Universidad Europea

GRADUATION PROJECT

Degree in Dentistry

MATRIX METALLOPROTEINASE INHIBITORS TO IMPROVE RESIN-COMPOSITE BOND STRENGTH

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ABSTRACT

Introduction: The durability of resin composite restoration relies heavily on the stability of the adhesive surface. One of the primary factors affecting the bond is the activation of matrix metalloproteinases (MPP), which degrade exposed collagen fibrils in the dentin hybrid layer. In response, matrix metalloproteinase inhibitors (MMPIs) have been studied to preserve the hybrid layer and maintain bond durability. **Objective:** This literature review aims to investigate the effect of MMPIs on the bond strength of resin composite to dentin, analyzing their role in preserving long-term adhesion, and assess their impact in one-step adhesive systems. **Methods:** A bibliographic search has been conducted using databases such as Pubmed and Medline Complete. The PICO model guided the selection of studies published between 2014 and 2024. After applying inclusion and exclusion criteria, 8 studies were selected for analysis. **Results:** The findings suggest that MMPIs do not significantly enhance immediate bond strength but play a crucial role is maintaining hybrid layer integrity over time. MMPIs such as chlorhexidine showed the most consistent results in preserving long-term bond strength, particularly when used with etch-and-rinse adhesives. Conversely, one-step self-etch systems may activate matrix metalloproteinase due to their acidity, limiting MMPI effectiveness. **Conclusion:** MMPIs show promise in enhancing the durability of resin-dentin bonds, though their clinical results depend on adhesive protocols. Further research is needed to confirm their efficacy in daily practice.

KEYWORDS

Dentistry; Matrix metalloproteinase; Inhibitors; Dentin; Bond strength

RESUMEN

Introducción: La longevidad de las restauraciones con composite de resina depende de la estabilidad de la interfaz adhesiva. Uno de los principales factores que compromete esta unión es la activación de las metaloproteinasas de matriz (MMP), que degradan las fibrillas de colágeno expuestas en la capa hibrida de la dentina. Como respuestas los inhibidores de metaloproteinasas de matriz (MMPIs) se han propuesto como estrategia para preservar esta capa hibrida y mantener la durabilidad del adhesivo. **Objetivo:** Esta revisión bibliográfica tuvo como objetivo investigar el efecto de los MMPIs en la fuerza de adhesión de los composites a la dentina, analizar su papel en la preservación de la adhesión a largo plazo y evaluar su impacto en los sistemas adhesivos simplificados de un paso. **Métodos:** Se realizo una búsqueda bibliográfica utilizando las bases de datos de PubMed, Medline Complete. El modelo PICO guio la selección de estudios publicados entre 2014 y 2024. Tras aplicar los criterios de inclusión y exclusión, se seleccionaron 8 estudios para el análisis. **Resultados:** Los MMPIs no mejoran significativamente la adhesión inmediata, pero contribuyen a mantener la integridad de la capa hibrida con el tiempo. MMPIs como clorhexidina fueron lo mas eficaces, especialmente en adhesivos de grabado total. En cambio, los sistemas autograbantes de un solo paso pueden reactivas las MMPs debido a su acidez. **Conclusión:** Los MMPIs representan una estrategia prometedora para mejorar durabilidad la unión resina-dentina, aunque su eficacia clínica depende del protocolo adhesivo utilizado. Se requiere más investigación clínica para validad estos resultados.

PALABRAS CLAVE

Odontología; Metaloproteinasas de matriz; Inhibidores; Dentina; Resistencia adhesiva

ÍNDICE

1. INTRODUCTION

Dental composite is part of a dentist's daily practice, used in a wide range of restorative and cosmetic procedure. They have revolutionized the aesthetic, durability, and biocompatibility of restorations, transforming both the techniques and the results of dental treatment.
Michael Buonocore introduced the acid etching technique in 1955 which marks a pivotal change in restorative dentistry (1). Etching helps expose and demineralize the dentin to improve the adhesive action. However, this process might increase the action of matrix metalloproteinases (MMPs)(1).
Matrix metalloproteinases (MMPs) were first discovered by Gross and Lapiere in 1962. They are part of a large family of enzymes that play a role in degrading the extracellular matrix(2). They also contribute to different physiological functions such as bone reconstructing, tissue repair or embryonic development (3). In the dentin, the extracellular matrix is primarily formed by collagen fibers which support the bond between dentin and the restorative material(4). During the process of tooth development, MMP contribute to the formation and mineralization of the collagen matrix. Once the dentin is mineralized, MMPs become dormant within the calcified tissue. However, exposure to acidic environments can reactivate those MMPs(3). Thus, MMPs are responsible for the hydrolysis of collagen fibers which reduce the bond strength of composite to the dentin(4).
As the knowledge increase on MMPs, different strategies to prevent their action has been developed using MMPs inhibitors(5). Generally, matrix metalloproteinase inhibitors (MMPIs) are either incorporated into adhesive, etchant or also primer. By preserving the hybrid layer, MPPIs ensure that bond strength is maintained overtime, preventing adhesive failure(6). Different MMPIs has been used such as Chlorhexidine, one of the most studied inhibitors, but also Epigallocatechin gallate or benzalkonium chloride(5).
For dentist, the aim is to restore the function, integrity and aesthetics of the tooth. This literature review is to study the use of MMPIs to improve resin strength bond.

Theoretical framework

1.1 Resin composite bonding

Since the mid-1900s, dental resin composite has redefined restorative dentistry by overcoming the limits of amalgams and metal restorations(7). They now provide an esthetically natural result while preserving healthy tooth structure(8). They are used to restore dental caries, for aesthetic restorations such as fluorosis or tooth discoloration or to close up space between tooth(9).

1.1.1 Composition

Dental resin composite consist of an organic matrix composed by resin monomers such as Bis-GMA or UDMA, initiators and accelerators used to start the polymerization process(7). These are coupled with inorganic fillers and coupling agent to bond it with the organic resin matrix (10). Color pigments has been added to the composition to match the color of the natural teeth(7).

1.1.2 Adhesion

Nowadays, the bonding of the dentin can be done through two distinct approaches: the etch-and-rinse adhesive system which eliminates the smear layer or the self-etch adhesive which maintain the smear layer as a substrate for bonding (4). During the adhesion process, a hybrid layer is formed and serves as an interface between the resin composite and the dentin. Its stability is essential for the durability of the adhesive bond (6). In the market, universal adhesives constitute the last generation. Even though, they are fabricated under the “all-in-one” concept with the one-step self-etch adhesive, the etch-and-rinse mode is also used (4,11).

1.1.2.1 *Etching*

Phosphoric acid is used as the etchant to demineralize and remove the smear layer. Depending on the dental tissue, etching time can vary between 15s and 60s. Excess acid should be eliminated by water rinse and then dry the remnant water(12).

1.1.2.2 *Priming and bonding*

First there is the etch-and-rinse technique: we demineralize the bonding area with the etchant, rinse and dry, and then apply the bonding agent to form a hybrid layer. On the other hand, the self-etch system doesn't require a separate etching step and there is a modification rather than a removal of the smear layer (13).

1.1.3 Bonding to dentin

Different physical and chemical factors can affect the adhesive interface which can compromise the durability. MMPs and cathepsin cysteine proteases influence the durability of adhesive bonding by degrading resin component through the breakdown of collagen and hydrolysis of the polymerized hydrophilic resin (4).

1.1.4 Microtensile bond strength (μTBS) testing

Microtensile bond strength is a method widely used to evaluate the bond strength of dental adhesives to tooth structures like dentin. Nowadays, it is recognized as one of the most standard and versatile bond strength test. It measures the force required to break the bond between dental adhesive and the tooth substrates (14).

1.2 MMPs

MMPs are a family of enzymes that are part of the extracellular matrix. They are part of a family dependent of zinc endopeptidases that are implicated in different physiological and pathological processes, including wound healing, tissue remodeling (15). Many cells type including endothelial cells, epithelial cells or fibroblast can produce MMPs (3).

1.2.1 MMPs in dentin

MMPs have an important role in the tooth development: studies have demonstrated that collagenase and gelatinase are present in dentin and predentin. Which underline that MMPs play an essential role in the organization phase of dentin formation and mineralization (16). Under physiological conditions, MMPs are entrapped in the extracellular matrix of the dentin in an inactive state(5). Compared to enamel bonding, the stability of the resin to the dentin

has always been a challenge. Dentin has a complex structure composed of around 70% of inorganic material, primarily hydroxyapatite crystals and about 20% of organic material which is mainly composed of collagen, and 10% of water. Therefore, the dentin present a hydrophilic and heterogeneous nature that influence the bonding of composite(17).

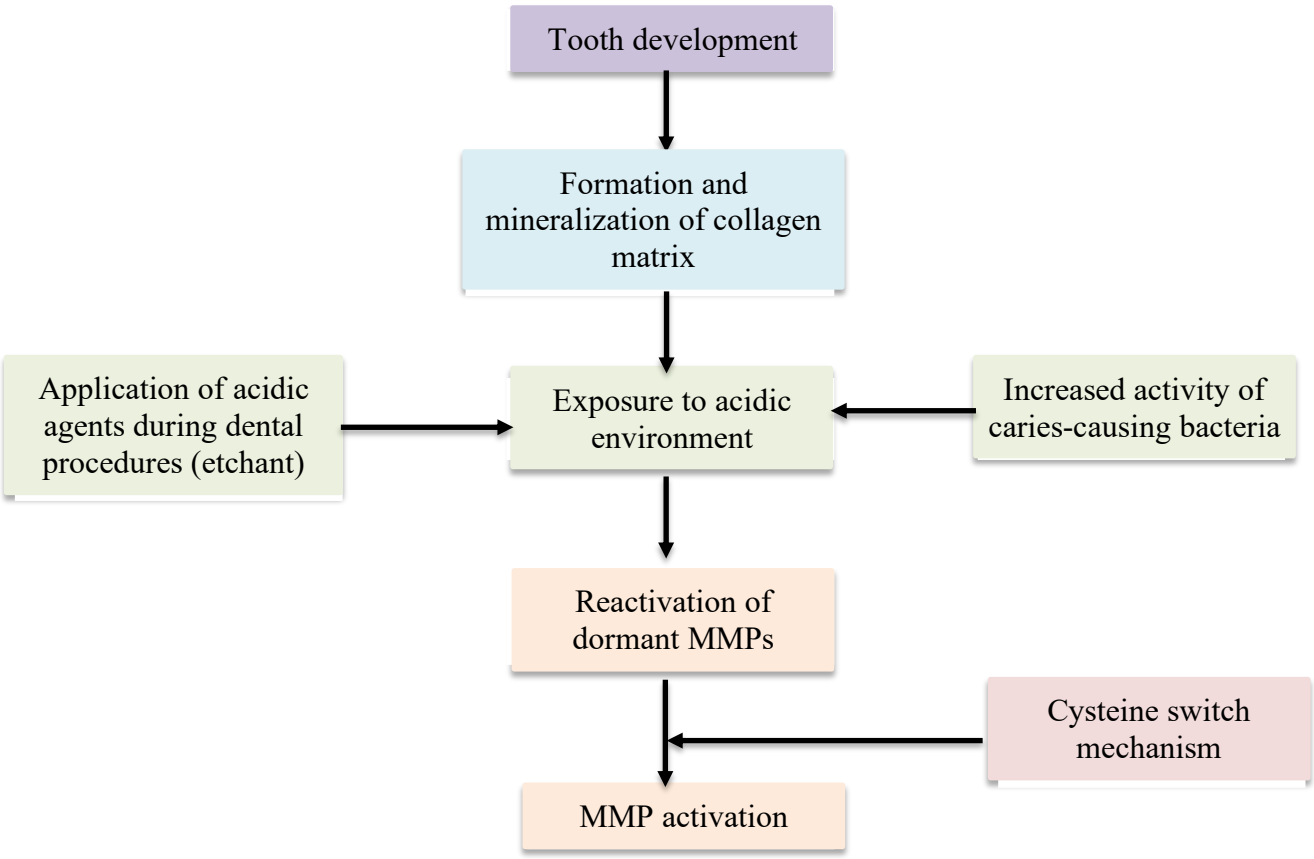


Figure 1: Mechanism of activation of matrix metalloproteinases in human tooth(3).

1.2.2 Degradation of the resin composite bond strength

Acid etching provoke a low pH, which activates the MPPs that are now responsible for the degradation of components of the extracellular matrix, mostly collagen type I(5,17). According to Nakabayashi, who introduced the notion of the hybrid layer, “the dentinal peptides (including collagen), should remain mineralized and not be decalcified. Additionally if the acid is too strong, it may expose collagen below the hybrid layer leaving a weakened dentin that is more vulnerable to long term degradation”(3). In other words, the use of phosphoric acid creates a demineralized surface that expose the organic matrix of the dentin. Then, this surface must be fully infiltrated by monomers provided by the adhesive system: this will create

the hybrid layer. However, when some collagen fibrils are unprotected, caused by an insufficient monomer infiltration, the interface is more susceptible to the activity of MMPs(5). The enzymatic degradation of the collagen by leads to a reduction in the adhesion of resin composites to dentin, resulting in a significant decrease in the microtensile bond strength(18).

1.3 MMPIs

Different natural or synthetic MMPIs has been experimented for dentin bonding to reduce enzymatic activity and preserve the hybrid layer. The aim of these agents is to enhance the strength and longevity of the adhesive surface(11).

1.3.1 Mechanisms of action

MMPIs are used to counteract the degradation of the of the collagen matrix within the hybrid layer. MMPIs function through several mechanisms of action to maintain the integrity of the resin-dentin bond. Some block the enzymes directly, while others strengthen the collagen structure, making it harder to break down. Thanks to these actions, MMPIs can help preserve the bond for a longer period, especially in situations where the restoration is exposed to moisture, acidity or mechanical stress (5).

1.3.1.1 *Inhibition by chelating*

To inhibit MMP activity, the agent act by chelating the zinc ion from the active site. (5) MMPs rely on zinc ion in their active site to catalyze the degradation of the collagen matrix. Effective MMPIs must contain a functional group, such as carboxylic acid capable of chelating the active site of the MMP molecules(19). By chelating the zinc ion, the MMPIs disrupt the catalytic mechanism of the enzyme, rendering it inactive. (5)

1.3.1.2 *Collagen crosslinking*

This process improves the structural and mechanical properties of dentin collagen by creating additional bonds between collagen fibrils. It stabilizes the collagen matrix and increases the resistance to enzymatic degradation. Natural MMPIs such as proanthocyanidins or epigallocatechin gallate (EGCG) have this properties(20).

1.3.1.3 *Competitive inhibition*

Another mechanism to inhibit the action of MMPs is through competitive inhibition where inhibitors bind to the active sites of the collagen molecule(21). When the MMPIs bind to the active site, it blocks the enzyme's ability to bind with collagen. This prevents the enzyme from cleaving the substrate and therefore inhibits its enzymatic function(5).

1.3.1.4 *Hydrophobic coating*

Certain MMPIs have the ability to create a protective, hydrophobic layer around collagen fibers or the adhesive interface, therefore preventing the degradation of the collagen matrix by MMPs. The hydrophobic layer reduces the hydrophilic nature of the collagen fibril, limiting the ability to absorb water(22).

1.3.2 Natural MMPIs

Natural MMPIs are usually derived from plants, and often work by promoting collagen cross-linking, providing an antioxidant effect. They are often used for their high compatibility and lower toxicity. However, their standardization for clinical use can be more complex(3).

1.3.2.1 *Epigallocatechin-3-gallate (EGCG)*

EGCG is a polyphenolic compound that can be found in green tea. This polyphenol can stabilize the collagen chain by promoting additional crosslinks between collagen fibrils, which helps reduce collagen degradation(5). Different studies have shown an improvement of stability and consistency of the bond strength when EGCG was added into the adhesive system(3).

1.3.2.2 *Proanthocyanidin (PA)*

Proanthocyanidin is a flavonoid typically found in grape seeds, pine bark and apples. Apart from having the ability to cross-link collagen fibrils, it is also known for their antioxidant properties. Which reduce collagen matrix from oxidative stress. In addition, this flavonoid has gained attention for his antimicrobial and anti-inflammatory properties(23).

1.3.3 Synthetic MMPIs

Synthetic MMPIs are chemically manufactured agents designed to target and reduce the activity of MMPs. Therefore their action is more reliable and predictable but have more risk of cytotoxic effects(3).

1.3.3.1 *Chlorhexidine*

Chlorhexidine (CHX) is an antiseptic compound commonly used in dentistry for its antimicrobial and antibacterial properties. CHX acts by chelating zinc ions, therefore preserving the integrity of the demineralized collagen matrix. It is capable of inactivating all dentinal MMPs even at low concentration such as 0,02%. Several studies show that CHX improves long-term stability of resin-bonds by reducing hydrolytic degradation(3).

1.3.3.2 *Galardin*

Galardin is a broad spectrum MPPIs, that act specifically by Zinc chelation located in the catalytic domain. Galardin inhibits a wide range of MMPs, making it effective against multiple enzymatic pathways of collagen degradation. This MMPIs shows potential as a clinically relevant option for stabilizing the hybrid layer. However, further research is needed to develop products with longer-lasting effects(11).

1.3.3.3 *Ethylenediaminetetraacetic acid (EDTA)*

EDTA is a chelating agent that binds to metal ions such as calcium and zinc, which are essential for MMP activation. It is commonly used as a dentin conditioner because of its ability to remove the smear layer, expose collagen fibrils, improve adhesive penetration into dentinal tubules and inhibits MMPs. Unlike CHX, EDTA modifies the dentin substrate without interfering with adhesive chemistry, making it a stable long-term option for MMP inhibition(5).

2. OBJETIVE

2.1 General objective.

To investigate the effect of matrix metalloproteinase inhibitors on bond strength of resin composite to dentin

2.2 Specific objective.

- To analyze the bond strength durability of resin composite using MMPI
- To study if there is a reduced risk of collagen degradation by MPPs using one step adhesive systems.

3. MATERIAL AND METHODS

3.1 Methodology

The bibliographic review was conducted using this following:

3.1.1 Data bases

This study was carried out using the online library of the University Europea de Madrid, PubMed and Medline Complete to find publications related to MMPIs.

3.1.2 Research question

This research is a literature review that has been conducted using a PICO question: are the MMPIs useful to improve resin composite bond strength in restored teeth using one step adhesive system?

Table 1. Pico Model of The Bibliographic Research

PICO ELEMENTS	KEYWORDS
P (Population or Patient)	Teeth restored with composite
I (**I**ntervention)	Use of MMPIs in adhesive protocol
C (**C**omparison)	No use of MMPIs
O (**O**utcome)	Improved composing resin bond strength

3.1.3 Research methods

The initial investigation focused on the topic of "matrix metalloproteinase inhibitors dentistry" to gain a broad understanding of the subject. 2147 articles were found (PubMed= 1015, Medline complete= 1132) without applying any filters.

To narrow down the results, Boolean Operators were used to incorporate additional keywords, resulting in the following search equation: ((matrix metalloproteinase inhibitors) AND (dentin)) AND (bond strength) AND (adhesive). This reduced the number of articles to 154. (PubMed= 69, Medline complete= 85)

From these 154 sources, a selection process was applied based on inclusion and exclusion criteria. Duplicate articles were removed, followed by an evaluation of the relevance of titles and abstracts. Finally, the full texts of the selected articles were assessed for eligibility and relevance to the research topic.

3.1.4 Timeframe

Throughout the bibliographic research, the articles have been chosen from 2014 to 2024. Focusing on the last 10 years ensures that the information follow the current standards and protocols.

3.2 Criteria selection

Table 2. Inclusion and exclusion criteria applied for this research

Inclusion criteria	Exclusion criteria
- Articles published between 2014 and 2024	- Articles published more than 10 years ago
- Articles available in English, Spanish or French	- Articles published in another language than English, Spanish or French
- Full article available	- Full article not available
- Title and abstract containing relevant content regarding our subject	- Title and abstract with irrelevant content regarding our subject
- Experimental studies	- Systematic review
	- Meta-analysis review

4. RESULTS

4.1 Flowchart

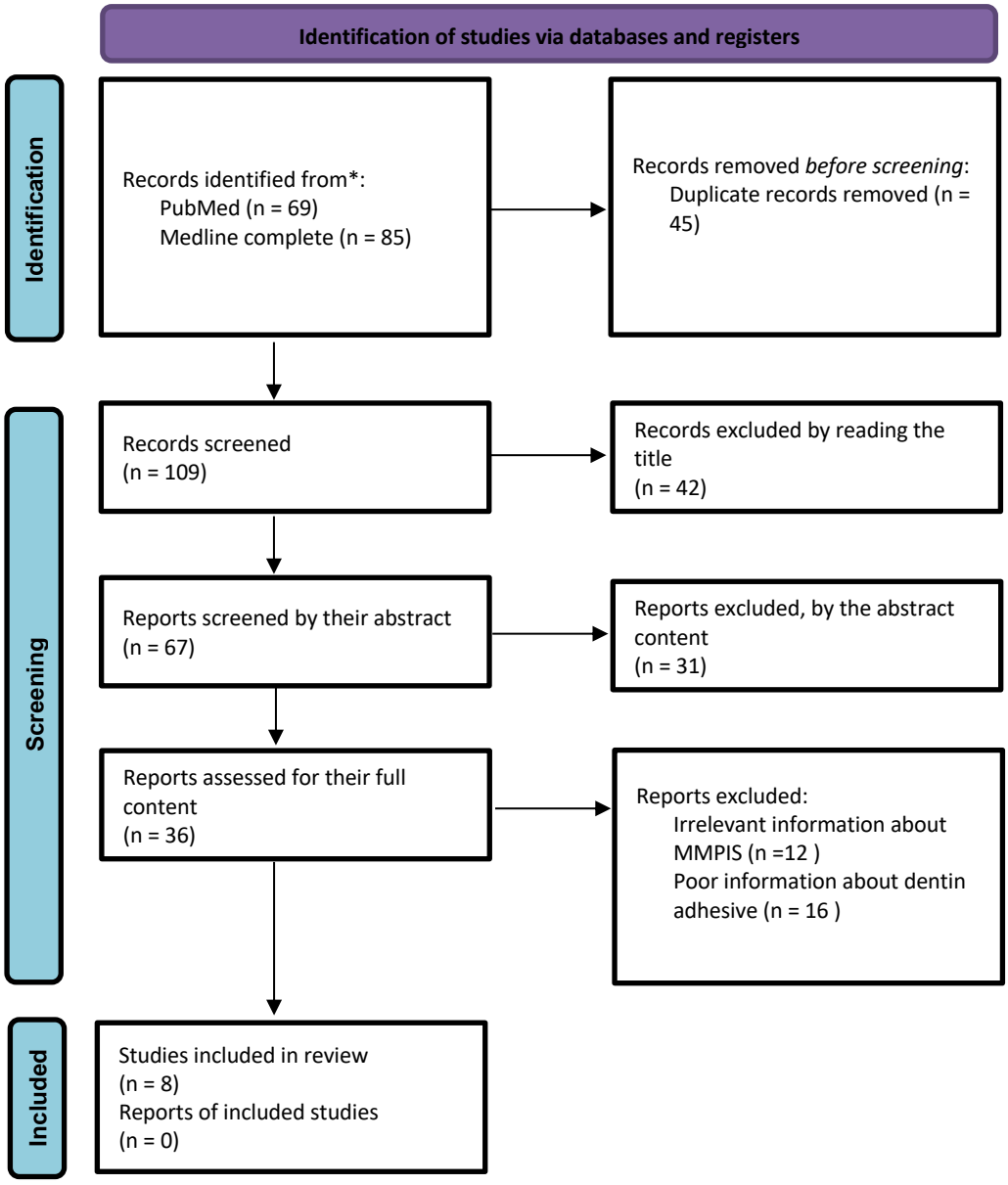


Figure 2. Flowchart of bibliographic research

4.2 Result tables

Table 3. **Studies collection about resin bond strength using MMPIs**

Authors, Years, Title and Type of study	Samples (n) and Groups (G)	Objectives and interventions	Significant findings
Sabatini C. et al. 2015 (24) Aging of adhesive interface treated with benzalkonium chloride and benzalkonium methacrylate Experimental studies	n = 35 G1: Control, using All-bond universal G2: 0,5% Benzalkonium chloride (BAC) G3: 1% BAC G4: 2% BAC G5: 0,5% Benzalkonium methacrylate (MBAC) G6: 2% MBAC G7: 2% MBAC(24)	Evaluate the long term stability of resin-dentin bonds by incorporating MMPIs (BAC and MBAC) into a adhesive system and measuring microtensile bond strength over time (at 24h, 6 months and 1 year) Determine if BAC can inhibit the activity of MMPs(24)	Table 3A. Mean micro-tensile bond strengths (μTBS) in MPa of BAC and MBAC at 24h, 6 months and 1 year of storage Study group / 24h / 6 months / 1 year ABU: 29,4 / 16,4 / 15,3 0,5% BAC: 30,6 / 31,4 / 30,1 1% BAC: 31,3 / 29,9 / 33,5 2% BAC: 29,1 / 27,5 / 25,2 0,5% MBAC: 25,8 / 28,7 / 32,6 1% MBAC: 26,0 / 27,4 / 32,0 2% MBAC: 30,5 / 28,8 / 30,2 A high microtensile bond strength value indicates that the adhesive interface between resin and dentin is strong. (24)
Tekce N. et al. 2016 (18) Do matrix metalloproteinase inhibitors improve the bond durability of universal dental adhesive	n = 70 G1: Single bond universal, self-etch mode G2: single bond universal, etch-and-rinse G3: single bond universal,	To determine whether incorporating MMPIs can improve the immediate and long-term microtensile bond strength of two universal dentin bonding agents: single bond universal and all bond universal To assess the effects of these interventions on the	Table 3B. Mean microtensile bond strength (MPa) Adhesive / Group / 24h storage / 12months storage Single bond universal: Self etch / 36,09 / 34,81 Etch-and-rinse / 43,33 / 37,67

Experimental studies	etch-and-rinse with 1% BAC G4: single bond universal etch-and-rinse with 2% Chlorhexidine G5: single bond universal, etch-and-rinse with 0,5 EDTA G6: all bond universal, self-etch mode G7: all bond universal, etch-and-rinse G8: all bond universal etch-and-rinse with 1%BAC G9: all bond universal, etch and rinse with 2% Chlorhexidine G10: all bond universal, etch-and rinse with 0,5 EDTA (18)	adhesive)dentin interface using scanning electron microscopy after 24hours and after 12 months of water storage (18)		Etch-and-rinse with 1% BAC	45,55	35,07
				Etch-and-rinse with 2% CHX	45,22	41,19
				Etch-and-rinse with 0,5 EDTA	43,60	43,97
			All bond Universal	Self-etch	38,68	30,07
				Etch-and-rinse	43,81	38,54
				Etch-and-rinse with 1% BAC	46,59	39,51
				Etch-and-rinse with 2% CHX	38,92	31,37
				Etch-and-rinse with 0,5 EDTA	49,29	38,13
			(18)			
Almahdy A. et al. 2015 (25) An MMP-inhibitor modified primer enhaces bond durability to carious dentin	n = 24 G1: Optibond FL (OB) primer and bond applied following manufacturer's instructions G2: Precontitioning of caries-	Determine whether incorporating a MPPI, Batimastast, into the primer of a three step etch-and-rinse adhesive system could reduce the MMP activity within caries-affected dentin and modify the chemical composition of the caries-affected	The in situ zymography revealed that BB94 lead to an inhibition of MMP activity. When applied as a precondition agent (group 2), it achieved up to a 93,3% inhibition, while incorporating BB94 directly into the primer (group 3) resulted in around 80% inhibition compared to the control (group 1) Raman micro-spectroscopy showed a 33% increase in resin infiltration with			

<table>
<tr><td>Experimental study</td><td>affected dentin with 500 μM Batimastat (BB94) before OB

G3: OB primer modified to contain 5 μM BB94 prior to bond application</td><td>dentin adhesive surface

MMP activity is assessed by in situ zymography and chemical changes at the interface were evaluated using Raman micro-spectroscopy (25)</td><td>the hybrid layer in the BB94-treated groups.

This study indicated that modifying the adhesive prime with the MMPI BB94 can enhance bon durability to caries-affected dentin by reducing enzyme degradation and promoting better resin incorporation into the hybrid layer(25)</td></tr>
<tr><td>Zheng P. et al. 2017 (23)

Evaluate the effect of different MMPS inhibitors on adhesive physical properties of dental adhesives, bond strength and MMP substrate activity

Experimental study</td><td>n = 160

G1: 2% chlorhexidined igluconate (CHX)

G2: 2% doxycycline solution

G3: 5% Proanthocyanidin (PA)

G4: control group</td><td>Evaluate and compare the effect of different MMPIs (CHX, DOX and PA) on the adhesive physical properties of dental adhesives, including bond strength and MMP substrate activity

All dentin blocks were etched with 37% phosphoric acid for 15secs
Immediately after etching, each group received its respective treatment (CHX, DOX, PA)
Then a resin adhesive was applied and then restored with composite resin

Tooth were sectioned and subjected to μTBS testing to determine bond strength and failure method

Field Emisssion Scanning Electron Microscopy (FESEM) and immunolabeling were used to assess the quality and</td><td>Table 3C. Mean tensile bind strength (MPa)
<table><tr><th>Group</th><th>Storage time</th><th>Mean (mpa)</th></tr><tr><td>CHX</td><td>24h</td><td>47,91</td></tr><tr><td>CHX</td><td>3 months</td><td>42</td></tr><tr><td>DOX</td><td>24h</td><td>32,11</td></tr><tr><td>DOX</td><td>3 months</td><td>30,76</td></tr><tr><td>PA</td><td>24h</td><td>28,98</td></tr><tr><td>PA</td><td>3 months</td><td>27,91</td></tr><tr><td>Control</td><td>24h</td><td>28,11</td></tr><tr><td>Control</td><td>3 months</td><td>27,89</td></tr></table>
Failure modes: the CHX group showed a mix of adhesive and cohesive failures, indicating improved interfacial integrity compared with the control, which exhibited 100% adhesive failure.

MMP inhibition: all MMP inhibitors treatments reduced MMP I and II activity compared to the control, with CHX having the most inhibitory effect

Micro-permeability: although variations in dye penetration were observed, differences among the groups were not significant (23)</td></tr>
</table>

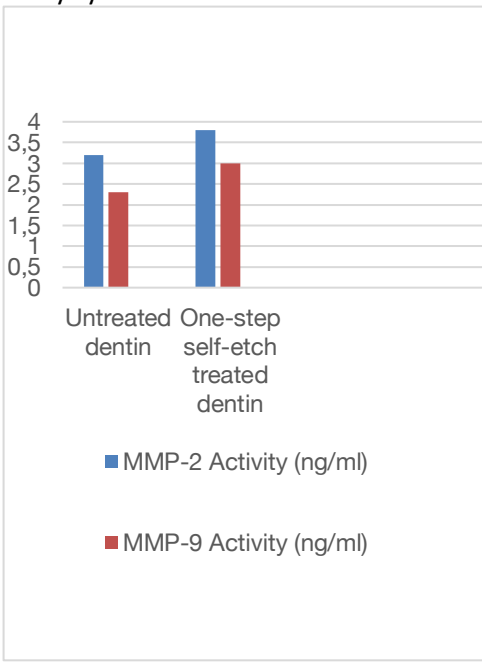
		density of the dentin hybrid layer Dye penetration tests evaluated the integrity of the hybrid layer Zymographic assays were performed to measure MMP activity after inhibitor application (23)	
Costa Perote L. et al. 2015 (26) Effect of Matrix Metalloproteinase-inhibiting solutions and aging methods on dentin bond strength Experimental study	n = 105 G1: control (CG) G2: 0,2% aqueous chlorhexidine solution (CHX) G3: 10% ethanolic propolis extract (EPE) G4: aqueous propolis extract (APE) G5= 70% ethanol Each group was subdivided into three aging subgroups: SI: sectioned and tested immediately S: stored in artificial saliva for 6 months at 37°C	To determine whether applying MMP-inhibiting solutions after acid-etching affects resin-dentin bond strength Dentin surfaces were etched with 37% phosphoric acid for 10s, rinse for 15s In the experimental groups, the respective solution was actively applied for 60s immediately after etching, then excess was removed before adhesive application All groups were bonded with Adper Single Bond 2 and restored with a resin composite The bond strength data were statically analyzed using two way ANOVA and Tuley's post-hoc tests (26)	Table 3D. Mean bond strengths (MPa) for all experimental groups Group / Aging method SI / S / T CG 28,6 24,0 25,4 CHX 31,6 26,5 27,0 EPE 29,1 23,1 25,8 APE 33,0 25,1 26,2 E 33,1 23,5 29,5 (26)

<table>
<tr><td></td><td>T: subjected to thermomechanical aging</td><td></td><td></td></tr>
<tr><td>Apolinio FM et al. 2017 (27)

Effect of a one-step self-etch adhesive on endogenous dentin matrix metalloproteinases

Experimental study</td><td>n = 18

G1: mineralized dentin powder without treatment

G2= mineralized dentin powder treated with the one-step adhesive Adper Easy Bond</td><td>To assess the impact of a one-step self-etch adhesive system on the activity of dentinal MPP (MMP-2 and MMP-9) using in situ zymography and enzymatic assays. As a negative controls, EDTA (250mM) and 1,10-phenanthroline (2mM) were used.(27)</td><td>Figure 3A. Expression of MMP-2 and MMP-9 activities using Biotrak activity assay system
 
</td></tr>
<tr><td>Montagner A. et al. 2015 (28)

Effect of Pre-treatment with Chlorhexidine on the retention of restorations: a randomized controlled trial

Randomized controlled clinical trial</td><td>n = 169

G1: (control group) placebo solution before adhesive application

G2: 2% CHX applied after etching before adhesive</td><td>To evaluate the effect of 2% chlorhexidine application after acid etching on the retention of restorations places on non-carious cervical lesions

Evaluations periods: 1 week and 6 months</td><td>Table 3E. Comparison between the treamtens considering the restoration remaining after 6month. Numbers separated by slash represent the number of evaluated restorations for each score, according to the FDI criteria: 1. Clinically excellent, 2. Clinically good, 3. Clinically sufficient/satisfactory, 4. Clinically unsatisfactory, 5. Clinically poor
<table>
<tr><th>Criteria evalutated</th><th>G1 restoration within each score</th><th>G2 restoration within each score</th></tr>
<tr><td>Fracture</td><td>80/1/0/0/0</td><td>87/1/0/0/0</td></tr>
<tr><td>Retention</td><td>81/0/0/0/3</td><td>88/0/0/0/3</td></tr>
<tr><td>Marginal adaptation</td><td>26/47/7/1/0</td><td>39/42/7/0/0</td></tr>
<tr><td>Patient perception</td><td>66/12/2/0/0</td><td>74/13/1/0/0</td></tr>
</table></td></tr>
</table>

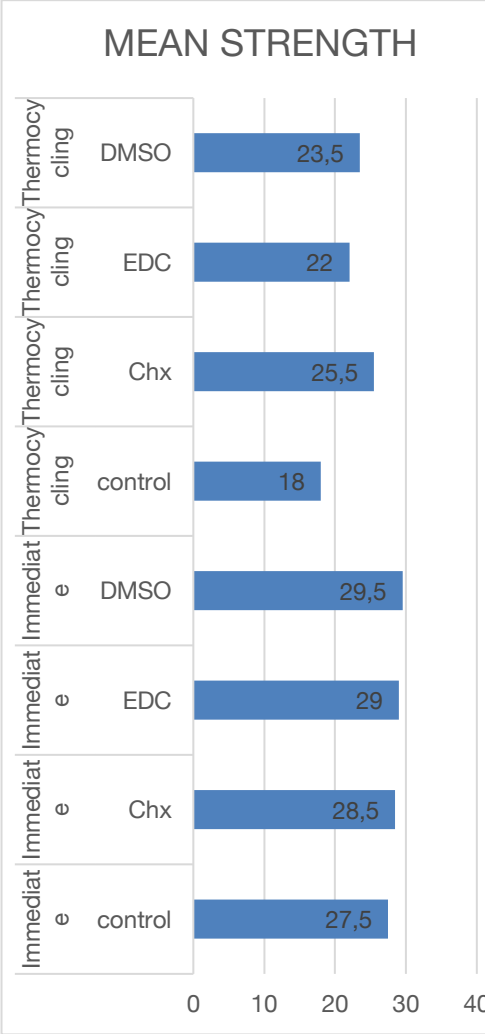
Saffarpour A. et al 2020 (4) Effect of matrix metallopro teinase inhibitors on microtensi le bond strength of dental composite restoratio ns to dentin in use of an etch-and-rinse adhesive system Experimen tal study	n = 88 G1: (control group): no inhibitor, adhesive applied directly G2: 0,2% Chlorhexidine G3: 0,3M Carbodiimide G4: 5% Dimethyl Sulfoxide applied	To assess and compare the effects of three MMPIs on microtensile bond strength of composite restorations to dentin. To determine whether these inhibitors improve resin-dentin bond durability after thermocycling (which simulated aging in a clinical environment) Bonding procedure: - Etch-and-rinse adhesive system: Adper Single Bond 2 (3M ESPE) - Composite resin build-up: Filtek Z350 (3M ESPE) Testing conditions: - Immediate µTBS testing - Post-thermocycling µTBS testing	

Figure 3B. Comparaison of mean microtensile bond strength of composite to dentin with and without thermocycling.

4.3. Analysis of the result

In the study of **Sabatini C.** where 35 extracted human molars teeth were used to create dentin specimens. The adhesive system was modified adding 0,5%, 1% or 2% of benzalkonium chloride and benzalkonium methacrylate, known for their MMP-inhibitory properties. Samples were subjected to microtensile bond strength testing using a universal testing machine. 15 additional molars were incubated in SensoLyte substrate to assess the total MMP activity. Bond strength comparisons showed that control group had a significant degradation over time, while BAC and MBAC-treated group maintained stable bond strength. MMP activity inhibition was dose-dependent, with 1,0% BAC reducing MMP activity by 54%(24).

Similar to the previous study, **Tekce N.** evaluated the effect of MMPIs such as BAC, Chlorhexidine and EDTA, on the microtensile bond strength and durability of two universal adhesives, Single Bond Universal and All Bond Universal. In addition to the microtensile bond strength testing, scanning electron microscopy analysis were performed, to confirm that resin penetration and hybrid layer stability were better in MMPIs groups(18).

Zheng P. used Chlorhexidine, Doxycycline and Proanthocyanidin as MMPIs to evaluate adhesive physical properties, bond strength and MMP substrate activity. With 160 extracted third molars, tests were performed at 24h and 3 months, including microtensile bond strength, scanning electron microscopy, immunolabeling and Rhodamine B dye penetration technique. Furthermore, zymography and fluorescence assays were used to measure MMP-1 and MMP-2 activity. This study successfully demonstrated that MMPIs enhance the durability of resin-dentin bonds, with CHX being the most effective(23).

Except from Chlorhexidine, different MMPIs were used for the study of **Costa Perote L**.: Ethanolic Propolis Extract, Aqueous Propolis Extract and Ethanol. 105 human molars were subjected to different aging conditions to stimulate long-term clinical degradation. (26) Similarly**, Saffarpour A**. used different MMPI such as Ethyl dimethylaminopropyl Carbodiimide and Dimethyl Sulfoxide to evaluate the microtensile bond strength when using an etch-and-rinse adhesive. 44 tooth were subjected to thermocycling to simulate one year of intraoral aging. In both studies, there were no significant differences between groups at 24h but after aging, CHX group had the highest bond strength. (4)

Almahdy A. focused his study on Batimastat, another MMPI, while using a three-step etch-and-rinse adhesive system. In situ zymography was performed to measure MMP activity and Raman micro-spectroscopy was used to analyze resin penetration and hybrid layer composition. Samples were observed using a confocal laser scanning microscope to detect MMP activity: Fluorescently labeled collagen substrate (FITC) fluorescence signals indicated active MMP degradation of collagen. Two-way ANOVA and Tukey's post hoc test were used to compare: effect of Batimastat on bond strength and chemical composition, and changes in hybrid layer properties over time. (25)

In the study conducted by **Montagner A.**, Chlorhexidine was the only MPPI studied. In this clinical trial, each patient had a non-carious cervical lesions treated with CHX and the other

with a placebo solution, in a split mouth design. Restorations were evaluated at 1 week post treatment and after 6 months using FDI World Dental Federation criteria. Retention was the primary outcome, meaning failure occurred if the restoration debonded. It was analyzed using Fisher's exact test. Chi-square tests and two-way ANOVA were used to assess the impact of: CHX application, cavity shape and depth on restoration failure and other patient-related factors(28).

Apolonio FM. Investigated the effect of a one-step self-etch adhesive on endogenous dentin MPPs, specifically MMP-2 and MMP-9. The enzymatic activities of MMP-2 and MMP-9 were measured using Biotrak activity assay systems. Eight additional extracted molars were used to analyze MMP activity directly in the hybrid layer using confocal laser scanning microscopy. In this experiment, EDTA , which is a known MMPI, was used as a negative control to confirm that any fluorescence observed in treated specimens was due to MMP activation rather than non-specific gelatin hydrolysis(27).

5. DISCUSION

5.1 Role of MMPI in improving resin bond strength

MMPIs have been proposed as a strategy to enhance resin-dentin bond durability by preventing enzymatic degradation of collagen fibrils. However, while MMPI application helps maintain bond integrity, its effectiveness varies across studies depending on various factors, such as the type of adhesive, aging method and experimental condition. Montagner et al. found that Chlorhexidine-treated groups showed higher bond strength retention after aging(28). Additionally, the type and concentration of the of the MMPI used also appear to significantly influence the effectiveness. For instance, Saffarpour et al. show that CHX at a concentration of 2% is more effective than lower concentration such as 0,2%. Higher concentration allow for deeper penetration into demineralized dentin and more sustained MMP inhibition(4).

Other MMPIs such as EDC and Proanthocyanidins provided long lasting stabilization of the hybrid layer, particularly when used with etch-and-rinse adhesive. Sabatini et al. demonstrated that BAC and MBAC treated adhesive maintained stable bond strength, supporting the idea of incorporating MMPIs directly into adhesive formulations rather than applying them separately(4).

Despite these findings, some studies such as Costa Perote et al. found that MMPIs did not significantly prevent bond strength after long-term aging, raising concerns about their real clinical impact(26).

5.2 Effect of MMPI on immediate vs long-term bond strength

5.2.1 Immediate bond strength

Most studies agree that MMPIs do not significantly improve immediate bond strength. Tekce et al. found no significant difference in immediate bond strength across different adhesive systems (Single Bond Universal and All Bond Universal) treated with different MMPI such as CHX, EDTA and BAC)(18). Similarly, Costa Perote et al. reported that CHX, EPE and APRE did not immediately enhance the bonding properties of resin adhesives(26).

However, Zheng et al. observed that CHX significantly increased immediate bond strength: it exhibited better resin infiltration into dentin tubules and fewer adhesive failures, suggesting a stronger resin-dentin interface(23).

5.2.2 Long-term bond strength

While MMPI may not enhance immediate bonding, many studies suggest they contribute to long-term bond durability. Sabatini et al. found that MBAC-treated adhesive provided sustained MMP inhibition, preventing enzymatic degradation and ensuring better long-term durability(24). Similarly, Almahdy et al. reported that BB94, a potent synthetic MMPI, achieved 80% inhibition of MMPs when incorporated into the adhesive primer, leading to significantly improved bond retention in caries-affected dentin, where MMP activity is higher(19). Saffarpour et al. further confirmed that CHX-treated dentin retained the highest bond strength after thermocycling, reinforcing its protective role in hybrid layer integrity. (4)
However, not all MMPIs exhibit the same long-term effectiveness; Tekce et al. found that while BAC-treated adhesive initially improved bond strength, their effect diminished after 12 months, suggesting that some MPPIs may leach out over time, reducing their protective function(18).

5.3 Influence of MMPI on different adhesive system

Montagner et al. found that etch-and-rinse adhesive treated with CHX had significantly higher bond retention after aging. This is explained because etch-and-rinse adhesives fully remove the smear layer and expose more collagen fibrils during acid etching. This collagen fibrils left exposed are more vulnerable to degradation by MPPs, making MMP inhibition more effective in this system. Since CHX and other MMPIs can penetrate deep into exposed collagen, they are able to prevent enzymatic degradation and slow down bond deterioration over time. (28).
On the contrary, one-step self-etch adhesive activate MMP-2 and MMP-9 within the hybrid layer. This happens because self-etch adhesive are acidic which is strong enough to activate latent MMPs in dentin. Unlike etch-and-rinse adhesive, self-etch adhesive do not fully remove the smear layer, meaning MMPs remain trapped within the hybrid layer. Consequently, collagen fibrils are less infiltrated by resin and are more exposed to enzymatic degradation. (27)

5.4 Clinical relevance and limitation of MMPI use

While in vitro studies provide valuable insights into the mechanisms and potential of MMPI, their clinical translation remains limited by real-world variables. Factors such as restoration site, cavity configuration and occlusal loading can significantly influence the long-term success of resin-dentin bonds. For example, non-carious cervical lesions often subjected to flexural

stress and poor enamel margins, may respond differently to MMPIs compared to occlusal restorations. Additionally, operator variability, including adhesive handling, application time, curing protocol can affect the MMPI result. Even when MMPIs are used correctly, the oral environment presents challenge such as humidity, salivary enzyme or thermal fluctuation which may compromise the durability of the adhesive interface. (28)

6. CONCLUSIONS

- MMPI such as chlorhexidine, EDC, OR BB94 do not significantly enhance immediate bond strength. However, they play an important role in preserving the hybrid layer and maintaining bond durability overtime.

- The effectiveness of MMPIs appears to be closely related to the adhesive system used.

- Findings consistently highlight that etch-and-rinse systems benefit more from MMPI application: they expose more collagen through acid etching, providing a better opportunity for inhibitors to stabilize the collagen network. In contrast, one-step self-etch adhesives, due to their high acidity, not only fail to inhibit MMP activity but may even trigger its reactivation, compromising bond stability over time.

- MMPIs clinical effectiveness remain a subject of debate: some studies support their long-term benefits, particularly with etch-and-rinse adhesives, while others report minimal or no improvement. Additional research are needed to assess the biocompatibility of MMPIs into adhesive systems to confirm their effectiveness in clinical practice.

7. SUSTAINABILITY

Sustainability regarding the use of matrix metalloproteinase inhibitors to improve resin bond strength is a growing consideration in restorative dentistry. By enhancing the longevity and durability of adhesive restorations, MMPI can reduce the need of frequent re treatment, thus decreasing material waste, energy consumption and overall environmental impact associated with repeated dental procedures. The selection of biocompatible MMPI formulations supports sustainable practice. As MMPI extend the lifespan of restorations, the overall demand for resin-based materials and associated packaging is reduced, leading to a diminished material footprint. Encouraging the integration of MMPI does not only enhances clinical outcomes but also supports ecological responsibility within adhesive dentistry.

8. REFERENCES

1. Burrer P, Dang H, Par M, Attin T, Tauböck TT. Effect of Over-Etching and Prolonged Application Time of a Universal Adhesive on Dentin Bond Strength. Polymers 2020;12(12):2902.

2. Li K, Tay FR, Yiu CKY. The past, present and future perspectives of matrix metalloproteinase inhibitors. Pharmacol Ther. marzo de 2020;207:107465.

3. Perarivalan I, Karunakaran J, Anbalagan N, Harishma S, Prasad V. Matrix metalloproteinase inhibitors in restorative dentistry. J Conserv Dent Endod. 2024;27(6):566-71.

4. Saffarpour A, Valizadeh S, Amini A, Kharazifard M, Rohaninasab M. Effect of matrix metalloproteinase inhibitors on microtensile bond strength of dental composite restorations to dentin in use of an etch-and-rinse adhesive system. Clin Exp Dent Res. 2020;6(6):686-92.

5. De Moraes IQS, Do Nascimento TG, Da Silva AT, De Lira LMSS, Parolia A, Porto ICCDM. Inhibition of matrix metalloproteinases: a troubleshooting for dentin adhesion. Restor Dent Endod. 2020;45(3):e31.

6. Da Silva Camin F, Santin DC, Mondelli RFLM, Wang L, Heitor MArques Honorio. Influence of Mmp Inhibitors on Bond Strength of Adhesive System: Systematic Review and Meta-Analysis. J Res Dent. 2023;11(3):28-41.

7. Saad Alsharif, Ahmed Alhareb, Asam Abudalazez. Components of Dental Resin Composites: A Literature Review. AlQalam J Med Appl Sci. 2024;427-40.

8. Cho K, Rajan G, Farrar P, Prentice L, Prusty BG. Dental resin composites: A review on materials to product realizations. Compos Part B Eng. 2022;230:109495.

9. Pratap B, Gupta RK, Bhardwaj B, Nag M. Resin based restorative dental materials: characteristics and future perspectives. Jpn Dent Sci Rev. 2019;55(1):126-38.

10. BediR MGA, Karadas M, BediR F. Effect of matrix metalloproteinase inhibitors on

bonding durability of universal adhesives. Dent Mater J. 2023;42(4):581-90.

12. Dr Xue Jun Gao. Guidelines for Direct Adhesive Composite Restoration. Chinese Stomatological Association. 2015;217-20.

13. Giannini M, Makishi P, Ayres APA, Vermelho PM, Fronza BM, Nikaido T, et al. Self-Etch Adhesive Systems: A Literature Review. Braz Dent J. 2015;26(1):3-10.

14. Sano H, Chowdhury AFMA, Saikaew P, Matsumoto M, Hoshika S, Yamauti M. The microtensile bond strength test: Its historical background and application to bond testing. Jpn Dent Sci Rev. 2020;56(1):24-31.

15. Pereira Prado V, Asquino N, Apellaniz D, Bueno Rossy L, Tapia G, Bologna Molina R. Metaloproteinasas de la matriz extracelular (mmps) en Odontologia. Odontoestomatola. 2016;18:20-9.

16. Sambandam V, Neelakantan P. Matrix Metalloproteinases (Mmp) in Restorative Dentistry and Endodontics. J Clin Pediatr Dent. 2014;39(1):57-9.

17. Sajjanhar I, Chandra A, Tikku A. MATRIX METALLOPROTEINASES: ITS EFFECT ON DENTIN BONDING. J Biol Nat. 2017;7:118-22.

18. Tekçe N, Tuncer S, Demirci M, Balci S. Do matrix metalloproteinase inhibitors improve the bond durability of universal dental adhesives? Scanning. 2016;38(6):535-44.

19. Almahdy A, Koller G, Sauro S, Bartsch JW, Sherriff M, Watson TF, et al. Effects of MMP Inhibitors Incorporated within Dental Adhesives. J Dent Res. 2012;91(6):605-11.

20. Sahadi BO, Sebold M, André CB, Nima G, Dos Santos A, Chiari MDESDC, et al. Effect of experimental dentin etchants on dentin bond strength, metalloproteinase inhibition, and antibiofilm activity. Dent Mater. 2024;40(4):e12-23.

21. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol ILS, Geraldeli S, et al. Optimizing dentin bond durability: Control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater. 2013;29(1):116-35.

22. Zheng P, Chen H. Evaluate the effect of different mmps inhibitors on adhesive physical properties of dental adhesives, bond strength and mmp substarte activity. Sci Rep. 2017;7(1):4975.

23. Sabatini C, Pashley DH. Aging of adhesive interfaces treated with benzalkonium chloride and benzalkonium methacrylate. Eur J Oral Sci. 2015;123(2):102-7.

24. Almahdy A, Koller G, Festy F, Bartsch JW, Watson TF, Banerjee A. An MMP-inhibitor modified adhesive primer enhances bond durability to carious dentin. Dent Mater. 2015;31(5):594-602.

25. Leticia C.C Costa Perote, Maria Beatriz Beber Kamozaki, Natalia C. Gutierrez, Franklin R. Tay, Cesar R. Pucci. Effect of Matrix Metalloproteinase-inhibiting Solutions and Aging Methods on Dentin Bond Strength. J Adhes Dent. 2015;17(4):347-52.

26. Apolonio FM, Mazzoni A, Angeloni V, Scaffa PMC, Santi S, Saboia VDPA, et al. Effect of a one-step self-etch adhesive on endogenous dentin matrix metalloproteinases. Eur J Oral Sci. 2017;125(2):168-72.

27. Montagner AF, Perroni AP, Corrêa MB, Masotti AS, Pereira-Cenci T, Cenci MS. Effect of Pre-treatment with Chlorhexidine on the Retention of Restorations: A Randomized Controlled Trial. Braz Dent J. 2015;26(3):234-41.

9. ANNEXES

ABBREVIATIONS

MMPs	Matrix Metalloproteinases
MMPIs	Matrix Metalloproteinases Inhibitors
EGCG	Epigallocatechin-3-gallate
PA	Proanthocyanidin
CHX	Chlorhexidine
EDTA	Ethylenediaminetetraacetic
MBAC	Benzalkonium Methacrylate
OB	Optibond FL
BB94	Batimastat
APE	Aqueous Propolis Extract

TABLES

FIGURE