Dental Adhesives—Surface Modifications of Dentin Structure for Stable Bonding

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KEYWORDS

• Dentin • Dentin bonding systems • Collagen • Matrix metalloproteinases
• Collagen cross-linking agents • MMP inhibitors • Bond strength • Zymography

KEY POINTS

- Resin hydrolysis and collagen degradation by endogenous enzymes influence the long-term integrity of bonded interfaces.
- Strategies to extend longevity of bonded interfaces include enzymatic inhibition, collagen cross-linking, and mineral precipitation within the hybrid layer.
- Proper and careful adhesive application by the dentist remains the foremost aspect to guarantee the longevity of bonded interfaces with currently available systems.

INTRODUCTION

Adhesive systems have been substantially improved over the years regarding their chemistry, interaction with dental substrates and with restorative materials, and clinical protocols.[1] Latest advancements have focused on reducing the number of steps and technique sensitivity, as well as on the development of strategies to extend the longevity of bonded interfaces. These included increasing the resistance of the resin components against hydrolysis and inhibiting enzymatic degradation of the collagen fibrils.[2–4]

Tjäderhane and colleagues (1998) first described the role of dentinal matrix metalloproteinases (MMPs), a group of endogenous peptidases, in the dentin matrix breakdown in caries lesions.[5] Pashley and colleagues (2004) then showed that the degradation of the collagen in acid-etched dentin is mediated by the activity of MMPs, activated during dentin bonding procedures[6] (**Fig. 1**). Besides MMPs, another

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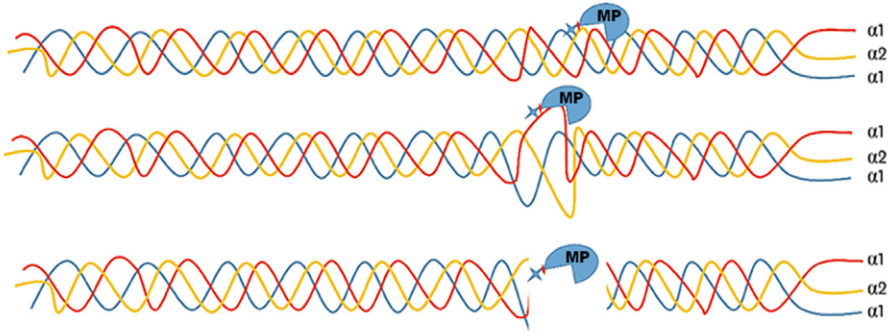

Fig. 1. Metalloproteinase (MP) breaks the collagen molecules, causing the loss of the triple helical conformation, resulting in fragments that will be slowly hydrolyzed by the proteolytic enzymes. (*Adapted from* de Moraes IQS, do Nascimento TG, da Silva AT, de Lira L, Parolia A, Porto I. Inhibition of matrix metalloproteinases: a troubleshooting for dentin adhesion. Restor Dent Endod. 2020;45(3):e31.)

class of enzymes has been identified in human dentin: cysteine proteases, which may contribute to the slow collagen degradation within the hybrid layer (HL) acting synergically with MMPs.[7] The elucidation of the role of endogenous proteases in the longevity of the HL prompted intense research activity aiming at inhibiting protease activity and/or increasing collagen resistance to enzymatic breakdown. This article describes the recent advances in dentin surface modification with the purpose of extending bond longevity.

PROTEASE INHIBITORS

Studies focused on MMPs inhibition in the last years show a great heterogeneity of molecules proposed: in a recent systematic review, 21 different inhibitors were identified.[8] Depending on the strategy chosen, the inhibitors can be used as an additional priming step after acid etching, included in primer formulations, or the adhesive resin.[9]

Chlorhexidine

A large portion of the literature on MMP inhibitors focuses on chlorhexidine (CHX), a common antimicrobial agent used in dentistry. The ability of CHX to inhibit the MMPs has been clearly demonstrated,[6] and since then, several studies have proven the efficacy of this biguanide compound in preserving the integrity and stability of the dentinal collagen in the HL and improving bond strength durability both in vivo and in vitro conditions.[10] CHX is readily available in clinical practice, and if applied at low concentrations (0.05%–0.2%) as an additional priming step on etched dentin, it effectively inhibits the enzymatic activity of MMPs[11] and improves the long-term bond strength.[12] Interestingly, it has been demonstrated that CHX can be retained within the HL even after 10 years of aging in vitro, maintaining its MMP-inhibitory properties.[11] Besides its use as a primer, CHX has also been added to adhesive formulations with encouraging results.[13]

The likely inhibitory mechanism of CHX against MMPs and cysteine cathepsins is linked to its ability to subtract zinc and calcium ions essential for the activity of these enzymes.[2,10] The cationic nature of CHX explains its ability to react with the anionic compounds of dentin contained in collagen but also in the inorganic matrix.[11]

Quaternary Ammonium Compounds

Similar to the CHX, other cationic molecules, such as quaternary ammonium compounds, may inhibit the collagenolytic activity of dentin endogenous enzymes. Benzalkonium chloride is a quaternary ammonium compound with antimicrobial and surfactant properties that has already been proved to be a MMPs inhibitor with an efficacy comparable to that of CHX, able to prolong the duration of the adhesive interface over time.[14,15] A quaternary ammonium methacrylate, 12-methacryloyloxydodecylpyridinium bromide (MDPB), integrated into a commercial adhesive system thanks to the presence of a resinous group, which has shown good ability to inhibit the MMPs activity.[16]

Therapeutic Drugs

Various therapeutic drugs used against different pathologic conditions have inhibiting properties on MMPs, and for this reason, their use in adhesive dentistry has been proposed. For instance, broad-spectrum antibiotics such as tetracycline and its analogs minocycline and doxycycline have cationic chelating properties and are capable of downregulation of MMPs mRNA expression, effectively blocking their activity.[2,10,17] The incorporation of nanotubes releasing doxycycline[18] and electrospun fibers containing doxycycline have been tested as fillers[19] in dental adhesives, leading to promising results in terms of MMPs inhibition. Nonetheless, the photo-oxidative stain of teeth caused by tetracyclines may impair the clinical use of these compounds in dentistry.[10]

Bisphosphonates are a class of drugs used in treating osteoporosis and Paget disease, which are effective inhibitors of MMPs produced by osteoclasts[17] by chelating zinc and calcium ions from the enzymes.[2] Their application in adhesive dentistry is promising,[20] even though the studies on bisphosphonates as MMP inhibitors in dentistry are still limited.

Other Inhibitors

Among the various molecules proposed, galardin is a specific synthetic MMP inhibitor effective against different MMPs at concentrations around 10 to 100 times lower than CHX.[2] When added to the primer of an E&R adhesive, galardin reduced the degradation of the HL after 1 year.[21]

Dimethyl sulfoxide (DMSO) has been recently proposed as a solvent in adhesive dentistry, being miscible in organic solvents, water and resin monomers commonly used in dentistry.[22] DMSO showed several beneficial properties for the adhesive interface, including a noteworthy inhibitory effect on MMPs when used as dentin pretreatment (**Box 1**).[23]

COLLAGEN CROSS-LINKING

Collagen strength and stiffness is related to its highly cross-linked structure. During the past 15 years, several synthetic and organic compounds capable of forming additional interfibrillar and intrafibrillar cross-links have been tested as a strategy to improve the longevity of the adhesive/dentin interface (**Box 2**). Although earlier investigations proposed long application times to effect maintenance of interfacial integrity,[24] cross-linkers soon started to be tested in clinically feasible protocols, for instance as an extra priming step after phosphoric acid etching or the application of the self-etching primer,[25] mixed to universal adhesive systems,[26] or mixed to phosphoric acid.[27]

How Do Collagen Cross-Linkers Contribute to Dentin Bond Stability?

The success of adhesive restorations, both in the short-term and long-term, relies on the effective adhesive infiltration within the collagen network. In that sense,

Box 1
Substances tested as protease inhibitors

Group	Inhibitor
Cationic compounds	• 0.2%–2% CHX gluconate
	• Quaternary ammonium compounds (MDPB)
Antibiotics	• Minocycline
	• Doxycycline
Bisphosphonates	• Polyvinylphosphonic acid
Other inhibitors	• Galardin
	• SB-3CT 2-[[(4-Phenoxyphenyl)sulfonyl]methyl]thiirane
	• E-64 L-trans-Epoxysuccinyl-leucylamido(4-guanidino)butane
	• Odanacatib
	• DMSO

cross-linking was shown to increase the stiffness of the collagen fibrils,[28] preventing their collapse when dried[29] and allowing for better resin infiltration. The superior quality of HLs obtained in dentin samples pretreated with different cross-linkers explains the higher initial bond strength values in comparison to nontreated dentin substrates.[30,31] Yet, the real benefits of collagen cross-linkers are observed after prolonged storage. Several in vitro studies testing different cross-linkers attested to the stability of the bonded interface after 1 to 5 years.[32,33] In part, this effect can be ascribed to better resin infiltration because poorly infiltrated demineralized collagen networks are more susceptible to endogenous proteases. Moreover, because voids in the HL are filled with dentinal fluid, suboptimal adhesive infiltration increases the hydrolysis of the resin component.[10]

Notwithstanding, to a large extent preservation of the bonded interfaces is explained by the inhibitory effect of cross-linkers over protease activity. The proposed inhibition mechanisms include (1) irreversible changes in their tertiary structure by the establishment of multiple cross-links in their catalytic sites, (2) alosteric control, that is, the modification of noncatalytic domains also involved in protease activity, and (3) indirectly, by cross-linking functional domains of noncollagen proteins present in dentin involved in MMP activity (eg, dentin matrix protein-1/DMP-1 and bone sialoprotein/BSP).[34] Protease inhibition by cross-linkers is a long-term effect, as demineralized dentin beams treated with cross-linkers showed less protease activity than the untreated control after 6 months.[35] In situ zymography, used to quantify MMP activity in bonded interfaces, showed lower protease activity in interfaces treated with cross-linkers after 1[30,36] and 5 years[37] (**Fig. 2**).

Box 2
Substances tested as collagen cross-linkers

Source	Group	Examples
Synthetic	Aldehydes	GA, acrolein
	Carbodiimides	EDC, DCC
Natural	Riboflavins	RF, riboflavin-5-phosphate
	Proanthocyanidins	GSE, GTE, CJE

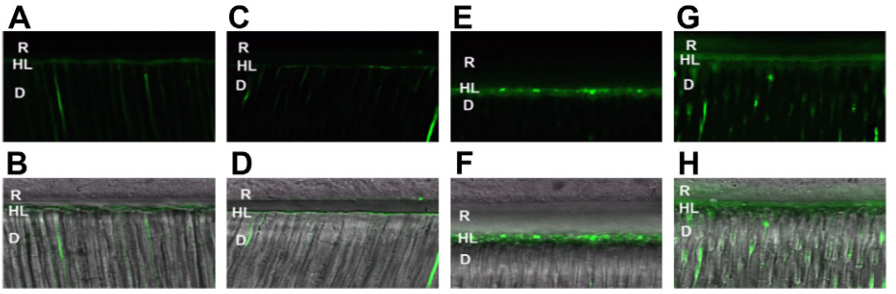

Fig. 2. Representative examples of in-situ zymography of the resin–dentin interfaces after 1 year in artificial saliva. Green fluorescence indicates protease activity. Dentin treated with a universal adhesive in self-etching (SE) mode (*A*, *B*); universal adhesive (SE) + 0.5 M carbodiimide, applied for 1 min between the application of 2 layers of the adhesive (*C*, *D*); universal adhesive in etch-and-rinse (ER) mode (*E*, *F*); and universal adhesive (ER)+ 0.5 M carbodiimide for 1 minute, after acid etching (*G*, *H*). D, dentin; HL, hybrid layer; R, resin composite. (*From* Comba A, Maravić T, Villalta V, et al. Effect of an ethanol cross-linker on universal adhesive. Dental Materials. 2020;36(12):1645-1654.)

Cross-Linkers

The characteristics of the main compounds tested as collagen cross-linkers are described below.

Glutaraldehyde

This is an aliphatic molecule containing 5 carbon atoms and one aldehyde (–COH) group in each extremity, capable of establishing chemical bonds with collagen amino ($-NH_2$) groups, forming intramolecular and intermolecular cross-links.[28] Unfortunately, glutaraldehyde (GA) is cytotoxic, which prevents its clinical use.[38]

Carbodiimides

N,N'-dicyclohexylcarbodiimide (DCC) and 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) (see **Box 2**) have a basic structure represented as R_1-N=C=N-R_2 (where R_1 and R_2 can be, for instance, a methyl group and an amino group). They are considered "zero-length" cross-linkers, which mediate the formation of an amide cross-link between collagen carboxyl and amine groups, without adding extra groups in between.[39]

Riboflavin

Riboflavin (RF) is a water-soluble, nontoxic photosensitizer capable of releasing oxygen-reactive species when exposed to ultraviolet A (UVA) light (368 nm) and induce the formation of new cross-links.[40] Blue light (470–480 nm) can also be used but it is less efficient than UVA.[41]

Proanthocyanidines

These belong to a group of naturally occurring plant metabolite bioflavonoids with complex molecular structures containing one aliphatic and multiple phenolic hydroxyl groups.[24,42] The cross-linking mechanisms promoted by proanthocyanidines (PACs) include hydrogen bonding and hydrophobic interactions with proline groups of the collagen at intrafibrillar and interfibrillar levels.[43] Collagen cross-linking with PACs leads to dehydration of the fibrils, improving its resistance to collagenases.[44]

Comparative Studies

Proteolytic activity

Cross-linkers were shown to reduce MMP-2, MMP-8, and MMP-9 activities by 21% to 70% after treatment times of 1 or 5 minutes, with grape seed extract (GSE) showing the highest percentages, and GA and RF, the lowest.[45] In situ zymography revealed statistically higher MMP inhibition for cranberry juice extract (CJE) in comparison to green tea extract (GTE) and GSE.[39]

Long-term stability of bonded interfaces

When applied as water-based primers on etched dentin, GSE and RF/UVA were able to maintain bond strength values of etch-and-rinse, 2-step adhesives to dentin during an 18-month period. When GA was used as primer, however, bond strength was stable only with one of the tested adhesives.[38] Indentation fracture toughness (iFT) test was used to assess the efficacy of water-based primers containing GSE, RF/UVA, or GA associated with universal adhesives. After 6-month storage in artificial saliva, only the specimens treated with GSE showed higher iFT in relation to the control.[25] Acid-eroded dentin samples treated with water-based primers containing GSE or RF/blue light following phosphoric acid etching showed stable microtensile bond strength values after 2-year storage in water.[32]

Is the Use of Collagen Cross-Linkers Clinically Viable?

Dentin biomodification using collagen cross-linkers seems to be a promising strategy to extend the durability of bonded interfaces. However, their application as primers would significantly increase chair-side time. In order to avoid adding an additional step to the bonding procedure, attempts have been made of adding cross-linkers to the adhesive or to the etchant.

Etchants

Proanthocianidins remain effective at lower pH and, therefore, can be mixed with phosphoric acid. Bonded interfaces obtained with the use of an experimental etchant containing 2% GSE, 20% ethanol, and 10% phosphoric acid associated with 2-step, etch-and-rinse commercial adhesive showed no reduction in bond strength after 6 months[46] and 1 year of storage,[31] as opposed to specimens where dentin was conditioned with 35% phosphoric acid. The same etchant formulation was shown to increase the immediate bond strength to caries-affected dentin by nearly 70% and significantly reduced MMP activity not only in relation to the control group (37% phosphoric acid) but also to specimens treated with CHX.[27] However, the incorporation of GSE to 37% phosphoric acid resulted in lower bond strength values in relation to the control etchant, even though the increase in cross-linking was verified by infrared spectroscopy.[47]

Adhesive systems

RF added to commercial universal adhesives resulted in less severe reductions in bond strength after 6 months of storage in artificial saliva in relation to the respective controls without the cross-linker.[26] A clinical study evaluated the effect of adding GSE to a commercial 2-step, etch-and-rinse adhesive system. After 2 years, the retention rates of restorations of noncarious cervical lesions placed with the modified adhesives were statistically lower than that of the control adhesive. Authors attributed the results to reduced degree of conversion of the adhesives, as proanthocyanidins are free-radicals scavengers.[48]

THE USE OF PARTICLES IN ADHESIVES

Several *in vitro* studies have demonstrated the beneficial effects of the addition of ion-releasing particles in experimental and commercial adhesives as a strategy to reduce adhesive degradation (**Box 3**).[3,9,49]

Silver ions can induce bacteria cell lysis, prevent bacterial DNA replication, and disrupt bacterial protein synthesis.[50] Adhesives containing silver-based particles present antibiofilm properties and lower risk for secondary caries development.[51,52] Other ions such as fluoride, zinc, and copper have been identified as MMPs inhibitors because they can bind to their specific sites and induce conformational changes that hinder enzyme activity.[53] Zinc ions can also bind to specific sites on the exposed collagen fibrils and modify their spatial configuration, protecting sensitive cleavage sites from the MMPs.[54] The addition of ZnO particles to a commercial etch-and-rinse, 2-step adhesive was shown to reduce collagen degradation after 4 weeks.[55]

Calcium-releasing particles, such as amorphous calcium phosphate (ACP), α-tricalcium phosphate, β-tricalcium phosphate (β-TCP), dicalcium phosphate dihydrate, octacalcium phosphate, and hydroxyapatite, have been tested as additives in experimental adhesives to promote mineral precipitation and replace water from poorly resin-infiltrated spaces within the HL.[56–60] Mineral precipitates may also reduce enzymatic activity by electrostatic interactions with some MMPs. Calcium-releasing particles can be also doped with metallic oxides as a strategy to increase the MMP inhibitory effect.[3]

Remineralization of the HL not only increases the mechanical properties of the interphase but was also shown to reduce hydrolytic degradation and to inhibit protease activity.[61] Bioactive glasses (BAGs) and calcium silicates (CaSi) release calcium ions that, along with phosphate ions present in physiologic fluids, promote mineral deposition. CaSi also releases hydroxyl ions from its crystalline calcium hydroxide phase, which increases local pH and favors mineral precipitation, antimicrobial action, and MMP inhibition.[62] Mineral precipitation (**Fig. 3**) within the HL reduced the micropermeability of dentin–resin interfaces for adhesives containing 30% to 40% of BAGs or CaSi fillers after 3 to 6 months storage in phosphate-containing medium, in comparison to unfilled adhesives.[56,57,62,63] The elastic modulus of the HL increased after 3 months for adhesives loaded with 40 wt% BAG or 33 wt% CaSi and β-TCP, whereas this property was reduced when particles were absent.[57,63] The application

Box 3
Particles and their effects on bonded interfaces

Particle	Observed Effects
Silver nanoparticles	Antimicrobial activity
Metallic oxides	Inhibition of enzymatic activity and reduced collagen degradation, antimicrobial activity, mineral deposition, higher long-term bond strength
BAGs	Reduction of micropermeability and nanoleakage of the HL, higher long-term bond strength, and remineralization of caries-affected dentin
CaSi	Mineral deposition, enhancement of HL mechanical properties, bond strength preservation, micropermeability and nanoleakage reduction
Calcium orthophosphates	Mineral precipitation, nanoleakage reduction, and improvement in bond strength

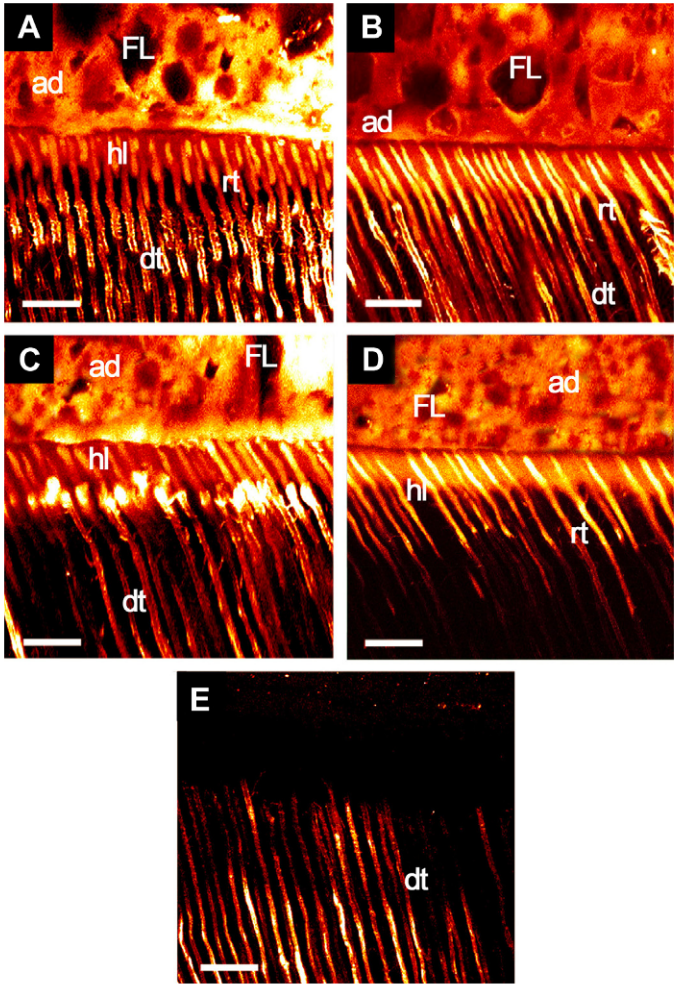

Fig. 3. Resin–dentin interfaces of experimental etch-and-rinse adhesive doped with calcium silicate-based microfillers immersed in simulated body fluid (SBF) solution for 24 hours or 6 months. Orange fluorescence is a calcium-chelator dye. Images indicate the remineralization of areas previously detected as poor-resin infiltrated zones of the resin–dentin interface. (*A*) Resin–dentin interface created with sodium–calcium–aluminum–magnesium silicate hydroxide fillers, where mineral deposition is identified within the adhesive layer (ad), the HL, and along the walls of dentinal tubules (dt), besides the fillers inside the resin tags (rt). (*B*) Resin–dentin interface created with aluminum–magnesium–carbonate hydroxide hydrates fillers showing Ca-deposits within the ad, HL, walls of the dt and rt after 6 months of SBF immersion. (*C*) Resin–dentin interface created with titanium oxide fillers demonstrate intense calcium deposition at bottom of HL, besides Ca-mineral within ad, HL, and dt. (*D*) Resin–dentin interface also with titanium oxide but after 6 months, showing Ca-mineral presence at the bottom and within the HL, dt, and rt. (*E*) Resin–dentin interface created with no-fillers adhesive (control) in which it is possible to note the absence of calcium deposition both within the HL and ad. Only the walls of the dentinal dt were stained by the fluorescent dye. (*From* Profeta AC, Mannocci F, Foxton R, et al. Experimental etch-and-rinse adhesives doped with bioactive calcium silicate-based micro-fillers to generate therapeutic resin-dentin interfaces. *Dent Mater.* 2013;29(7):729-741.)

of BAG particles to dentin as pretreatment primers in combination with a commercial adhesive also resulted in higher elastic modulus values of the HL.[64]

The association of ion-releasing particles with biomimetic analogs of noncollagenous proteins such as poli(acrylic) and poly(aspartic) acids has been suggested.[3] These molecules act as stabilizers and templates to guide apatite growth in an oriented manner along within collagen matrix. Dental adhesives containing ion-releasing fillers treated with polyacrylic acid have been shown to regulate apatite precipitation.[56] Polyaspartic acid in combination with Si-ACP nanoparticles added into self-etch adhesives promoted the remineralization of the bottom of the HL after 3 months.[60]

Studies on long-term dentin bond strength involving particle-containing adhesives have shown inconsistent results. Some studies report no loss in microtensile bond strength, whereas adhesion values are significantly decreased for control unfilled groups.[55,62,64] However, no difference between adhesives with or without particles was found in other studies.[56,57] Indeed, particles size and concentration are determinants for the mechanical behavior and bond strength of adhesives.[59,65] Besides, the lack of a strong chemical interaction between the resin matrix and of the particles jeopardizes stress distribution and can significantly reduce the material's mechanical properties.[59,66]

Two clinical studies evaluated the use of particles in adhesive systems. The addition of 0.1 wt% copper nanoparticles in a commercial one-bottle universal adhesive applied in etch-and-rinse mode improved the retention rate and marginal sealing of noncarious cervical lesions restorations after 18 months. These findings were attributed to copper protective action against collagen degradation.[67] However, the use of biosilicate particles (BAG) in different classes of adhesives did not improve the clinical performance of posterior restorations after 18 months.[68]

FINAL REMARKS

Adhesive systems have been substantially improved over the years regarding their chemistry, interaction with dental substrates and restorative materials, and technique sensitivity. Notwithstanding, the longevity of bonded interfaces remains a clinical concern. The strategies described here to reduce collagen degradation represent pathways to increase the long-term success of adhesive procedures. However, clinical studies are necessary to confirm some of the promising results obtained in vitro.

CLINICS CARE POINTS

- Most of the strategies to improve the durability of the adhesive interface are still experimental and need further investigations to be transferred to the clinical practice.
- CHX applied in low concentrations (0.05–0.2%) as an additional priming step on etched dentin to inhibit the MMPs is the only protocol that can be easily applied in clinical practice.
- The dentist should be aware that careful placement of the adhesive system is the first step to guarantee a stable bonding.

DISCLOSURE

The authors have nothing to disclose.

REFERENCES

1. Van Meerbeek B, Yoshihara K, Van Landuyt K, et al. From Buonocore's Pioneering Acid-Etch Technique to Self-Adhering Restoratives. A Status

Perspective of Rapidly Advancing Dental Adhesive Technology. J Adhes Dent 2020;22(1):7–34.
2. Frassetto A, Breschi L, Turco G, 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(2):e41–53.
3. Braga RR, Fronza BM. The use of bioactive particles and biomimetic analogues for increasing the longevity of resin-dentin interfaces: A literature review. Dent Mater J 2020;39(1):62–8.
4. Perdigão J. Current perspectives on dental adhesion: (1) Dentin adhesion - not there yet. Jpn Dent Sci Rev 2020;56(1):190–207.
5. Tjäderhane L, Larjava H, Sorsa T, et al. The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions. J Dent Res 1998; 77(8):1622–9.
6. Pashley DH, Tay FR, Yiu C, et al. Collagen degradation by host-derived enzymes during aging. J Dent Res 2004;83(3):216–21.
7. Mazzoni A, Tjäderhane L, Checchi V, et al. Role of dentin MMPs in caries progression and bond stability. J Dent Res 2015;94(2):241–51.
8. Kiuru O, Sinervo J, Vähänikkilä H, et al. MMP Inhibitors and Dentin Bonding: Systematic Review and Meta-Analysis. Int J Dent 2021;2021:9949699.
9. Münchow EA, Bottino MC. Recent Advances in Adhesive Bonding - The Role of Biomolecules, Nanocompounds, and Bonding Strategies in Enhancing Resin Bonding to Dental Substrates. Curr Oral Health Rep 2017;4(3):215–27.
10. Breschi L, Maravic T, Cunha SR, et al. Dentin bonding systems: From dentin collagen structure to bond preservation and clinical applications. Dent Mater 2018;34(1):78–96.
11. Breschi L, Maravic T, Comba A, et al. Chlorhexidine preserves the hybrid layer in vitro after 10-years aging. Dent Mater 2020;36(5):672–80.
12. Zhang X, Wang L, Liu S, et al. Evaluation of the bond strength of chlorhexidine incorporated into the adhesive system composition: A PRISMA guided meta-analysis. J Dent Sci 2020;15(3):315–28.
13. da Silva EM, de Sá Rodrigues CU, de Oliveira Matos MP, et al. Experimental etch-and-rinse adhesive systems containing MMP-inhibitors: Physicochemical characterization and resin-dentin bonding stability. J Dent 2015;43(12):1491–7.
14. Sabatini C, Scheffel DL, Scheffel RH, et al. Inhibition of endogenous human dentin MMPs by Gluma. Dent Mater 2014;30(7):752–8.
15. Comba A, Maravic T, Valente L, et al. Effect of benzalkonium chloride on dentin bond strength and endogenous enzymatic activity. J Dent 2019;85:25–32.
16. Tezvergil-Mutluay A, Agee KA, Mazzoni A, et al. Can quaternary ammonium methacrylates inhibit matrix MMPs and cathepsins? Dent Mater 2015;31(2): e25–32.
17. Boelen GJ, Boute L, d'Hoop J, et al. Matrix metalloproteinases and inhibitors in dentistry. Clin Oral Investig 2019;23(7):2823–35.
18. Palasuk J, Windsor LJ, Platt JA, et al. Doxycycline-loaded nanotube-modified adhesives inhibit MMP in a dose-dependent fashion. Clin Oral Investig 2018;22(3): 1243–52.
19. Münchow EA, da Silva AF, Piva E, et al. Development of an antibacterial and anti-metalloproteinase dental adhesive for long-lasting resin composite restorations. J Mater Chem B 2020;8(47):10797–811.
20. Tezvergil-Mutluay A, Agee KA, Hoshika T, et al. The inhibitory effect of polyvinylphosphonic acid on functional matrix metalloproteinase activities in human demineralized dentin. Acta Biomater 2010;6(10):4136–42.

21. Breschi L, Martin P, Mazzoni A, et al. Use of a specific MMP-inhibitor (galardin) for preservation of hybrid layer. Dent Mater 2010;26(6):571–8.
22. Stape THS, Tjäderhane L, Abuna G, et al. Optimization of the etch-and-rinse technique: New perspectives to improve resin-dentin bonding and hybrid layer integrity by reducing residual water using dimethyl sulfoxide pretreatments. Dent Mater 2018;34(7):967–77.
23. Stape THS, Mutluay MM, Tjäderhane L, et al. The pursuit of resin-dentin bond durability: Simultaneous enhancement of collagen structure and polymer network formation in hybrid layers. Dent Mater 2021;37(7):1083–95.
24. Bedran-Russo AK, Pereira PN, Duarte WR, et al. Application of crosslinkers to dentin collagen enhances the ultimate tensile strength. J Biomed Mater Res B Appl Biomater 2007;80(1):268–72.
25. Parise Gré C, Pedrollo Lise D, Ayres AP, et al. Do collagen cross-linkers improve dentin's bonding receptiveness? Dent Mater 2018;34(11):1679–89.
26. Fu C, Deng S, Koneski I, et al. Multiscale in-vitro analysis of photo-activated riboflavin incorporated in an experimental universal adhesive. J Mech Behav Biomed Mater 2020;112:104082.
27. Hass V, da Maceno Oliveira TB, Cardenas AFM, et al. Is it possible for a simultaneous biomodification during acid etching on naturally caries-affected dentin bonding? Clin Oral Investig 2021;25(6):3543–53.
28. Bedran-Russo AK, Pashley DH, Agee K, et al. Changes in stiffness of demineralized dentin following application of collagen crosslinkers. J Biomed Mater Res B Appl Biomater 2008;86(2):330–4.
29. Liu R, Fang M, Xiao Y, et al. The effect of transient proanthocyanidins preconditioning on the cross-linking and mechanical properties of demineralized dentin. J Mater Sci Mater Med 2011;22(11):2403–11.
30. Comba A, Maravić T, Villalta V, et al. Effect of an ethanol cross-linker on universal adhesive. Dental Mater 2020;36(12):1645–54.
31. Loguercio AD, Malaquias P, Dos Santos FP, et al. Acid Etching with Modified Phosphoric Acid to Increase the Longevity of the Bonded Interface. J Adhes Dent 2017;195–201.
32. de Siqueira FSF, Hilgemberg B, Araujo LCR, et al. Improving bonding to eroded dentin by using collagen cross-linking agents: 2 years of water storage. Clin Oral Investig 2020;24(2):809–22.
33. Mazzoni A, Angeloni V, Comba A, et al. Cross-linking effect on dentin bond strength and MMPs activity. Dent Mater 2018;34(2):288–95.
34. Liu Y, Tjaderhane L, Breschi L, et al. Limitations in bonding to dentin and experimental strategies to prevent bond degradation. J Dent Res 2011;90(8):953–68.
35. Seseogullari-Dirihan R, Mutluay MM, Pashley DH, et al. Is the inactivation of dentin proteases by crosslinkers reversible? Dent Mater 2017;33(2):e62–8.
36. Mazzoni A, Angeloni V, Sartori N, et al. Substantivity of Carbodiimide Inhibition on Dentinal Enzyme Activity over Time. J Dent Res 2017;96(8):902–8.
37. Maravic T, Mancuso E, Comba A, et al. Dentin Cross-linking Effect of Carbodiimide After 5 Years. J Dent Res 2021;100(10):1090–8.
38. Hass V, Luque-Martinez IV, Gutierrez MF, et al. Collagen cross-linkers on dentin bonding: Stability of the adhesive interfaces, degree of conversion of the adhesive, cytotoxicity and in situ MMP inhibition. Dent Mater 2016;32(6):732–41.
39. Wang Y, Green A, Yao X, et al. Cranberry juice extract rapidly protects demineralized dentin against digestion and inhibits its gelatinolytic activity. Materials (Basel) 2021;14(13):3637.

40. Spoerl E, Huhle M, Seiler T. Induction of Cross-links in Corneal Tissue. Exp Eye Res 1998;66(1):97–103.
41. Fawzy AS, Nitisusanta LI, Iqbal K, et al. Riboflavin as a dentin crosslinking agent: ultraviolet A versus blue light. Dent Mater 2012;28(12):1284–91.
42. Reis M, Zhou B, Alania Y, et al. Unveiling structure-activity relationships of proanthocyanidins with dentin collagen. Dent Mater 2021;37(11):1633–44.
43. Vidal CMP, Leme AA, Aguiar TR, et al. Mimicking the Hierarchical Functions of Dentin Collagen Cross-Links with Plant Derived Phenols and Phenolic Acids. Langmuir 2014;30(49):14887–93.
44. Liu Y, Chen M, Yao X, et al. Enhancement in dentin collagen's biological stability after proanthocyanidins treatment in clinically relevant time periods. Dental Mater 2013;29(4):485–92.
45. Seseogullari-Dirihan R, Apollonio F, Mazzoni A, et al. Use of crosslinkers to inactivate dentin MMPs. Dent Mater 2016;32(3):423–32.
46. Hass V, Luque-Martinez I, Muñoz MA, et al. The effect of proanthocyanidin-containing 10% phosphoric acid on bonding properties and MMP inhibition. Dent Mater 2016;32(3):468–75.
47. De-Paula DM, Lomonaco D, Ponte AMP, et al. Influence of collagen cross-linkers addition in phosphoric acid on dentin biomodification and bonding of an etch-and-rinse adhesive. Dent Mater 2020;36(1):e1–8.
48. de Souza LC, Rodrigues NS, Cunha DA, et al. Two-year clinical evaluation of proanthocyanidins added to a two-step etch-and-rinse adhesive. J Dent 2019; 81:7–16.
49. Farooq I, Ali S, Al-Saleh S, et al. Synergistic Effect of Bioactive Inorganic Fillers in Enhancing Properties of Dentin Adhesives-A Review. Polymers (Basel) 2021; 13(13):2169.
50. Noronha VT, Paula AJ, Duran G, et al. Silver nanoparticles in dentistry. Dent Mater 2017;33(10):1110–26.
51. Kramer N, Mohwald M, Lucker S, et al. Effect of microparticulate silver addition in dental adhesives on secondary caries in vitro. Clin Oral Investig 2015;19(7): 1673–81.
52. Dutra-Correa M, Leite A, de Cara S, et al. Antibacterial effects and cytotoxicity of an adhesive containing low concentration of silver nanoparticles. J Dent 2018;77: 66–71.
53. de Souza AP, Gerlach RF, Line SR. Inhibition of human gingival gelatinases (MMP-2 and MMP-9) by metal salts. Dent Mater 2000;16(2):103–8.
54. Osorio R, Yamauti M, Osorio E, et al. Zinc-doped dentin adhesive for collagen protection at the hybrid layer. Eur J Oral Sci 2011;119(5):401–10.
55. Toledano M, Yamauti M, Ruiz-Requena ME, et al. A ZnO-doped adhesive reduced collagen degradation favouring dentine remineralization. J Dent 2012; 40(9):756–65.
56. Wang Z, Shen Y, Haapasalo M, et al. Polycarboxylated microfillers incorporated into light-curable resin-based dental adhesives evoke remineralization at the mineral-depleted dentin. J Biomater Sci Polym Ed 2014;25(7):679–97.
57. Sauro S, Osorio R, Osorio E, et al. Novel light-curable materials containing experimental bioactive micro-fillers remineralise mineral-depleted bonded-dentine interfaces. J Biomater Sci Polym Ed 2013;24(8):940–56.
58. Garcia IM, Leitune VCB, Samuel SMW, et al. Influence of Different Calcium Phosphates on an Experimental Adhesive Resin. J Adhes Dent 2017;19(5):379–84.
59. Al-Hamdan RS, Almutairi B, Kattan HF, et al. Influence of Hydroxyapatite Nanospheres in Dentin Adhesive on the Dentin Bond Integrity and Degree of

Conversion: A Scanning Electron Microscopy (SEM), Raman, Fourier Transform-Infrared (FTIR), and Microtensile Study. Polymers (Basel) 2020;12(12):2948.
60. Wu Z, Wang X, Wang Z, et al. Self-Etch Adhesive as a Carrier for ACP Nanoprecursors to Deliver Biomimetic Remineralization. ACS Appl Mater Inter 2017;9(21):17710–7.
61. Gu LS, Huffman BP, Arola DD, et al. Changes in stiffness of resin-infiltrated demineralized dentin after remineralization by a bottom-up biomimetic approach. Acta Biomater 2010;6(4):1453–61.
62. Profeta AC, Mannocci F, Foxton R, et al. Experimental etch-and-rinse adhesives doped with bioactive calcium silicate-based micro-fillers to generate therapeutic resin-dentin interfaces. Dent Mater 2013;29(7):729–41.
63. Sauro S, Osorio R, Watson TF, et al. Therapeutic effects of novel resin bonding systems containing bioactive glasses on mineral-depleted areas within the bonded-dentine interface. J Mater Sci Mater Med 2012;23(6):1521–32.
64. Bauer J, Silva ESA, Carvalho EM, et al. Dentin pretreatment with 45S5 and niobophosphate bioactive glass: Effects on pH, antibacterial, mechanical properties of the interface and microtensile bond strength. J Mech Behav Biomed Mater 2019;90:374–80.
65. Belli R, Kreppel S, Petschelt A, et al. Strengthening of dental adhesives via particle reinforcement. J Mech Behav Biomed Mater 2014;37:100–8.
66. Balbinot GS, Collares FM, Herpich TL, et al. Niobium containing bioactive glasses as remineralizing filler for adhesive resins. Dent Mater 2020;36(2):221–8.
67. Matos TP, Gutierrez MF, Hanzen TA, et al. 18-month clinical evaluation of a copper-containing universal adhesive in non-carious cervical lesions: A double-blind, randomized controlled trial. J Dent 2019;90:103219.
68. Pintado-Palomino K, de Almeida C, da Motta RJG, et al. Clinical, double blind, randomized controlled trial of experimental adhesive protocols in caries-affected dentin. Clin Oral Investig 2019;23(4):1855–64.