CLINICAL REVIEW

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MMP Inhibitors on Dentin Stability: A Systematic Review and Meta-analysis

ABSTRACT

The aim of this study was to systematically review the literature for *in vitro* and *ex vivo* studies that evaluated the effect of matrix metalloproteinase (MMP) inhibitors during the adhesive procedure on the immediate and long-term resin-dentin bond strength. The search was conducted in 6 databases with no publication year or language limits, following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement. From 1,336 potentially eligible studies, 48 were selected for full-text analysis, and 30 were included for review, with 17 considered in the meta-analysis. Two reviewers independently selected the studies, extracted the data, and assessed the risk of bias. Pooled effect estimates were expressed as the weighted mean difference between groups. The most used MMP inhibitor was chlorhexidine (CHX). Immediate bond strength results showed no difference between 2% CHX and control; however, a difference was found between 0.2% CHX and control at baseline. After aging, CHX presented higher bond strength values compared to control groups ($p < .05$). However, this was not observed for longer periods of aging. High heterogeneity was found in some comparisons, especially for the water storage aging subgroup. Subgroup analyses showed that self-etching and etch-and-rinse adhesives are benefited by the CHX use. From the studies included, only 1 presented low risk of bias, while the others showed medium or high risk of bias. The use of MMP inhibitors did not affect the immediate bond strength overall, while it influenced the aged bond strength. Aging procedures influenced bond strength values of the dentin adhesion stability.

KEY WORDS: chlorhexidine, dental bonding, bond strength, adhesive system, long-term, protease inhibitor.

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INTRODUCTION

It is well established that adhesive systems lose their bond to dentin through time, and there is a consensus that the hybrid layer degradation is related to that loss of bond strength (Hashimoto *et al.*, 2000; Tjäderhane *et al.*, 2013). Typically, bond strength decreases after 100 days or 6 months of aging, although not down to zero, as an amount of bond strength is retained even after water storage for a long time (Hashimoto *et al.*, 2010). This decrease in bond strength is related to a hydrolysis of collagen matrix of the hybrid layer combined with the degradation of the hydrophilic polymers of the adhesive systems (Manso *et al.*, 2009). Studies have shown that the adhesive interface may be negatively affected through time by the collagen fibril degradation. As plastification degradation is an inherent characteristic of the polymers, other factors involved in the resin-dentin interface degradation (collagen destruction) could be mediated to promote a more stable interface. The preservation of the collagen matrix integrity is decisive to improve the dentin bond durability (Tjäderhane *et al.*, 2013).

The exposed collagen network after phosphoric acid or acidic primer etching in etch-and-rinse and self-etching adhesive systems, respectively, is vulnerable to degradation also by endogenous metalloproteinases present in human dentin (Mazzoni *et al.*, 2006; Nishitani *et al.*, 2006). Enzymatic degradation of the collagen matrix by host-derived enzymes is shown to play a significant role in the destruction of the bonded interface (Carrilho *et al.*, 2007a). Some matrix metalloproteinases (MMPs) have been identified in human dentin and suggested to be responsible for that damage (Nishitani *et al.*, 2006). It is not new that some substances are able to inhibit those proteinases (MMP inhibitors), and the most used inhibitor is chlorhexidine (CHX). CHX has been shown to inhibit another class of collagen-degrading enzymes (cysteine cathepsins) also present in dentin (Tersariol *et al.*, 2010; Scaffa *et al.*, 2012).

MMP inhibitor application on dentin surface after acid etching or incorporation into the adhesive system could result in improvement of the integrity and stability of the tooth restoration through time (Carrilho *et al.*, 2007b; Loguércio *et al.*, 2009; De Munck *et al.*, 2010). An interesting topic is the effectiveness of the MMP inhibitor application on reducing the bond strength loss through time and the significance of this reduction when compared with restorations without the use of MMP inhibitors (Collares *et al.*, 2013).

Thus, the aim of this study was to systematically review the literature for *in vitro* and *ex vivo* studies that evaluated the resin-dentin bond strength immediately and after aging with the use of MMP inhibitors applied on dentin after etching. The hypothesis tested was that there would be no difference in bond strength values with or without the use of MMP inhibitors after aging.

MATERIALS & METHODS

Data Sources

This systematic review was performed according to the PRISMA statement (*i.e.*, preferred reporting items for systematic reviews and meta-analysis) (Liberati *et al.*, 2009). Six electronic databases (Medline via PubMed, TRIP, LILACS, Scielo, Cochrane, and ISI Web of Science) were searched to identify manuscripts that could be included. The following search strategies were performed: computer search of databases, review of reference lists of all articles included, and contact with authors and experts on the issue.

Regarding computer searches of databases, no publication year or language limit was used, and the last search was made in June 2013. Search words/terms were as follows: *(matrix metalloproteinase* OR protease inhibitor* OR MMPs inhibitor OR chlorhexidine) AND (dentin* adhesive OR adhesive system* OR bond*) AND (ag* OR stability OR durability OR long-term OR storage)*.

In terms of reviewing reference lists, the references of all included articles were manually searched for further relevant studies that could fulfill the inclusion criteria.

The inclusion criteria were *in vitro* or *ex vivo* studies that evaluated the influence of MMP inhibitor application on dentin during the adhesive step (application after acid etching or incorporated within adhesive composition) on immediate bond strength of resin-dentin and after aging of the adhesive interface (at least 6 mo of any type of aging). Only studies that had a control group (without MMP inhibitor application) of comparison were included. The outcome bond strength, in megapascals, was required for inclusion. Papers that did not provide such data were excluded, even after e-mail request to authors (at least twice).

Studies that did not evaluate the immediate and aged bond strength and that did not present a control group without the use of any MMP inhibitor were excluded from evaluation. Also, studies in which the MMP inhibitor was applied before etching were excluded, as were studies with the storage time shorter than 6 mo.

Search Steps: Screening and Selection

Step 1: Titles and abstracts were reviewed by 2 authors (A.F.M. and T.P.-C.) and selected for further review if they met the inclusion criteria.

Step 2: Abstracts were reviewed independently by 2 authors (A.F.M. and T.P.-C.) and selected per their consensus according to the same inclusion criteria used in step 1. If consensus was not reached, the abstract was set aside for further evaluation.

Step 3: Full-text articles of abstracts selected in step 2 were retrieved and reviewed by 1 author (A.F.M.). Inclusion was based on consensus between 2 investigators (A.F.M. and T.P.-C.). Disagreements were discussed with a third author (M.S.C.). The reference lists of all articles selected in step 3 were reviewed, and the full texts of potentially interesting studies were examined.

Data Extraction

A protocol for data extraction was defined and evaluated by 2 authors (A.F.M. and T.P.-C.) and divided into studies that used MMP inhibitor after acid etching or incorporated within the composition of the adhesive systems. Data were extracted from full-text articles by one author (A.F.M.) and reviewed by a second author (T.P.-C.) using a standardized outline.

To make the identification of variables found in the papers easier, the authors categorized similar information into 2 or 3 groups (*e.g.*, type of adhesive system, type of aging). For studies that did not report the precise bond strength values and that showed the results in graphs or figures, the authors were contacted via e-mail if data were missing or more information was needed.

In the selected studies, only the data of interest were extracted to be analyzed in the meta-analysis. For instance, one study had more than one control group, for which an arithmetic average of the values was used (Zhou *et al.*, 2009); the data from carious (Komori *et al.*, 2009) and eroded (Francisconi *et al.*, 2012) dentin were not extracted; aging with substances with MMP inhibitors (Carrilho *et al.*, 2007b) and data from CHX-containing acid were discarded (Stanislawczuk *et al.*, 2011); and for studies that, apart from the use of the MMP inhibitors in the adhesive procedure, also used it before that, the data were not extracted (Shafiei *et al.*, 2010; Kim and Shin, 2012; Luhrs *et al.*, 2013). To determine pooled estimates, each study contributed with the interested estimate.

Assessment of Risk of Bias

The risk of bias evaluation was based on and adapted from a previous study (Sarkis-Onofre *et al.*, 2014) and evaluated according to the description of the following parameters for the study's quality assessment: randomization of teeth, use of teeth free of caries or restoration, materials used according to the manufacturer's instructions, adhesive procedures performed by the same operator, description of sample size calculation, and blinding of the operator of the testing machine. If the authors reported the parameter, the paper had a *Y* (yes) on that specific parameter; if it was not possible to find the information, the paper received an *N* (no). Papers that reported 1 or 2 items were classified as high risk of bias, 3 or 4 as medium risk, and 4 to 6 as low risk.

Data Analyses

First, each possible comparison of the bond strength of both CHX concentrations used and the control data was carried out—for example, a study using 2% CHX and 0.2% CHX and 1 control resulted in 2 comparisons. Pooled effect estimates were obtained by comparing the means of each bond strength value of the adhesive systems and were expressed as the raw mean difference among the groups. A p value $\leq .05$ was considered statistically significant (Z test).

Statistical heterogeneity of the treatment effect among studies was assessed via the Cochran Q test, with a threshold p value of .1, and the inconsistency I^2 test, in which values > 50% were considered indicative of high heterogeneity (Higgins and Green, 2013).

For the meta-analysis, only the data from the studies that used CHX as a MMP inhibitor applied for 60 s, with 2% and/or 0.2% CHX concentrations and submitted to 6 mo, 12 mo, or more of aging, were included because they were the most usual data from all the selected studies. The analyses were carried out via a random effect model. The following analyses were carried out:

2% CHX *vs.* control at baseline (immediate bond strength values);
0.2% CHX *vs.* control at baseline;
2% CHX *vs.* control at 6 mo of aging;
0.2% CHX *vs.* control at 6 mo of aging;
2% CHX *vs.* control at 12 mo of aging;
2% CHX *vs.* control at 12, 14, 18, and 24 mo of aging considering the aging as subgroup; and
2% CHX *vs.* control at 6, 12, 14, 18, and 24 mo of aging considering the adhesive system as subgroup.

All analyses were conducted with Review Manager Software 5.1 (Copenhagen, Nordic Cochrane Centre, Cochrane Collaboration). The amount of specimens was considered as the amount of experimental units.

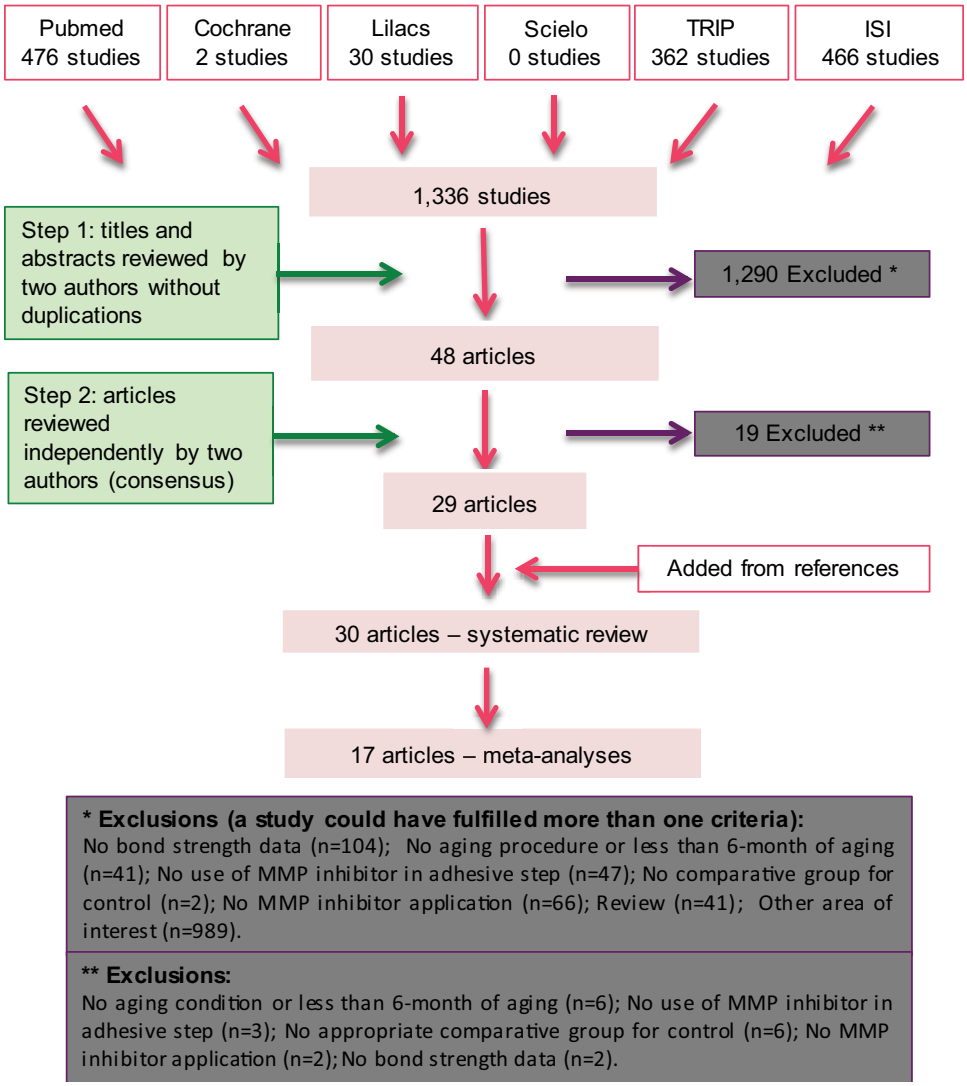


Figure 1. Flow diagram of study selection according to PRISMA statement.

Because the analyses of aging comparison could present a high heterogeneity, subgroup analyses considering the aging protocol (artificial saliva, water storage, and others) and the adhesive system category (self-etching and etch-and-rinse) were carried out to explore that influence on the results.

The studies that used MMP inhibitors other than CHX (Breschi *et al.*, 2010b; Cova *et al.*, 2011) or applied the solution for < 60 s (Breschi *et al.*, 2009; Breschi *et al.*, 2010a; Leitune *et al.*, 2011; Manfro *et al.*, 2012) and the studies that incorporated the MMP inhibitors within the composition of the adhesive system (Zhou *et al.*, 2009; De Munck *et al.*, 2010; Yiu *et al.*, 2012; Sabatini, 2013; Zhou *et al.*, 2013) were not included in the meta-analysis.

RESULTS

From 1,336 potentially eligible studies, 48 were selected for full-text analysis, and 30 were included for the systematic review (Figure 1). Twenty-eight studies were in English, 1 in Chinese, and 1 in Portuguese. The most used MMP inhibitor was CHX at 2% and 0.2% concentrations. The most used aging protocol was artificial saliva and water storage. The characteristics of the included studies are presented in Table 1.

Regarding the descriptive analysis, it was possible to observe a trend toward the decrease of the bond strength through time mostly for the control groups (without MMP inhibitors) regardless of the use of MMP inhibitors. This trend was observed even with the studies not described in Table 1. However, the use of MMP inhibitors did not negatively affect the immediate bond strength values.

Risk of Bias

Of the 30 studies included, only 1 (3.3%) presented low risk of bias, while the majority (21%-70%) showed medium risk of bias and 8 (26.7%) showed high risk of bias. The results are described in Table 2, according to the parameters considered in the analysis.

Table 1. Percentage of Bond Strength Reduction: Data from Studies Included in the Systematic Review That Used Matrix Metalloproteinase Inhibitors with 6 or 12 mo of Aging

				Bond Strength Reduction, %	
Paper	Type of Aging	Bond Strength Test	MMP Inhibitor	0-6 mo	0-12 mo
Data from included studies that used MMP inhibitors as a pre-treatment solution					
Carrilho *et al.*, 2007a	Artificial saliva without inhibitor	Microtensile	CHX 2%	23.4	—
			Control (no MMPs)	45.3	—
Campos *et al.*, 2009	Distilled water and thermocycling	Microtensile	CHX 0.2%	37.1	—
			CHX 2%	22.1	—
			Control (no MMPs)	40.9	—
			CHX 0.2%	23.1	—
			CHX 2%	26.4	—
			Control (no MMPs)	43.6	—
Komori *et al.*, 2009	Artificial saliva	Microtensile	CHX 2%	17.0	—
			Control (no MMPs)	44.6	—
			CHX 2%	28.3	—
			Control (no MMPs)	47.7	—
Breschi *et al.*, 2009	Artificial saliva	Microtensile	CHX 0.2%	16.4	20.7
			CHX 2%	10.9	24.5
			Control (no MMPs)	28.0	54.2
			CHX 0.2%	13.0	30.8
			CHX 2%	14.3	24.2
			Control (no MMPs)	33.1	64.1
Stanislawczuk *et al.*, 2009	Water storage (distilled water)	Microtensile	CHX 2%	0	—
			Control (no MMPs)	25	—
			CHX 2%	0	—
			Control (no MMPs)	33.6	—
Loguércio *et al.*, 2009	Water storage (distilled water)	Microtensile–Exp 1	CHX 0.002%	11.6	—
			CHX 0.02%	9.6	—
			CHX 0.2%	0	—
			CHX 2%	12.6	—
			CHX 4%	7.6	—
			Control (no MMPs)	29.0	—
			CHX 0.002%	10.9	—
			CHX 0.02%	0	—
			CHX 0.2%	11.3	—
			CHX 2%	8.4	—
			CHX 4%	20.9	—
			Control (no MMPs)	33.4	—
		Microtensile–Exp 2	CHX 0.002%	7.2	—
			CHX 2%	8.7	—
			Control (no MMPs)	38.8	—
			CHX 0.002%	7.5	—
			CHX 2%	10.2	—
			Control (no MMPs)	34.5	—
Breschi *et al.*, 2010a	Artificial saliva	Microtensile	Galardin	—	26.5
			Control (no MMPs)	—	45.4
Shafiei *et al.*, 2010	Water storage (distilled water)	Shear	CHX 2%	—	10.8
			Control (no MMPs)	—	36.0
Zhang *et al.*, 2010	Water storage (distilled water)	Microtensile	CHX 0.02%	32.0	—
			CHX 0.2%	6.5	—
			CHX 2%	17.4	—
			CHX 20%	18.6	—
			Control (no MMPs)	31.8	—
Ricci *et al.*, 2010	Oral function	Microtensile	CHX 2%	—	26.3
			Control (no MMPs)	—	43.9
Leitune *et al.*, 2010	Water storage (distilled water)	Push-out	CHX 0.2%	21.9	—
			CHX 2%	32.0	—
			Control (no MMPs)	36.1	—

(continued)

Table 1. (continued)

Paper	Type of Aging	Bond Strength Test	MMP Inhibitor	Bond Strength Reduction, % 0-6 mo	0-12 mo
Cova *et al.*, 2011	Artificial saliva	Microtensile	Riboflavin 0.1%	19.8	30.4
			Control (no MMPs)	41.0	52.5
Leitune *et al.*, 2011	Water storage (distilled water)	Microshear	CHX 2%	0	—
			Control (no MMPs)	10.9	—
Manfro *et al.*, 2012	Artificial saliva	Tensile	CHX 0.5%	—	34.5
			CHX 2%	—	21.3
			Control (no MMPs)	—	59.8
Sacramento *et al.*, 2012	Water storage (distilled water)	Microshear	CHX 2%	76.7	85.8
			Control (no MMPs)	76.3	89.1
			CHX 2%	78.8	83.9
			Control (no MMPs)	85.7	93.1
Ali *et al.*, 2013	Artificial saliva	Microtensile	CHX 2%	13.7	—
			Control (no MMPs)	36.5	—
Luhrs *et al.*, 2013	Water storage (distilled water)	Microtensile	CHX 2%	30	—
			Galardin 0.2mM	9.2	—
			Control (no MMPs)	0	—
Santiago *et al.*, 2013	3 mmol/L sodium azide solution	Microtensile	CHX 2%	8.8	—
			EGCG 0.02%	0	—
			EGCG 0.1%	0	—
			EGCG 0.5%	0	—
			Control (Water)	19.0	—
Francisconi *et al.*, 2012	Water storage (deionized water)	Microtensile	CHX 0.004 %	35.2	61.2
			CHX 2%	15.9	67.8
			Control (no MMPs)	40.2	78.2
Data from included studies that used MMP inhibitors incorporated into the adhesive system composition					
Zhou *et al.*, 2009	0.9 NaCl containing 0.02 sodium azide	Microtensile	CHX 0.05%	—	28.8
			CHX 0.1%	—	0.7
			CHX 0.5%	—	5.9
			CHX 1.0%	—	2.8
			Control (no MMPs)	—	18.0
De Munck *et al.*, 2010	Water storage (distilled water)	Microtensile	CHX 0.05%	41.4	78.9
			SB-3CT	76.5	93.6
			Control (no MMPs)	42.9	49.5
			CHX 0.05%	35.2	79.1
			SB-3CT	62.2	60.8
			Control (no MMPs)	52.1	66.2
			CHX 0.05%	33.0	48.2
			SB-3CT	30.4	57.5
			Control (no MMPs)	20.9	33.4
Sabatini, 2013	Water storage (distilled water)	Shear	CHX 2% adhesive	5.21	—
			CHX 2% surface	0	—
			Control (no MMPs)	0	—
			CHX 0.2% adhesive	0	—
			CHX 0.2% surface	0	—
			Control (no MMPs)	0	—
Yiu *et al.*, 2012	Artificial saliva	Microtensile	CHX 2.0 wt	—	14.2
			Control (no MMPs)	—	37.1
			CHX 2.0 wt	—	12.9
			Control (no MMPs)	—	24.1
			CHX 2.0 wt	—	28.7
			Control (no MMPs)	—	64.8

All studies were conducted in coronal dentin, except for Zhou *et al.* (2013) and Leitune *et al.* (2010), which used radicular dentin. All studies were conducted using permanent teeth, except Leitune *et al.* (2011) and Manfro *et al.* (2012), which used primary teeth.
MMP, matrix metalloproteinase; CHX, chlorhexidine.

Table 2. Risk of Bias of the Studies Considering Aspects Reported in the Materials & Methods Section

Study	Random	Caries	Materials	Adhesive	Sample	Blinding	Risk
Carrilho *et al.*, 2007a	Y	Y	Y[a]	N	N[b]	N	Medium
Carrilho *et al.*, 2007b	N	Y	Y[a]	N	N[b]	N	High
Breschi *et al.*, 2009	Y	Y	Y	N	N[b]	N	Medium
Campos *et al.*, 2009	Y	Y	Y	N	N[b]	N	Medium
Komori *et al.*, 2009	N	Y	N	N	N[b]	N	High
Loguércio *et al.*, 2009	Y	Y	Y	Y	N[b]	Y	Low
Stanislawczuk *et al.*, 2009	Y	Y	N	Y	N[b]	N	Medium
Zhou *et al.*, 2009	Y	Y	Y	N	N[b]	N	Medium
Breschi *et al.*, 2010a	Y	Y	Y	N	N	N	Medium
Breschi *et al.*, 2010b	Y	Y	Y	N	N	N	Medium
Chang *et al.*, 2010	Y	Y	Y	N	N	N	Medium
De Munck *et al.*, 2010	N	N	Y[a]	N	N	N	High
Leitune *et al.*, 2010	Y	—[a]	Y	Y	N	N	Medium
Ricci *et al.*, 2010	Y	N[c]	Y	Y	N	N	Medium
Sadek *et al.*, 2010	Y	—[a]	Y	N	N	N	High
Shafiei *et al.*, 2010	Y	Y	Y	N	N	N	Medium
Zhang *et al.*, 2010	Y	Y	Y	N	N	N	Medium
Cova *et al.*, 2011	Y	Y	Y	N	N	N	Medium
Leitune *et al.*, 2011	Y	Y	Y	N	N	N	Medium
Stanislawczuk *et al.*, 2011	Y	Y	Y[a]	Y	N	N	Medium
Francisconi *et al.*, 2012	Y	Y	Y	N	N	N	Medium
Kim and Shin, 2012	Y	Y	Y	N	N	N	Medium
Manfro *et al.*, 2012	Y	Y	Y	Y	N	N	Medium
Sacramento *et al.*, 2012	Y	Y	Y	N	N	N	Medium
Yiu *et al.*, 2012	Y	Y	NA	N	N	N	High
Ali *et al.*, 2013	N	Y	Y	N	N	N	High
Luhrs *et al.*, 2013	Y	N	N	N	N	N	High
Sabatini, 2013	Y	Y	Y	N	N	N	Medium
Santiago *et al.*, 2013	Y	Y	Y	N	N	N	Medium
Zhou *et al.*, 2013	Y	N	Y	N	N	N	High

Random, teeth randomization; caries, teeth free of caries/restoration; materials, materials used according to manufacturers' instructions; adhesive, adhesive procedures performed by a single operator; sample, sample size calculation; blinding, blinding of the operator of the testing machine; Y, yes; N, no; NA, not applicable.

[a]Not mentioned.

[b]This aspect was not considered for the first published studies on the topic.

[c]Carious teeth.

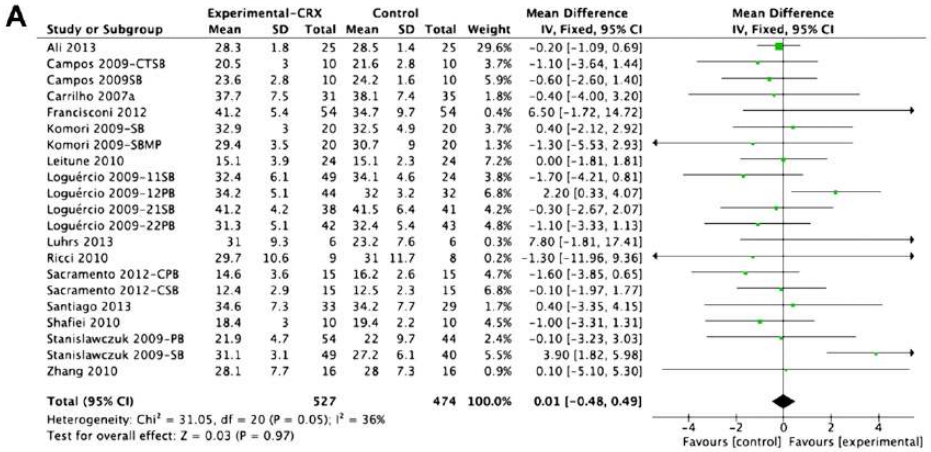
Meta-analysis

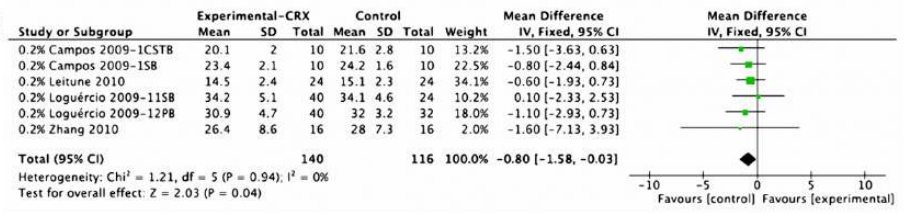
Of the 30 studies, data from 17 were subjected to further evaluation for meta-analysis (Appendix Table). The meta-analysis results are presented in Figure 2. For the first analysis (2% CHX *vs.* control at the baseline—immediate bond strength values), 21 data sets were considered, although 14 studies were included (Figure 2A). The values of the Cochran Q and Z tests were > .05 showing no statistically significant difference between groups, and the I^2 tests were 36%.

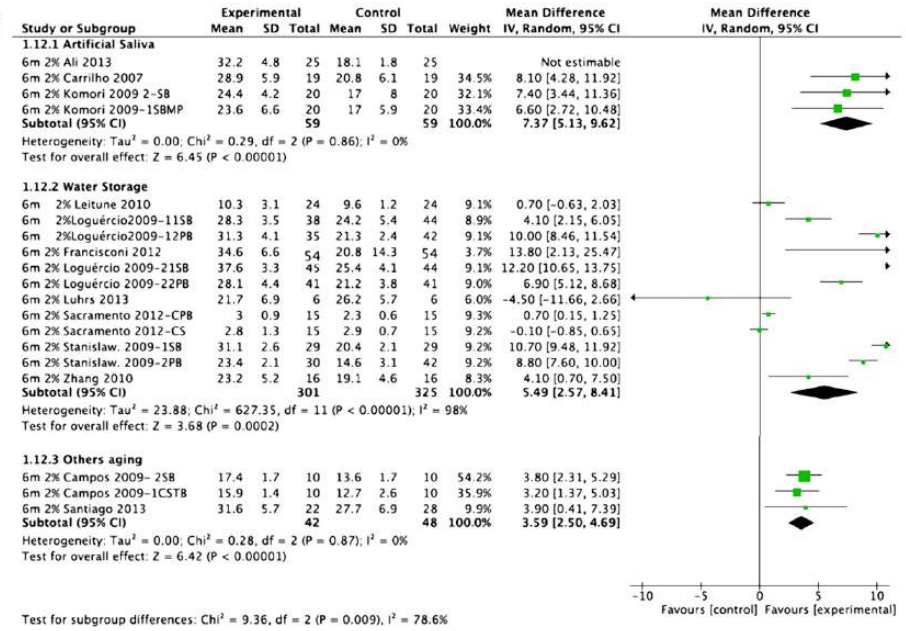
For the second analysis (0.2% CHX *vs.* control in the baseline—immediate bond strength values), 6 data sets were considered, with 4 studies included (Figure 2B). Regarding the results, the value of the Cochran Q test was > .05. However, the Z test was < .05, with the control group showing higher values of bond strength compared with the experimental group (0.2% CHX), and the I^2 test was 0%.

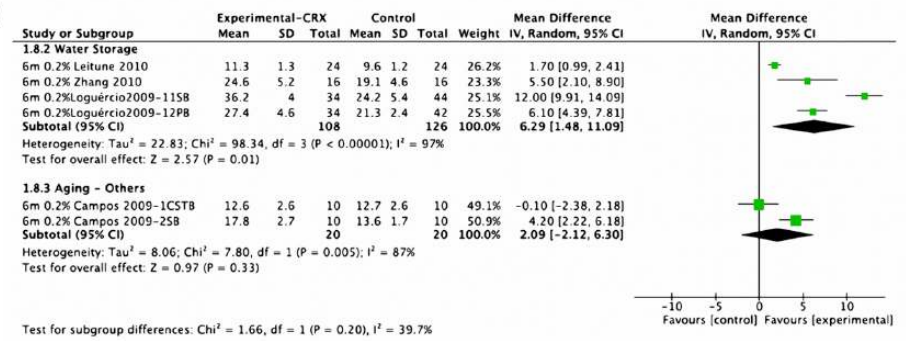
For the third analysis (2% CHX *vs.* control at 6 mo of aging), 19 data sets were considered, although 12 studies were included (Figure 2C). In this analysis, the values of the Cochran Q and Z tests were < .05, favoring the experimental group (2% CHX), which showed higher bond strength values compared with the control group (no MMP inhibitors), and the I^2 test was 78.6%. Subgroup analysis was performed considering the aging protocol. For the artificial saliva and other aging protocols, the result was the same but with zero heterogeneity. For the artificial saliva subgroup, one study (Ali *et al.*, 2013) was excluded because at the sensitivity analysis it overestimated the results and presented high risk of bias. For water storage aging, the result was the same, and the heterogeneity was higher (I^2 = 98%), showing a great influence of the aging protocol on the results.

For the fourth analysis (0.2% CHX *vs.* control in 6 mo of aging), the same data sets of the second analysis were considered

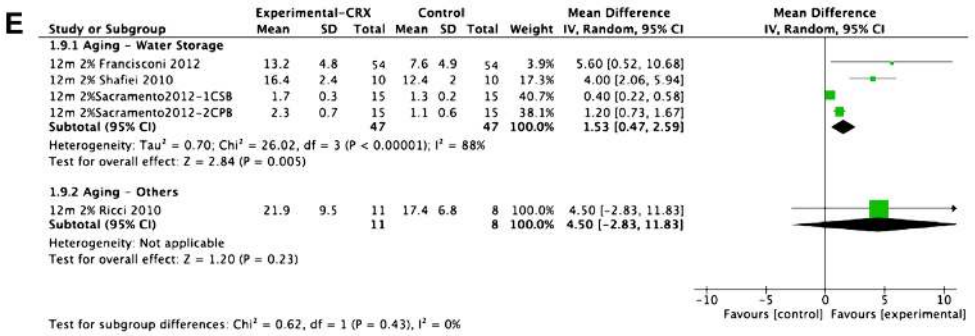

A
Experimental-CRX
Control
Mean Difference
Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Fixed, 95% CI
IV, Fixed, 95% CI
Ali 2013 28.3 1.8 25 28.5 1.4 25 29.6% -0.20 [-1.09, 0.69]
Campos 2009-CTSB 20.5 3 10 21.6 2.8 10 3.7% -1.10 [-3.64, 1.44]
Campos 2009SB 23.6 2.8 10 24.2 1.6 10 5.9% -0.60 [-2.60, 1.40]
Carrilho 2007a 37.7 7.5 31 38.1 7.4 35 1.8% -0.40 [-4.00, 3.20]
Francisconi 2012 41.2 5.4 54 34.7 9.7 54 0.4% 6.50 [-1.72, 14.72]
Komori 2009-SB 32.9 3 20 32.5 4.9 20 3.7% 0.40 [-2.12, 2.92]
Komori 2009-SBMP 29.4 3.5 20 30.7 9 20 1.3% -1.30 [-5.53, 2.93]
Leitune 2010 15.1 3.9 24 15.1 2.3 24 7.2% 0.00 [-1.81, 1.81]
Loguércio 2009-11SB 32.4 6.1 49 34.1 4.6 24 3.8% -1.70 [-4.21, 0.81]
Loguércio 2009-12PB 34.2 5.1 44 32 3.2 32 6.8% 2.20 [0.33, 4.07]
Loguércio 2009-21SB 41.2 4.2 38 41.5 6.4 41 4.2% -0.30 [-2.67, 2.07]
Loguércio 2009-22PB 31.3 5.1 42 32.4 5.4 43 4.8% -1.10 [-3.33, 1.13]
Luhrs 2013 31 9.3 6 23.2 7.6 6 0.3% 7.80 [-1.81, 17.41]
Ricci 2010 29.7 10.6 9 31 11.7 8 0.2% -1.30 [-11.96, 9.36]
Sacramento 2012-CPB 14.6 3.6 15 16.2 2.6 15 4.7% -1.60 [-3.85, 0.65]
Sacramento 2012-CSB 12.4 2.9 15 12.5 2.3 15 6.8% -0.10 [-1.97, 1.77]
Santiago 2013 34.6 7.3 33 34.2 7.7 29 1.7% 0.40 [-3.35, 4.15]
Shafiei 2010 18.4 3 10 19.4 2.2 10 4.5% -1.00 [-3.31, 1.31]
Stanislawczuk 2009-PB 21.9 4.7 54 22 9.7 44 2.4% -0.10 [-3.23, 3.03]
Stanislawczuk 2009-SB 31.1 3.1 49 27.2 6.1 40 5.5% 3.90 [1.82, 5.98]
Zhang 2010 28.1 7.7 16 28 7.3 16 0.9% 0.10 [-5.10, 5.30]
Total (95% CI) 527 474 100.0% 0.01 [-0.48, 0.49]
Heterogeneity: Chi² = 31.05, df = 20 (P = 0.05); I² = 36%
Test for overall effect: Z = 0.03 (P = 0.97)
-4 -2 0 2 4
Favours [control] Favours [experimental]

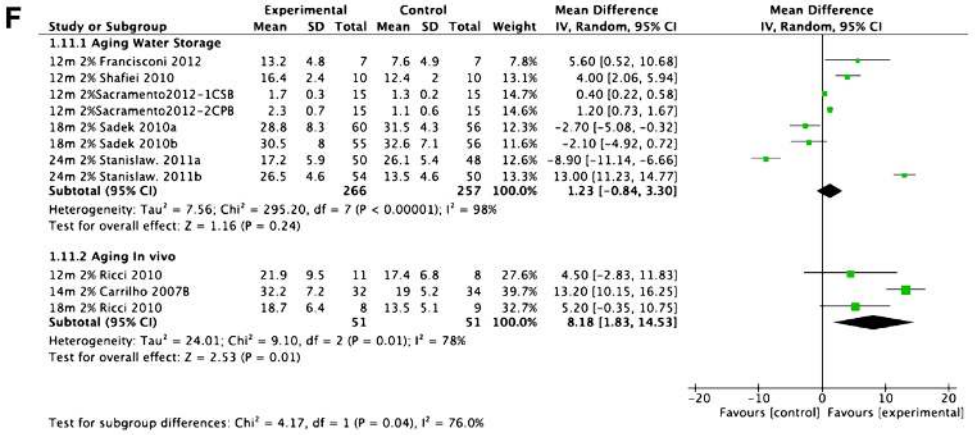

B
Experimental-CRX
Control
Mean Difference
Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Fixed, 95% CI
IV, Fixed, 95% CI
0.2% Campos 2009-1CSTB 20.1 2 10 21.6 2.8 10 13.2% -1.50 [-3.63, 0.63]
0.2% Campos 2009-1SB 23.4 2.1 10 24.2 1.6 10 22.5% -0.80 [-2.44, 0.84]
0.2% Leitune 2010 14.5 2.4 24 15.1 2.3 24 34.1% -0.60 [-1.93, 0.73]
0.2% Loguércio 2009-11SB 34.2 5.1 40 34.1 4.6 24 10.2% 0.10 [-2.33, 2.53]
0.2% Loguércio 2009-12PB 30.9 4.7 40 32 3.2 32 18.0% -1.10 [-2.93, 0.73]
0.2% Zhang 2010 26.4 8.6 16 28 7.3 16 2.0% -1.60 [-7.13, 3.93]
Total (95% CI) 140 116 100.0% -0.80 [-1.58, -0.03]
Heterogeneity: Chi² = 1.21, df = 5 (P = 0.94); I² = 0%
Test for overall effect: Z = 2.03 (P = 0.04)
-10 -5 0 5 10
Favours [control] Favours [experimental]


C
Experimental
Control
Mean Difference
Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
1.12.1 Artificial Saliva
6m 2% Ali 2013 32.2 4.8 25 18.1 1.8 25 Not estimable
6m 2% Carrilho 2007 28.9 5.9 19 20.8 6.1 19 34.5% 8.10 [4.28, 11.92]
6m 2% Komori 2009 2-SB 24.4 4.2 20 17 8 20 32.1% 7.40 [3.44, 11.36]
6m 2% Komori 2009-1SBMP 23.6 6.6 20 17 5.9 20 33.4% 6.60 [2.72, 10.48]
Subtotal (95% CI) 59 59 100.0% 7.37 [5.13, 9.62]
Heterogeneity: Tau² = 0.00; Chi² = 0.29, df = 2 (P = 0.86); I² = 0%
Test for overall effect: Z = 6.45 (P < 0.00001)
1.12.2 Water Storage
6m 2% Leitune 2010 10.3 3.1 24 9.6 1.2 24 9.1% 0.70 [-0.63, 2.03]
6m 2%Loguércio2009-11SB 28.3 3.5 38 24.2 5.4 44 8.9% 4.10 [2.15, 6.05]
6m 2%Loguércio2009-12PB 31.3 4.1 35 21.3 2.4 42 9.1% 10.00 [8.46, 11.54]
6m 2% Francisconi 2012 34.6 6.6 54 20.8 14.3 54 3.7% 13.80 [2.13, 25.47]
6m 2% Loguércio 2009-21SB 37.6 3.3 45 25.4 4.1 44 9.1% 12.20 [10.65, 13.75]
6m 2% Loguércio 2009-22PB 28.1 4.4 41 21.2 3.8 41 9.0% 6.90 [5.12, 8.68]
6m 2% Luhrs 2013 21.7 6.9 6 26.2 5.7 6 6.0% -4.50 [-11.66, 2.66]
6m 2% Sacramento 2012-CPB 3 0.9 15 2.3 0.6 15 9.3% 0.70 [0.15, 1.25]
6m 2% Sacramento 2012-CS 2.8 1.3 15 2.9 0.7 15 9.2% -0.10 [-0.85, 0.65]
6m 2% Stanislaw. 2009-1SB 31.1 2.6 29 20.4 2.1 29 9.2% 10.70 [9.48, 11.92]
6m 2% Stanislaw. 2009-2PB 23.4 2.1 30 14.6 3.1 42 9.2% 8.80 [7.60, 10.00]
6m 2% Zhang 2010 23.2 5.2 16 19.1 4.6 16 8.3% 4.10 [0.70, 7.50]
Subtotal (95% CI) 301 325 100.0% 5.49 [2.57, 8.41]
Heterogeneity: Tau² = 23.88; Chi² = 627.35, df = 11 (P < 0.00001); I² = 98%
Test for overall effect: Z = 3.68 (P = 0.0002)
1.12.3 Others aging
6m 2% Campos 2009- 2SB 17.4 1.7 10 13.6 1.7 10 54.2% 3.80 [2.31, 5.29]
6m 2% Campos 2009-1CSTB 15.9 1.4 10 12.7 2.6 10 35.9% 3.20 [1.37, 5.03]
6m 2% Santiago 2013 31.6 5.7 22 27.7 6.9 28 9.9% 3.90 [0.41, 7.39]
Subtotal (95% CI) 42 48 100.0% 3.59 [2.50, 4.69]
Heterogeneity: Tau² = 0.00; Chi² = 0.28, df = 2 (P = 0.87); I² = 0%
Test for overall effect: Z = 6.42 (P < 0.00001)
Test for subgroup differences: Chi² = 9.36, df = 2 (P = 0.009), I² = 78.6%
-10 -5 0 5 10
Favours [control] Favours [experimental]


D
Experimental-CRX
Control
Mean Difference
Mean Difference
Study or Subgroup Mean SD Total Mean SD Total Weight IV, Random, 95% CI
IV, Random, 95% CI
1.8.2 Water Storage
6m 0.2% Leitune 2010 11.3 1.3 24 9.6 1.2 24 26.2% 1.70 [0.99, 2.41]
6m 0.2% Zhang 2010 24.6 5.2 16 19.1 4.6 16 23.3% 5.50 [2.10, 8.90]
6m 0.2%Loguércio2009-11SB 36.2 4 34 24.2 5.4 44 25.1% 12.00 [9.91, 14.09]
6m 0.2%Loguércio2009-12PB 27.4 4.6 34 21.3 2.4 42 25.5% 6.10 [4.39, 7.81]
Subtotal (95% CI) 108 126 100.0% 6.29 [1.48, 11.09]
Heterogeneity: Tau² = 22.83; Chi² = 98.34, df = 3 (P < 0.00001); I² = 97%
Test for overall effect: Z = 2.57 (P = 0.01)
1.8.3 Aging - Others
6m 0.2% Campos 2009-1CSTB 12.6 2.6 10 12.7 2.6 10 49.1% -0.10 [-2.38, 2.18]
6m 0.2% Campos 2009-2SB 17.8 2.7 10 13.6 1.7 10 50.9% 4.20 [2.22, 6.18]
Subtotal (95% CI) 20 20 100.0% 2.09 [-2.12, 6.30]
Heterogeneity: Tau² = 8.06; Chi² = 7.80, df = 1 (P = 0.005); I² = 87%
Test for overall effect: Z = 0.97 (P = 0.33)
Test for subgroup differences: Chi² = 1.66, df = 1 (P = 0.20), I² = 39.7%
-10 -5 0 5 10
Favours [control] Favours [experimental]

(continued)





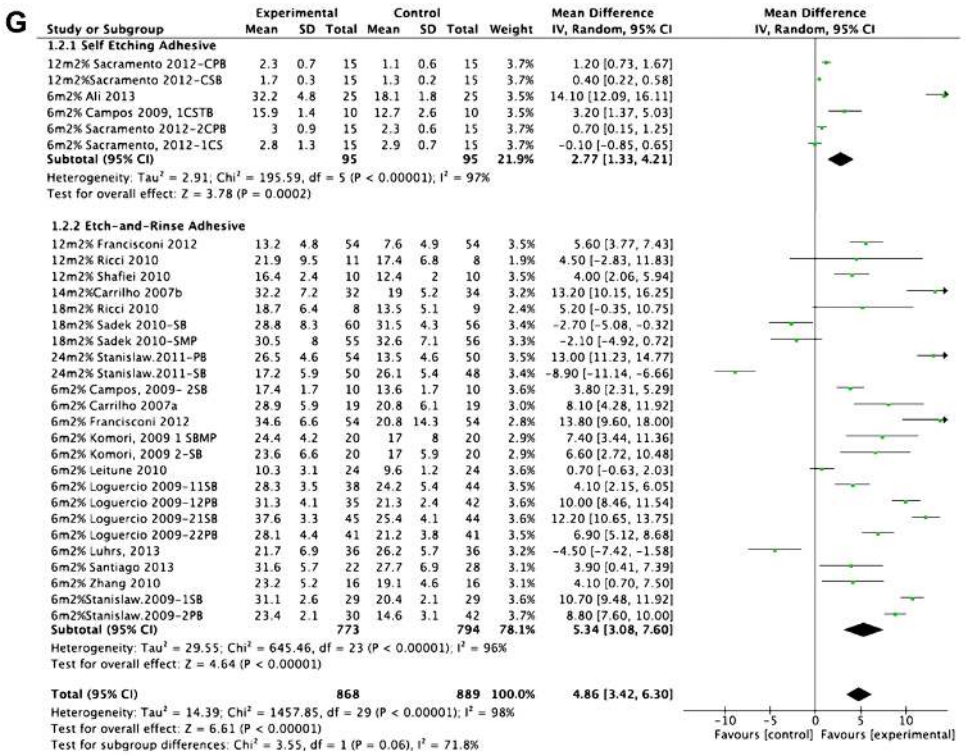


Figure 2. Forest plots according to the analyses. *No aging*: (**A**) 2% chlorhexidine (CHX) *vs.* control; (**B**) 0.2% CHX *vs.* control. *Aging subgroup analysis*: (**C**) 2% CHX *vs.* control, 6 mo aging; (**D**) 0.2% CHX *vs.* control, 6 mo aging; (**E**) 2% CHX *vs.* control, 12 mo aging; (**F**) 2% CHX *vs.* control, 12 mo of aging or more. *Adhesive system subgroup analysis*: (**G**) 2% CHX *vs.* control after 6 mo, 12 mo, or more of aging.

(Figure 2D). The values of the Cochran *Q* and *Z* tests were < .05, presenting a trend for the experimental group (0.2% CHX), which showed higher bond strength values than the control group, and the I^2 test was 39.7%. At the subgroup analysis, the same results were found for water storage. Yet, artificial saliva aging presented no difference between 0.2% CHX and control groups. For the subgroup analysis, there were no artificial saliva aging data.

For the fifth analysis (2% CHX *vs.* control at 12 mo of aging), 5 data sets were considered, with 4 studies included (Figure 2E). The values of the Cochran *Q* and *Z* tests were < .05, presenting a trend for the experimental group (2% CHX), which showed higher bond strength values, and the I^2 test was 0%. For water storage, the same trend was found, with a high heterogeneity (I^2 = 88%). However, for the other aging protocols, there was no statistically significant difference between groups. For the subgroup analysis, there were no artificial saliva aging data. There was no possible way to evaluate 0.2% CHX *vs.* control at 12 mo of aging because of the limited data available.

The sixth analysis combined all the aging data ≥ 12 mo (2% CHX *vs.* control at 12, 14, 18, and 24 mo of aging), and 11 data sets were considered, with 7 studies included (Figure 2F). The values of the Cochran *Q* and *Z* tests were < .05, and the I^2 test was 76%. At the subgroup analysis, for *in vivo* aging, there was a trend for the experimental group (2% CHX), which showed higher bond strength values than the control group. However, water storage aging showed a different result: no difference between 2% CHX and control groups was observed, yet the results were different according to each aging time (Figure 2F). For the subgroup analysis, there were no artificial saliva aging data. For 0.2% CHX, it was not possible to perform a similar extra analysis, because of the absence of available data.

The seventh analysis (2% CHX *vs.* control at 6, 12, 14, 18, and 24 mo of aging) was performed considering adhesive system categories as subgroups (self-etching and etch-and-rinse), and 30 databases were considered, with 18 studies included (Figure 2G). The values of the Cochran *Q* and *Z* tests were < .05, and the I^2 test was 71.8%. At the subgroup analysis, for both self-etching and etch-and-rinse adhesive systems, there was a trend for the experimental group (2% CHX), which showed higher bond strength values than the control group. For 0.2% CHX, it was not possible to perform a similar analysis, because of the absence of available data.

DISCUSSION

This review showed that the use of MMP inhibitors promoted different effects on immediate and aged bond strength values. Some *in vitro* studies have shown that MMP inhibitors, especially CHX, are capable of increasing the stability of resin-dentin adhesion through time (Figure 2). This was corroborated in the meta-analysis considering the aged bond strength values for both CHX concentrations evaluated, which presented higher values than control ones (without use of CHX) after aging. Thus, the hypothesis was rejected. The analysis for longer aging time (> 12 mo) showed that this trend did not remain for longer aging periods (Figure 2F).

For the immediate results (without aging), 2% CHX did not affect the bond strength values and was similar to the control group. In contrast, 0.2% CHX negatively affected the bond strength values compared with control (without use of CHX). The meta-analysis showed lower bond strength values for 0.2% CHX when compared with the control group for immediate results. Yet, this result was not confirmed after the aging of the samples with 0.2% and 2% CHX showing favorable results after aging. An inhibitory effect of CHX on MMPs seems to be dose dependent (Gendron *et al.*, 1999), although some studies have shown that the association between the concentration of CHX and the bond strength is apparently not clear (Collares *et al.*, 2013). A previous study showed that even low concentrations of CHX offer a desirable metalloproteinase inhibition property for MMPs 2, 8, and 9 (Gendron *et al.*, 1999), proven by meta-analysis after 6 mo of aging for both CHX concentrations.

Like the present review, other studies have suggested the use of MMP inhibitors (*e.g.*, CHX) in an effort to improve the stability of resin-dentin adhesive along time (Carrilho *et al.*, 2007a; Tjäderhane *et al.*, 2013). CHX digluconate is an effective and nonspecific MMP inhibitor (Carrilho *et al.*, 2007a; Breschi *et al.*, 2008; Loguércio *et al.*, 2009) that can be applied in different modes: (1) incorporated into the acid etching agent, which is rinsed away from the surface, (2) incorporated within the adhesive system composition, or (3) applied as a solution directly on the dentin surface after the etching, which remains in contact with the surface (the most used mode).

CHX application has been proposed and presented a less hybrid-layer degradation and a stability of resin-dentin adhesion long-term (Hebling *et al.*, 2005; Carrilho *et al.*, 2007b; Ricci *et al.*, 2010), indicating that the MMP inhibition can preserve the interface integrity, corroborating results with our meta-analysis. A previous study performed a metaregression to evaluate the influence of different variables on CHX and showed that the effect may be dependent on the adhesive system used, with the nonsimplified more stable through time than the simplified ones (Collares *et al.*, 2013). As the MMP inhibitors act on the exposed collagen, the sequential application of phosphoric acid, CHX, and an etch-and-rinse adhesive may be more effective than the self-etching adhesives. However, both categories of adhesive systems (self-etching and etch-and-rinse) showed to be benefited by the 2% CHX technique in *in vitro* studies after aging (Figure 2G). It could be hypothesized that also self-etching adhesive could be beneficial by this technique, since some CHX residual effect could remain in the dentin by its substantivity. Moreover, other aspects related to the adhesive systems composition could influence it. However, it is necessary to emphasize that (1) this analysis does not include studies that incorporated CHX on the composition of the adhesive system, (2) the adhesives were categorized just in self-etching and etch-and-rinse, and (3) it does not consider the number of steps involved. Because of these limitations, further studies should focus more on the type of adhesive system regarding the CHX action.

The present study was able to show the linearity of the use of CHX through time and summarize the *in vitro* data on the influence of CHX on the adhesion stability, which could predict the clinical behavior of restorations and give support for the clinician

on an evidence-based decision-making process. We performed a broad search and a strict selection for meta-analysis to decrease heterogeneity of the data. In several of the analyses performed, there was high heterogeneity of the data (97%), corroborating the literature showing the wide range of bond strength values comparing *in vitro* studies (De Munck *et al.*, 2012). However, that high heterogeneity occurred only for longitudinal (aging) meta-analysis, not immediate (without aging). This probably occurred because of the different types of adhesive systems used and the different types of aging methods applied. Based on this heterogeneity, subgroup analyses were performed, and a relevant result was found: aging plays a role on the heterogeneity of the data. Water storage protocol presented the higher heterogeneity, while artificial saliva and other aging methods presented lower heterogeneity.

Few studies evaluated the effect of CHX on bond strength aged in oral function conditions (*in vivo*) (Figure 2F). The most commonly used artificial aging technique is water storage. It has been shown that the use of water instead of Ca- and Zn-containing artificial saliva as an aging medium may underestimate the hydrolytic activity of endogenous dentin MMPs (Tezvergil-Mutluay *et al.*, 2010). The high heterogeneity of water storage could be explained by the different solutions used to immerse the samples (distillate and deionized water), the size of the specimens that were stored (microsticks or restored teeth), the temperature, the pH of the water, and the number of times that the solution is changed. To prevent bacterial growth during the storage period, some specific solution addition is recommended such as sodium azide, chloramine, or even antibiotics (De Munck *et al.*, 2005), and perhaps those solutions could act in a different way on the resin-dentin degradation. The temperature may also play a role. It is usually fixed at 37°C, although room temperature is also used to mimic the intraoral temperature. Thus, the high heterogeneity of water storage aging can be influenced by numerous factors, and this aspect is important when comparing such varying data among studies and when considering water storage as a gold standard aging protocol (De Munck *et al.*, 2005).

The heterogeneity could have also occurred because the included studies presented, in their majority, medium and high risk of bias, a small number of samples, and (consequently) high standard deviations and a high number of covariables, favoring the heterogeneity. This did not occur at only the 12-mo aging analysis, where a low heterogeneity was found (Figure 2E), probably because of the few papers included. It is likely that the results may have been influenced by publication bias, once negative results were probably not published or published in low-impact factor journals. Nonetheless, this aspect is present in all studies, not only *in vitro* studies. A broad search was used to try to overcome this problem, which is current in any systematic review (Gillies, 2009; Song *et al.*, 2013).

In this review, only *in vitro* data from bond strength were analyzed. However, it is important to highlight that even with the association between the clinical and laboratory results (Van Meerbeek *et al.*, 2010), the bond strength is just one factor that can directly influence the effectiveness of dentin adhesion. By reducing the metalloproteinase interference, CHX may provide better clinical performance of the restorations over time. In this context, 2 clinical trials that tested CHX in the adhesive step did not present any benefit of CHX (Dutra-Correa *et al.*, 2013; Sartori *et al.*, 2013), although more randomized clinical trials with longer follow-up times are needed.

CONCLUSION

The use of 2% CHX did not negatively affect the immediate bond strength values, and both 2% and 0.2% CHX decreased the loss of bond strength after aging, showing a beneficial effect for the stability of dentin adhesion after aging. However, this trend does not stand for longer periods of aging. There is considerable heterogeneity across different aging protocols used, and this brings some limitations to the meta-analysis approach. Both self-etching and etch-and-rinse systems showed to be benefited by the CHX *in vitro*. Because of the limited number of clinical data, further research is required to confirm the advantageous use of MMPs inhibitors.

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