Research Article

Check for updates

Is dentin biomodification with collagen cross-linking agents effective for improving dentin adhesion? A systematic review and meta-analysis

Julianne Coelho Silva [iD],[1] Edson Luiz Cetira Filho [iD],[1]
Paulo Goberlânio de Barros Silva [iD],[2] Fábio Wildson Gurgel Costa [iD],[1]
Vicente de Paulo Aragão Saboia [iD] [1*]

[1]Department of Restorative Dentistry, Postgraduate Program in Dentistry, Federal University of Ceará, Fortaleza, Ceará, Brazil
[2]Department of Dentistry, Unichristus, Fortaleza, Ceará, Brazil

OPEN ACCESS

*Correspondence to
Vicente de Paulo Aragão Saboia, DDS, MSc, PhD
Full Professor, Department of Restorative Dentistry, Postgraduate Program in Dentistry, Federal University of Ceará, Monsenhor Furtado Street, 1273, Rodolfo Teófilo, Fortaleza, Ceará 60430-355, Brazil.
Email: vpsaboia@yahoo.com

ABSTRACT

Objectives: The aim of this investigation was to evaluate the effectiveness of collagen cross-linking agents (CCLAs) used in combination with the adhesive technique in restorative procedures.
Materials and Methods: In this systematic review, the authors followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses checklist. An electronic search was performed using PubMed, Scopus, Web of Science, Cochrane Library, LILACS, and DOSS, up to October 2020. The gray literature was also researched. Only randomized clinical trials were selected.
Results: The selection process yielded 3 studies from the 838 retrieved. The addition of CCLAs in the retention of restorations increased the number of events. The postoperative sensitivity scores and marginal adaptation scores showed no significant difference between the CCLA and control groups, and the marginal pigmentation scores showed a significant increase in the CCLA group. There were no caries events in any group throughout the evaluation period.
Conclusions: This systematic review showed that there is no clinical efficacy to justify the use of CCLAs in the protocols performed.

Keywords: Adhesive; Collagen; Cross-linking agents; Dentin; Dentin-bonding agents

INTRODUCTION

Despite countless advances in the development of adhesive systems, the degradation of bonding interfaces over time remains a challenge for restorative dentistry [1]. Several factors are related to the degradation of the resin-dentin interface, which mainly occurs through the hydrolysis and leaching of the adhesive monomers that form the hybrid layer. These processes are facilitated by the presence of water and its penetration into hydrophilic or loosely cross-linked sites [1,2]. Besides that, the hydrophilicity of the monomers and the presence of residual solvent and nanoleakage zones originating from naked collagen may

FWG; Investigation: Silva JC, Cetira Filho EL;
Methodology: Saboia VPA, Silva JC, Cetira
Filho EL, Costa FWG; Project administration:
Saboia VPA; Supervision: Saboia VPA;
Validation Saboia VPA, Silva PGB; Writing
- original draft: Silva JC; Writing - review &
editing: Saboia VPA, Costa FWG.

ORCID iDs
Julianne Coelho Silva
https://orcid.org/0000-0002-7004-4848
Edson Luiz Cetira Filho
https://orcid.org/0000-0002-5985-8955
Paulo Goberlânio de Barros Silva
https://orcid.org/0000-0002-1513-9027
Fábio Wildson Gurgel Costa
https://orcid.org/0000-0002-3262-3347
Vicente de Paulo Aragão Saboia
https://orcid.org/0000-0002-1143-6390

contribute to the degradation of the resin-dentin interface [1,3]. In addition, regions of unprotected collagen may undergo a degradation process caused by matrix metalloproteinase enzymes and cysteine cathepsins, which can reduce the longevity of restorations [4].

Dentin biomodification through collagen cross-linking agents (CCLAs) is a strategy to improve the biomechanical and biochemical properties that can reduce the biodegradation of this tissue and preserve adhesion stability over time [5,6]. Multiple interactions between these agents and various extracellular components of the dentin matrix are the basis for tissue biostability and enhanced biomechanical properties of the dentin matrix. Their use has shown promising initial results in laboratory studies [7-11].

CCLAs can be applied to dentin as a pretreatment, incorporated into the adhesive system, or added to the phosphoric acid etchant [8-11]. Various CCLAs have been tested, such as proanthocyanidins (PACs), epigallocatechin-3-gallate (EGCG), riboflavin and glutaraldehyde. Fawzy *et al.* [9] evaluated the effect of riboflavin photoactivated by ultraviolet A or visible blue light as a dentin pretreatment, and showed its ability to improve the mechanical properties of dentin collagen. This agent was able to increase and preserve the bond strength and interface integrity after short-term water storage. Daood *et al.* [10] assessed the effectiveness of adhesives incorporated with riboflavin and showed that they were able to increase and maintain the bond strength and interface integrity after storage in artificial saliva. Moreover, there were no adverse effects on the degree of conversion of the adhesive monomers. Subsequently, the effects of CCLAs were evaluated in clinical trials, but there is still a need for a well-established protocol regarding the application and clinical efficacy of these agents under clinical conditions [12-14].

Hitherto, no systematic reviews have been published on the use of CCLAs as a strategy to improve the performance of adhesive interfaces, which could provide substantial evidence on the use of these agents in restorative dentistry.

In this context, the objective of this study was to answer the following research question through a systematic review of the literature and meta-analysis of randomized clinical trials (RCTs): are CCLAs capable of improving dentin adhesion?

MATERIALS AND METHODS

Study protocol and search information strategy
This systematic review was based on the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist [15]. Therefore, its research question was organized according to the PICOS strategy:

1. Population (P): subjects undergoing restorative treatment with composite resin;
2. Intervention (I): application of CCLAs either separately or incorporated into the adhesive;
3. Comparison (C): no use of CCLAs;
4. Outcome (O): performance of a restorative procedure evaluated by postoperative sensitivity, the retention rate, marginal adaptation, marginal staining, or recurrence of caries;
5. Study design (S): RCTs.

The information searched was individualized for each adopted database with a combination of specific terms. **Supplementary Table 1** shows additional information regarding the search

strategies in this review paper. EndNote X8 (Thompson Reuters, New York, NY, USA) was chosen to manage all references and exclude duplicate articles.

Inclusion and exclusion criteria

Potential studies were required to have included an experimental group (*i.e.*, any composite resin restorative procedure that used any CCLA associated with the adhesive strategy) and a comparative group (*i.e.*, composite resin restorative procedure without adhesive technique-related biomimetic agents).

The following exclusion criteria were considered during the selection process: (1) non-RCT study designs (case reports, meeting abstracts, letters to the editor, book chapters, authors' personal opinions, clinical observations, and literature reviews); (2) studies that concomitantly used a technique that may interfere with adhesion, in addition to CCLAs; (3) studies with a control group that used an adhesive system differing from that of the experimental group; and (4) studies not written in languages using a Latin (Roman) alphabet (**Table 1**).

Database sources

Individual search strategies were performed in PubMed, Web of Science, Scopus, LILACS, Cochrane, and DOSS. The gray literature was assessed through Google Scholar and OpenGrey. The search included all articles published until October 7, 2020, without time limitation.

Study selection and data extraction

Initially, 2 investigators (JCS and ELCF) independently evaluated the titles and abstracts of all studies obtained with the search strategy. They used a web application for systematic reviews (Rayyan, Qatar Computing Research Institute, Doha, Qatar) [16]. Papers that did not appear to meet the inclusion criteria were excluded. After that, the full texts of selected articles were independently analyzed to check their eligibility. A third examiner (VPAS) critically assessed the reference lists of the selected studies. Any disagreement during the study selection process was resolved when the 2 authors reached an agreement. However, in case of disagreement, the other authors (VPAS and FWGC) participated in the final decision.

One investigator (JCS) collected data from the included studies, and a second investigator (ELCF) cross-checked all the obtained information. Any disagreements between the 2 authors were discussed until their complete resolution. The third investigator (VPAS) made the final decision when the 2 authors failed to reach an agreement. Thereafter the statistical analysis was performed by another author (PGBS).

Table 1. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
· Randomized clinical trials. · Potential studies should have included an experimental group (*i.e.*, any restorative procedure with a composite resin that could be using any CCLAs associated with the adhesive strategy) and a comparative group (*i.e.*, composite resin restorative procedure without adhesive technique-related biomimetic agents).	· Non-adopted study designs (case reports, meeting abstracts, letters to the editor, book chapters, authors' personal opinions, clinical observations, and literature reviews). · Studies that also performed a technique that may interfere with adhesion, in addition to the use of CCLAs. · Studies with a control group that used an adhesive system differing from the experimental group. · Studies not written in languages using a Latin (Roman) alphabet.

CCLA, collagen cross-linking agent.

Data items

The following aspects were evaluated for further data synthesis: (1) publication year; (2) methodological design; (3) participants (sample size, sex, and age); (4) type of cavity; (5) cavity characteristics; (6) restorative material-associated variables (CCLAs, adhesive system, composite resin, restorative technique, and curing light); and (7) outcomes of interest for the present systematic review.

Risk of bias (RoB) in individual studies

The RoB was assessed independently by 2 authors (JCS and ELCF), guided by the Cochrane checklist for RCTs [17]. Any disagreement between them over the RoB items was resolved through discussion with a third reviewer (VPAS). The following questions were used: (1) random sequence generation (selection bias); (2) allocation concealment (selection bias); (3) blinding participants and personnel (performance bias); (4) blinding outcome assessment (detection bias); (5) incomplete outcome data (attrition bias); (6) selective reporting (reporting bias); (7) other sources of bias (other bias).

The RoB was classified as high (when the study had a "yes" score of less than 49%), moderate (50%–69%), or low (70% or more), according to Haas *et al.* [18]. The RevMan software (Review Manager, version 5.3, Cochrane Collaboration, Copenhagen, Denmark) was used to generate the RoB figures.

Meta-analysis

After extraction, the data were imported into the RevMan software to calculate the combined relative risks of each quality parameter of restorations (Fédération Dentaire Internationale [FDI] scores) in the evaluated study periods. The outcome used was the incidence of restorations with events described by mean values from the analysis of fixed effects by the inverse variance method. The I^2 coefficient assessed heterogeneity, and leave-one-out analysis (removal of studies one by one from each period) was used to assess the influence of the weight of each data on the final outcome. All analyses were conducted using a 95% confidence threshold.

Quality of the evidence

The Grading of Recommendations, Assessment, Development and Evaluation (GRADE) profiler was used to summarize the evidence quality using the GRADE pro-GDT software (http://gdt.guidelinedevelopment.org). Depending on the study design, RoB, consistency, directness, heterogeneity, precision, publication bias, and additional relevant aspects, the quality of the evidence could be downgraded by 1 or 2 levels for each aspect [19].

RESULTS

Study selection

Initially, 893 articles were identified from the electronic databases. Fifty-five duplicated studies were excluded. Then, the titles and abstracts of 838 articles were screened, and 10 potentially relevant studies were selected for full-text reading. No studies were selected from the gray literature (Google Scholar and OpenGrey). Eleven articles were read for eligibility assessment according to the inclusion criteria, from which 8 were excluded due to the main exclusion criteria. Additional records identified through other sources were included ($n = 1$). Finally, 3 studies satisfied the inclusion criteria and were selected for this systematic review (**Figure 1**).
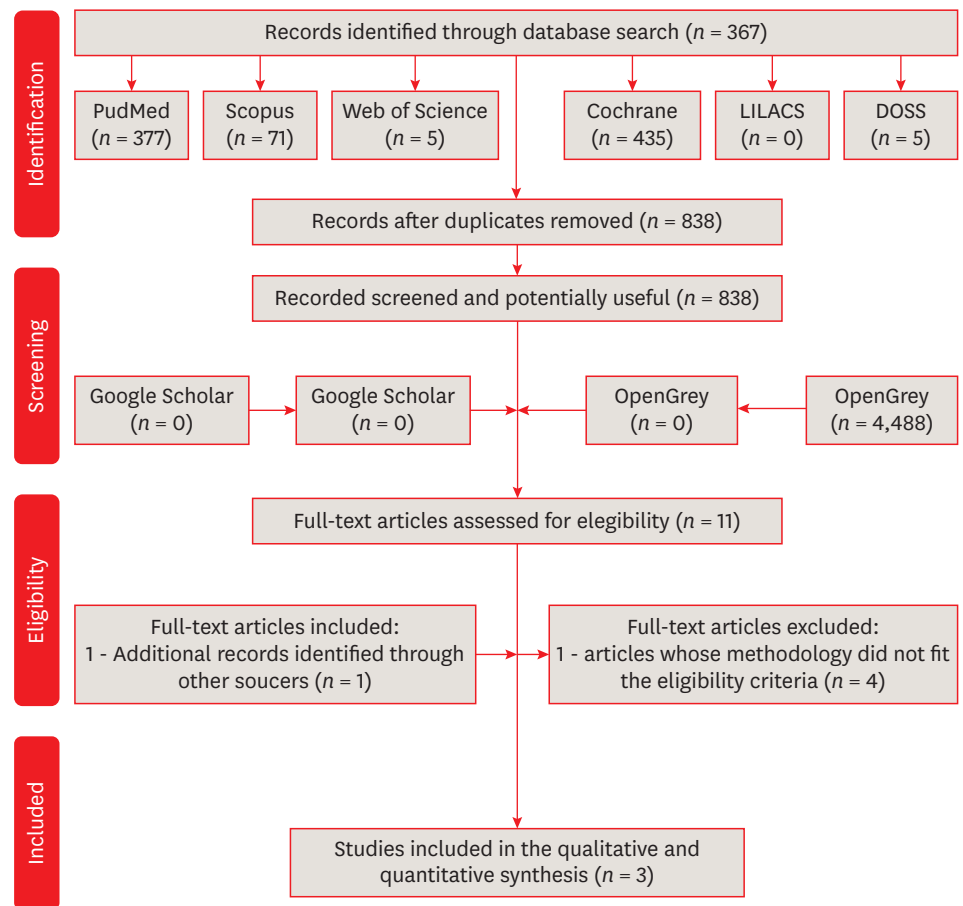
Figure 1. Flow diagram of the study identification, screening, and inclusion process. Adapted from Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA). References for the 9 excluded articles are listed in **Supplementary References**.

Study characteristics

The 3 included studies were from Brazil. They were published in the period from 2019 through 2020 (**Table 2**) and amounted to 123 participants. All restorative procedures were performed on noncarious cervical lesions (NCCLs), and the CCLAs were obtained from a natural source.

RoB within individual studies

In general, the included articles showed low RoB, with 85% of items classified as low RoB and 15% showing high RoB (**Supplementary Figure 1**). Regarding the individual questions of the checklist, a high RoB was observed for the blindness of the performer of the procedure, and a low RoB was attributed to the other items (**Figure 2**).

Results of individual studies

Methodologically, the analyzed studies were randomized and reported a double-blinded design. They performed restorative procedures on NCCLs. The composite resin insertion technique performed in all studies was the incremental type.

In 2 of these studies, CCLA application was performed as a pretreatment [13,14]. In the other study, the application was performed through the incorporation of the cross-linking agent within the adhesive system. The CCLAs used were PACs and EGCG [12-14].

Table 2. Main data of selected articles

Reference	Follow-up	No. of subjects	Placement technique	Type of adhesive + adhesive brand + composite resin brand	No. of restorations per group	CCLAs	Method of application	Criteria		Results
de Souza et al., 2019 [12]	Baseline, 6 and 24 mon	45	Incremental	ER - ExciTE F (Ivoclar Vivadent AG, Schaan, Liechtenstein) IPS Empress Direct (Ivoclar Vivadent AG)	45	PACs (Vitis vinifera, Meganatural Gold, Madera, CA, USA)	Incorporated	RT		The CT showed a significantly higher RT rate after 24 mon than the groups with PAC
								PS		After 24 mon no restorations showed PS
								MA		Significantly worse marginal adaptation was observed within all groups after 24 mon, without a significant difference detected between any pairs of groups
								RC		No restorations showed recurrent caries lesions after 24 mon
de Souza et al., 2020 [14]	Baseline, 6 and 24 mon	45	Incremental	ER - ExciTE F IPS Empress Direct	45	PACs (Vitis vinifera, Meganatural Gold, Madera)	Pretreatment	RT		After 24 mon, the group with the highest concentration of PAC showed a significant difference when compared with their respective baseline findings and when compared to the other groups
								PS		After 24 mon, no restorations showed PS
								MS		A significant difference between baseline vs. 24 mon was observed for all groups, although no significant differences were observed among groups after 24 mon
								MA		Significantly worse MA was observed within all groups after 24 mon, although none of the restorations was considered to have clinically relevant discrepancies
								RC		No restorations showed recurrent caries lesions after 24 mon
Costa et al., 2020 [13]	Baseline, 6,12, 18, and 24 mon	33	Incremental	ER or SE - Single Bond Universal 3M ESPE, 3M ESPE, St. Paul, MN, USA) Filtek Z350 XT 3M ESPE (3M ESPE)	39	0.1% EGCG (Sigma-Aldrich, St. Louis, MO, USA) for 60 sec	Pretreatment	RT		The results of RT showed no statistically significant difference between any pairs of groups after 24 mon, and for each group when the baseline and 24 mon results were compared
								PS		After 24 mon no restorations showed PS
								MS		No significant difference was found between groups at 24 mon and within each group when the baseline and 24 mon findings were compared
								MA		No significant difference was detected between any pair of groups after 24 mon, but a significant difference was detected when the baseline and 24 mon data were compared within the SE control group
								RC		No restorations showed recurrent caries lesion after 24 mon

CCLA, collagen cross-linking agent; RT, retention; PS, postoperative sensitivity; MS, marginal staining; MA, marginal adaptation; RC, recurrence of caries; CT, control group; PAC, proanthocyanidins; EGCG, epigallocatechin-3-gallate; ER, etch-and-rinse; SE, self-etch.

All studies had control groups with the same adhesive system without the use of a CCLA. Two investigations used ExcitTE F (Ivoclar Vivadent AG, Schaan, Liechtenstein), and the other one used Single Bond Universal 3M ESPE (3M ESPE, St. Paul, MN, USA) [12-14]. Two studies used IPS Empress Direct (Ivoclar Vivadent) composite resin, and the other study used Filtek Z350 XT 3M ESPE (3M ESPE) [12-14].

The curing light devices were the Emitter A FIT (Schuster Equipamentos Odontológicos, Santa Maria, RS, Brazil) and the DB-685 (1,100 mW/ cm², Dabi Atlante, Ribeirão Preto, Brazil) [12-14].

A total of 426 restorations were performed, distributed into experimental and control groups: 39 restorations per group in Costa et al. [13] and 45 restorations per group in both de Souza et al.

Figure 2. Risk of bias (RoB) summary: review of the authors' assessment of each RoB item, presented as percentages across all included studies in the meta-analysis. Symbol colors: green, low RoB; yellow, unclear RoB; red, high RoB.

[12] and de Souza *et al.* [14]. In all studies, 2 blinded examiners performed clinical evaluations independently. The evaluators were previously calibrated and used the FDI criteria to evaluate retention, the presence of fractures, marginal discoloration, marginal adaptation, caries, and postoperative sensitivity [12-14]. In the study performed by Costa *et al.* [13] these variables were classified as follows: clinically very good or good (scores 1 + 2), clinically sufficient/satisfactory (score 3), clinically unsatisfactory (score 4), and clinically poor (score 5) at baseline and after 6, 12, 18, and 24 months. In the studies carried out by de Souza *et al.* [12] and de Souza *et al.* [14], these variables were classified as follows: clinically very good (score 1), clinically good (score 2), clinically sufficient/satisfactory (score 3), clinically unsatisfactory (score 4), and clinically poor (score 5) at baseline and after 6 and 24 months.

In the studies performed by de Souza *et al.* [12] and de Souza *et al.* [14], the restorations were evaluated with the classical United States Public Health Service criteria (adapted by Dalton Bittencourt *et al.* [20] and Perdigão *et al.* [21]), in the same periods. **Table 2** shows the main results of the selected articles.

Meta-analysis results

1. Retention
The scores for the retention of restorations showed there was a significant increase in the number of events in the CCLA group, with a relative risk of 2.06 (95% confidence interval [CI], 1.29–3.27) times greater in that group. There was no significant heterogeneity ($p = 0.480$, $I^2 = 0\%$) or differences between the subgroups ($p = 0.630$). In the first week of evaluation, there was no loss of retention ($p = 1.000$). After 6 months, the relative risk for loss of retention was 3.38 times higher (95% CI, 1.14–10.02) in the CCLA group ($p = 0.030$). After 12 and 18 months, only Costa *et al.* [13] had evaluations, which showed no increased risk, whereas after
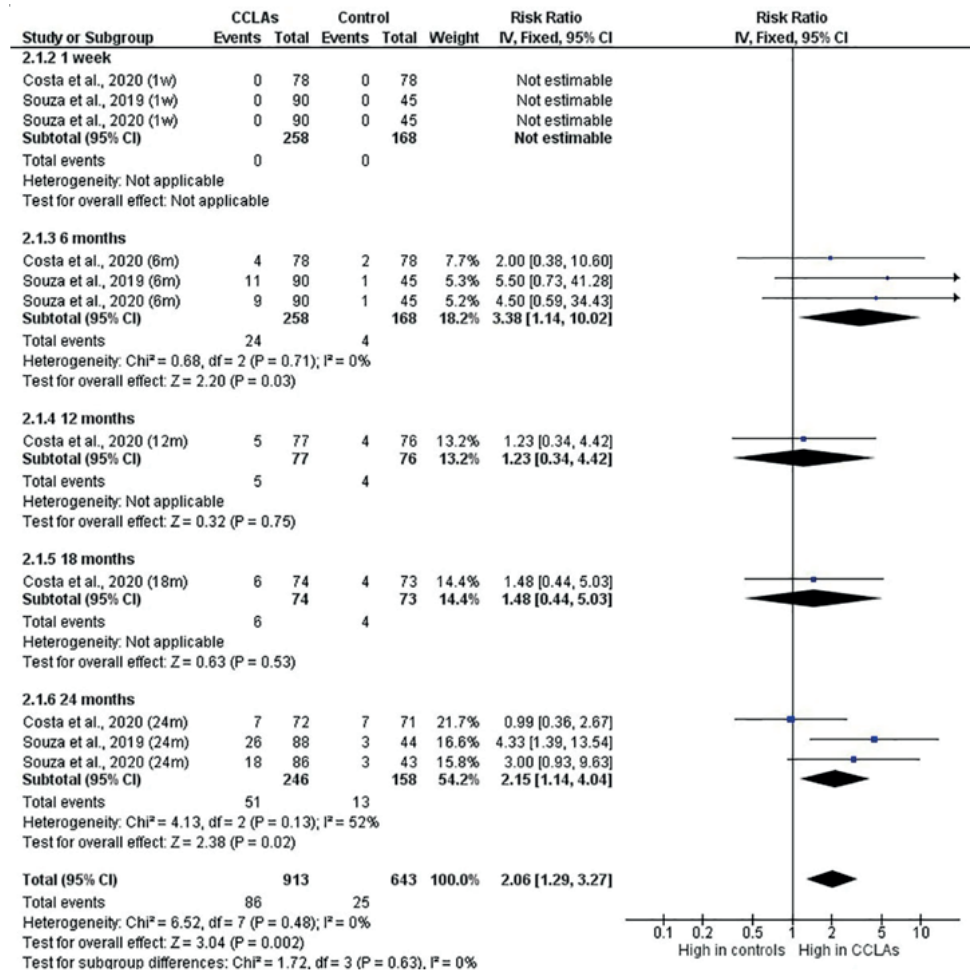
Figure 3. Forest plot of the retention rates.
CCLA, collagen cross-linking agent; CI, confidence interval.

24 months, there was a 2.15-fold increase (95% CI, 1.14–4.04) in the risk of loss of retention in the CCLA group (p = 0.020). In the leave-one-out analysis, the removal of de Souza *et al.* [12] (p = 0.120) and de Souza *et al.* [14] (p = 0.090) in the 6-month period diluted the increased risk of restoration loss. The removal of these 2 works in the 24-month period also diluted the increased risk of loss of restorations (p = 0.240 and p = 0.100), respectively (**Figure 3**).

2. Postoperative sensitivity
No significant difference was found in postoperative sensitivity between the CCLA and control groups (p = 0.300). There was no significant influence of the evaluated period (p = 0.370) or significant heterogeneity between the studies (p = 0.370, I^2 = 4%). There were no significant differences between groups in any of the evaluated periods. The leave-one-out analysis showed no significant favoring of any individual study (**Figure 4**).

3. Marginal staining
Pigmentation scores showed a significant increase in the CCLA group, with a relative risk of 1.75 (95% CI, 1.10–2.78) (p = 0.020). There were no significant differences between subgroups (p = 0.670) or significant heterogeneity between the studies (p = 0.670, I^2 = 0%). There were
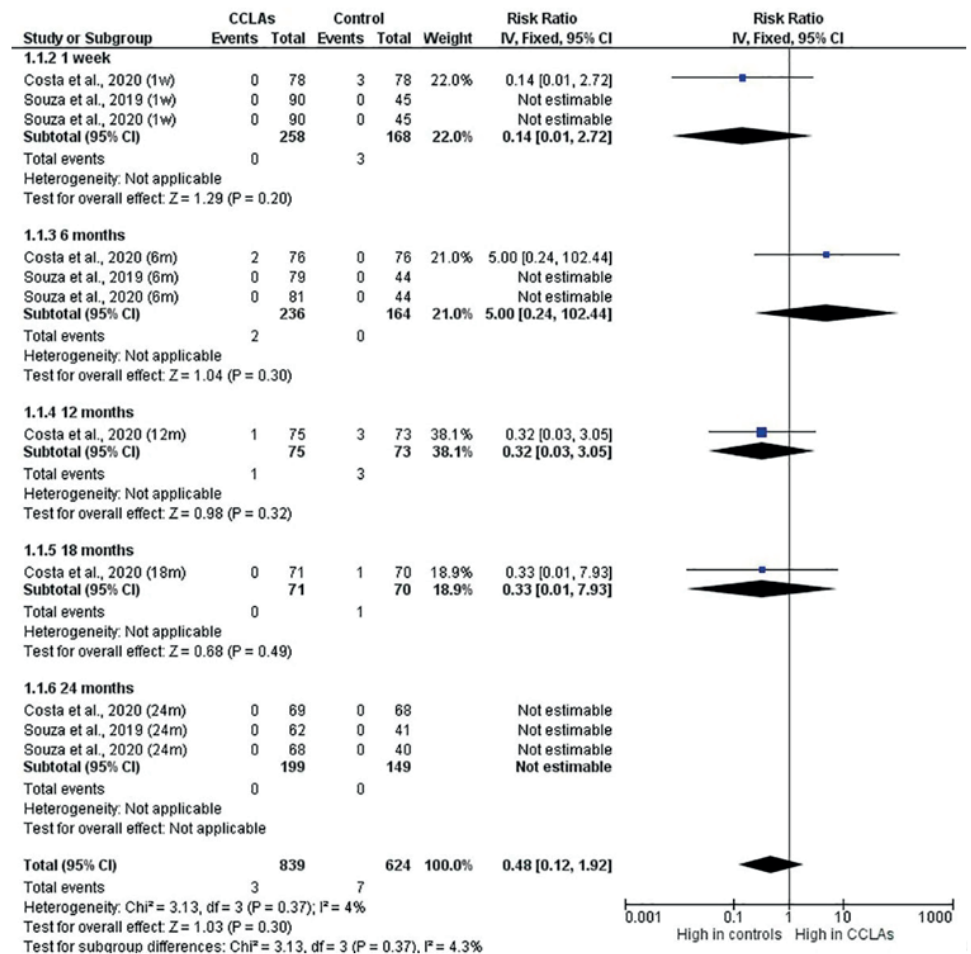
Figure 4. Forest plot of the postoperative sensitivity rates.
CCLA, collagen cross-linking agent; CI, confidence interval.

no events in the first week ($p = 1.000$), nor a significant increase in the risk of pigmentation after 6 ($p = 0.650$), 12 ($p = 0.580$), or 18 ($p = 0.140$) months. After 24 months, the CCLA group showed a 1.79 times increase (95% CI, 1.05–3.05) in the risk of pigmentation ($p = 0.030$). The leave-one-out analysis showed no significant effects at the 6-month period, but significant effects were found at 24 months for the removal of Costa *et al.* [13] ($p = 0.080$) and de Souza *et al.* [12] ($p = 0.330$), but not de Souza *et al.* [14] ($p = 0.010$) (**Figure 5**).

4. Marginal adaptation
The adaptation failure scores showed no significant difference between the CCLA and control groups ($p = 0.860$). There were no differences between subgroups ($p = 0.750$), nor significant heterogeneity ($p = 0.850$, $I^2 = 0\%$). There were no events in the first week ($p = 1.000$), nor a significant increase in the risk of failure on marginal adaptation after 6 ($p = 0.880$), 12 ($p = 0.350$), 18 ($p = 0.660$), or 24 ($p = 0.700$) months. The leave-one-out analysis showed no significant favoring of any individual study (**Figure 6**).

5. Incidence of caries
There were no caries events in any group during the 24-month evaluation period (**Appendix 1**).
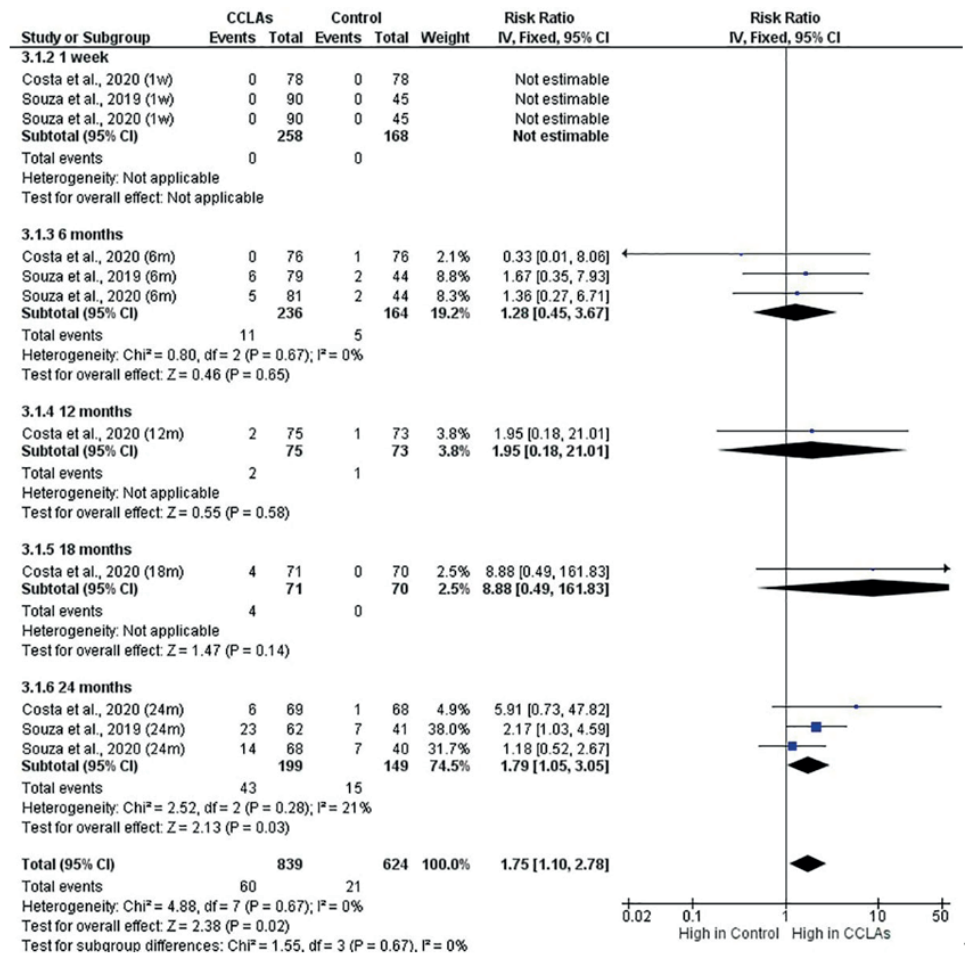
Figure 5. Forest plot of the marginal staining rates.
CCLA, collagen cross-linking agent; CI, confidence interval.

6. Additional analysis and confidence in cumulative evidence

According to the GRADE statements, which were based on certain aspects (*e.g.*, RoB, inconsistency, and imprecision), certainty was estimated as high for all outcomes (retention of restorations, marginal staining, postoperative sensitivity, and marginal adaptation). Further explanations of evidence appraisal are available in **Table 3**.

DISCUSSION

The achievement of a stable and lasting dentin-resin bonding interface is one of the main challenges of adhesive dentistry, and it has been the subject of several studies. One of the strategies to improve the quality of this interface is the use of CCLAs. Among these agents, glutaraldehyde, which is of synthetic origin, and EGCG and PACs, which are of natural origin, stand out [8,22-24].

The present systematic review compared the efficiency of procedures performed with the use of CCLAs, combined or not with the adhesive technique. Thus, the main objective of this investigation was to assess the effectiveness of CCLAs in combination with the adhesive
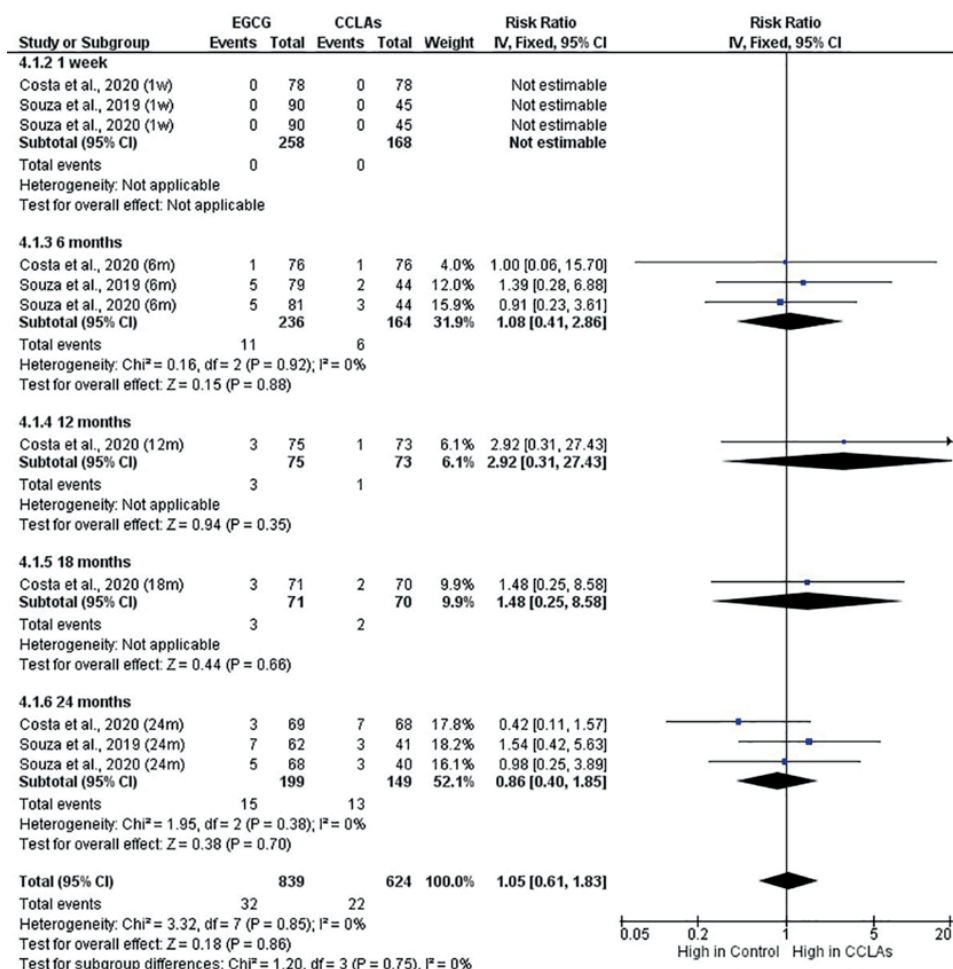
Figure 6. Forest plot of the marginal adaptation rates.
EGCG, epigallocatechin-3-gallate; CCLA, collagen cross-linking agent; CI, confidence interval.

technique in the performance of the restorative procedure. The articles included in this study have in common the use of CCLAs of natural origin. The studies performed by de Souza *et al.* [12] and de Souza *et al.* [14] used PACs from grape-seed extract as CCLAs, whereas the study conducted by Costa *et al.* [13] used EGCG from green tea. These substances contain polyphenols, which can interact with the collagen in dentin by forming multiple hydrogen bonds [25]. The meta-analysis showed no significant clinical benefit for the evaluated outcomes in the restorative procedures performed with the use of CCLAs when compared to those performed without the use of CCLAs.

The results obtained in the present meta-analysis showed that the addition of CCLAs had no significant clinical benefit on the retention of restorations. All studies included in this review reported that no additional mechanical preparation was performed, so the retention was based only on the adhesive system performance. It is worth mentioning that the presence of micromechanical retentions could positively interfere with this outcome. It is important to highlight that, 6 and 24 months after the use of CCLAs, there was a significant decrease in the quality of the retention of the restorations in the CCLA group. However, after 1 week, and at the 12- and 18-month time points evaluated only by Costa *et al.* [13], CCLA use showed

Table 3. Grading of Recommendations, Assessment, Development and Evaluation (GRADE) evidence profile

			Certainty assessment				No. of patients		Effect		Certainty	Importance
No. of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	CCLAs	Control	Relative (95% CI)	Absolute (95% CI)		
Retention of restorations												
3	Randomized trial	Not serious	Not serious	Not serious	Not serious	None	86/913 (9.4%)	25/643 (3.9%)	RR 2.06 (1.29–3.27)	41 more per 1,000 (from 11 more to 88 more)	⊕⊕⊕⊕ HIGH	CRITICAL
Postoperative sensitivity												
3	Randomized trial	Not serious	Not serious	Not serious	Not serious	None	3/839 (0.4%)	7/624 (1.1%)	RR 0.48 (0.12–1.92)	6 fewer per 1.000 (from 10 fewer to 10 more)	⊕⊕⊕⊕ HIGH	CRITICAL
Marginal staining												
3	Randomized trial	Not serious	Not serious	Not serious	Not serious	None	60/839 (7.2%)	21/624 (3.4%)	RR 1.75 (1.10–2.78)	25 more per 1.000 (from 3 more to 60 more)	⊕⊕⊕⊕ HIGH	CRITICAL
Marginal adaptation												
3	Randomized trial	Not serious	Not serious	Not serious	Not serious	None	32/839 (3.8%)	22/624 (3.5%)	RR 1.05 (0.61–1.83)	2 more per 1.000 (from 14 fewer to 29 more)	⊕⊕⊕⊕ HIGH	CRITICAL

GRADE Working Group grades of evidence - High certainty: We are very confident that the true effect lies close to that of the estimate of the effect. Moderate certainty: We are moderately confident in the effect estimate. The true effect is likely to be close to the estimate of the effect, but there is a possibility that it is substantially different. Low certainty: Our confidence in the effect estimate is limited. The true effect may be substantially different from the estimate of the effect. Very low certainty: We have very little confidence in the effect estimate. The true effect is likely to be substantially different from the estimate of the effect.
CCLA, collagen cross-linking agent; CI, confidence interval; RR, risk ratio.

no influence on retention. These findings raise the hypothesis that the studies conducted by de Souza et al. [12] and de Souza et al. [14] showed a major decrease in the retention of restorations in the CCLA group. However, the study of Costa et al. [13] supports the hypothesis of the clinical benefit caused by the addition of CCLAs to the adhesive technique. This investigation, unlike the others included in this meta-analysis, used EGCG, which is the major polyphenol present in green tea, as the CCLA; the effects of EGCG on reducing biodegradation and stabilizing collagen fibrils have already been demonstrated in laboratory studies [26,27]. Furthermore, Du et al. [28] showed that, depending on the concentration, EGCG increased the immediate bond strength compared to the control group. In addition, Costa et al. [13] used the Single Bond Universal adhesive system (3M ESPE), which contains a functional monomer with a phosphate group, known as 10-methacryloyloxydecyl dihydrogen phosphate, that is able to bind to hydroxyapatite, providing a chemical adhesion mechanism [29]. A polyalkenoic acid copolymer is also present in the composition of this adhesive system, which has functional carboxylic groups capable of chemically interacting with hydroxyapatite [30,31]. High retention rates with the use of this adhesive system have been previously demonstrated by other clinical trials [31,32]. Therefore, the presence of these components may have improved the retention rate.

Marginal staining demonstrated that using CCLAs in combination with the adhesive technique had no significant clinical benefits within the evaluation periods used in the studies. After 1 week and 6, 12 and 18 months, the latter 2 time points evaluated only by Costa et al. [13], the use of these agents had no influence on marginal staining. After 24 months, the use of CCLAs increased the risk of pigmentation. The leave-one-out analysis showed that the

removal of all studies within 6 months and the study of Costa *et al.* [13] and de Souza *et al.* [12] within 24 months did not change the outcomes. Conversely, the removal of the study of de Souza *et al.* [14] (within 24 months) demonstrated had a significant impact. The literature has described that dentin specimens treated with PAC agents presented a brownish color [33]. This pigmentation may be attributed to the oxidative properties of these compounds, the dark color of solutions, and the content of high-molecular-weight polymeric polyphenols [33-36]. The application mode seems to be associated with staining, once only the application as pretreatment resulted in increased pigmentation, as demonstrated by de Souza *et al.* [14].

The studies conducted by Costa *et al.* [13] and de Souza *et al.* [12] used CCLAs through the pretreatment approach. This technique has been the target of several laboratory studies [8,24,27]. The time of application of CCLAs as a pretreatment is an important topic to be evaluated. Costa *et al.* [13] and de Souza *et al.* [12] carried out the application for 60 seconds, as well as Neri *et al.* [37] and Zheng and Chen [38], which showed *in vitro* that the application of EGCG and PAC, respectively, for that time was effective in preserving the resin-dentin bond durability. Although this application period is clinically feasible, it involves adding a step to the adhesive technique, thereby increasing the clinical time of the procedure, which goes against the trend of simplifying materials and restorative techniques [12,13].

In order to simplify the use of CCLAs with the adhesive technique, several studies have investigated their incorporation into adhesive systems [28,29,39-42]. Daood *et al.* [10] showed that the incorporation of 3% riboflavin in an adhesive improved the immediate bond strength and maintained the durability of the resin-dentin bond, without adversely affecting its degree of conversion. Another study showed that the degree of conversion of a commercial adhesive was not affected by the addition of 200 mg/mL EGCG [28]. In contrast, Epasinghe *et al.* [41] observed that up to 1% of grape seed extract can be incorporated into a dental adhesive resin without interfering with the mechanical properties or solubility of the resins. These adverse effects are associated with the antioxidant potential of CCLAs, which have free radicals that negatively interfere with the polymerization reaction of adhesives [39,42]. During the polymerization process, PACs donate hydrogen radicals present in the phenolic groups, making it difficult to initiate and propagate the polymerization chain [39]. For EGCG, it was assumed that the interference in the polymerization reaction was due to its effect of scavenging free radicals [28].

There were no caries events in any group within 24 months of evaluation, and this result may be related to the multifactorial etiology of caries, the development of which requires the presence of specific microorganisms and frequent consumption of fermentable carbohydrates that facilitate their activity [43]. Furthermore, the studies used in this systematic review reported that, to be included in RCTs, all patients were required to have an acceptable or satisfactory standard of oral hygiene, which eliminates the RoB for this outcome.

When evaluating the GRADE, good levels of certainty were observed in the scientific evidence, in which the certainty was classified as high for all outcomes (**Table 3**). This certainty of scientific evidence is related to the quality of the selected articles, the study methodology (RCTs), and the number of restorative procedures performed. This result reflects a low RoB in the selected studies, which presented only a single criterion with a high RoB (blinding of participants and personnel). Due to the methodological design of the primary studies, the operator could not be blinded to the interventions performed, since the technique included the addition of an extra step in the application of the adhesive system or an adhesive system with changes in the commercial composition.

There are some other RCTs using other CCLAs; for instance, glutaraldehyde, a chemical compound of synthetic origin, has already demonstrated a potential biomodifying effect during *in vitro* studies, and it is one of the components of Gluma, a dental adhesive system available on the market [44]. Some RCTs have evaluated the performance of this adhesive system in restorative procedures; however, these works compared Gluma to other commercial adhesive systems with different formulations [45-49]. Therefore, it would be impracticable to attribute effects to the use of this CCLA, without considering effects arising from the different compositions of the evaluated products. As previously mentioned, all articles included in this review compared the use or non-use of CCLA with the same adhesive system [13,14].

The main methodological limitation of this systematic review is related to the small number of studies available in the literature and the short follow-up time, as well as the origin of the same country. Systematic reviews with meta-analysis are studies of great relevance to clinical practice, as they are able to summarize the best evidence with regard to clinical conduct and interventions [17]. Although few studies have been included in the present work, it followed all the steps of PRISMA and, according to GRADE, all papers presented a high certainty of the scientific evidence. Furthermore, this investigation shows a gap in the literature that can help guide new research in order to obtain more solid scientific evidence on the subject.

CONCLUSIONS

This systematic review showed that there is no clinical efficacy to justify the use of CCLAs in the protocols performed. However, this investigation shows a gap in the literature that can help guide new research in order to obtain more solid scientific evidence on the subject.

SUPPLEMENTARY MATERIALS

Supplementary Table 1
Search terms specific for each database and truncations

Click here to view

Supplementary Figure 1
Risk of bias (RoB) graph: review of the authors' assessment of each RoB item, presented as percentages across all included studies in the meta-analysis. Symbol colors: green, low RoB; yellow, unclear RoB; red, high RoB.

Click here to view

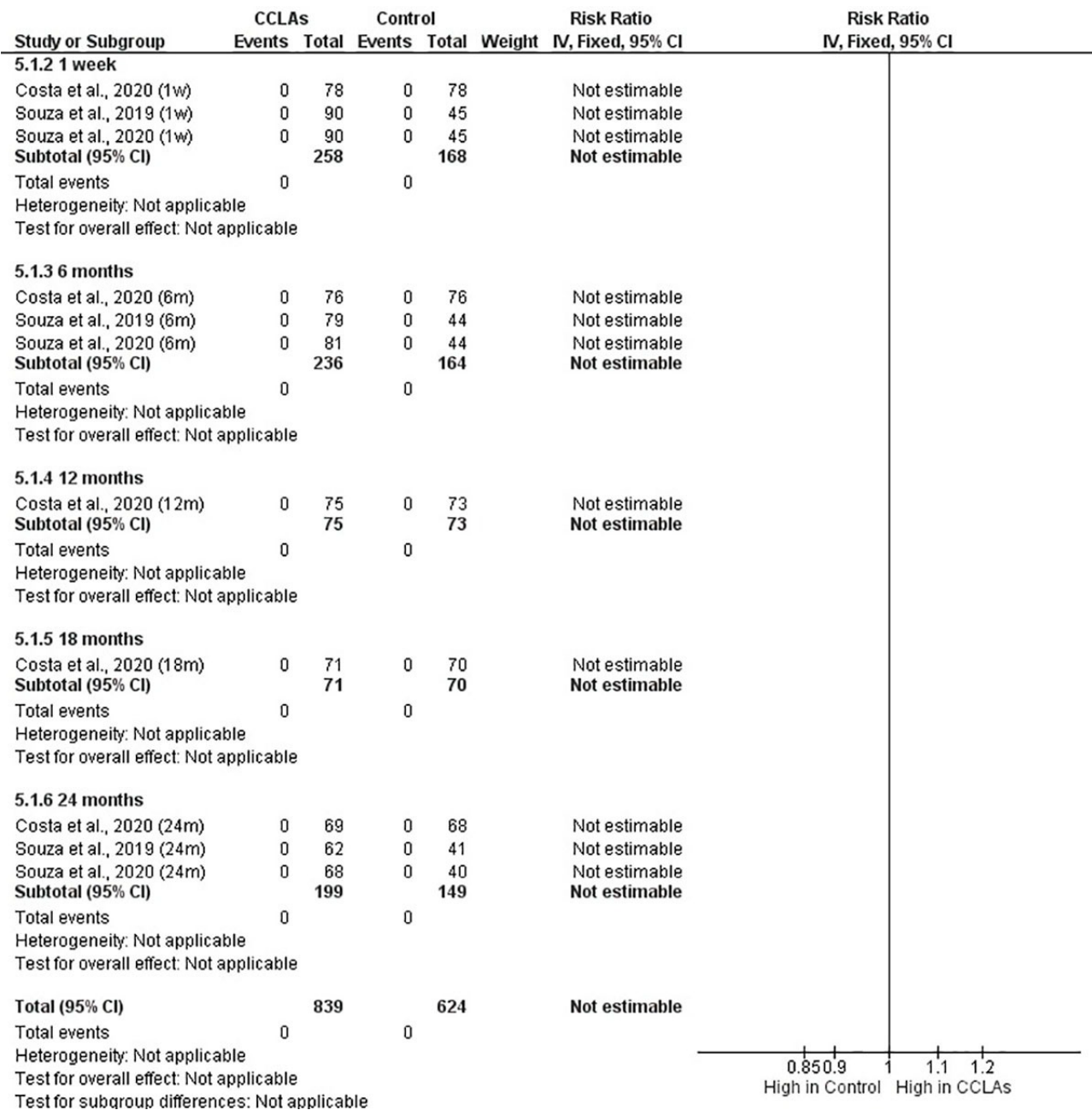### Supplementary References
References of the 4 excluded articles

Click here to view

REFERENCES

1. Frassetto A, Breschi L, Turco G, Marchesi G, Di Lenarda R, Tay FR, Pashley DH, Cadenaro M. Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability--A literature review. Dent Mater 2016;32:e41-e53.
   PUBMED | CROSSREF

2. Spencer P, Wang Y. Adhesive phase separation at the dentin interface under wet bonding conditions. J Biomed Mater Res 2002;62:447-456.
   PUBMED | CROSSREF

3. Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. J Dent Res 2002;81:472-476.
   PUBMED | CROSSREF

4. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, Tezvergil-Mutluay A, Carrilho MR, Carvalho RM, Tay FR, Pashley DH. Optimizing dentin bond durability: control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater 2013;29:116-135.
   PUBMED | CROSSREF

5. Bedran-Russo AK, Pauli GF, Chen SN, McAlpine J, Castellan CS, Phansalkar RS, Aguiar TR, Vidal CM, Napotilano JG, Nam JW, Leme AA. Dentin biomodification: strategies, renewable resources and clinical applications. Dent Mater 2014;30:62-76.
   PUBMED | CROSSREF

6. Van Meerbeek B, Yoshihara K, Van Landuyt K, Yoshida Y, Peumans M. From Buonocore's pioneering acid-etch technique to self-adhering restoratives. a status perspective of rapidly advancing dental adhesive technology. J Adhes Dent 2020;22:7-34.
   PUBMED | CROSSREF

7. Singh P, Nagpal R, Singh UP, Manuja N. Effect of carbodiimide on the structural stability of resin/dentin interface. J Conserv Dent 2016;19:501-509.
   PUBMED | CROSSREF

8. Al-Ammar A, Drummond JL, Bedran-Russo AK. The use of collagen cross-linking agents to enhance dentin bond strength. J Biomed Mater Res B Appl Biomater 2009;91:419-424.
   PUBMED | CROSSREF

9. Fawzy AS, Nitisusanta LI, Iqbal K, Daood U, Neo J. Riboflavin as a dentin crosslinking agent: ultraviolet A versus blue light. Dent Mater 2012;28:1284-1291.
   PUBMED | CROSSREF

10. Daood U, Swee Heng C, Neo Chiew Lian J, Fawzy AS. *In vitro* analysis of riboflavin-modified, experimental, two-step etch-and-rinse dentin adhesive: Fourier transform infrared spectroscopy and micro-Raman studies. Int J Oral Sci 2015;7:110-124.
    PUBMED | CROSSREF

11. De-Paula DM, Lomonaco D, Ponte AM, Cordeiro KE, Moreira MM, Mazzetto SE, Feitosa VP. Influence of collagen cross-linkers addition in phosphoric acid on dentin biomodification and bonding of an etch-and-rinse adhesive. Dent Mater 2020;36:e1-e8.
    PUBMED | CROSSREF

12. de Souza LC, Rodrigues NS, Cunha DA, Feitosa VP, Santiago SL, Reis A, Loguercio AD, Matos TP, Saboia VP, Perdigão J. Two-year clinical evaluation of proanthocyanidins added to a two-step etch-and-rinse adhesive. J Dent 2019;81:7-16.
    PUBMED | CROSSREF

13. Costa C, Albuquerque N, Mendonça JS, Loguercio AD, Saboia V, Santiago SL. Catechin-based dentin pretreatment on the clinical performance of a universal adhesive: a two-year randomized clinical trial. Oper Dent 2020;45:473-483.
    PUBMED | CROSSREF

14. de Souza LC, Rodrigues NS, Cunha DA, Feitosa VP, Santiago SL, Reis A, Loguercio AD, Perdigão J, Saboia VP. Two-year clinical evaluation of a proanthocyanidins-based primer in non-carious cervical lesions: a double-blind randomized clinical trial. J Dent 2020;96:103325.
    PUBMED | CROSSREF

15. Moher D, Liberati A, Tetzlaff J, Altman DG; PRISMA Group. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. PLoS Med 2009;6:e1000097.
    PUBMED | CROSSREF

16. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan-a web and mobile app for systematic reviews. Syst Rev 2016;5:210.
    PUBMED | CROSSREF

17. Tufanaru C, Munn Z, Aromataris E, Campbell J, Hopp L. Chapter 3: Systematic reviews of effectiveness. In: Aromataris E, Munn Z, editors. Joanna Briggs Institute reviewer's manual. Adelaide: The Joanna Briggs Institute; 2017.

18. Haas LF, Dutra K, Porporatti AL, Mezzomo LA, De Luca Canto G, Flores-Mir C, Corrêa M. Anatomical variations of mandibular canal detected by panoramic radiography and CT: a systematic review and meta-analysis. Dentomaxillofac Radiol 2016;45:20150310.
   PUBMED | CROSSREF

19. Schünemann H, Brożek J, Guyatt G, Oxman A. GRADE handbook for grading quality of evidence and strength of recommendations. [place unknown]: The GRADE Working Group; 2013.

20. Dalton Bittencourt D, Ezecelevski IG, Reis A, Van Dijken JW, Loguercio AD. An 18-months' evaluation of self-etch and etch & rinse adhesive in non-carious cervical lesions. Acta Odontol Scand 2005;63:173-178.
   PUBMED | CROSSREF

21. Perdigão J, Dutra-Corrêa M, Saraceni CH, Ciaramicoli MT, Kiyan VH, Queiroz CS. Randomized clinical trial of four adhesion strategies: 18-month results. Oper Dent 2012;37:3-11.
   PUBMED | CROSSREF

22. Aguiar TR, Vidal CM, Phansalkar RS, Todorova I, Napolitano JG, McAlpine JB, Chen SN, Pauli GF, Bedran-Russo AK. Dentin biomodification potential depends on polyphenol source. J Dent Res 2014;93:417-422.
   PUBMED | CROSSREF

23. Bedran-Russo AK, Pashley DH, Agee K, Drummond JL, Miescke KJ. Changes in stiffness of demineralized dentin following application of collagen crosslinkers. J Biomed Mater Res B Appl Biomater 2008;86:330-334.
   PUBMED | CROSSREF

24. Cova A, Breschi L, Nato F, Ruggeri A Jr, Carrilho M, Tjäderhane L, Prati C, Di Lenarda R, Tay FR, Pashley DH, Mazzoni A. Effect of UVA-activated riboflavin on dentin bonding. J Dent Res 2011;90:1439-1445.
   PUBMED | CROSSREF

25. Cai J, Palamara JE, Burrow MF. Effects of collagen crosslinkers on dentine: a literature review. Calcif Tissue Int 2018;102:265-279.
   PUBMED | CROSSREF

26. Yun JH, Pang EK, Kim CS, Yoo YJ, Cho KS, Chai JK, Kim CK, Choi SH. Inhibitory effects of green tea polyphenol (-)-epigallocatechin gallate on the expression of matrix metalloproteinase-9 and on the formation of osteoclasts. J Periodontal Res 2004;39:300-307.
   PUBMED | CROSSREF

27. Jackson JK, Zhao J, Wong W, Burt HM. The inhibition of collagenase induced degradation of collagen by the galloyl-containing polyphenols tannic acid, epigallocatechin gallate and epicatechin gallate. J Mater Sci Mater Med 2010;21:1435-1443.
   PUBMED | CROSSREF

28. Du X, Huang X, Huang C, Wang Y, Zhang Y. Epigallocatechin-3-gallate (EGCG) enhances the therapeutic activity of a dental adhesive. J Dent 2012;40:485-492.
   PUBMED | CROSSREF

29. Yoshida Y, Yoshihara K, Nagaoka N, Hayakawa S, Torii Y, Ogawa T, Osaka A, Meerbeek BV. Self-assembled nano-layering at the adhesive interface. J Dent Res 2012;91:376-381.
   PUBMED | CROSSREF

30. Mitra SB, Lee CY, Bui HT, Tantbirojn D, Rusin RP. Long-term adhesion and mechanism of bonding of a paste-liquid resin-modified glass-ionomer. Dent Mater 2009;25:459-466.
   PUBMED | CROSSREF

31. Perdigão J, Kose C, Mena-Serrano AP, De Paula EA, Tay LY, Reis A, Loguercio AD. A new universal simplified adhesive: 18-month clinical evaluation. Oper Dent 2014;39:113-127.
   PUBMED | CROSSREF

32. Lawson NC, Robles A, Fu CC, Lin CP, Sawlani K, Burgess JO. Two-year clinical trial of a universal adhesive in total-etch and self-etch mode in non-carious cervical lesions. J Dent 2015;43:1229-1234.
   PUBMED | CROSSREF

33. Moreira MA, Souza NO, Sousa RS, Freitas DQ, Lemos MV, De Paula DM, Maia FJ, Lomonaco D, Mazzetto SE, Feitosa VP. Efficacy of new natural biomodification agents from *Anacardiaceae* extracts on dentin collagen cross-linking. Dent Mater 2017;33:1103-1109.
   PUBMED | CROSSREF

34. Scheffel DL, Hebling J, Scheffel RH, Agee K, Turco G, de Souza Costa CA, Pashley D. Inactivation of matrix-bound matrix metalloproteinases by cross-linking agents in acid-etched dentin. Oper Dent 2014;39:152-158.
   PUBMED | CROSSREF

35. Machado AC, Dezan Junior E, Gomes-Filho JE, Cintra LT, Ruviére DB, Zoccal R, Damante CA, Jardim Junior EG. Evaluation of tissue reaction to Aroeira (*Myracrodruon urundeuva*) extracts: a histologic and edemogenic study. J Appl Oral Sci 2012;20:414-418.
   PUBMED | CROSSREF

36. Santiago SL, Osorio R, Neri JR, Carvalho RM, Toledano M. Effect of the flavonoid epigallocatechin-3-gallate on resin-dentin bond strength. J Adhes Dent 2013;15:535-540.
   PUBMED | CROSSREF

37. Neri JR, Yamauti M, Da Silveira F, Mendonça JS, Carvalho RM, Santiago SL. Influence of dentin biomodification with epigallocatechin-3-gallate on the bond strength of self-etch adhesive: twelve-month results. Int J Adhes Adhes 2016;71:81-86.
   CROSSREF

38. Zheng P, Chen H. Evaluate the effect of different MMPs inhibitors on adhesive physical properties of dental adhesives, bond strength and MMP substarte activity. Sci Rep 2017;7:4975-4980.
   PUBMED | CROSSREF

39. Liu Y, Wang Y. Effect of proanthocyanidins and photo-initiators on photo-polymerization of a dental adhesive. J Dent 2013;41:71-79.
   PUBMED | CROSSREF

40. Epasinghe DJ, Yiu CK, Burrow MF, Tay FR, King NM. Effect of proanthocyanidin incorporation into dental adhesive resin on resin-dentine bond strength. J Dent 2012;40:173-180.
   PUBMED | CROSSREF

41. Epasinghe DJ, Yiu CK, Burrow MF. Mechanical properties, water sorption characteristics, and compound release of grape seed extract-incorporated resins. J Appl Oral Sci 2017;25:412-419.
   PUBMED | CROSSREF

42. Pallan S, Furtado Araujo MV, Cilli R, Prakki A. Mechanical properties and characteristics of developmental copolymers incorporating catechin or chlorhexidine. Dent Mater 2012;28:687-694.
   PUBMED | CROSSREF

43. Innes NP, Frencken JE, Bjørndal L, Maltz M, Manton DJ, Ricketts D, Van Landuyt K, Banerjee A, Campus G, Doméjean S, Fontana M, Leal S, Lo E, Machiulskiene V, Schulte A, Splieth C, Zandona A, Schwendicke F. Managing carious lesions: consensus recommendations on terminology. Adv Dent Res 2016;28:49-57.
   PUBMED | CROSSREF

44. Perdigão J, Reis A, Loguercio AD. Dentin adhesion and MMPs: a comprehensive review. J Esthet Restor Dent 2013;25:219-241.
   PUBMED | CROSSREF

45. Hörsted-Bindslev P, Knudsen J, Baelum V. 3-year clinical evaluation of modified Gluma adhesive systems in cervical abrasion/erosion lesions. Am J Dent 1996;9:22-26.
   PUBMED

46. Gallo JR, Burgess JO, Ripps AH, Walker RS, Winkler MM, Mercante DE, Davidson JM. Two-year clinical evaluation of a posterior resin composite using a fourth- and fifth-generation bonding agent. Oper Dent 2005;30:290-296.
   PUBMED

47. Oz FD, Ergin E, Canatan S. Twenty-four-month clinical performance of different universal adhesives in etch-and-rinse, selective etching and self-etch application modes in NCCL - a randomized controlled clinical trial. J Appl Oral Sci 2019;27:e20180358.
   PUBMED | CROSSREF

48. Chermont AB, Carneiro KK, Lobato MF, Machado SM, Silva e Souza Junior MH. Clinical evaluation of postoperative sensitivity using self-etching adhesives containing glutaraldehyde. Braz Oral Res 2010;24:349-354.
   PUBMED | CROSSREF

49. Çakır NN, Demirbuga S. The effect of five different universal adhesives on the clinical success of class I restorations: 24-month clinical follow-up. Clin Oral Investig 2019;23:2767-2776.
   PUBMED | CROSSREF

Study or Subgroup	CCLAs Events	Total	Control Events	Total	Weight	Risk Ratio IV, Fixed, 95% CI	Risk Ratio IV, Fixed, 95% CI
5.1.2 1 week							
Costa et al., 2020 (1w)	0	78	0	78		Not estimable	
Souza et al., 2019 (1w)	0	90	0	45		Not estimable	
Souza et al., 2020 (1w)	0	90	0	45		Not estimable	
Subtotal (95% CI)		**258**		**168**		**Not estimable**	
Total events	0		0				
Heterogeneity: Not applicable							
Test for overall effect: Not applicable							
5.1.3 6 months							
Costa et al., 2020 (6m)	0	76	0	76		Not estimable	
Souza et al., 2019 (6m)	0	79	0	44		Not estimable	
Souza et al., 2020 (6m)	0	81	0	44		Not estimable	
Subtotal (95% CI)		**236**		**164**		**Not estimable**	
Total events	0		0				
Heterogeneity: Not applicable							
Test for overall effect: Not applicable							
5.1.4 12 months							
Costa et al., 2020 (12m)	0	75	0	73		Not estimable	
Subtotal (95% CI)		**75**		**73**		**Not estimable**	
Total events	0		0				
Heterogeneity: Not applicable							
Test for overall effect: Not applicable							
5.1.5 18 months							
Costa et al., 2020 (18m)	0	71	0	70		Not estimable	
Subtotal (95% CI)		**71**		**70**		**Not estimable**	
Total events	0		0				
Heterogeneity: Not applicable							
Test for overall effect: Not applicable							
5.1.6 24 months							
Costa et al., 2020 (24m)	0	69	0	68		Not estimable	
Souza et al., 2019 (24m)	0	62	0	41		Not estimable	
Souza et al., 2020 (24m)	0	68	0	40		Not estimable	
Subtotal (95% CI)		**199**		**149**		**Not estimable**	
Total events	0		0				
Heterogeneity: Not applicable							
Test for overall effect: Not applicable							
Total (95% CI)		**839**		**624**		**Not estimable**	
Total events	0		0				
Heterogeneity: Not applicable							
Test for overall effect: Not applicable							
Test for subgroup differences: Not applicable							

0.85 0.9 1 1.1 1.2
High in Control   High in CCLAs

Appendix 1. Forest plot of the incidence of caries rates.
CCLA, collagen cross-linking agent; CI, confidence interval.