INFLUENCE OF MMP INHIBITORS ON BOND STRENGTH OF ADHESIVE SYSTEM: SYSTEMATIC REVIEW AND META-ANALYSIS

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ABSTRACT

Aims: This systematic review with meta-analysis aimed to evaluate the effect of metalloproteinase (MMPs) inhibitors on in vitro bond strength using initial (24 hours) and long-term (6, 12 months or longer) microtensile tests.

Materials and methods: A search was carried out in 7 databases and in the gray literature, limited to Portuguese, English and Spanish languages without publication year limit, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyzes (PRISMA) 2009.

Results: Only *in vitro* studies assessing the use of MMP inhibitors in adhesive procedures were included (Kappa = 0.86). Meta-analyses were conducted with the extracted data and the studies were evaluated for quality. Data: Of 5,134 potentially eligible studies, 112 were selected for full-text reading, 48 were reviewed, and 43 were included in the meta-analysis. Two independent evaluators selected the studies and assessed the risk of bias. Estimates of the combined effect were reported as means and standard deviation between groups.

Conclusion: The use of 2% CHX affected positively the initial bond strength, but no inhibitor was effective in maintaining the bond strength after the aging process. The use of MMP inhibitors during adhesive procedures to promote greater longevity to adhesive restorations is controversial. This study contributes to the understanding of the influence of these inhibitors in bond strength of restorations, which may aid clinical applicability.

KEYWORDS: Matrix metalloproteinases. Dental bonding. Bond strength. Adhesive system. Long-term. Protease inhibitor.
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INTRODUCTION

Since it was first reported by Nakabayashi et al.[1] resin adhesion to the dentin substrate has been the target of several studies[2,3], During the adhesion process, a hybrid layer is formed by the infiltration of resinous monomers into the network of collagen fibrils that are exposed by acid etching and the removal of minerals[4]. The maintenance of a stable hybrid layer over time could promote greater longevity of the resin / dentin bond, and thus, greater effectiveness of the restoration[5].

However, the bond between resin and dentin is not stable and suffers significant loss of strength with aging[6-8]; it is believed that this loss is related to the degradation of the hybrid layer[9]. The two main mechanisms reported are the degradation of the collagen matrix by the action of endogenous metalloproteinases (MMPs) present in dentin[10] or the

hydrolytic degradation of the resinous polymers that make up the hybrid layer[11,12].

The main endogenous MMPs of dentin are collagenase-2 (MMP-8), gelatinase-B (MMP-9)[13,14], and gelatinase-A (MMP-2)[13,14], which belong to the family of calcium and zinc-dependent proteolytic enzymes[15] found most frequently in dentin tissue affected by caries[15,16]. In its latent form, these MMPs are not able to degrade collagen; however, after the pH decrease during acid conditioning this MMPs is activated and followed by neutralization are able causing degradation of exposed collagen fibrils[15,17]. Thus, exposed collagen fibrils lead to decreased bond strength and cohesive failures in demineralized dentin below the hybrid layer, both in etch-and-rinse and self-etching adhesives[18,19].

Consequently, the inhibition of collagen degradation by MMPs would be beneficial to the hybrid layer[20]. Thus, antiproteolytic substances have been proposed to inhibit the action of MMPs and promote greater longevity of the adhesive bonds, such as the solutions applied in demineralized dentin prior to the application of adhesive systems[6,21-23]. Several inhibitory agents have been studied, including chlorhexidine digluconate (CHX) solutions[6,7,21,24,25], proanthocyanidin (PA)[26-28], glutaraldehyde (GD)[26,28], epigallocatechin-3-gallate (EGCG)[29], galardin[30], green tea extract[30,31], among others. However, many studies show favorable results regarding the use of these inhibitors[6,21,29,32] and others present unfavorable results[30,33], tested soon after application[34-38] or after an aging process[7,33,35-37,39].

In 2014, Montagner et al.[40] conducted a systematic review with meta-analysis at use of metalloproteinase inhibitor solutions, mainly emphasizing the influence of the use of CHX initial and after aging by means of bond strength tests. Differently from the Montagner study, this study conducted meta-analyzes covering different MMP inhibitors, since several inhibitors can be found in the literature.

Therefore, the objective of this study was to evaluate the effect of specific or non-specific MMP inhibitors applied to dentin after acid etching for an etch-and-rinse adhesive system or after the application of the acidulated primer of self-etching systems, through a systematic review and meta-analysis. Microtensile tests were performed soon after the application and after the aging process. For the PICO structure was: P (patient- adapted for in vitro studies) - healthy dentin, I (intervention) – use of different MMP inhibitors; C (control) - without MMP inhibitors; and O (outcome) - bond strength by microtensile test. The key question guiding this review was: "What is the effect of specific or non-specific MMP inhibitors in the initial or long-term bond strength between resin and dentin, assessed by microtensile tests, compared to control?". The null hypothesis tested was that the use of MMP inhibitors does not affect initial and long-term resin/dentin bond strength.

MATERIALS AND METHODS

This systematic review was based on the PRISMA strategy (Preferred Reporting Items for Systematic Reviews and Meta-Analyze) 2009[41]. The best scientific evidence for studies aimed at verifying bond strength is provided by laboratory studies using microtensile tests. Randomized clinical trials and in vivo studies do not allow this type of analysis with exact numerical values. Therefore, this review aimed at in vitro studies that carried out microtensile test in dentin. For the PICO structure was adapted as follows: P (patient) - healthy dentin, I (intervention) – use of different MMP inhibitors; C (control) - without MMP inhibitors; and O (outcome) - bond strength by microtensile test. The key question guiding this review was: "What is the effect of specific or non-specific MMP inhibitors in the initial or long-term bond strength between resin and dentin, assessed by microtensile tests, compared to control?".

Registration and protocol

This study was registered in the PROSPERO base (International prospective register of systematic reviews) with ID CRD42017077516, obtained when the protocol was sent to https://www.crd.york.ac.uk/prospero/.

Search strategy

An electronic search in the major scientific databases (PubMed, Web of Science, Scielo, Lilacs, Cochrane, Embase and Scopus) and in the gray literature BDTD (http://bdtd.ibict.br/vufind/) was performed based on the eligibility criteria. Languages were limited to English, Spanish, and Portuguese. Studies were included until January 29, 2018. The search strategy defined for the PubMed database was as follows: (((dentin*[tw] OR adhesi*[tw] OR ("dentin-bonding agents" OR "dental bonding" [MeSH Terms] OR (metalloproteinase* OR "metalloproteinase" OR "metalloproteinase" OR "metalloproteinase" OR "metalloprotease" OR "metalloprotease" OR protease "OR" metallo proteases "OR metalloprotease* OR mmp[tw] OR mmps[tw] OR protease* [tw] OR proteinase* [tw] [tw] OR storage* [tw] OR time factor*[tw] OR aging[tw] OR longevity[tw])). The strategy was adapted for the other databases.

Eligibility criteria

The inclusion criteria were in vitro studies that evaluated the influence of specific or non-specific MMP inhibitors applied to human or bovine (sound) dentin after acid etching, prior to the application of an etch-and-rinse adhesive system or prior to the application of primer in self-etching systems (external application) that were tested for adhesive strength (microtensile) at 24 hours after application or after an aging process (6 months or longer). Only studies that had a control group (without application of MMP inhibitors) submitted to the same aging conditions as the other test groups were included. Once the adhesive interface is being evaluated, studies evaluating resin cements by microtensile tests were also included. The test of microtensile is a technique is ideal for evaluating the long-term durability of resin interfaces once this technique produce better stress distribution at the true interface.

The authors of studies that did not provide the complete numerical data were contacted as an attempt to obtain the missing data and if the data were not provided the study was excluded. Studies that did not present a control group were excluded. Studies that applied the inhibitor prior to acid conditioning or in which the inhibitor was incorporated into the adhesive system or phosphoric acid were also excluded or only the data of interest were collected. Descriptive studies about MMP inhibitors were not used for quantitative purposes but were included for the qualitative analysis of this review. In addition, studies that used carious, clarified, eroded, or divided dentin were excluded. A single study could be included more than once in the quantitative analysis if it reported results for different types of inhibitors, as in studies that studied more than one type of inhibitor.

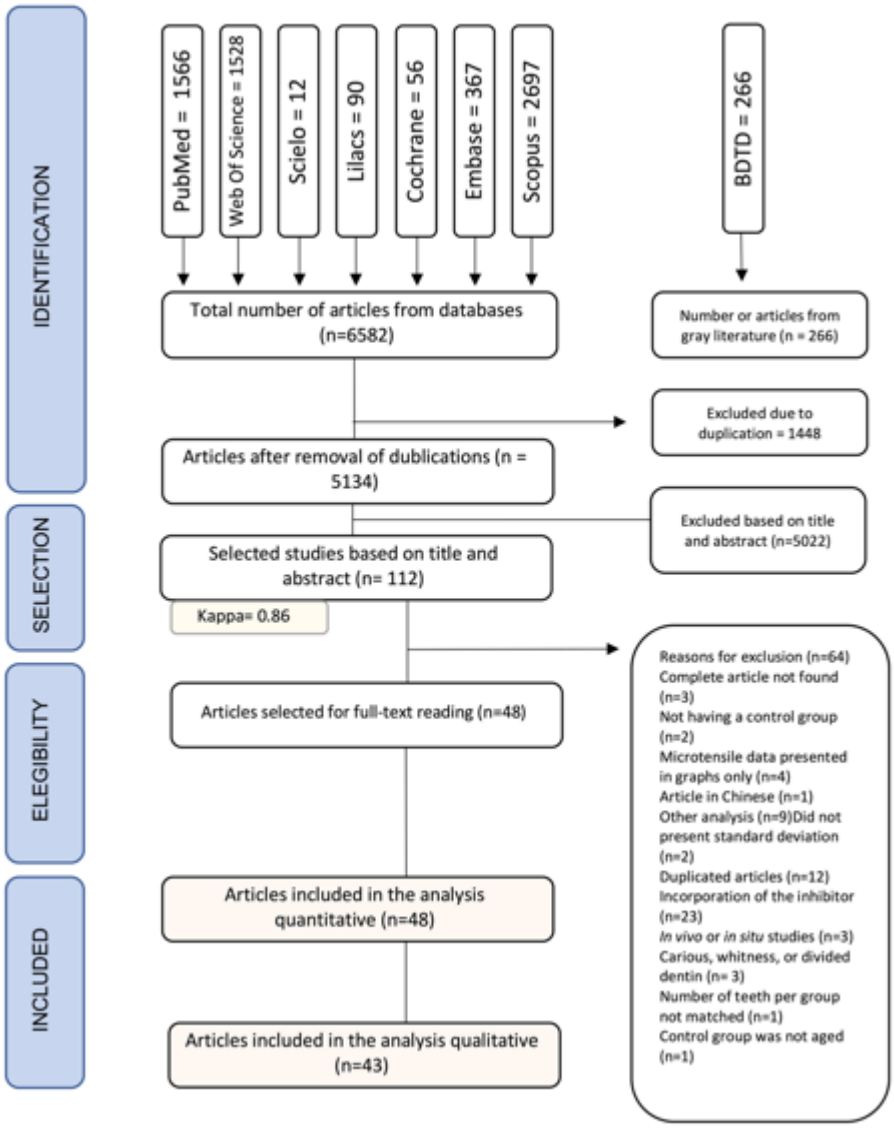

Figure 1. Flowchart of study selection based on the PRISMA strategy.

Screening and selection

The collected data were inserted into the reference management software Endnote Web (https://access.clarivate.com/login?app=endnote) so that duplicate studies were excluded. Two independent reviewers (FSC and DCS) assessed the titles based on the eligibility criteria. If selected, the article was submitted to full-text reading (Kappa = 0.86). In case of disagreement among the two reviewers, a third reviewer (HMH) decided whether the study should be included. The complete reading of selected studies was then performed by a reviewer (FSC) to verify the eligibility criteria. The complete flowchart of the selection process of the included articles for qualitative and quantitative analysis the process is shown in **Figure 1**.

Data extraction

A standardized protocol for data collection was developed by two authors (HMN and FSC) using a Microsoft Excel spreadsheet. The main data were extracted for the meta-analysis, including surname of first author and year of publication, type of study, type of tooth, aging time, aging process, method of application, MMP inhibitor, and mean and standard

Table 1. Relation between each inhibitor and its concentrations according to the aging time.

Initial	6 months	12 or more months
CHX 0.002% vs. control	CHX 0.002% vs. control	CHX 0.2% vs. control
CHX 0.004% vs. control	CHX 0.02% vs. control	CHX 1% vs. control
CHX 0.02% vs. control	CHX 0.2% vs. control	CHX 2% vs. control
CHX 0.12% vs. control	CHX 1% vs. control	CHX 2.2% vs. control
CHX 0.2% vs. control	CHX 2.2% vs. control	CHX 22% vs. control
CHX 1% vs. control	CHX 2% vs. control	EDC vs. control
CHX 2.2% vs. control	EDC vs. control	Riboflavin 0.1% vs. control
CHX 2% vs. control	EGCG 0.02% vs. control	
CHX 4% vs. control	EGCG 0.1% vs. control	
CHX 5% vs. control	EGCG 0.5% vs. control	
CHX 22% vs. control	EGCG 2% vs. control	
EDC vs. control		
EGCG 0.1% vs. control		
EGCG 0.02% vs. control		
EGCG 0.5% vs. control		
EGCG 2% vs. control		
GD 5-10% vs. control		
GD 5-8% vs. control		
GD 5% vs. control		
PA 10 vs. control		
PA 15% vs. control		
PA 6.5% vs. control		
Riboflavin 0.1% vs. control		

CHX, chlorhexidine; PA, proanthocyanidin; GD, glutaraldehyde; EDC, carbodiimide; EGCG, epigallocatechin-3-gallate.

deviation of bond strength values in the test and control groups.

Due to the great variability of the adhesive systems used in the selected studies, these were divided according to the mode of application (etch-and-rinse and self-etching), not considering whether these systems were simplified or not. Only studies that reported data of inhibitors using both modes of application were included.

Risk of bias assessment

Two independent authors performed the risk of bias assessment (FSC and DCS) based on the Montagner et al.[40] and Sarkis-Onofre et al.[41] studies considering the following criteria: randomization of teeth, caries-free teeth or restorations, materials used according to manufacturer's instructions, single operator for the adhesive procedure, sample size calculation, and blinding of the test machine operator. Studies that reported 2 or less criteria were considered as having high risk of bias, 3 or 4 as medium risk of bias, and 5 or 6 as low risk of bias. A quantitative analysis including only studies that presented medium and low risk of bias was performed and results were compared with those of the analysis with high risk of bias studies.

Data analysis

All the available data were used for analysis, allowing one or more data combinations in a single article if aging time varied, such as chlorhexidine 0.2% versus control (initial) and chlorhexidine 0.2% versus control (at 6 months). For the quantitative analysis between control and experimental groups, the gross mean and standard deviation of each article were used. The Comprehensive Meta-Analysis (Biostat, Englewood, NJ, USA) software was used, considering a level of significance of 0.05.

The heterogeneity between studies was assessed by the inconsistency test (I2) in which values greater than 75% (range 0 to 100) indicate high heterogeneity[42].

Thus, when heterogeneity was less than 75% the fixed-effect model was used and when 75% or greater the random effect model was used. The

analyzes performed for each MMP inhibitor and their concentrations are described in **Table 1**.

Due to the great variability in inhibitor concentrations, only the inhibitory agent was used in the meta-analysis, and not its concentration. Subgroup analyzes were performed for each tested agent, so that their individual influence on the result could be evaluated.

RESULTS

Initially, 6582 studies were found and 5134 remained after removal of 1448 duplicates. After assessment of titles and abstracts, 112 were selected for full-text reading. Finally, 43 studies[8,23-25,27-29,35,43-77] from 2008 to 2018 were included in the meta-analysis (**Figure 1**) and another 5 articles[32,79-81] were included in the qualitative analysis.

Characteristics of included studies

Most of the selected studies used permanent teeth, except the studies by Soares, et al.[72] and Lenzi et al.[58] that used bovine and deciduous teeth, respectively. Regarding MMP inhibitors, the most used solution was chlorhexidine digluconate at 2 and 0.2% concentrations. The most used aging solutions were deionized water and artificial saliva. The most used adhesive system was Adper Single Bond 2 (3M ESPE), an etch-and-rinse two-step adhesive system. Of the selected studies, 36 were published in English and seven in Portuguese, the latter being theses and dissertations found in the gray literature. The characteristics of the included studies are presented in table included in supplemental files.

Risk of bias assessment

Of the 43 studies included, none had a low risk of bias, 53.4% (23) presented a medium risk, and 46.6% (20), a high risk of bias. These results are described in **Table 2**.

Meta-analysis

The analysis was performed at three time-points: initial (at 24 hours), at 6 months, and at 12 months or longer. The first meta-analysis for the initial time-point (inhibitors versus controls) included 117 comparisons from 41 studies that grouped inhibitors of various concentrations (**Table 1**) evaluating the initial bond strength. Concerning subgroup analysis, the results of bond strength were higher for the test group only when chlorhexidine was used ($p<0.05$). For the group that used proanthocyanidin solutions, bond strength values were lower when compared to the control group; the other tested inhibitors presented bond strength values like the control group. However, the overall analysis showed no significant difference between groups, with $p = 0.748$ (95% confidence interval: 0.075-0.105 and fixed effect model, $I2 = 64.72\%$) (**Supplementary Figure 2**).

The second meta-analysis for the 6-month time-point included 56 comparisons from 18 studies (**Table 1**). At 6 months, lower values of bond strength were found for groups using CHX and EDC solutions when compared to the control group. The EGCG group presented similar bond strength to the control group (**Supplementary Figure 3**). Thus, the overall analysis showed significant differences between the groups ($p<0.05$, 95% confidence interval: 0.487-0.785 and fixed effect model, $I2 = 68.70\%$), with bond strength significantly higher for the control group **(Supplementary Figure 3**).

The meta-analysis for the 12-month+ time-point included 41 comparisons from 20 studies. Inhibitors were used in various concentrations as shown in Table 1. Subgroup analysis showed that all groups with inhibitors had lower bond strength values than the control group (**Supplementary Figure 4**). Therefore, the overall analysis showed a significant difference between the groups ($p <0.05$), in which the control group presented higher bond strength results than the test group (95% confidence interval: 0.541-2.651 and random effect model, $I2 = 82.162\%$).

Another meta-analysis (**Supplementary Figure 5**) was done to verify the effect of excluding studies with a high risk of bias. For the baseline analysis, 59 comparisons from 22 studies were included. Bond strength was higher for the test group compared to the control group only when chlorhexidine was used. For the EDC group, the bond strength values were similar to the control. The general analysis showed a significant difference between groups with $p <0.05$ (confidence interval -0.083-0.197, fixed-effect model and $I2 = 50.45\%$), with values of bond strength significantly higher for the test group. At 6 months, 31 comparisons from 8 studies were included and showed lower bond strength values for the chlorhexidine and EDC groups compared to the control group, with $p <0.05$ (confidence interval 0.802-1.190, fixed-effect model and $I2 = 43.90\%$). At 12 months or more, 21 comparisons from 11 studies were included, from which similar results to the 6-month analysis were found, with significantly lower bond strength values for chlorhexidine and EDC solutions (confidence interval 0.635-2.325, random effect model and $I2 = 75.27\%$). The results found in the above meta-analyzes are like those in which all studies were included.

A meta-analysis (**Supplementary Figure 6**) was also performed to compare the application modes of the adhesive systems (etch-and-rinse and self-etching) used in the selected studies. Inhibitors that had enough data to allow comparison (minimum of 2 different studies) for this analysis were CHX and EGCG at baseline.

Table 2. Bias risk of included studies considering the factors described in Materials and Methods.

Study	TR	TFCR	MUAMI	APSP	SZC	BOTM	RISCK
Abu Nawareg et al., 2016 [49]	Y	Y	Y	N	N	N	MEDIUM
Breschi et al., 2009 [50]	Y	Y	Y	N	N	N	MEDIUM
Breshi et al., 2010 [23]	Y	Y	Y	N	N	N	MEDIUM
Costa, 2013 [48]	Y	Y	Y	N	N	N	MEDIUM
Cova et al., 2011 [51]	Y	Y	Y	N	N	N	MEDIUM
Daood et al., 2017 [52]	Y	Y	Y	N	N	N	MEDIUM
Delgado, 2015 [43]	Y	Y	Y	N	N	N	MEDIUM
Ekambaram et al., 2014 [53]	Y	Y	N	Y	N	N	MEDIUM
Erhardt et al., 2008 [54]	N	Y	Y	N	N	N	HIGH
Francisconi-dos-Rios et al., 2015 [25]	N	Y	N	N	N	N	HIGH
Gerhardt et al., 2016 [55]	Y	N	Y	N	N	N	HIGH
Giacomini et al., 2017 [56]	N	Y	Y	N	N	N	HIGH
Gunaydin, Yazici, and Cehreli, 2016 [57]	Y	Y	Y	N	N	N	MEDIUM
Hass et al., 2016 [28]	Y	Y	N	N	N	N	HIGH
Hiraishi et al., 2009[58]	Y	Y	Y	N	N	N	MEDIUM
Lee and Sabatini, 2017 [8]	Y	Y	Y	N	N	N	MEDIUM
Lenzi et al., 2014 [59]	Y	Y	N	N	N	N	HIGH
Lin et al., 2013 [60]	N	Y	Y	N	N	N	HIGH
Liu et al., 2014 [27]	Y	Y	N	N	N	N	HIGH
Loguercio et al., 2016/1 [61]	N	Y	Y	N	N	N	HIGH
Loguercio et al., 2016/2 [24]	Y	Y	Y	Y	N	N	MEDIUM
Loguercio et al., 2009 [62]	N	Y	Y	Y	N	N	MEDIUM
Luhrs et al., 2013 [63]	Y	N	Y	N	N	N	HIGH
Manso et al. 2014 [64]	Y	Y	N	N	N	N	HIGH
Mazzoni et al., 2013 [65]	Y	Y	Y	N	N	N	MEDIUM
Mazzoni et al., 2018 [66]	Y	Y	Y	N	N	N	MEDIUM
Montagner, 2013 [46]	Y	Y	Y	N	N	Y	MEDIUM
Perote, 2016 [45]	N	N	N	N	N	N	HIGH
Perote et al., 2015 [67]	Y	Y	Y	N	N	N	MEDIUM
Sabatini, Kim, and Alias, 2014 [69]	Y	Y	Y	N	N	N	MEDIUM
Sabatini, Ortiz, and Pashley, 2015 [70]	Y	Y	Y	N	N	N	MEDIUM
Sadek et al., 2010 [71]	Y	N	Y	N	N	N	HIGH
Sanabe, 2009 [69]	Y	Y	Y	N	N	N	MEDIUM
Santiago et al., 2013 [29]	N	Y	Y	N	N	N	HIGH
Scaffa, 2012 [47]	Y	N	Y	N	N	N	HIGH
Scheffel et al., 2015 [72]	Y	Y	Y	N	N	N	MEDIUM
Soares et al., 2008 [73]	Y	N	Y	N	N	N	HIGH

Sousa, 2015 [44]	Y	Y	Y	N	N	N	MEDIUM
Stanislawczuk et al., 2009 [74]	N	Y	N	Y	N	N	HIGH
Stape et al., 2012 [75]	Y	Y	N	N	N	N	HIGH
Stape et al., 2014 [39]	Y	Y	N	N	N	N	HIGH
Talungchit et al., 2014 [76]	Y	Y	N	N	N	N	HIGH
Tekce et al., 2016 [77]	N	Y	Y	Y	N	N	MEDIUM

Y (yes); N (no).

* TR, randomization of teeth; TFCR, caries-free teeth or restorations; MUAMI, materials used according to the manufacturer's instructions; APSP, adhesive procedure performed by a single operator; SZC, calculation of sample size; BOTM blinding operator's test machine

For CHX at the initial time-point (inhibitor versus control), the etch-and-rinse adhesive systems presented higher bond strength values when compared to the control (p = 0.00). For the self-etching mode, there was no significant difference when compared to the control group (p = 0.338). The general analysis showed a difference between groups with p = 0.00 (confidence interval -0.366-0.127, fixed-effect model and I2 heterogeneity = 54.73%), in which the test group (CHX) had higher values when compared to the control group. For EGCG analysis, EGCG showed no significant difference for both modes of application when compared to the control (etch-and-rinse p = 0.259, self-conditioning p = 0.711). As for the general analysis, there was no difference between the application modes (p = 0.720 confidence interval -0.772, -0.221, fixed-effect model and I2 = 0%).

Since chlorhexidine was the only inhibitor to present higher bond strength values when compared to control, a more detailed analysis was performed (**Supplementary Figure 7**) to verify which concentrations would demonstrate significant values compared to the control group. In the initial time-point, only the 2% concentration showed higher bond strength values than the control group. The general analysis showed a significant difference between groups with p <0.05 (95% confidence interval: 0.387, -0.146 and fixed effect model, I2 = 50.22%), with bond strength significantly higher for the test group.

At 6 months, the 0.002, 0.2, and 2% concentrations resulted in lower bond strength values than the control group; the other groups had similar results among them. The general analysis showed significant differences between the groups, p <0.05 (95% confidence interval: 0.509-0.860 and fixed effect model, I2 = 73.20%), with bond strength significantly higher for the group control.

At 12 months or more, 0.2, 2.2, and 2% concentrations showed lower bond strength values than the control group. The other groups showed no difference compared to the control group. The general analysis showed no significant difference between the groups studied, with p=0.063 (95% confidence interval: 0.049-1.906 and random effect model, I2 = 82.25%).

The qualitative analysis included 5 studies[32,79-82] and the results showed that the use of MMP inhibitors does not affect initial bond strength values. However, the studies that performed a long-term evaluation[32,78,79,82] showed that the bond strength values were higher for the inhibitor-treated groups compared to untreated groups.

DISCUSSION

This systematic review showed that the use of MMP inhibitors to maintain the adhesion numbers between sound dentin and resin present different results at the initial time-point and after aging. Several inhibitors have been studied to assess their effect on collagen degradation and consequently increase the bond strength of the restorative material to the dental structure; however, only chlorhexidine at the initial analysis presented higher values of bond strength compared to control groups. Thus, the null hypothesis was partially rejected, since in the 6- and 12-month meta-analyses test and control groups did not differ statistically.

This study performed individual and collective comparisons of the influence of MMP inhibitors in bond strength through meta-analyses. Since randomized clinical studies on the bond resistance of restorative materials to the dental structure are not possible, the available evidence is mostly from *in vitro* studies, which might be a limiting factor of this investigation that should be considered in the interpretation of its results[57]

Although studies on the use of natural solutions[27,28] for inhibiting proteases and strengthening collagen fibrils through more stable bonds have shown promising results[82], this review showed that the use of proanthocyanidin negatively affects the initial bond strength. Perhaps this happens because the effectiveness and stability of the crosslinking treatment depend mainly on the type of PA rich extract and the adhesive system employed, since acetone and ethane can cause dehydration of the dentin matrix, making it even more difficult for the penetration of the adhesive. In addition, the concentration and the time of application are important for the performance of this inhibitor[83,84]. On the other hand, glutaraldehyde, EGCG, EDC and riboflavin provide similar results to control groups. Moreover, after 6 months of aging, CHX and EDC groups had lower bond strength than controls, and the EGCG group was like the control group. After 12 months or more of aging all included inhibitors presented worse results than the control group. These findings contrast with several other studies[22-24,40,50,61,82], that show stable bond strength values in the long-term when compared to groups not treated with inhibitors.

For years the most studied MMP inhibitors were CHX, EDC, EGCG and GD with great expectation of success in promoting more stable adhesive interfaces. Currently, several data can be found, and the synthesis of these results does not show such promising results after aging. This can be explained by the mechanism of action of these inhibitors, since CHX acts by a mechanism of cation chelation, sequestering metal ions such as zinc and calcium thus inhibiting the catalytic activity of MMPs[85]. Already EDC promotes biomodification of collagen, this is due to the formation of intra and inter molecular ionic

covalent bonds, but these ionic bonds do not demonstrate stability after aging[86]. The inhibitory effect of EGCG has also been attributed to zinc chelation, since polyphenols have high affinity for metal ions[29,55]. Like most inhibitors, GD acts by chemical interaction with collagen to increase its resistance, presenting as a disadvantage the difficulty of penetration into the tissue[87,88]. The main action of the MMP inhibitors occurs by chemical interaction and this does not act permanently being this mechanism reversed.

Due to the great variation in inhibitor concentrations, the first analysis did not consider this parameter. Afterwards, an analysis to evaluate the effectiveness of each concentration of CHX was performed, as CHX presented significant results (**Supplementary Figure 7**). The non-specific inhibitory effect of CHX solutions on MMPs has been extensively studied and its action proven[6,7,23,24,34,35]. Some studies have demonstrated the inhibitory effect of CHX at low concentrations, especially after specimen aging[40,61,89] which was not confirmed in this systematic review, in which only the 2% concentration showed higher bond strength values at the initial time-point compared to the control group. This might be explained by the leaching of CHX through the interfaces, thus decreasing its inhibitory potential[2,7].

MMPs are a Ca+ and Zn+ dependent enzyme family capable of degrading various components of the extracellular organic matrix. These enzymes, synthesized and secreted by odontoblasts[90] and activated during adhesive procedures[17], deteriorate the exposed collagen fibrils at the base of the hybrid layer by both etch-and-rinse or self-etching adhesives[17,91]. To decrease degradation, MMP inhibitors are used, and a meta-analysis (**Supplementary Figure 6**) was developed to compare the effectiveness of these inhibitors. However, in this study, only CHX, at initial evaluation, was effective when used with the etch-and-rinse mode, with no difference to the control group when self-etching adhesives were used. The reason for this result might be that the self-etching systems produce a more regular hybrid layer with smaller area of exposed collagen fibrils when compared to etch-and-rinse systems[91,92]. In addition, many self-etching systems have a chemical interaction with the dental substrate by the action of functional monomers. These monomers may interfere with the action of CHX because, due to the high pH, they form precipitates and because monomers and CHX, which can bind to organic and inorganic components of the substrate, can both bind to calcium[56,89,93,94].

The results of the group treated with EGCG (**Supplementary Figure 6-B**) showed no difference in the mode of application when compared to the control group. This might have happened because of the various concentrations used, since this parameter was not considered in this analysis. As EGCG is a natural inhibitor that has not yet been thoroughly assessed, further studies should be carried out at various concentrations. Moreover, in this analysis, the results were grouped by the mode of application (etch-and-rinse and self-etching), and not by the number of steps, which is a limitation of this analysis but may help plan future studies on adhesive systems and their effectiveness.

Because many studies presented a high risk of bias, an analysis including only studies with a medium risk of bias was performed (**Supplementary Figure 5**). The results did not differ from the results with the overall analyzes (**Supplementary Figures 2, 3,** and **4**), indicating that studies with a high risk of bias did not negatively interfere with the results.

To avoid a large methodological variability that could compromise the analysis of the data, this systematic review included only studies that evaluated bond strength through microtensile tests. However, some of the analyses presented high heterogeneity, probably due to the studies being carried out by different teams and centers, with different aging protocols, test machines, adhesive systems, and composite resins.[40] In this review, only in vitro studies were considered. However, despite the association between laboratory and clinical data, other factors, such as marginal adaptation and loss of retention[95,96], should also be analyzed to evaluate the effectiveness of an adhesive system, for which further systematic reviews are needed.

The qualitative analysis showed no difference in the initial bond strength between tests and controls, but after aging, the groups treated with MMP inhibitors showed higher values of bond strength. These findings contrast with the results of the quantitative analysis. This may have occurred because the inhibitors used are still poorly studied, in addition to the different aging media and adhesive systems used. However, despite the importance of the qualitative data, the greater number of papers included in the quantitative analysis guarantees the production of stronger scientific evidence.

To test the effectiveness of using MMP inhibitors to increase the maintenance of adhesive restorations, four randomized clinical studies with follow-up of up to 36 months[97-100] have shown no difference between test and control groups using the FDI and USPHS criteria. These clinical findings are in line with those found in the present meta-analysis. It is worth emphasizing the importance of the analysis of the laboratory data of these meta-analyzes together with data from clinical studies so that evidence of greater reliability is generated.

The inhibition of collagen degradation and consequent production of more stable and long-lasting hybrid layers by applying MMP inhibitors was a landmark in Restorative Dentistry. Early in vitro and in vivo studies showed that the use of CHX prior to the application of adhesive systems promoted more stable adhesive restorations in the long term[6,7,21-23,101]. Based on such studies, several dental schools adopted clinical protocols that included the use of MMP inhibitors as part of the adhesive step in resin restorations. Later studies with stronger scientific evidence, however, showed that although the initial inhibitory effects were promising, in the long term, the application of these inhibitors was not relevant to the longevity of restoration[38,90-92]. Thus, the use of MMP inhibitors in clinical practice was gradually abandoned, and the results of this systematic review corroborate this trend, since it did not generate strong scientific evidence of the beneficial effect to long-term adhesive stability.

CONCLUSION

Among the various inhibitors studied CHX 2% presented higher bond strength values than the control group in an initial analysis (up to 24 hours), but not after aging. On the other hand, the PA negatively affected the initial values. None of the inhibitors resulted in increased bond strength in the long term. Etch-and-rinse adhesive systems had better results than self-etching systems when using CHX.

The results of previous clinical studies combined to this systematic review with meta-analysis of in vitro studies indicate that there is no evidence to support the use of metalloproteinase inhibitors to increase bond strength between resin and dentin.

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