Original Article

# Enhancing adhesive performance with N, N, N′, N′-tetrakis (2-pyridyl methyl) ethylenediamine matrix metalloproteinase inhibitors: A comprehensive study of degree of conversion, microleakage, and micro-tensile bond strength in dental adhesives

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## Abstract

**Aim:** Matrix metalloproteinases (MMPs) play a significant role in the degradation of dentin collagen within hybrid layers, affecting the longevity of resin-bonded restorations. The incorporation of MMP inhibitors into dental adhesives has been explored to address this issue. This study aimed to assess the impact of the MMP inhibitor, N, N, N′, N′-Tetrakis (2-pyridyl methyl) ethylenediamine (TPEN), on key adhesive properties, including the degree of conversion (DC), microleakage, and micro-tensile bond strength, shedding light on their potential in enhancing bond durability.

**Subjects and Methods:** Microleakage evaluations were conducted on 24 premolar specimens, while micro-tensile bond strength measurements were performed on the buccal surface of dentin samples. The DC was determined using Fourier Transform Infrared spectroscopy (FTIR) spectroscopy.

**Results:** The findings revealed no significant difference in DC between the adhesive with MMP inhibitors and the control group ($P = 0.998$). Remarkably, the adhesive containing the MMP inhibitor, TPEN, exhibited significantly higher micro-tensile bond strength than the control group ($P = 0.008$). However, there was no notable distinction between the two groups concerning microleakage ($P = 0.085$).

**Conclusion:** The results suggest that including TPEN can effectively enhance micro-tensile bond strength in dental adhesives without compromising DC or exacerbating microleakage. This highlights the potential of MMP inhibitors in improving bond durability in restorative dentistry.

**Keywords:** Adhesive; bond strength; matrix metalloproteinase inhibitors; matrix metalloproteinases; microleakage; N, N, N′, N′-Tetrakis (2-pyridyl methyl) ethylenediamine

## INTRODUCTION

Adhesive systems are pivotal in advancing restorative dentistry, enabling the effective restoration of damaged or

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decayed teeth. However, a persistent challenge in this field pertains to the long-term stability and durability of adhesive bonds within the complex tooth structure.[1] Over time, these bonds may deteriorate, particularly when restorations extend below the cementoenamel junction.[2] This phenomenon can result in a range of complications, including margin staining, gap formation, bacterial microleakage, recurrent decay beneath restorations, postrestoration sensitivity, and, ultimately, the failure of the restoration itself.[3]

At the heart of the challenge of adhesive bond stability lies the creation and preservation of a resilient resin-infiltrated hybrid layer within the dentin. This hybrid layer, composed of collagen fibrils embedded with methacrylate-based resins, serves as a critical interface between the tooth structure and the adhesive material. The integrity of this layer is indispensable for the longevity of resin-bonded restorations. However, the hydrophilic nature of certain resin components can lead to the breakdown of this crucial interface over time.[4]

Matrix metalloproteinases (MMPs) and cathepsin are the integral components within dentin composition and are widely recognized as the key culprits in the degradation of collagen fibers. The enzymatic activity of MMPs and cathepsin can have a detrimental impact on the stability of the hybrid layer, subsequently compromising the effectiveness of adhesive bonds in restorative dentistry.[5] To mitigate these challenges, synthetic MMP inhibitors, such as quaternary ammonium methacrylate compounds and benzalkonium chloride, have been investigated for their potential to enhance the durability of the resin bondings.[6,7] These inhibitors hold promise in addressing the adverse effects of the MMP and cathepsin activity, ultimately contributing to the long-term success of adhesive dental restorations. They can be categorized as endogenous inhibitors, known as tissue inhibitors of metalloproteinases, or exogenous inhibitors.[8] Among the exogenous inhibitors, chlorhexidine, tetracyclines, and derivatives have been investigated for their ability to compete with MMPs for essential ions such as calcium and zinc, thus inhibiting their enzymatic activity.[9,10]

In this context, our study delves into the potential of N, N, N', N'-Tetrakis (2-pyridyl methyl) ethylenediamine (TPEN) as an MMP inhibitor within the realm of adhesive dentistry. TPEN has the capacity to chelate zinc and calcium ions present in MMPs, which could potentially impede their destructive effects on the hybrid layer.[11] To the best of our knowledge, this avenue of research is unexplored, offering promising prospects for enhancing adhesive stability. Our study takes a multifaceted approach, exploring MMP inhibition, degree of conversion (DC), microleakage, and micro-tensile bond strength. In doing so, we aim to contribute to the advancement of adhesive dental techniques, ultimately enhancing the longevity and reliability of dental restorations.

## SUBJECTS AND METHODS

### Preparation of the experimental adhesive systems

The experimental adhesive systems in this study were meticulously prepared to meet the desired composition and properties. The formulations consisted of key components, including Bisphenol A Glycidyl Methacrylate (Bis-GMA), 2-Hydroxyethyl Methacrylate (HEMA), urethane dimethacrylate (UDMA), and trimethylolpropane trimethacrylate (TMPTMA), camphorquinone (CQ), N, N-Dimethylaminoethyl Methacrylate (DMAEMA), TPEN, and ethanol (all purchased from Sigma-Aldrich, Germany). Precise ratios were used: Bis-GMA (14%), HEMA (26%), UDMA (12%), TMPTMA (8%), CQ (0.5%), DMAEMA (0.5%), and ethanol (39%). TPEN was then added to the base adhesive in different concentrations of 0.5, 1, and 2 phr. These components were accurately weighed mixed and homogenized in a controlled environment. The prepared adhesive systems were stored in dark airtight containers to maintain stability.

### Degree of release

To determine the optimal concentration of TPEN, the adhesives with different percentages of TPEN. Release tests were conducted to assess the TPEN release rates. Based on the results, 1% TPEN concentration was selected as optimal due to its comparable release profile to 2% TPEN while causing minimal impact on the adhesive's physical and chemical properties.

For the degree of release evaluation, five disk-shaped cured adhesive specimens were prepared for each TPEN concentration (0.5%, 1%, and 2%). 100 mL of dissolution medium (comprising a blend of ethanol and distilled water) was dispensed into each chamber of the apparatus. The device was then configured to operate at 150 rotations per minute (rpm), while the temperature of the dissolution medium was maintained at 37°C ± 0.5°C. After achieving thermal equilibrium, a disk was placed inside each compartment and the device started working. At designated intervals of 1, 2, 3, 4, 5, 6, and 7 h, 3 mL of the specimen was extracted from each chamber using a syringe fitted with a filter holder. Simultaneously, an equal volume of dissolution medium at the same temperature was introduced to replace the withdrawn sample. Then, one ml of the sample was diluted with 10 ml of the relevant fresh dissolution medium. The absorption spectra of the samples were measured at a wavelength of 292 nm using a ultraviolet-visible spectrophotometer (Shimadzu Corporation, Duisburg, Germany). Considering the dilution factor and based on the standard curve, the concentration of each sample and the percentage of TPEN released were calculated.

## Degree of conversion

To this aim, the DC of adhesive resins was measured using FTIR spectroscopy (EQUINOX 55, Bruker, Germany). A small amount of each adhesive resin was placed between two layers of polyethylene cover, creating a thin layer. The absorption spectrum of uncured samples was recorded. Each sample was then cured for 20 s using an LED light unit (Woodpecker, China) with an intensity of 850 mW/cm$^2$, and the absorption spectrum of the cured specimen was collected. The test was repeated three times for each sample.

DC was calculated by comparing the absorptions of the aliphatic carbon-carbon double bonds (C = C) at 1638 cm$^{-1}$ and aromatic carbon-carbon bonds (C-C) at 1608 cm$^{-1}$ before and after curing, using the following equation:

$$DC\% = \left(1 - \frac{\left(1637\ cm^{-1} / 1608\ cm^{-1}\right) \text{peak area after curing}}{\left(1637\ cm^{-1} / 1608\ cm^{-1}\right) \text{peak area before curing}}\right) \times 100$$

## Microleakage

In this experimental study, to evaluate microleakage, 24 maxillary premolar teeth that were extracted for orthodontic reasons, free of any fractures, structural anomalies, decay, or restoration were collected. The teeth were washed and purified with pumice powder. In addition, the remnants of periodontal ligaments, plaque, and calculus were removed from them. Then, the teeth were stored at the room temperature in normal saline. The teeth were disinfected for 2 h in formaldehyde solution (Yekta Chem Co., Tehran, Iran) and then stored in distilled water at the room temperature for 3 months after being extracted. Using a turbine with air-water spray as a cooling and straight diamond fissure (D and Z, Frankfurt, Germany), a class 5 cavity was prepared in the cervical area of the facial surface of the samples. The cavities had a mesiodistal width of three millimeters an occlusogingival length of three millimeters and an axial depth of 1.5 mm, no pulp of the teeth was exposed during preparation. Depth, length, and width were measured by a periodontal-graded probe. After preparing seven teeth, a new bur was replaced. Then, all the teeth were washed with water and dried. The samples were randomly divided into two groups with 12 teeth in each group. Group 1: Etch and Rinse bonding and Group 2: Etch and Rinse bonding-containing TPEN (Sigma-Aldrich, Germany).

The teeth specimens were etched with a 37% phosphoric acid gel (Bisco, Schaumburg, IL, USA) for 15 s, then completely washed and dried. Adhesives were applied with small brushes on the exposed dentin surface and cured (Woodpecker, China) for 20 s. The Filtek Z250 A2 (3M ESPE, St. Paul, USA) methacrylate base composite resin was placed in bulk in a cavity and cured for 40 s. Microfine diamond bur (D and Z, Berlin, Germany) was used to create a suitable contour and remove remnants of restoration, and to use aluminum oxide discs (KerrHawe, Bioggio, Switzerland) to polish immediately after restoration. Samples were tested at 37°C ± 1°C temperature and 1500 times thermocycles after 6 months of storage in distilled water. According to the ISO/TR 11405 standard for thermocycling tests, all samples were kept in separate containers containing physiological serum for 24 h. The teeth were thermocycled 1500 times at 55°C–5°C temperature. Then, the root end of each tooth was sealed with sticky wax and the entire tooth surface, except one millimeter around the restoration was covered by two layers of nail varnish, to ensure that the upper layer of the lacquer was completely placed on the underlayer, two different colored varnishes were used to penetrate the color through the walls of the cavity. The samples were immersed in 0.5% methylene blue at 37°C for 24 h. Then, the samples were washed with water and dried. Samples were cut in faciolingual form by a diamond disk in a cutting machine (Nemo, Mashhad, Iran) at a low speed under water spray. The depth of penetration in the occlusal and cervical margins of the restoration was measured individually with digital images (Canon, Japan) from each of the samples by using the Photoshop software version 8 with a magnification of 20 and a precision of a tenth of a millimeter. The zero number means the lack of microleakage, the number 1 means the penetration of the color in less than half of the axial wall, the number two means the penetration of the color is more than half of the axial wall and the number three, meaning the penetration of the color in the axial wall.

## Micro-tensile bond strength

This test evaluates the strength of bonds between adhesive materials and dental tissues, especially vital when dealing with different dental surfaces.[12,13] In this study, 12 third molar teeth without any caries or cracks were selected for the micro-tensile bond strength test, all of the debris was removed from them and they were kept in the physiologic serum until the test was performed. The dentin surfaces were prepared by cutting the facial surfaces with fine diamond disks (Horico-PFINGST, New Jersey, USA) to create 4 mm × 4 mm standard dentin sections. Dentin surfaces were flattened by abrasive sheets of aluminum oxide (300–600 seeds) underwater rinse. All dentin surfaces were screened by the SMZ 1500 stereomicroscope (Nikon, Tokyo, Japan) and samples that were not cracked or defective were selected.

The samples were randomly divided into two groups with 6 teeth in each group. Group 1: Etch and Rinse bonding and Group 2: Etch and Rinse bonding Containing TPEN (Sigma-Aldrich, Germany).

Samples were etched with a 37% phosphoric acid gel (Bisco, Schaumburg, IL, USA) for 15 s, then completely washed and dried. Adhesives were applied with small brushes on the exposed dentin surface and cured (Woodpecker, China) for 20 s. Then, the Filtek-Z250 A2 (3M, ESPE, St. Paul, MN, USA) methacrylate base composite resin was placed in two layers and at a height of 5 mm on the dentin surface. Each layer was cured for 40 s by an LED device (Demetron A.2, Kerr Italia, S.p.A., Scafati, Italy). Samples were tested at 37°C ± 1°C and 1500 times Thermocycles after 6 months of storage in distilled water. The samples were cut by a metallographic cutting machine (Automatic Section Saw, SYJ-200 Beijing, China), and 15 smaller pieces with a cross-section of 1 mm block for each group were obtained. To measure the micro-tensile bond strength, each piece was first attached to a movable jig by cyanoacrylate adhesive and then a universal test machine at a speed of 1 mm/min was used. The amount of force was recorded at the moment of breaking of the samples and the bond strength was calculated from the ratio of force applied to the composite resin cylinder to the cross-sectional area of the samples and was reported in Mega Pascal.

Patterns of sample breaking were determined by one person and by a stereo microscope with a magnification of ×80. The type of fracture of the samples was divided into three groups: (1) Adhesive (between adhesive and dentin), (2) cohesive (inside dentin or composite), and (3) mixed (combination of both adhesive and cohesive fracture).

### Statistical analysis

Data were analyzed using the SPSS software version 22 (SPSS Inc., Chicago, IL, U.S.A.). Normal distribution of the data was confirmed using the Shapiro-Wilk test ($P > 0.05$); while homogeneity of variances was not verified using Levene's test ($P = 0.002$). Therefore, one-way analysis of variances was used for comparison among all groups and *post hoc* Tamhane's T2 test for pairwise comparisons in each storage times. The failure mode frequencies were analyzed using the Fisher's exact test. A significance level of $P < 0.05$ was considered statistically significant.

## RESULTS

Figure 1 shows the release rate of the MMP inhibitor (TPEN) from the samples containing the adhesives with different concentrations of TPEN (0.5%, 1%, and 2%). The release rate of TPEN in 1% and 2% concentrations was the same and the highest value was at 7 h. All further tests were conducted using the 1% TPEN concentration.

The DC of the bonding agents containing TPEN was evaluated compared to control bonding agents. The mean

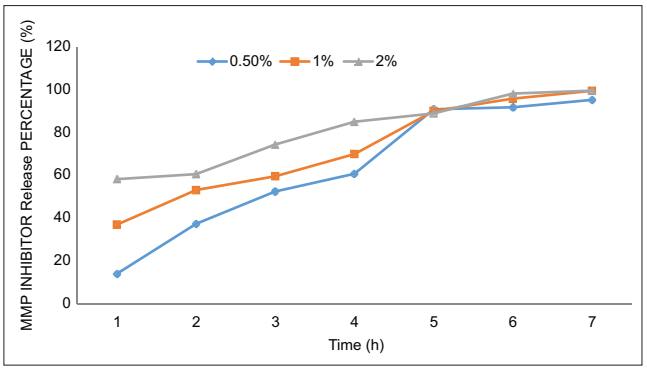


**Figure 1:** Matrix metalloproteinase inhibitor (TPEN) release rate of the cured adhesive samples with different concentrations (0.5%, 1%, and 2%) over time using a ultraviolet-visible spectrophotometer to measure the absorption spectra

DC for the TPEN-containing bonding agents was 36.33% with a standard deviation (SD) of 6.51%, while the control bonding agents exhibited a mean DC of 36.33% with a SD of 4.04%. The independent *t*-test analysis showed that there was no significant difference in DC between the conventional bonding agents and the bonding agents containing TPEN ($P = 0.998$).

### Micro-tensile bond strength

The mean micro-tensile bond strength for TPEN-containing bonding agents was 20.86 MPa with a SD of 6.39 MPa, while the control bonding agents had a mean micro-tensile bond strength of 15.13 MPa with a SD of 4.36 MPa. The independent *t*-test analysis revealed that the micro-tensile bond strength was significantly higher in the TPEN-containing bonding agents compared to the control bonding agents ($P = 0.008$). All types of sample fractures observed during testing were adhesive in nature.

### Microleakage

Table 1 shows the microleakage values for both the control bonding and the TPEN-containing bonding, categorized by the depth of penetration. The data indicate that the highest rate of microleakage in both groups occurred at the midpoint of the outer wall. In addition, microleakage was lower in the TPEN-containing group compared to the control group, although this difference was not statistically significant according to the Chi-square test ($P = 0.085$).

## DISCUSSION

The present study delves into the intricate dynamics of adhesive dentistry, particularly regarding the inclusion of TPEN as a potential MMP inhibitor. MMP inhibitors have gained attention for their role in preserving adhesive bond stability by countering enzymatic collagen

**Table 1: Microleakage value in control bonding and bonding containing Tetrakis (2-pyridylmethyl) ethylene diamine (*n* [%])**

| Bonding | Leak | | | | |
|---|---|---|---|---|---|
| | 0 | 1 | 2 | 3 | Total |
| Control | 2 (16.7) | 5 (41.7) | 3 (25) | 2 (16.7) | 12 (100) |
| Containing TPEN | 5 (41.7) | 7 (58.3) | 0 | 0 | 12 (100) |

TPEN: Tetrakis (2-pyridylmethyl) ethylene diamine

degradation within the hybrid layer.[14] Our investigation thoughtfully examines the various critical aspects of adhesive performance, including DC, microleakage, and micro-tensile bond strength, both with and without the inclusion of TPEN.

DC is a critical parameter in adhesive dentistry, as it directly influences the mechanical properties, solubility, dimensional stability, and biocompatibility of the resin composite.[15] In our study, the addition of TPEN did not result in a significant difference in DC between control bonding and TPEN-containing bonding. This finding is congruent with the observations of da Silva *et al*.[16] who found no substantial disparity in DC when comparing various bonding agents, including those with MMP inhibitors.

The study results showed no significant difference in the DC between conventional bonding agents and those containing TPEN ($P = 0.998$). This suggests that adding TPEN does not affect the formation of C = C bonds. da Silva *et al*.[16] found that the highest DC was observed in single bond 2 bonding without MMP inhibitors and there is no significant difference between the DC of bondings included Galardin-GAL, Batimastat-BAT, GM1489-GM1 and CHX. Although Cadenaro *et al*.[17] showed that adding chlorhexidine to the bonding process could interfere with the conversion of the C-C bonds to C = C bonds and thus decrease the bond strength. Therefore, chlorhexidine may inhibit the MMPs, but because of the reduction in the DC, it may not be a suitable substance for inhibiting the enzymes. Mohan and Kumar conducted a systematic review to evaluate the influence of MMP inhibitors on the longevity of adhesive restorations. Their findings highlighted that the use of MMP inhibitors significantly improves the durability of the resin-dentin bond, reducing bond degradation over time and enhancing restoration longevity.[18] Sharma *et al*. found that the application of MMP inhibitors significantly reduced microleakage in restorative procedures compared to restorations without MMP inhibitors, suggesting that the use of these inhibitors can enhance the longevity and sealing ability of adhesive restorations.[19]

Moving to the micro-tensile bond strength assessment, our study unveils an intriguing trend. The incorporation of TPEN into the adhesive formulation led to a significantly higher micro-tensile bond strength compared to conventional bonding. This outcome aligns with the findings of Almahdy *et al*.[6] who observed enhanced bond strength and improved sealing ability with the introduction of MMP inhibitors, specifically BB94 and GM6001, within primers. However, it is essential to acknowledge the divergent results seen in prior research. Kapdan and Öztaş[20] reported no significant increase in bond strength with the application of ozone, an MMP inhibitor. Conversely, Zheng *et al*.[21] noted that MMP inhibitors effectively prevented the decrease in micro-tensile bond strength upon aging of the etch-and-rinse adhesive but did not yield the same effect for self-etching adhesives. These variations emphasize the nuanced relationship between MMP inhibition and bond strength, which may vary depending on the adhesive system employed.

The most important factor for the long-term clinical success of a restoration is providing an effective and permanent plugging between the restorative material and tooth surfaces. Microgaps may develop between a tooth and filling because of contraction during the polymerization of the esthetic restorative material in the same color as a tooth that is widely and recently used. Bacteria, ions, and fluids may easily pass through these gaps and lead to microleakage; this causes secondary caries, pulp inflammation, sensitivity, and coloring on the interfaces. The results of this study showed that the microleakage in the bonding agents containing TPEN was lower than the conventional bonding, but this difference was not significant ($P = 0.05$). Kapdan and Öztaş[20] showed when the occlusal and gingival microleakage rates among the groups (control, ozone, and chlorhexidine) were compared, the difference was insignificant ($P > 0.05$) but the microleakage in the ozone group was lower than other groups. Meiers and Kresin[22] also state that a 2% chlorhexidine solution can be used to disinfect the cavity without causing a change in microleakage values of dentin bonding agents. Whereas Tulunoglu *et al*.[23] Carrilho *et al*. (2007) showed that the chlorhexidine solution preserved the hybrid layer and inhibited its degradation over time.[24] investigated the effect of chlorhexidine as an MMP inhibitor on the bond strength of resin adhesive systems and found that the use of chlorhexidine significantly enhanced bond durability, especially in the etch-and-rinse adhesive systems, by inhibiting MMPs and preventing collagen degradation.

A recent study explored the effects of the MMP inhibitor GM1489 at different concentrations on both an experimental and a commercial adhesive system. The research evaluated the DC, dentin bond strength (μTBS) immediately and after 1 year of storage, as well as nanoleakage. The findings revealed that incorporating GM1489 did not adversely affect the DC %, and certain concentrations (5 μM and 10 μM) significantly enhanced μTBS after 1 year of storage without causing negative effects on nano leakage.[25] In

another study, a novel prime-and-rinse mode involving MMP inhibitors in adhesive primers significantly improved both short-term and long-term dentin micro-tensile bond strengths compared to controls. SEM/TEM analyses confirmed stable resin-dentin interfaces over time, highlighting the potential value of MMP inhibitors in enhancing contemporary dentin bonding techniques.[26] In a related study, the effect of MMP inhibitors on μTBS was investigated with an etch-and-rinse adhesive system. The study involved various MMP inhibitors, including CHX, 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide and dimethyl sulfoxide, applied to dentin surfaces. The results demonstrated significantly improved thermocycler μTBS values in the groups treated with MMP inhibitors compared to the control group. This finding aligns with our emphasis on the potential benefits of MMP inhibition in adhesive dentistry.[27] A recent systematic review of *in vitro* studies delved into the incorporation of MMP inhibitors into adhesive systems to evaluate their influence on dental composite bond strength to dentin. The review encompassed numerous studies that employed various MMP inhibitors. Notably, the results revealed that the incorporation of MMP inhibitors had no adverse impact on immediate bond strength, aligning with our findings. Moreover, the review highlighted a favorable effect on longer-term bond strength, which supports the notion that optimizing adhesive techniques with MMP inhibitors can enhance the durability of dental restorations. It's worth noting that the review identified a range of inhibitors, some of which demonstrated similar or even superior effects on bond strength compared to the commonly used CHX. This collective evidence underscores the potential benefits of MMP inhibitors in adhesive dentistry, aligning with our study's implications for long-term bond stability.[28] Another study systematically reviews the effects of MMP inhibitors on immediate and aged dentin bond strengths, emphasizing the role of MMPs in the loss of bond strength over time.[29] Tjäderhane L *et al*.[30] reported that MMPs play a crucial role in the degradation of the hybrid layer, emphasizing the importance of utilizing specific inhibitors to prevent bond failure. Their findings suggest that incorporating these inhibitors can enhance the longevity of adhesive restorations, thereby improving the clinical outcomes.

## CONCLUSION

In conclusion, our study contributes to the evolving landscape of adhesive dentistry by investigating the potential of TPEN as an MMP inhibitor. While the results are promising, highlighting enhanced micro-tensile bond strength and reduced microleakage, they also underscore the intricate interplay of multiple variables within adhesive systems. Future research should continue to explore the complex relationships between MMP inhibition, DC, bond strength, and microleakage, to optimize adhesive techniques for the long-term success of dental restorations.

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### Conflicts of interest
There are no conflicts of interest.

## REFERENCES

1. Gwinnett AJ, Yu S. Effect of long-term water storage on dentin bonding. Am J Dent 1995;8:109-11.
2. Oz FD, Ergin E, Canatan S. Twenty-four-month clinical performance of different universal adhesives in etch-and-rinse, selective etching and self-etch application modes in NCCL – A randomized controlled clinical trial. J Appl Oral Sci 2019;27:e20180358.
3. Mjör IA, Moorhead JE, Dahl JE. Reasons for replacement of restorations in permanent teeth in general dental practice. Int Dent J 2000;50:361-6.
4. Frassetto A, Breschi L, Turco G, Marchesi G, Di Lenarda R, Tay FR, *et al*. Mechanisms of degradation of the hybrid layer in adhesive dentistry and therapeutic agents to improve bond durability – A literature review. Dent Mater 2016;32:e41-53.
5. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Tersariol IL, Geraldeli S, *et al*. Optimizing dentin bond durability: Control of collagen degradation by matrix metalloproteinases and cysteine cathepsins. Dent Mater 2013;29:116-35.
6. 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;91:605-11.
7. Tezvergil-Mutluay A, Agee KA, Uchiyama T, Imazato S, Mutluay MM, Cadenaro M, *et al*. The inhibitory effects of quaternary ammonium methacrylates on soluble and matrix-bound MMPs. J Dent Res 2011;90:535-40.
8. Trojanek JB, Niemirska A, Grzywa R, Wierzbicka A, Obrycki Ł, Kułaga Z, *et al*. Leukocyte matrix metalloproteinase and tissue inhibitor gene expression patterns in children with primary hypertension. J Hum Hypertens 2020;34:355-63.
9. Scannevin RH, Alexander R, Haarlander TM, Burke SL, Singer M, Huo C, *et al*. Discovery of a highly selective chemical inhibitor of matrix metalloproteinase-9 (MMP-9) that allosterically inhibits zymogen activation. J Biol Chem 2017;292:17963-74.
10. Levin M, Udi Y, Solomonov I, Sagi I. Next generation matrix metalloproteinase inhibitors – Novel strategies bring new prospects. Biochim Biophys Acta Mol Cell Res 2017;1864:1927-39.
11. Zhu B, Wang J, Zhou F, Liu Y, Lai Y, Wang J, *et al*. Zinc depletion by TPEN induces apoptosis in human acute promyelocytic NB4 cells. Cell Physiol Biochem 2017;42:1822-36.
12. Kazem NE, El-Refai DA, Alian G. Assessment of physical properties of bioactive glass-modified universal multimode adhesive and its bonding potential to artificially induced caries affected dentin. BMC Oral Health 2024;24:423.
13. Bourgi R, Kharouf N, Cuevas-Suárez CE, Lukomska-Szymańska M, Devoto W, Kassis C, *et al*. Effect of modified triple-layer application on the bond strength of different dental adhesive systems to dentin. J Funct Biomater 2023;14:522.
14. Mazzoni A, Tjäderhane L, Checchi V, Di Lenarda R, Salo T, Tay FR, *et al*. Inhibition of matrix metalloproteinases by quaternary ammonium methacrylates prevents bond degradation. Dental Materials 2013;29: 221-8.
15. Yılmaz Atalı P, Doğu Kaya B, Manav Özen A, Tarçın B, Şenol AA, Tüter Bayraktar E, *et al*. Assessment of micro-hardness, degree of conversion, and flexural strength for single-shade universal resin composites. Polymers (Basel) 2022;14:4987.
16. da Silva EM, de Sá Rodrigues CU, de Oliveira Matos MP, de Carvalho TR, dos Santos GB, Amaral CM. Experimental etch-and-rinse adhesive systems containing MMP-inhibitors: Physicochemical characterization and resin-dentin bonding stability. J Dent 2015;43:1491-7.
17. Cadenaro M, Pashley DH, Marchesi G, Carrilho M, Antoniolli F, Mazzoni A, *et al*. Influence of chlorhexidine on the degree of conversion and E-modulus of experimental adhesive blends. Dent Mater 2009;25:1269-74.
18. Mohan S, Kumar P. Influence of MMP inhibitors on the longevity of adhesive restorations: A systematic review. J Conserv Dent

2018;21:485-91.
19. Bhati M, Kumar S, Mittal M, Singh R, Koul V. Effect of Chlorhexidine as an MMP Inhibitor on the Bond Strength of Resin Adhesive Systems: An *In Vitro* Study. J Conserv Dent 2020; 23:361-6.
20. Kapdan A, Öztaş N. Effects of chlorhexidine and gaseous ozone on microleakage and the bond strength of dentin bonding agents with compomer restoration on primary teeth. J Dent Sci 2015;10:46-54.
21. Zheng P, Zaruba M, Attin T, Wiegand A. Effect of different matrix metalloproteinase inhibitors on microtensile bond strength of an etch-and-rinse and a self-etching adhesive to dentin. Oper Dent 2015;40:80-6.
22. Meiers JC, Kresin JC. Cavity disinfectants and dentin bonding. Oper Dent 1996;21:153-9.
23. Tulunoglu O, Ayhan H, Olmez A, Bodur H. The effect of cavity disinfectants on microleakage in dentin bonding systems. J Clin Pediatr Dent 1998;22:299-305.
24. Carrilho MR, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjäderhane L, *et al*. In vivo preservation of the hybrid layer by chlorhexidine. J Dent Res 2007;86:529-33. [doi: 10.1177/154405910708600608].
25. Miranda ME, Silva EM, Oliveira MF, Simmer FS, Santos GB, Amaral CM. Resin-dentin bond stability of etch-and-rinse adhesive systems with different concentrations of MMP inhibitor GM1489. J Appl Oral Sci 2020;28:e20190499.
26. Xu J, Li M, Wang W, Wu Z, Wang C, Jin X, *et al*. A novel prime- and -rinse mode using MDP and MMPs inhibitors improves the dentin bond durability of self-etch adhesive. J Mech Behav Biomed Mater 2020;104:103698.
27. Saffarpour A, Valizadeh S, Amini A, Kharazifard MJ, 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;6:686-92.
28. Yaghmoor RB, Jamal H, Abed H, Allan E, Ashley P, Young A. Incorporation of MMP inhibitors into dental adhesive systems and bond strength of coronal composite restorations: A systematic review and meta-analysis of *in vitro* studies. Jpn Dent Sci Rev 2022;58:298-315.
29. Kiuru O, Sinervo J, Vähänikkilä H, Anttonen V, Tjäderhane L. MMP inhibitors and dentin bonding: Systematic review and meta-analysis. Int J Dent 2021;2021:9949699.
30. Tjäderhane L, Nascimento FD, Breschi L, Mazzoni A, Pashley DH. The Role of Matrix Metalloproteinases in Hybrid Layer Degradation: Strategies to Prevent Bond Failure. J Conserv Dent 2021;24:256-62.