Review Article

The Effect of Matrix Metalloproteinase Inhibitors on the Microtensile Bond Strength of Dentin Bonding Agents in Caries Affected Dentin: A Systematic Review

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ABSTRACT

Aims and Objectives: Matrix metalloproteinases (MMPs) cause degradation of the dentinal matrix, as they act actively on collagen fibrils, leading to their deterioration and collapse. MMP inhibitors are known to be used for the pre-treatment of human dentin before bonding. Most studies on the MMP inhibitors examined the effect of MMP inhibitors on bonding to sound dentin (SD), but few examine their effect on bonding to caries affected dentin (CAD). This systematic review aims to identify and summarize studies that have applied MMP inhibitors for pre-treatment of CAD, and examine the microtensile bond strength (μTBS), bond durability, and the mode of failure. **Materials and Methods:** A systematic review was performed using the PubMed database according to the PRISMA guidelines. A total of 785 original articles published between 2010 and 2022 were initially retrieved. Six studies were selected based on predefined inclusion–exclusion criteria, and their outcomes were extracted and analyzed. The methodological quality assessment was performed using a combined checklist that utilizes the reporting criteria mentioned in the checklist for reporting in-vitro studies guidelines and guidelines for reporting pre-clinical *in vitro* studies on dental materials. **Results:** All six studies included here showed a definitive increase of the μTBS when MMP inhibitors were applied to the CAD. The mode of failure was found to be predominantly adhesive in nature. The deviation in the values of μTBS was approximately 2–5 MPa on immediate and delayed testing. **Conclusion:** MMP-inhibiting agents could be considered for the pretreatment of teeth with CAD as a part of their tooth preparation area, thereby allowing the clinician to retain CAD and bond to the CAD without endangering the vital pulp.

Keywords: *Caries affected dentin (CAD), collagen cross linkers, microtensile bond strength (μTBS), MMP inhibitors, resin dentin bonding stability*

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Introduction

In carious lesions, matrix metalloproteinases (MMPs) present within the dentin matrix induce collagenolytic activity in addition to the collagen degradation caused by active caries. MMPs cleave collagen at a neutral pH, and their levels are seen to be

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higher in the saliva of humans with active caries than in individuals with no caries.[1]

The continued presence of these endogenous collagenolytic enzymes in the mineralized dentin, that is to be hybridized with dental adhesives, threatens both the stability and the durability of the hybrid layer.

In CAD, this threat increases manifold due to the presence of MMPs. In order to minimize pulpal injury and retain CAD as part of the tooth preparation, it is imperative to improve the hybridization of the already damaged dentin by treating it with chemicals that would prevent or, at the very least, delay the degradation of the newly formed hybrid layer.

Proanthocyanins are naturally available phytochemical bioactive agents and they serve a dual function as MMP inhibitors and collagen crosslinkers in human dentin. MMP inhibitors can be used to nullify the activity of MMPs in caries-affected dentin (CAD) and prevent further collagen degradation. This would improve the bonding stability and durability in adhesive procedures.[1-4]

It is not common practice to either retain CAD as a part of the tooth preparation or pretreatment of CAD with MMP inhibitors in the clinical situation. However, the benefits of retaining the CAD far outweigh its removal, and thus a systematic review was conducted on the ability of MMP inhibitors used as a dentin pre-treatment protocol to improve the microtensile bond strength (μTBS) in CAD during adhesive procedures. This study also evaluated the bond durability immediately and at delayed time periods, along with the mode of failure.

Materials and Methods

This systematic review was performed according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) guidelines. The registration is under the number: CRD4202124908 in PROSPERO (International Prospective Register of Systematic Reviews), at the UK's National Institute for Health Research (NHS), University of York, Centre for Reviews and Dissemination. The PICO model [Table 1] was applied to structure the research question "Is the μTBS altered by the application of MMP inhibitors when applied to affected dentin?"

Inclusion criteria

All the studies which have reported the use of MMP Inhibitors as a pre-treatment protocol applied to caries affected dentin/simulated caries affected dentin and measurement of the μTBS of the dental adhesive used were selected for this systematic review. Articles published in English Language or those having summary in English and articles published from January 1, 2010 to September 9, 2022 were included in the review [Figure 1].

Table 1: PICOS: (P) population; (I) intervention/exposure; (C) comparator; (O) outcomes; (S) study design

PICO	Included studies	Excluded Studies
1. Participants/ population	Simulated caries affected dentin (*in vitro*)	*In vivo* studies
2. Intervention	MMP Inhibitors directly applied to simulated caries affected dentin	Studies in which pre-treatment of dentin was not performed
3. Comparator/ control	MMP inhibitors directly applied to normal sound dentin or caries affected dentin/ no pre-treatment	–
4. Outcomes	An altered microtensile bond strength (μTBS) in dentin bonding agents	–
5. Others	Only original and full text studies written in English	Written in other language than English. Other article types than original (e.g., reviews, letters to editors, trial registrations, proposals for protocols etc.).

Exclusion criteria

Reviews, abstracts, letters to editors, *in vivo* studies and any *in vitro* studies done before January 1, 2010 were excluded.

Search strategy for the identification of the studies

An electronic search on PubMed/Medline was carried out on September 9, 2022 using multiple different keywords: Microtensile Bond Strength AND Dentin Bonding Agent AND Caries Affected Dentin, Microtensile Bond Strength and Dentin Bonding Agent, Microtensile Bond Strength AND Dentin Bonding Agent AND MMP Inhibitors AND Caries Affected Dentin, MMP Inhibitors AND Collagen Cross Linkers AND Caries Affected Dentin, Tooth Substrate AND Dentin AND Microtensile Bond Strength AND Caries Affected Dentin, Resin Dentin Bonding Stability AND Caries Affected Dentin AND MMP Inhibitors.

The search performed included restrictions such as language (English), publication status restrictions (Abstract), and a date limitation (2010–2022). Additionally, manual screening was performed amidst

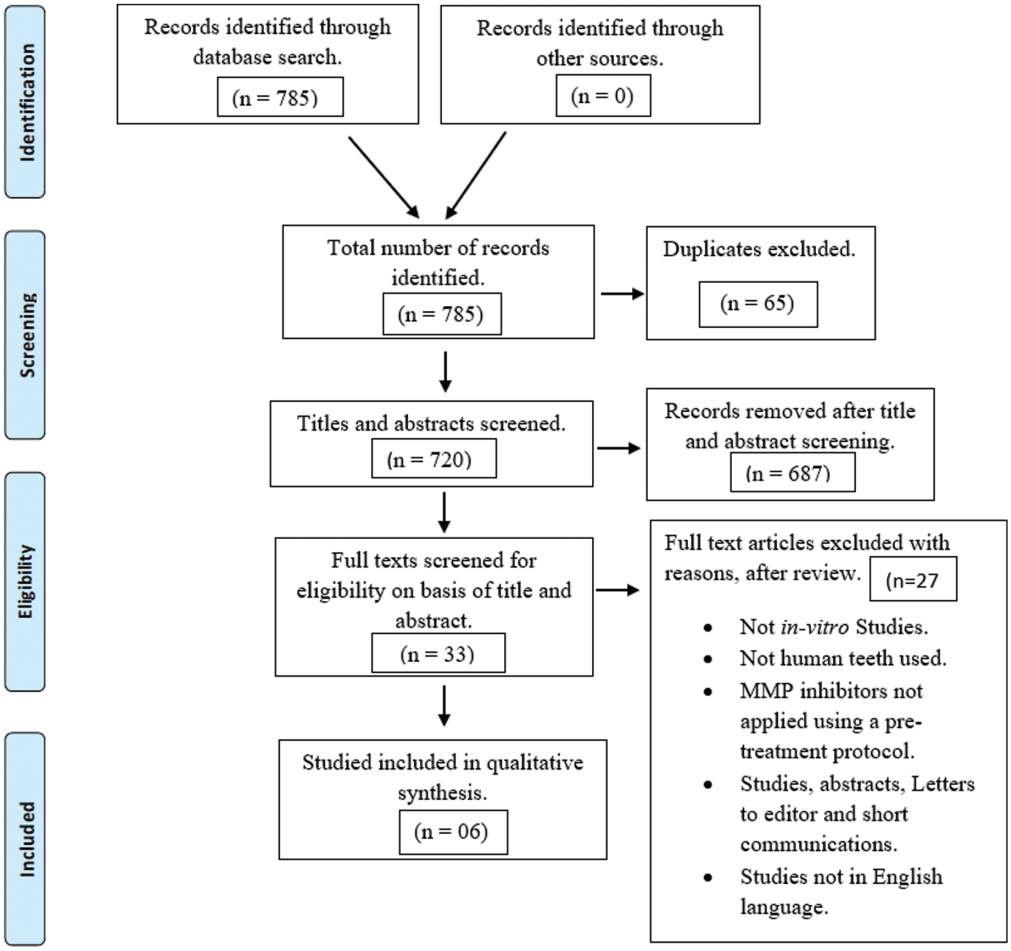

Figure 1: PRISMA flow diagram for systematic review

the references of selected articles to retrieve further relevant publications. Five authors independently performed the initial screening of the studies to check for eligibility in succession of titles and abstracts. The full text articles were then retrieved for further assessment when they met the inclusion criteria. Studies that had insufficient data were excluded. Any disputes were resolved through discussion and consensus between the two primary authors. The PRISMA flow chart [Figure 1] has been used to report the included studies according to the eligibility criteria and the excluded studies during the study selection process with reasons.

Data extraction

A spreadsheet was specifically designed in Microsoft Excel (Microsoft Corporation, Redmond, Washington) for processing the data extraction. This spreadsheet contained information about the inclusion and exclusion requirements and the reasons were detailed. The selection of studies was performed independently by five authors and in case of any disagreements, a discussion was then carried out to reach a consensus.

Risk of bias assessment

The methodological quality assessment was performed using a combined checklist that utilizes the reporting criteria mentioned in the checklist for reporting in-vitro studies guidelines and guidelines for reporting pre-clinical *in vitro* studies on dental materials. A thorough examination of the selected studies was done by the two primary authors, to examine for risk of bias [Table 2]. It was ascertained that all the *in vitro* studies selected for this review evaluated the μTBS of the adhesive when applied to SD and compared it with that of CAD. Additionally, it was confirmed that there was an intervention made by the application of MMP inhibitors. None of the studies reported random allocation of samples and only Fialho reported sample size calculation. The two primary authors agreed to include these six studies without any details about sample allocation as the other criteria were met. The study design was scrutinized to look for the use of contemporary agents and the establishment of baseline values. The statistical analysis of selected studies was

Table 2: Methodological assessment using a combined checklist[5,6]

S. No.	Reporting criteria	Lenzi *et al.*[7]	Giacomini *et al.*[8]	Carvalho *et al.*[9]	Fialho *et al.*[10]	Sanchez *et al.*[11]	Costa *et al.*[12]
1.	Title						
	a. Contains the study design "*in-vitro.*"	Yes	Yes	Yes	Yes	Yes	Yes
	b. Conveys the aims of the study.	Yes	Yes	Yes	Yes	Yes	Yes
2.	Abstract						
	a. Structured abstract.	Yes	Yes	Yes	Yes	Yes	Yes
	b. Conveys the relevant information.	Yes	Yes	Yes	Yes	Yes	Yes
3.	Introduction						
	a. Scientific background and explanation of rationale and hypothesis.	Yes	Yes	Yes	Yes	Yes	Yes
	b. Statement cite appropriately.	Yes	Yes	Yes	Yes	Yes	Yes
4.	Materials and Methods						
	a. Ethical statements as relevant.	Yes	No	Yes	Yes	Yes	Yes
	b. Sample size calculation/justification.	No	No	No	Yes	No	No
	c. Sample preparation and handling.	Yes	Yes	Yes	Yes	Yes	Yes
	d. Allocation sequence, randomization and blinding.	Yes	Yes	Yes	Yes	Yes	Yes
	e. Intervention for each group.	Yes	Yes	Yes	Yes	Yes	Yes
	f. Outcomes- Primary and secondary outcomes assessment.	Yes	Yes	Yes	Yes	Yes	Yes
	g. Statistical analysis.	Yes	Yes	Yes	Yes	Yes	Yes
5.	Results						
	a. Analysis of outcomes with appropriate illustration.	Yes	Yes	Yes	Yes	Yes	Yes
6.	Discussion						
	a. Interpretation of results.	Yes	Yes	Yes	Yes	Yes	Yes
	b. Limitation.	Yes	Yes	Yes	Yes	Yes	Yes
	c. Applicability to clinical practice.	Yes	Yes	Yes	Yes	Yes	Yes
	d. Generalizability	Yes	Yes	Yes	Yes	Yes	Yes
7.	Other information						
	a. Funding	Yes	Yes	Yes	Yes	Yes	Yes
	b. Conflict of interest.	No	No	No	No	No	No

examined to check for adequacy, and statistically significant results were confirmed.[13,14]

Results

A total of 785 original articles were initially retrieved from the above-mentioned databases, of which 65 were duplicates. A total of 720 articles were checked by title and abstract, and 687 were excluded as they did not match the criteria for inclusion in our protocol; the remaining 33 articles were assessed in full text. A total of six articles met all the inclusion criteria and were finally included in the qualitative synthesis. All the steps that were followed for the selection of the articles are available in Figure 1. The six selected studies were *in vitro* studies. The methodological quality assessment was evaluated according to the combined checklist[13,14] for reporting *in vitro* studies. This systematic review aims to analyze if there is any correlation between the application of MMP inhibitors to CAD, and the μTBS achieved by these hybridized samples mentioned.

The data related to methodological quality[13,14] of the studies are presented in Table 2. All the selected studies had a clearly stated aim, and their study design followed the protocol for non-randomized control trials, as the studies were all *in vitro*. Each study design had a finite endpoint and yielded the data necessary for this systematic review. As per the combined checklist, the authors were able to include methodologically sound studies in this systematic review.[13,14]

Table 3 represents the characteristics of the studies regarding the adhesive system used, type of intervention (MMP inhibitors), mode of failure, bond durability, and μTBS. Of the six studies included, the study conducted by Carvalho *et al.*[9] used only two MMP inhibitors as an intervention protocol. Giacomini *et al.*[8] conducted a similar study comparing a 2% CHX solution and E-64. The study conducted by Fialho *et al.*[10] also used two primary MMP inhibitors, and one of which was utilized in three different concentrations. Lenzi *et al.*[7] and Costa *et al.*[12] only compared the effects of 12%

Table 3: Study characteristics of each study comparing caries affected dentin (CAD)

Author	Adhesive system	Intervention (MMP inhibitors)	Mode of failure	Bond durability and microtensile bond strength (μTBS)
1. Lenzi *et al.*[7]	Adper™ Single Bond 2 (3M ESPE, St. Paul, Minnesota)	**a) Control**	**24 h storage in water** - Adhesive/Mixed failure (est. 72%) - Cohesive in dentin (est. 14%) - Cohesive in resin (est. 12%) - Premature failure (est. 2%)	**Control** 24 h 29.1 ± 6.0
		b) 2% CHX	**24 h storage in water** - Adhesive/Mixed failure (est. 77%) - Cohesive in dentin (est. 4%) - Cohesive in resin (est. 13%) - Premature failure (est. 6%)	**CHX** 24 h 36.4 ± 1.3
2. Giacomini *et al*.[8]	Adper™ Single Bond Universal (3M ESPE, St. Paul, Minnesota)	**a) Control**	**24 h storage in artificial saliva** - Failure mode not analyzed	**Control** 24 h 23.42 (4.95)
			After 6 months of storage in artificial saliva - Adhesive Failure (52%) - Mixed Failure (48%)	6 months 20.28 (3.55)
		b) 2% CHX (Pharmacia Specıfica, Bauru, Sao Paulo, Brazil)	**24 h storage in artificial saliva** - Failure mode not analysed	**CHX** 24 h 18.31 (3.50)
			After 6 months of storage in artificial saliva - Adhesive failure (51%) - Mixed failure (47%) - Cohesive in resin (2%)	6 months 16.50 (3.90)
		c) E-64 (Sigma-Aldrich, St. Louis, Missouri)	**24 h storage in artificial saliva** Failure mode not analyzed	**E-64** 24 h 24.51 (4.41)
			After 6 months of storage in artificial saliva - Adhesive failure (51%) - Mixed failure (47%) - Cohesive in resin (2%)	6 months 20.80 (3.71)

Table 3: Continued

Author	Adhesive system	Intervention (MMP inhibitors)	Mode of failure	Bond durability and microtensile bond strength (μTBS)
3. Carvalho *et al.*[9]	Adper™ Single Bond 2 (3M ESPE, St. Paul, Minnesota)	**a) Control**	**24 h storage in water** - Adhesive failure (63%) - Mixed (18%), - Cohesive in resin (15%) - Cohesive in dentin (5%)	**Control** 24 h 24.3 (8.6)
			After months of storage in water - Adhesive type (35%) - Mixed failures (33%) - Failure of the resin (25%) - Cohesive failure in dentin (8%)	6 months 21.6 (6.4)
		b) Green tea extract (2% GTE) (Magistral Farmácia Homeopática, Joaçaba, SC, Brazil)	**24 h storage in water** - Cohesive in dentin (45%) - Mixed (23%) - Cohesive in resin (20%) - Adhesive (13%)	**2% GTE** 24 h 23.0 (6.3)
			After 6 months of storage in water - Adhesive (35%) - Mixed (33%) - Cohesive in resin (25%) - Cohesive in dentin (8%)	6 months 35.7 (8.4)
		(c) Chlorhexidine (2% CLX) (FGM, Joinville, SC, Brazil)	**24 h storage in water** - Adhesive Type (48%) - Cohesive in resin composite (25%) - Mixed (18%) - Cohesive in dentin (10%)	**2% CLX** 24 h 23.0 (6.0)
			6 months of water storage in water - Mixed (45%) - Adhesive (33%) - Cohesive in dentin (13%)	6 months 23.0 (7.2)
4. Fialho *et al.*[10]	Adper™ Single Bond 2 (3M ESPE, St. Paul, Minnesota)	**a) Control**	**24 hours storage in water** - Adhesive failure (41.9%) - Mixed (6.5%), - Cohesive in resin (45.2%) - Cohesive in dentin (6.5%) **After 12 months of storage in water** - Adhesive type (65.5%) - Mixed failures (10.3%) - Failure of the resin (20.7%) - Cohesive failure in dentin (3.4%).	**Control** 24 h 35.81 (8.25) 12 months 26.17 (12.28)

Table 3: Continued

Author	Adhesive system	Intervention (MMP inhibitors)	Mode of failure	Bond durability and microtensile bond strength (μTBS)
		b) Chlorhexidine (2% CHX)	**24 h storage in water** - Adhesive Type (48.4%) - Cohesive in resin (29%) - Mixed (12.9%) - Cohesive in dentin (9.7%) **After 12 months storage in water** - Mixed (10.3%) - Adhesive (86.2%) - Cohesive in resin (3.4%)	**2%CLX** 24 h 33.33 (11.26) 12 months 19.98 (7.01)
		c) Epigallocatechin-3-gallate (EGCG) 0.02%	**24 h storage in water** - Mixed (26.7%) - Cohesive in resin (23.3%) - Adhesive (50%) **After 12 months of storage in water** - Adhesive (78.6%) - Cohesive in resin (14%) - Cohesive in dentin (7.1%)	**0.02% EGCG** 24 h 32.65 (9.97) 12 months 22.75 (9.38)
		d) Epigallocatechin-3-gallate (EGCG) 0.2%	**24 h storage in water** - Mixed (51.7%) - Cohesive in resin composite (17.2%) - Adhesive (31%) **After 12 months** - Adhesive (92.3%) - Cohesive in resin composite (7.7%)	**0.2% EGCG** 24 h 29.16 (11.52) 12 months 17.15 (10.61)
		e) Epigallocatechin-3- gallate (EGCG) 0.5%	**24 h storage in water** - Mixed (38.7%) - Cohesive in resin composite (25.8%) - Adhesive (35.5%) **After 12 months in water** - Adhesive (83.9%) - Cohesive in resin composite (9.7%) - Cohesive in dentin (3.2%) - Mixed (3.2%)	**0.5% EGCG** 24 h 28.57 (6.30) 12 months 23.65 (7.19)

Table 3: Continued

Author	Adhesive system	Intervention (MMP inhibitors)	Mode of failure	Bond durability and microtensile bond strength (μTBS)
5. Sanchez *et al.*[11]	Scotchbond Universal™ (3M Oral Care, St. Paul, Minnesota)	**a) Control**	**24-h storage in water** - Adhesive failure (100%)	**Control** 24 h 14.42 (4.43)
			Thermocycling - Adhesive failure (98%) - Cohesive in resin (2%)	TC 9.43 (4.29)
		b) Placebo	**24-h storage in water** - Adhesive failure (100%)	**PA** 24 h 14.42 (4.43)
			Thermocycling - Adhesive failure (96%) - Cohesive in dentin (2%) - Cohesive in resin (2%)	TC 17.11 (5.27)
		c) 6.5% Hesperidin solution	**24-h storage in water** - Adhesive failure (100%)	**HES** 24 h 18.41 (5.30)
			Thermocycling - Adhesive failure (98%) - Cohesive in resin (2%)	TC 15.73 (6.07)
		d) 6.5% Proanthocinydin solution	**24-h storage in water** - Adhesive failure (97%) - Mixed failure (3%)	**PRO** 24 h 20.66 (4.88)
			Thermocycling - Adhesive failure (94%) - Cohesive in dentin (3%) - Cohesive in resin (3%)	TC 17.20 (2.72)
		e) 6.5% Quercetin solution	**24-h storage in water** - Adhesive failure (97%) - Mixed failure (3%)	**QUE** 24 h 24.58 (4.90)
			Thermocycling - Adhesive failure (100%)	TC 12.02 (5.21)
		f) 6.5% Naringin solution	**24-h storage in water** - Adhesive failure (92%) - Cohesive in dentin (5%) - Mixed failure (3%)	**NAR** 24 h 24.64 (3.70)
			Thermocycling - Adhesive failure (100%)	TC 22.12 (2.92)
		g) 6.5% Rutin solution	**24 h storage in water** - Adhesive failure (100%)	**RUT** 24 h 26.00 (5.51)
			Thermocycling - Adhesive failure (94%) - Cohesive in dentin (6%)	TC 21.08 (4.75)

Table 3: Continued

Author	Adhesive system	Intervention (MMP inhibitors)	Mode of failure	Bond durability and microtensile bond strength (μTBS)
6. Costa *et al.*[12]	Adper™ Single Bond 2 (3M ESPE, St. Paul, Minnesota)	**a) Control**	**24 h storage in water** - Adhesive failure (37.8%) - Mixed failure (32.4%) - Cohesive in resin (5.4%) - Cohesive in dentin (24.3)	**Control** 24 h 36.3 (9.2)
			After 6 months of storage in water - Adhesive failure (41.2%) - Mixed failure (23.5%) - Cohesive in resin (14.7%) - Cohesive in dentin (20.6%)	6 months 29.6 (9.1)
			After 12 months of storage in water - Adhesive failure (29.0%) - Mixed failure (38.7%) - Cohesive in resin (9.7%) - Cohesive in dentin (22.5%)	12 months 28.1 (9.6)
		b) 2% CHX	**24 h storage in water** - Adhesive failure (66.7%) - Mixed failure (28.6%) - Cohesive in resin (4.7%)	**CHX** 24 h 39.2 (10.5)
			After 6 months of storage in water - Adhesive failure (33.3%) - Mixed failure (51.3%) - Cohesive in resin (12.8%) - Cohesive in dentin (2.5%)	6 months 40.0 (14.6)
			After 12 months of storage in water - Adhesive failure (65.8%) - Mixed failure (26.3%) - Cohesive in resin (7.9%)	12 months 36.8 (11.4)

CHX. The study by Sanchez *et al.*[11] evaluated the effects of five different flavonoids by preparing them in 6.5% solutions and using them as a pretreatment protocol to compare their effectiveness.

Discussion

This systematic review gathered information evaluating the effect that MMPs would have on the hybridization of CAD on both the μTBS and the long-term integrity of the hybrid layer. There are MMPs that have marked their presence in dentin. There is a wide gamut of collagenolytic enzyme inhibitor protocols; the use of which has shown significant resistance to the loss of bond strength, which is regularly caused by the action of the MMPs present in human dentin.

Studies that examine the hybridization of CAD are few; even lesser articles examine the effect of MMP inhibitors on CAD. Knowing that CAD forms a part of the tooth preparation, which is hybridized by dental adhesives,[15,16] it is imperative to know the effect that an MMP inhibitor would have on the morphology of the hybrid layer formed in CAD and on its durability.[17] These inhibitor protocols have also been shown to

be capable of, sustaining the bond durability when evaluated over a period of time.[18]

The studies examined in this systematic review showed an appreciable increase in the μTBS and the durability of the hybrid layer over a period for SD and CAD, albeit this increase was more statistically significant for SD. The mode of failure (adhesive) was found to be the same in five of the six studies and changed to the cohesive mode of failure only in the study by Costa *et al.*[12]

Lenzi *et al.*[7] tested the effect of CHX, on the adhesive performance of a 6th gen. bonding agent, when used on SD or CAD. They further evaluated the mode of failure of the adhesive bond. The testing done was on the adhesive bonds after 24 h water storage. The application of CHX, found by them, was seen to not have any significant effect on the immediate bond strengths of the 6th generation adhesive to either SD or CAD. An appreciable difference in bond strengths was seen b/w sound dentin (SD), and caries-affected dentin for permanent and primary teeth, with the values for SD being significantly higher than those for carious dentin. Lenzi *et al.*[7] found that the mode of failure of all their groups was primarily adhesive in nature. The use of CHX to condition the dentin, both SD and CAD, showed no change in this mode of failure.

Giacomini *et al.*[8] tested the effect of CHX and E-64 on SD, artificially simulated caries-affected dentin (ACD) and artificially eroded dentin (ERO). The SD was used as the control among the substrates, and water was used as the control among the dentin pre-treatments. Their usage of CHX as one of the pre-conditioners of dentin is in accordance with the study done by Lenzi *et al.*[7] Additionally, they have also tested E-64 (trans-epoxysuccinyl-L-leucylamido-[4-guanidino] butane) and CHX on the μTBS and the mode of failures. E-64 inhibits cysteine cathepsins, thereby, slowing down the proteolytic activity caused by these cathepsins, as observed by Vidal *et al.*[19] and Hass *et al.*[20] Giacomini *et al*.[8] used orange juice to establish an erosive challenge, and they used the protocol established by Vieira *et al.*[21] to simulate carious affected dentin. All the variables tested by them—substrate, conditioning and time of strength testing showed statistically significant results. Consistent with other studies, their results showed the lowest values for all the variables in CAD, irrespective of the pre-treatment protocol and time lapsed. E-64 was able to inhibit the degradation of bond strength over time (6 months) for both ACD and ERO. E-64 was not shown to have any effect on SD, and in fact, there was deterioration of bond strengths when E-64 was used for SD as seen at the end of 6 months. The mode of failure was predominantly adhesive and mixed in all the groups.

Sanchez *et al.*[11] tested the effect of pre-treatment application of quercetin (QUE), hesperidin (HES), rutin (RUT), naringin (NAR), proanthanocyanidin (PRO) and placebo (PLA) on CAD. The control group established was only CAD and did not include pre-treatment. They found that QUE, RUT, and NAR, at the end of 24 h showed the highest μTBS, whereas, at the end of thermocycling, it was the RUT and NAR groups that showed the highest μTBS. The control and que group had the lowest μTBS after thermocycling. Adhesive failures were the prominent mode of failure across all the groups, both before and after thermocycling. Sanchez *et al*.[11] also evaluated the nano-hardness (NH) within adhesive layer, hybrid layer (HL) and dentin. They found a statistically significant drop in the values, across all groups, after thermocycling. It was the QUE group that had the highest values (NH) at the adhesive layer, while the HES and NAR groups showed the highest values (NH) at the HL. The control group had the least values in the adhesive layer. Imaging of the hybrid layer using CLSM showed the presence of uniform HL in the control group. This hybrid layer had a high number of blisters which were absent in the usage of RUT, PRO, NAR, and PLA groups. When the HL was evaluated at the end of thermocycling, there appeared to be a lack of HL in many regions of the interface, in all the groups. The HL was markedly absent in the specimens of the QUE group. The authors concluded that a pre-treatment protocol employing certain flavonoids such as RUT and NAR could improve both the immediate and long-term bonding performance of the universal adhesive system used on CAD.

While Carvalho *et al*.[9] concluded that the use of green tea extract at a concentration of 2%, when applied subsequent to the acid etching of CAD, increased the long-term bond strength to a statistically significant level, while CHX at a similar concentration, showed a sustained bond strength. The control group, which received no intervention, showed a decrease in the μTBS. An examination of the failure mode also showed that cohesive failures dominated when examined immediately and was mostly adhesive at the 6-month.[18]

The study conducted by Fialho *et al.*,[10] used varying concentrations of EGCG, for evaluation against 2% CHX. They attributed the deterioration of the bond in CAD, to the deeper zones of demineralization occurring in CAD, post-etching, along with a poor infiltration of

the adhesive monomer.[5,22-24] The high humidity in the depths of the CAD competes with the monomer for infiltration of the demineralized zone and could cause deficient polymerization of the bonding agent and increased hydrolytic degradation of the formed bonds.

Costa *et al.*[12] evaluated the effect of CHX on the μTBS of a sixth-generation dental adhesive when applied to SD, caries infected dentin (CID) or caries affected dentin (CAD). They employed a partial caries removal technique to remove the caries infected dentin to expose CAD and then used a caries detector solution to identify CAD. After etching with phosphoric acid (PA), CHX was used for all types of dentin substrates, followed by the application of the adhesive system and composite resin restorations. μTBS showed that there was a marked difference b/w the strengths of CAD and CID regardless of the storage time or pre-treatment. In the control group, the strength was found to have deteriorated at the end of 6 months, regardless of the type of dentin. In CAD, there was no reduction in the bond strength at the end of 6 months, but there was a mild depreciation at the end of 1 year. Application of CHX, irrespective of the type of dentin, showed a decrease in cohesive failures. Examination of the hybrid layer formed within SD, CID, and CAD showed that there was degradation of the hybrid layer as time progressed irrespective of the type of substrate or application of CHX.

All the studies examined in this study used different methods to simulate caries to form CAD, but this did not seem to have any bearing on the change in the properties of the bonding layers. Any change in the properties of the bonding layer with and without the application of MMP inhibitors was examined immediately and after a delay (thermocycling). It was observed that the durability of the bonds was marginally better in CAD that had been pre-treated with MMP inhibitors. There was evidence of hybridization in the CAD, whether any pre-treatment was used, and the bonds formed here were found to have appreciable bond strengths, even though they were lesser than that achieved in SD, which was as expected.

No paper that was examined here reported a lowering of the bond strength after the application of MMP inhibitors. Carvalho *et al.*[9] did report an inadequate monomer infiltration in the CAD treated with MMP inhibitors, but the bond strengths did not show a negative trend despite it.

Conclusions

All the studies examined here showed an appreciable positive trend when pre-treatment was done with MMP inhibitors, even when used at low concentrations. It could be concluded that the application of MMP inhibitors as a pre-treatment for CAD would lead to bond stability and bond durability in tooth preparations that have included CAD as a part of the final tooth preparations. The mode of failure was found to be predominantly adhesive in nature. This shall enable clinicians to provide restorations in proximity to the vital dental pulp with better long-term outcomes and cause the least damage during the operative procedure itself. The use of MMP inhibitors could be considered when bonding to tooth preparations that have CAD as a part of their bonding surfaces.

Future study recommendations

Studies, henceforth, could evaluate the Shear bond strengths of CAD pre-treated with MMP inhibitors in addition to a detailed study of the hybrid layer that is formed in such cases, along with the evaluation of nanoleakage within the hybrid layer.

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Conflicts of interest

There are no conflicts of interest.

Authors contributions

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Ethical policy and institutional review board statement

The authors fully adhere and comply to the policies and principles of Committee on Publication Ethics (COPE). The following research was conducted in compliance with the institutional guidelines (Dr. D.Y Patil Dental College, Dr. D.Y Patil Vidyapeeth, Pune-411018, India.).

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Data availability statement

Not applicable.

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