreshing it three times, and corroborated a negative influence on the bond strength of Resilon/Epiphany SE (Resilon Research LLC, Madison, CT, USA); they found no significant differences with EndoSolv R (Septodont, Saint-Maur, France). Conversely, Palhais *et al.* [27], when depositing 1 drop of solvent before removing the root filling with D1, D2 and D3 (ProTaper Universal retreatment system; Dentsply Maillefer, Ballaigues, Switzerland), found that the Xylol, eucalyptol and orange oil had a negative impact on the bond strength of AH Plus to root canal dentin, with eucalyptol having the lowest values. Nevertheless, they obtained similar results in the group retreated only with files (without solvent), but different from the control group (teeth not retreated); the refinement of the root canals in all groups occurred until F5.

Nasim *et al.* [25] studied the effect of 5 min of exposure to xylene and EndoSolv E, before root filling, and reported a negative result on the bond strength of both sealers - AH Plus and Epiphany. In that experiment, teeth were filled only with sealer and were not retreated.

Table 1. Risk of bias assessment

Author	Sample size calculation	Randomization	Allocation concealment	Blinding	Standardization of samples selection (tooth type)	Standardization preparation (single operator)	Reporting of data
Nasim <i>et al.</i> [25]	High	Unclear	High	High	Low (Single-rooted maxillary canines)	High (NS)	Low
Rached-Junior <i>et al.</i> [29]	High	Unclear	High	High	Unclear (Incisors without calcifications or accentuated curvature)	High (NS)	Low
Shokouhinejad <i>et al.</i> [23]	High	Unclear	High	High	High (Single-rooted teeth)	Low (Yes)	Low
Topçuoğlu <i>et al.</i> [26]	High	Unclear	High	High	Low (Straight, single-rooted mandibular premolars with completely formed apex and absence of previous root filling, resorption or calcifications)	High (NS)	Low
Bayram <i>et al.</i> [24]	High	Unclear	High	High	Low (Single-rooted mandibular premolars, with canal curvatures less than 5°)	High (NS)	Low
Palhais <i>et al.</i> [27]	High	Unclear	High	High	Low (Maxillary canines with completely formed apices and a single canal without calcifications)	High (NS)	Low
Yavari <i>et al.</i> [28]	High	Unclear	High	High	Unclear (Single-rooted mandibular premolars with similar morphologies)	High (NS)	Low

NS: not stated

Table 2. Study Characteristics

Author	Root filling materials	Retreatment procedures	Solvents/contact time/volume	Push-out parameters				Main results
				Canal segments	Slice thickness	Plunger diameter	Crosshead speed	
Nasim <i>et al.</i> [25]	AH Plus, Epiphany	Not performed	Xylene, EndoSolv E/ 5 min/5 mL	Coronal, middle, apical	1 mm	0.8	1 mm/min	Negative effect of Xylene and EndoSolv E on PBS to the AH Plus and Epiphany
Rached-Junior <i>et al.</i> [29]	GP+Endofill	G1: PTR+ProTaper F5+GP/AH-Plus G2: PTR+xylo+ ProTaper F5+GP/AH Plus G3: Ultrasound+ ProTaper F5+GP/AH Plus G4: Ultrasound+ xylo+ProTaper F5+GP/AH Plus	Xylo/ a drop	First slice of each third	1 mm	1-coronal; 0.6-middle; 0.4-apical	0.5 mm/min	No influence of Xylo on PBS of AH Plus sealer
Shokouhinejad <i>et al.</i> [23]	Resilon+ Epiphany SE	G1: without retreatment G2: Mtwo Retreatment/Mtwo G3: Mtwo Retreatment/Mtwo+Chloroform G4: Mtwo Retreatment/Mtwo+EndoSolv R	Chloroform, EndoSolv R/ NS/ 1 mL	Middle	1 mm	0.7	0.5 mm/min	Negative effect of Chloroform on PBS of Resilon/Epiphany. EndoSolv R did not influence
Topçuoğlu <i>et al.</i> [26]	GP+AH Plus or MTA Fillapex or Sealapex	Not performed	Chloroform, Eucalyptol, and Orange oil/ 2 or 5 min /0.2 mL	Coronal, middle, apical	1 mm	0.7-coronal; 0.8-middle; 0.9-apical	1 mm/min	Chloroform (for 5 min) reduced the PBS of all sealers. Orange oil and eucalyptol did not affect
Bayram <i>et al.</i> [24]	WMTA, Biodentine, and CMTA	Not performed	Chloroform, EndoSolv E, Eucalyptol, and orange oil/ 5 min/ 0.1 mL	Middle	3 mm	1.2	0.5 mm/min	Negative effect of solvents on the PBS of Biodentine and CMTA. WMTA was not affected.
Palhais <i>et al.</i> [27]	GP+AH Plus	PTR with solvents+ProTaper F5+GP/AH-Plus)	Xylo, Orange oil, Eucalyptol/ NS	Coronal, middle, apical	1 mm	1-coronal; 0.6-middle; 0.4-apical	0.5 mm/min	The solvents reduced the PBS of the AH Plus
Yavari <i>et al.</i> [28]	GP+AH Plus or MTA Fillapex	PTR with solvent+ProTaper F3+GP/AH Plus or MTA Fillapex	Chloroform/ 3 drops	Coronal, apical	2 mm	0.8	0.5 mm/min	No effect of Chloroform on the PBS of AH Plus and MTA Fillapex

GP: gutta-percha; PTR: ProTaper retreatment system, PBS: push out bond strength; NS: not stated; MTA: mineral trioxide aggregate; CMTA: capsule-form mineral trioxide aggregate; WMTA: white mineral trioxide aggregate

Rached-Junior *et al.* [29] first obturated the teeth with Endofill and then used one drop of Xylol between each change of instrument during retreatment before refilling them with AH Plus. They concluded that the application of Xylol did not increase the bond strength of AH Plus to dentin but, when combined with ultrasound, the adhesion increased. Nevertheless, the experimental groups, independently of the solvent, presented lower bond strengths than the control (refilled with the same sealer-AH Plus).

Bayram *et al.* [24], who used a different time-slicing methodology and also used only sealer for filling, reported the decrease of bond strength of all the silicate sealers studied, Biodentine and capsule-form mineral trioxide aggregate (CMTA), but not of the white mineral trioxide aggregate (WMTA) with 5 minutes exposure to each solvent - chloroform, EndoSolv E, orange oil and eucalyptol.

Yavari *et al.* [28] compared not retreated teeth with retreated teeth in which three drops of chloroform were applied before the use of ProTaper Universal retreatment files, and reported no differences between the groups, independently of the sealer (AH Plus, MTA Fillapex).

Retreatment procedures

Four studies [23, 27-29] obturated the roots and then removed the initial root filling with a combination of retreatment instruments/Gates-Glidden burs and solvents. Three studies [24-26] investigated the direct effect of the solvents (before filling), without performing the retreatment procedures.

Push-out test parameters

Thickness, diameter and taper: The thickness of the slices varied between 1 mm [23, 25-27, 29], 2 mm [28] and 3 mm [24]. Bayram *et al.* [24] determined the diameter of the canal (1.35 mm) because they used burs for the preparation. In the remaining studies where rotatory NiTi files were used for preparation, only the size and taper of the instruments (F3, F4, F5 ProTaper Universal system), which cannot accurately describe the diameter of the canal, were indicated.

Timing of sectioning: When NiTi files were used [23, 25-29], the root canals were obturated before slicing/sectioning. When burs were used [24], the root filling was performed after sectioning.

Canal segments: Four studies [25-27, 29] evaluated each root third, and the apical third had the lowest bond strength values in all. Shokouhinejad *et al.* [23] and Bayram *et al.* [24] only used the middle third for the measurements of the push-out test. Yavari *et al.* [28] were unclear, reporting the use of

dentin disks at a distance of 2 mm from the root canal surface.

Plunger size, speed and direction: Three studies [26, 27, 29] used three plunger sizes to equal the diameter of each root third. Yavari *et al.* [28] used a plugger of 0.8 mm, selecting only samples with a canal diameter of 0.88 ± 0.22 mm. All except Bayram *et al.* [24], who were unclear, used an apical-coronal plunger loading direction.

Discussion

The aim of the present systematic review was to assess the influence of gutta-percha solvents on the bond strength of endodontic sealers to intraradicular dentin. The role of solvents during endodontic retreatment has been disregarded with the advent of rotary instrumentation. Nevertheless, micro-CT analyses have highlighted the presence of remnants of filling materials, gutta-percha and sealers after retreatment procedures, which might worsen the prognosis of non-surgical endodontic retreatments [27, 30].

As endodontic solvents are mainly organic, the concern arises with the exposure of intraradicular dentin to these compounds, particularly when they are recommended as a supplementary irrigation step, during retreatment [6, 8].

Two main philosophies are evident in the selected papers. In most of the studies, teeth were filled with a core, usually gutta-percha, and a sealer, which is the closest to the clinical method generally used. In two studies [24, 25], teeth were filled only with sealer: silicate sealers in the first study and epoxy and methacrylate sealers in the second. However, there is no consensus on the best procedure for PBT [31]. Most of the studies assessed the bond strength of the sealers after filling the canal, to simulate the clinical situation where solvents are used to remove root filling material. The remaining two studies [24, 25] did not perform initial root filling to try to avoid confounding factors such as remnants of filling material, which could interfere with the bond strength. Different methodologies were identified, and this was particularly relevant regarding the period of contact of the solvent with the dentin. Other differences were also identified, such as the variety of solvents and filling materials and the variability in the set-up of the PBT, including specimen selection, randomization, identification of the statistical unit (root or slice), root thirds sampled, the preparation size/taper or method (slice thickness and time filling) and the standardization of operator protocol. Due to this variability, further statistical analysis could not be performed, and that

constitutes a limitation of the present study. Hence, our general analysis might result in a report of data with an impact on the outcome of retreatment procedures that should be confirmed in future researches.

The authors agree on the adverse influence of chloroform on the bond strength of different sealers such as Resilon/Epiphany SE [23], AH Plus, Sealapex, MTA Fillapex [26], CMTA and Biodentine [24]. Even though both Shokouhinejad *et al.* [23] and Topçuoğlu *et al.* [26] reported an adverse effect of chloroform, their methodologies differed, as the first re-obtured the teeth and the second did not. That effect did not occur with the other solvents, which are potentially less aggressive to the dentin [32], including EndoSolv R [23], eucalyptol or orange oil [26], regardless of the exposure time. Bayram *et al.* [24] used a different methodological approach concerning the time of slicing; they found a reduced bond strength to dentin after 5 min of exposure to chloroform, when using some silicate cements (CMTA and Biodentine), contrary to others (WMTA). Nevertheless, this effect also occurred with other solvents, like EndoSolv E, orange oil and eucalyptol. Those authors claim that the bond strength depends on the sealer's and solvent's chemistry [24]. Other variables concerning calcium silicate sealers, such as the obturation technique-single cone or thermoplastic, must also be addressed when assessing bond strength results [13]. Furthermore, different sealers seem to have different mechanisms of adhesion, either based on self-adhesiveness or a reaction with exposed amino groups in collagen [12].

In another study [25], which included a 5-min exposure to xylene and EndoSolv E before filling the root canal system, the authors reported an unfavorable effect on the bond of the sealers related to the solvent's and sealer's chemistry. The bond strength of AH Plus was significantly higher after exposure to EndoSolv E compared to xylene. The authors of that study argued that solvents may modify the chemical composition of the dentin surface, thus changing its bond strength to sealer. Nevertheless, when comparing the effect of the different solvents on the bond strength of the different sealers (epoxy and methacrylate resin-based), they also emphasized that epoxy-resin sealers (AH Plus) bonded chemically to the collagen of dentin [11] and, thus, EndoSolv E might not produce a significant modification of the dentin collagen fibers, contrary to xylene.

Using a similar methodology, Shokouhinejad *et al.* [23], Palhais *et al.* [27] and Rached-Junior *et al.* [29] assessed the bond strength of the sealer to dentin after re-obturation. In the first two cases [23, 27], the same sealer (Resilon/ Epiphany and

AH Plus) was used for the filling and refilling. In the last [29], the teeth were first filled with a zinc oxide eugenol-based sealer (Endofill) and refilled with AH Plus, not only interfering with the penetration of AH Plus but also stressing the chemical interaction between zinc oxide-eugenol and epoxy-resin sealers. In all these studies [23, 27, 29], the bond strength of the control (not retreated) was higher than the retreated canals, with or without solvents. Palhais *et al.* [27] argued that solvents reduce the bond strength, but this explanation is not coherent with the similar lower bond strength of the group retreated with files alone (without solvent), which was different from the control group. Rached-Junior *et al.* [29], who also found higher bond strength values in the control group (not retreated) than in the experimental groups, regardless of the use of solvents, stressed the interaction between sealers of different chemical formulations as the main factor for the reduced the bond strength of the refilling material to dentin. Furthermore, those authors emphasized that the canal refinement until larger files (F4 and F5) might have a rather decisive effect in the elimination of the sealer tags of the previous filling, regardless of the solvent [23]. This argument may introduce another factor, besides the solvent's effect, for the bond strength of the new filling: the role of the remnants of the previous filling and the way they can be removed.

However, increased sealer penetration into dentinal tubules was not always related to an increase in bond strength [33]. In the set-up by Yavari *et al.* [28], the exposure time of the solvent-chloroform, was not registered. That study only reported that root canals had received three drops of chloroform after the removal of 3 mm of the coronal filling with Gates-Glidden drills, and then ProTaper Universal retreatment files were used to remove the filling and the refinement of the canal preparation was done with F1, F2 and F3 finishing files from ProTaper Universal System. Those authors assumed that the differences from other studies might have resulted from different methodologies, the amount of solvent used and the period of contact to dentin. Assessing different methods, materials and protocols must be done cautiously to avoid bias in the analysis of the results.

Another result generally found was the higher bond strength of AH Plus as compared to other sealers, such as MTA Fillapex [28] and Sealapex [26], detected using the PBT. This finding is corroborated by other studies [14, 34]. Nevertheless, it was reported that, in oval canals, both the instrumentation system and the filling materials might affect the bond strength. Differences in the chemistry of the sealers may also justify the

results [15]. AH Plus is reported to bind with the dentinal collagen [11], for instance, while the hydrophilic nature of a silicate-based sealer such as BC sealer [35] requires other conditions to have close contact with the canal walls [36]. These aspects should influence the selection of the irrigation protocol as follows: when using an epoxy-resin sealer, a final wash with sodium hypochlorite after ethylenediaminetetraacetic acid (EDTA) should be avoided to preserve the collagen fibers; when using Endosequence® BC sealer™ (BC sealer), some moisture should be kept for the root canal filling to benefit the slow-setting [15]. Furthermore, it will also prevent dentin erosion on the main root canal wall [37].

Regarding the limitations of this systematic review, grey literature was not searched, which may have reduced the number of potentially eligible studies. Besides, a restricted number of studies was found, and the methodological variation between them did not allow a meta-analysis to be performed. The risk of bias of the included studies, which was considered globally high, affected the strength of this systematic review.

In-vitro and *ex-vivo* laboratory studies need to be effectively planned and designed to establish guidance standards in Endodontology [38]. Researchers are encouraged to follow guidance standards to prevent wasting time, avoid bias on their results and not affect the ultimate goal of their investigations, which is disseminating reliable information to be further evaluated in animal studies and clinical trials.

Conclusion

Overall, we have highlighted the adverse effect of chloroform and xylene on decreasing bond strength of various sealers, and the generally higher bond strength of epoxy resin-based sealers to intraradicular dentin, detected with push-out bond test. The inconsistent and almost erratic methodologies are an impediment to reaching concrete conclusions. Future studies should follow a standardized protocol to draw clinically valuable results.

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Conflict of Interest: 'None declared'.

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