Dentin Adhesion and MMPs: A Comprehensive Review

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ABSTRACT

This review examines the fundamental processes responsible for the aging mechanisms involved in the degradation of resin-bonded interfaces, as well as some potential approaches to prevent and counteract this degradation. Current research in several research centers aims at increasing the resin–dentin bond durability. The hydrophilic and acidic characteristics of current dentin adhesives have made hybrid layers highly prone to water sorption. This, in turn, causes polymer degradation and results in decreased resin–dentin bond strength over time. These unstable polymers inside the hybrid layer may result in denuded collagen fibers, which become vulnerable to mechanical and hydrolytical fatigue, as well as degradation by host-derived proteases with collagenolytic activity. These enzymes, such as matrix metalloproteinases and cysteine cathepsins, have a crucial role in the degradation of type I collagen, the organic component of the hybrid layer. This review will also describe several methods that have been recently advocated to silent the activity of these endogenous proteases.

INTRODUCTION

After Dr. Buonocore introduced enamel chemical etching with 85% phosphoric acid,[1] ensuing research suggested that the micromechanical interweaving of resin into enamel microporosities, through the formation of tag-like resin extensions, was the foundation for dental bonding.[2,3]

The goal of inserting an adhesive restoration is to achieve a sealed and tight adaptation between the restorative material and the dental substrate. This ideal intimate attachment is difficult to accomplish, as dentin contains a significant amount of water and organic material, whereas enamel is composed of over 90% weight of hydroxyapatite.[4] Enamel bonding is reliable when enamel is etched with phosphoric acid[5] (Figure 1). Dentin is a humid porous biologic composite in which the filler is composed of apatite crystal particles embedded in a proteinaceous matrix that includes type I collagen[6] (Figure 2). Dentin is extremely difficult to bond because of its humid and organic nature.[7]

Many dental adhesives combine hydrophilic and hydrophobic monomers in the same bottle. Hydrophilic groups enhance the wettability to the dental hard tissues; hydrophobic groups interact and copolymerize with the restorative material. Because vital dentin is intrinsically humid, it is virtually impossible to dry dentin completely in a clinical situation. Consequently, manufacturers have developed dentin adhesives that are compatible with humid environments.

Upon polymerization of the monomers in the adhesive, the mixture of collagen, resin, residual water, and hydroxyapatite crystallites forms a hybrid layer that bonds the resin restoration to the dentin substrate.[8,9] In spite of the formation of a hybridized tissue at the bonding interface, both etch-and-rinse and self-etch adhesives have their bonding ability compromised over

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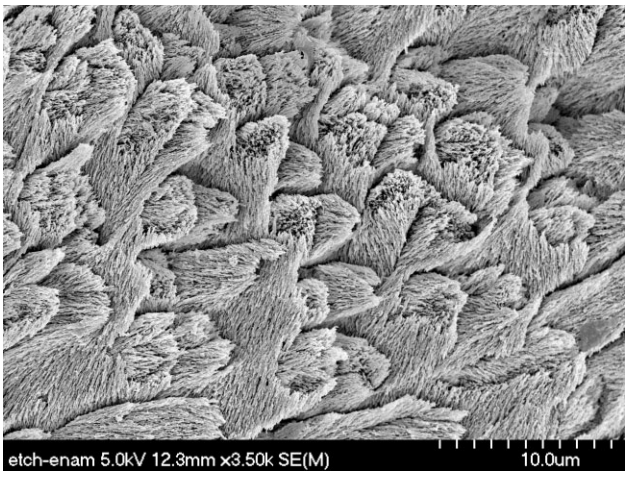

FIGURE 1. Scanning electron microscopy (SEM) micrograph of enamel etched with 34% phosphoric acid for 15 seconds.

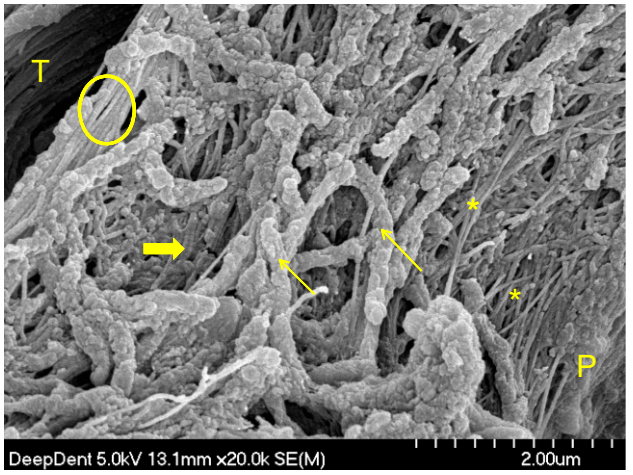

FIGURE 2. Scanning electron microscopy (SEM) micrograph of dentin etched with 34% phosphoric acid for 5 seconds. T = dentin tubule; P = peritubular dentin; Thin arrow = collagen fiber enveloped by hydroxyapatite; Block arrow = collagen fiber with its characteristic striation; Asterisk = collagen fiber around dentin tubule.

time.[10,11] Several factors contribute to the degradation of dentin bonding materials: (1) The hydrophilic nature of some monomers used in the composition of dentin adhesives[12]; (2) The water concentration required for ionization of the acidic monomers in self-etch adhesives[12]; (3) The wet-bonding or moist-bonding technique[13,14] associated with etch-and-rinse adhesives; and (4) The tubular fluid in the anastomoses that permeate dentinal tubules (Figure 3).

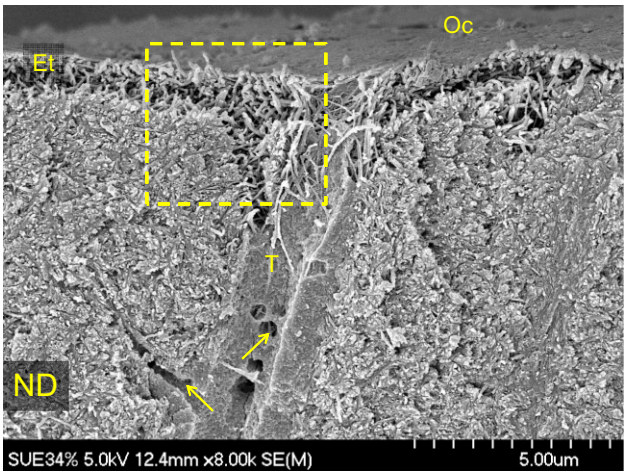

FIGURE 3. Scanning electron microscopy (SEM) micrograph of dentin etched with 34% phosphoric acid for 15 seconds. The arrows point to tubular anastomoses. Oc = occlusal surface; Et = etched dentin with exposed collagen fibers; T = dentin tubule; ND = normal dentin.

Water plays an important role in the partial hydrolytical degradation of adhesive polymers, decreasing their physical properties.[15,16] Absorption of water leads to plasticization of the adhesive resulting in lower bond strengths.[17] For example, 2-hydroxyethyl methacrylate (HEMA) undergoes a decline in physical properties at 24 hours as a result of water sorption after polymerization and extraction of water-soluble unreacted monomers.[18] Additionally, the percentage of water in the adhesive solution influences the degree of conversion of bisphenol A diglycidyl methacrylate (BisGMA)/HEMA mixtures as they undergo phase separation with the increase in water content.[19] The elution of resin from hydrolytically unstable polymers inside the hybrid layer may also cause exposure of the collagen fibers. These newly exposed fibrils, along with the collagen fibrils not fully enveloped by resin monomers during the bonding protocol, are vulnerable to mechanical and hydrolytical fatigue as well as degradation by collagenolytic enzymes,[20] which may compromise the integrity of dentin–resin bonds[11,17,21,22] (Figure 4).

Recent research on dentin noncollagenous proteins has demonstrated that dentin collagen fibrils contain inactive proforms of proteolytic enzymes called matrix metalloproteinases (MMPs). These enzymes have been

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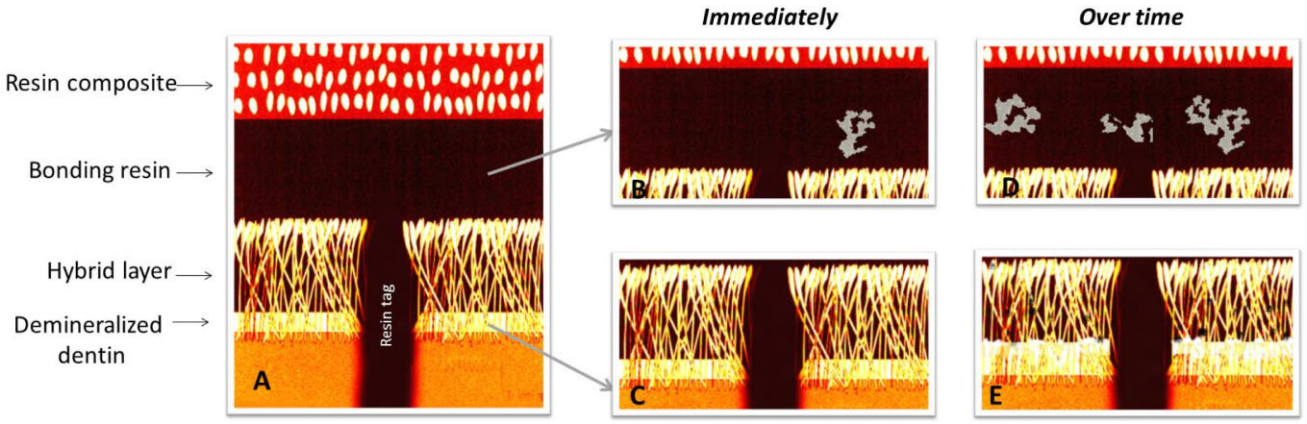

FIGURE 4. Schematic drawing representing the sequence of resin–dentin bonding degradation over time. (Adapted from Hashimoto *et al.*[22]) A, Resin–dentin bonding interface. B, The presence of silver nitrate deposits (in gray) in the adhesive layer soon after bonding indicates the presence of retained water/solvent, as well as areas of inadequate polymerization. C, Areas of denuded collagen fibers, incompletely infiltrated by resin monomers, are present at the base of the hybrid layer. These collagen fibers are prone to degradation by host-derived proteinases. D, Degradation continues over time because of water sorption. The polymer swelling leads to the release of oligomers and residual monomers, which are not cross-linked to the main polymer chains. This can be identified by an increase in the number of silver nitrate deposits in the adhesive layer. E, The area of incomplete collagen infiltration also increases with time.

identified in both odontoblasts and mineralized or demineralized human dentin[23–25] and have been claimed to play a role on the degradation of resin–dentin bonds, which is the focus of this current review.

WHY ARE DENTIN ADHESIVES VULNERABLE TO DEGRADATION?

Etch-and-rinse Adhesives

Upon rinsing the etchant, a scaffold of water-filled collagen network (Figure 5) is created wherein resin monomers infiltrate to form a hybrid complex substrate capable to bond restorative materials to the dental structure. In order to achieve this goal, dentin must be kept fully hydrated to be able to support the collagen fibers and prevent their collapse.[26] Air-drying etched dentin prevents the monomers from penetrating the labyrinth of nanochannels formed by the dissolution of hydroxyapatite crystals between collagen fibers (Figure 5). When dentin is air-dried, the collagen molecules are arranged more compactly because of the lack of water in the interfibrillar spaces. Rewetting this dentin in vitro increases bond strengths and expands the collapsed collagen network to a level similar to a moist condition.[14,27,28]

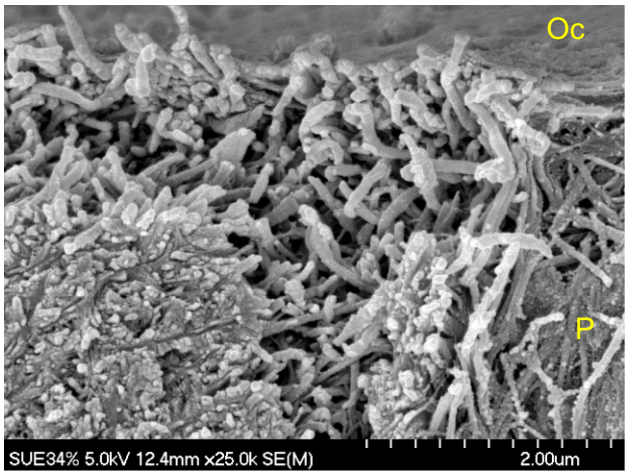

FIGURE 5. Higher magnification of dashed area in Figure 3. After rinsing the etchant, the spaces between the collagen fibrils are filled with water.

The "moist-bonding" technique with etch-and-rinse adhesives has been shown to enhance bond strengths, as water preserves the porosity of collagen network available for monomer interdiffusion in vitro.[13,29] The use of adhesive systems on moist etched dentin is made possible by dissolving hydrophilic monomers in organic solvents, such as acetone or ethanol, in the primers or in the adhesive solutions. Because the solvent can

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displace water from both the dentin surface and the moist collagen network, it promotes the infiltration of resin monomers throughout the nanospaces of the dense collagen web. Ideally, these monomers would replace all the water. However, the complete replacement of the rinsing water by adhesive monomers is unachievable, resulting in hybrid layers that contain voids.[21,30] Additionally, the application of solvated commercial bonding agents to moist collagen matrices causes shrinkage of the collagen network between 23% and 28%, depending on the concentration of ethanol or acetone in the adhesive solution.[31]

Although water is essential at the early stage of resin infiltration, its presence within the interfibrillar spaces of the collagen matrices may trigger, not only the hydrolysis of resin matrices by esterases, but also hydrolysis of collagen by endogenous and exogenous collagenolytic and gelatinolytic enzymes.[32] The residual water in the collagen network may also result in adhesive phase separation at the resin–dentin interface[19] (Figure 4B), which weakens the polymer within hybrid layer making it more susceptible to degradation by enzymes[33] (Figure 4D).

Another unsolved problem associated with etch-and-rinse adhesives is that there is a decreasing gradient of monomer impregnation of collagen fibers with depth,[34] meaning that collagen is less infiltrated with resin toward the base of the hybrid layer[35] (Figure 4C). This is even more pronounced in caries-affected dentin,[35] which is a more clinically relevant substrate than ideal unaffected dentin used in in vitro bond testing. Denuded collagen fibrils resulting from incomplete resin infiltration (Figure 6) cannot be protected against denaturation and fatigue breakdown after function,[36,37] which may result in an area of uninfiltrated collagen network at the bottom of the hybrid layer[38] (Figure 7).

In vitro research has shown that resin–dentin bonds deteriorate over time,[11,39–41] as a result of the degradation of the hybrid layer at the dentin-adhesive interface.[42] Besides the hydrolytic degradation of adhesive resins,[17] proteolytic degradation of collagen fibrils may be also responsible for the decline in dentin bond strengths over time.[43,44] In vivo studies have also demonstrated that the collagen component of the hybrid layer undergoes a gradual hydrolytic degradation[43,45,46] (Figure 4E) (Table 1).

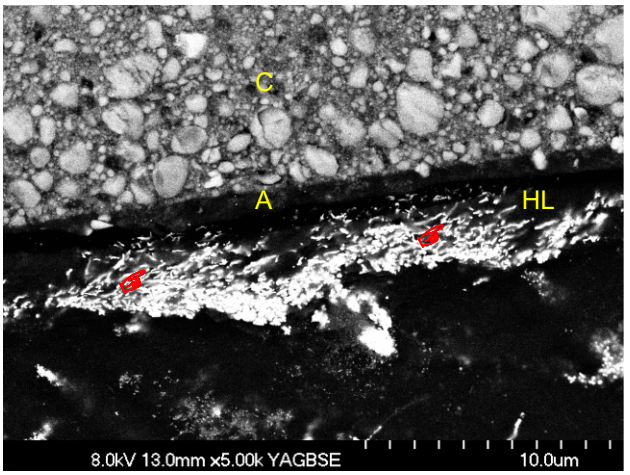

FIGURE 6. Scanning electron microscopy (SEM) micrograph (backscattered mode) of resin–dentin interface formed with a two-step etch-and-rinse adhesive applied on moist dentin. C = resin composite; A = adhesive; HL = hybrid layer; Pointer = the reticular pattern corresponds to collagen fibers in the hybrid layer enveloped by silver nitrate.

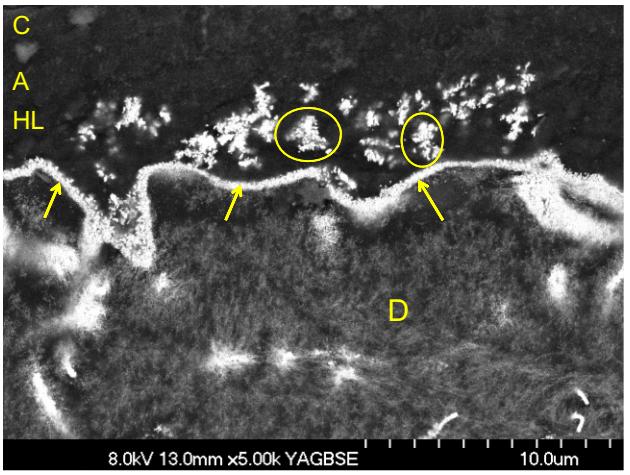

FIGURE 7. Scanning electron microscopy (SEM) micrograph (backscattered mode) of resin–dentin interface formed with a two-step etch-and-rinse adhesive applied on dried dentin. The arrows point to accumulation of silver nitrate at the bottom of the hybrid layer. The circles show "water trees." C = resin composite; A = adhesive; HL = hybrid layer; D = dentin.

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TABLE 1. Degradation mechanisms and prevention

	Collagen	Resin monomers
Degradation mechanism	Hydrolysis by residual water in the composition of the adhesive	Hydrolysis and plasticization by residual water and by dentin intrinsic humidity
	Hydrolysis by residual water not removed after rinsing the etchant	Enzymatic hydrolysis
	Exposed collagen by elution of resin from hydrolytically unstable polymers in the hybrid layer	Decreasing gradient of monomer impregnation of exposed collagen fibers (hybrid layer) with depth
	Denuded collagen fibers have increased susceptibility to mechanical fatigue and denaturation after function	Phase separation from residual water in self-etch adhesives
	Dentin and saliva collagenolytic enzymes (MMPs, cathepsins) digest unprotected collagen fibers	Self-etching primers behave as semi-permeable membranes—water blisters result in mechanical disruption of the bond between the adhesive and the composite resin
		Continuing etching of dentin underneath the interface caused by uncured acidic monomers in self-etch adhesives
How to prevent degradation?	Exogenous inhibitors of collagenolytic enzymes (chlorhexidine, EGCG, and others)	Water-free solvated primers + hydrophobic bonding resins
	Endogenous tissue inhibitors of MMPs (TIMPs)	New monomers with increased conversion rate
	Water-free solvated primers + hydrophobic bonding resins	New initiators that may increase the polymerization of monomers even in the presence of water
	Cross-linking agents (glutaraldehyde, riboflavin, carbodiimides, and others)	Resin monomers with antibacterial agents, such as quaternary ammonium methacrylates
	Biomimetic remineralization	Self-adhesive restorative materials that are able to incorporate water in their setting reaction
	Use of iron gel to inhibit Zn^{2+} and Ca^{2+} present in the MMP structure	
	Quaternary ammonium methacrylates may inhibit MMPs	

EGCG = epigallocatechin-3-gallate; MMP = matrix metalloproteinase.

Self-etch Adhesives

It has been reported that self-etch adhesives result in a simultaneous demineralization and infiltration of the dentin substrate[5] (Figure 8). Nevertheless, incomplete infiltration of dentin by self-etch adhesives occurs[47,48] in particular for some mild self-etch adhesives that may possess a reduced etching potential of the acidic monomers toward the base of hybrid layers[49] (Figure 4C). Self-etch adhesives are highly hydrophilic; thereby, they attract water, which may increase the potential for degradation[15,50] (Figure 4B), as the water sorption of adhesive resins is proportional to their hydrophilic characteristics.[51,52] The self-etching ability of self-etching primers is commonly achieved by the incorporation of sufficient water for adequate ionization of the acidic monomers without lowering the monomer concentration to a threshold that would compromise the bonding efficacy.

Water is an important ingredient because it ionizes the acidic groups, allowing the formation of hydronium ions (H_3O^+), which etch hydroxyapatite.[12] Water also facilitates solubilization of the reaction products resulting from the etching process. Self-etching adhesives typically contain 30% to 40% water.[12]

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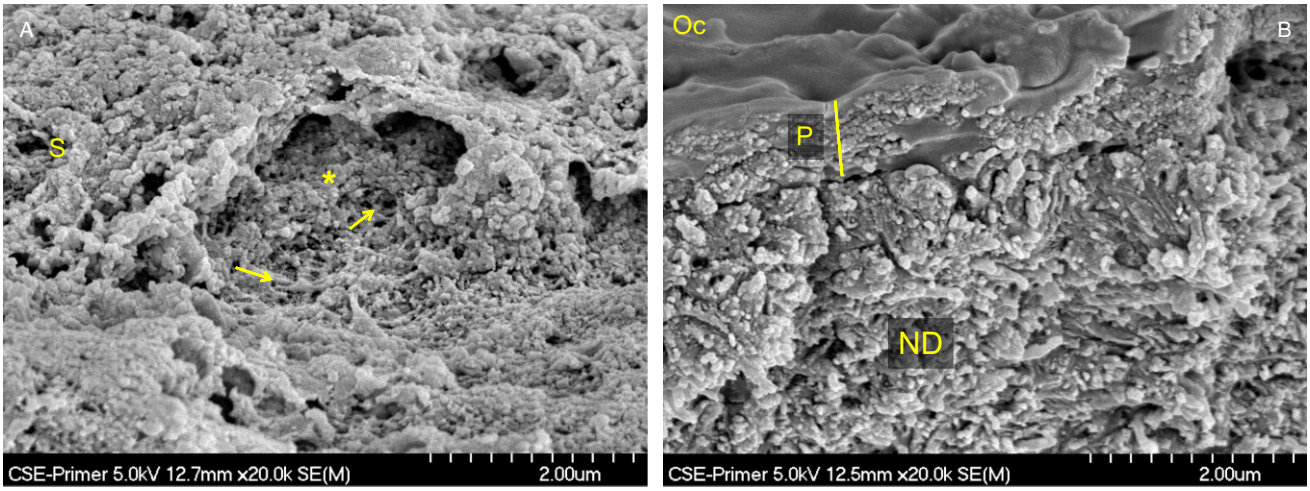

FIGURE 8. A, Scanning electron microscopy (SEM) micrograph of smear layer-covered dentin treated with a self-etching primer. In order to visualize the thickness of the smear layer, this micrograph was recorded at a 45° angle. The arrows denote collagen fibers exposed by the mild acidity of the primer. S = primer-impregnated smear layer. B, SEM micrograph of smear layer-covered dentin (lateral view) treated with a self-etching primer. The line represents the depth of primer penetration (0.6 μm). P = mixture of self-etching primer with smear layer and hydroxyapatite crystals; ND = normal dentin; Oc = occlusal surface.

Increasing the water concentration dilutes the concentration of the acidic monomer and may decrease the bonding effectiveness of the corresponding adhesive system. Although water is essential for the ionization of acidic resin monomers, it may prevent the formation of a strong polymer within the hybrid layer. The mechanical properties of one-step self-etch adhesives decrease significantly in the presence of water, which is less likely to occur with two-step self-etch adhesives.[52] In fact, one-step self-etch adhesives undergo higher water sorption than two-step self-etch adhesives,[53] as the hydrophobic resin layer tends to make two-step self-etch adhesives more impermeable to water sorption, therefore increasing the respective bond strengths and clinical longevity.[54,55]

One-step self-etch adhesives absorb and retain water through hydrogen bonds when applied to hydrated dentin, behaving like semipermeable membranes[56] (Figure 4B). Even after polymerization, these adhesives allow the movement of water and fluids from the intertubular dentin and from the dentinal tubules.[57] This water flux is responsible for an intricate pattern of water-filled channels within the adhesive layer known as water trees,[58] which are also observed for two-step etch-and-rinse adhesives (Figures 4B, D, and 7).

Water that migrates to the interface adhesive/composite resin will be trapped by the overlying hydrophobic composite, forming water blisters.[56] The water blisters have a detrimental role, as they result in mechanical disruption of the bond between the adhesive and the composite resin.[58] Water permeability and blister formation may also occur in light-cured composites when coupled to one-step self-etch adhesives applied to hydrated dentin, in case there is a delay in the composite light polymerization.[59] Additionally, the higher hydrophilicity of self-etch adhesives, especially one-step self-etch solutions, makes this bonding strategy more vulnerable to water sorption/solubility. As a consequence, polymer swelling facilitates the elution of low molecular weight oligomers from the hybrid layer, which may, in turn, expose collagen for cleavage by endogenous proteases (Figure 4E). Although bond degradation by endogenous proteases appears to be less of an issue for mild self-etch adhesives,[42] one cannot completely rule out the role of dentin proteases in the degradation of bonded interfaces created with ultrasimplified one-step adhesives. In fact, the addition of MMP inhibitors to self-etch primers prevented resin–dentin bond degradation in vitro.[60] This phenomenon may be even more significant for acidic self-etch systems, in which the continuing etching of

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dentin underneath the interface may occur caused by uncured acidic components, even long after the initial application.[49,61]

ENDOGENOUS PROTEASES AS COLLAGENOLYTIC ENZYMES

MMPs

In 1962, Gross & Lapiere described an "activity" that was observed during metamorphosis in tadpoles and had the ability to degrade collagen.[62] This activity was later described as triggered by interstitial collagenase, an enzyme that degrades the collagen triple helix. This initial observation would progress to include a new family of enzymes—MMPs.[63]

One of the first MMPs to be extracted directly from tissues was collagenase (later known as MMP-13), which was obtained from rat uterus. This isolation of a collagenase from rat tissue suggested at the time that the increased production occurred in response to a particular biological process, in this particular case, uterine resorption after pregnancy.[64]

MMPs or matrixins are a family of over 20 host-derived proteolytic enzymes, a class of zinc- and calcium-dependent endopeptidases[63,65] that are capable of degrading extracellular matrix (ECM) proteins, as well as clotting factors, lipoproteins, latent growth factors, and chemotactic and cell adhesion molecules.[63,66,67]

MMPs are secreted as proenzymes (zymogens) and are activated by proteinases or some chemical agents, including reactive oxygen species. MMPs can also be activated by low pH,[68] probably through the disruption of cysteine–zinc binding.[69] MMP activities are inhibited by endogenous inhibitors or tissue inhibitors of metalloproteinases (TIMPs).[63,69] Thus, the balance between MMPs and TIMPs is critical for the eventual ECM remodeling in the tissue. Timely degradation of ECM is an important feature of development, tissue repair, and remodeling. When the activity of MMPs is deregulated, it may become a cause of many diseases such as nephritis, cardiac diseases, cancer, chronic ulcers, arthritis, and fibrosis.[69,70]

Odontoblasts synthesize MMPs that participate in tooth development,[71] dentin caries process,[71,72] and degradation of the hybrid layer in dentin–resin interfaces.[20,73] MMPs also contribute to the organization and mineralization of the dentin matrix.[72] Several MMPs have been identified in mineralized human dentin, in an inactive state—MMP-8 collagenase,[25] MMP-2 and MMP-9 gelatinases,[24,74] and MMP-20 enamelysin.[75] Although MMP-2 and MMP-9 have been considered as gelatinases, other research has described collagenolytic activity associated with these two MMPs.[76,77] As early as 1995, it was reported that both human and chicken MMP-2, when free of TIMPs, were capable of cleaving soluble, triple helical type I collagen.[76] Further research identified MMP-2 and MMP-9 as playing a role in bone resorption.[77]

MMPs are exposed and activated by acidic agents during adhesive bonding procedures. If these matrix-bound, activated MMPs are not fully infiltrated with adhesive resin, they can slowly degrade the collagen fibrils at the resin–dentin-bonded interface.[78] When dentin MMPs are exposed and activated by self-etch or total-etch adhesives,[68,73] these enzymes degrade type I collagen.[79] As collagen fibrils are incompletely infiltrated with resin monomers,[21,34] MMPs may degrade the collagen within incompletely resin-infiltrated hybrid layers, decreasing the longevity of bonded restorations.[42,80] Intense MMP-2 and MMP-9 activities were detected at the basal part of the hybrid layer.[42,80]

New strategies to prevent the degradation of dentin bonds may be crucial to increase the longevity of bonded restorations. Therefore, the use of exogenous MMP inhibitors, such as chlorhexidine, gallardin, and flavonols[81–83] may be an effective strategy to improve the longevity of adhesive restorations (Table 1). Synthetic MMP inhibitors must contain a functional group such as a carboxylic acid that can interact ionically with the Zn^{2+} ion in the MMP molecule.

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Cysteine Proteases (Cathepsins)

Cathepsins are papain-like cysteine proteases that are widely expressed throughout the animal and plant kingdoms.[84] There are approximately 12 members of this family, which are distinguished by their structure, catalytic mechanism, and which proteins they target. Although most cathepsins are lysosomal enzymes that become activated in lysosomes by low pH, cathepsin K works extracellularly after secretion by osteoclasts in bone resorption.[84] This protease represents 98% of the total cysteine protease activity.[85] The physiologically relevant substrate of osteoclast-expressed cathepsin K is type I collagen which constitutes 95% of the organic bone matrix and 90% of the dentin matrix.[86,87]

Cathepsins play an important role in specific functions in ECM turnover, antigen presentation, and processing events. They may also represent viable drug targets for major diseases such as osteoporosis, arthritis, immunorelated diseases, atherosclerosis, cancer, and for a wide variety of parasitic infections.[84] Papain-like cysteine proteases are expressed as preproenzymes, being synthesized at the rough endoplasmic reticulum. The activity of cathepsins is regulated by a large number of endogenous protein-based inhibitors.

Proteoglycans and phosphoproteins are the main constituents of dentin noncollagenous proteins.[88,89] The high affinity of proteoglycans for water molecules and their close association with dentin collagen fibrils gives proteoglycans a significant role in controlling the properties of the demineralized dentin matrix, which is the typical bonding substrate. Proteoglycans have attached one or more glycosaminoglycan chains,[89,90] among which is chondroitin sulfate. Cathepsin activities are regulated by chondroitin sulphate.[91] For example, the collagenolytic activity of cathepsin K requires the formation of a complex between this protease and chondroitin sulfate. In the absence of chondroitin sulfate, which is a major component of ECM including dentin, monomeric cathepsin K does not degrade the triple helical collagen.[91]

The relationship between the activities of MMPs and cysteine cathepsin in intact or carious dentin has been recently reported.[92,93] The activities of MMPs and cysteine cathepsin in carious lesions showed a tenfold increase compared with those of intact dentin. The role of cysteine cathepsins and MMPs in dentinal tubules remains unclear, but they may play a role in tubular occlusion and peritubular dentin formation.[92] Cathepsin K is the only known mammalian collagenase with activity capable to cleave inside the triple helical collagens at multiple sites and to completely hydrolyze collagen fibrils into small peptides.[94,95] This activity is only comparable to that of bacterial collagenases.[96] Therefore, one of the pathways to preserve dentinal collagen fibers at the bonded interface from proteolytic digestion may be the inactivation of dentin cathepsins.

INHIBITORS OF PROTEOLYTIC/ COLLAGENOLYTIC ACTIVITY

Chlorhexidine

Chlorhexidine (CHX) is a widely used antimicrobial agent that possesses a broad spectrum of activity against oral bacteria.[97] This cationic bisbiguanide has a unique effect of dental plaque inhibition, mainly due to its substantivity[98] and its antimicrobial property.[99]

It has been demonstrated that CHX is an efficient adjunct to periodontal therapy by controlling supragingival plaque and gingival inflammation.[99] Consequently, CHX mouthrinses are widely used in the prophylaxis and treatment of periodontal diseases.

In 1999, it was reported[100] that CHX inhibits some MMPs, which became a valuable effect in the treatment of periodontal disease. The ECM components, including collagen and proteoglycans, are proteins responsible for the structural integrity of the periodontal tissues. Destruction of the periodontal tissues involves the degradation of the ECM components, leading to irreversible loss of periodontal tissues.

Even before being tested as a potential MMP inhibitor, CHX was first used in dental adhesion as a dentin disinfectant prior to the application of dental adhesives.

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Under the scanning electron microscope, CHX residues were firmly attached to the etched dentin surface after rinsing, but the application of CHX during the adhesive procedure did not influence dentin bond strengths.[101]

CHX has been recently studied as a protease inhibitor to preserve the hybrid layer through the inhibition of MMPs[20] and cysteine cathepsins.[102] Dentinal collagen fibrils may undergo degradation by MMPs if they are not fully enveloped by resin.[73] The use of CHX-containing aqueous primer results in the preservation of dentin bond strengths and the integrity of the hybrid layer with time,[103–105] similarly to when CHX is included in the composition of commercial phosphoric acid etchants,[106,107] or incorporated into the composition of some bonding resins.[108] However, in spite of the demineralized dentin being able to bind more CHX than mineralized dentin, rinsing with water easily removes CHX, as opposed to ethanol or even HEMA, which do not remove as much CHX from demineralized dentin.[109] The dissolution with water may preclude the future use of CHX in the composition of phosphoric acid gels.

The effect of including two MMP inhibitors in the primers of three commercial adhesives has been recently tested.[110] Although the immediate bond strengths were increased when MMP inhibitors were added to Optibond FL (Kerr Corporation, Orange, CA, USA) and G-Bond (GC America Inc., Alsip, IL, USA), after 3 months, there was no difference in bond strengths. Nonetheless, zymographic analyses showed that the activity of the dentin MMPs was inhibited in all experimental groups.[110]

Other Potential MMP Exogenous Inhibitors

Epigallocatechin-3-gallate

The MMP-inhibitory potential of several natural substances has recently received increased attention from researchers.[111] Although CHX has been extensively studied, very little is known about the potential utility of green tea polyphenol epigallocatechin-3-gallate (EGCG) in resin–dentin bonds.

The tumor-inhibiting property of green tea polyphenol EGCG is well documented.[112,113] In addition to the protective effects of green tea against certain types of cancer, its consumption enhanced the efficiency of cancer chemotherapy in mice.[82] Polyphenols were reported to be the most effective chemopreventive agents against the initiation, promotion, and progression stages of multistage skin carcinogenesis.[113]

EGCG inhibits the activity of MMP-2 and MMP-9 by the degradation of the MMP molecule.[83] Studies revealed that MMPs play a fundamental role in cancer progression, facilitating an access of tumor cells to vasculature and lymphatic systems, as well as in tumor invasion through the degradation of basement membranes and ECM.[114,115] EGCG may have a therapeutic effect in activation, secretion, and signaling molecules that might be involved in the regulation/inactivation of MMP-2 in some forms of cancer.[116]

In dentistry, EGCG may inhibit the activity and expression of MMP-9 involved in the formation of osteoclasts in periodontal disease[117] apart from its inhibitory effect on the growth of *Streptococcus mutans* when added to a bonding adhesive.[118] Green tea extract has also been reported to reduce dentin erosion-abrasion by inhibiting MMPs.[111] Because of its anti-MMP properties, EGCG-containing adhesives may have a potential to increase the resin–dentin bond durability, although this is yet to be fully investigated.

Galardin

Galardin is a potent and broad-spectrum hydroxamate-type synthetic MMP inhibitor designed as a molecular mimic of MMP substrates, which allows it to enter the active site of MMPs, where it binds the critical zinc atom.[119] Galardin is active against several MMPs.[120] Galardin suppresses MMP activity in vivo, inhibiting wound healing[121,122] and mammary gland development.[123] It also inhibits tumor growth and metastasis in mice.[124]

The inhibitory effect of galardin on dentin MMPs was confirmed by zymographic analysis, as the complete inhibition of both MMP-2 and MMP-9 was observed,[80]

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without compromising bond strengths. Galardin resulted in a 27% reduction in bond strength after 1 year of aging, which was significantly less than the 45% reduction measured in untreated controls.[80]

Tetracyclines and Analogues

Although tetracyclines (TCs) are commonly known as broad-spectrum antibiotics acting at the ribosomal level, where they interfere with protein synthesis, they also exert other biological actions such as inflammation, immunomodulation, cell proliferation, and angiogenesis.[125]

TCs and their analogues inhibit MMPs by mechanisms independent of the antimicrobial property of these antibiotics.[126,127] Although it is claimed that Ca^{2+}- and Zn^{2+}-binding site of TCs and analogues is responsible, at least in part, for inhibiting the extracellular activity of MMPs, multiple nonantimicrobial mechanisms at the ECM and intracellular region are involved in such therapeutic property of TCs and their analogues.[125,127]

Three groups of TCs are currently available: TC and natural products, semisynthetic TC compounds, and chemically modified TC.[128] The latter have therapeutic potential but do not appear to induce antibiotic side effects,[125] such as gastrointestinal disturbance. Minocycline and doxycycline, semisynthetic TCs, have widespread medical and dental applications. Doxycycline is safer than minocycline and is one of the common antimicrobial TCs studied as an anticancer agent[129–131] and as adjunctive treatment for the management of chronic periodontitis.[132,133]

Although the application of aqueous solutions of minocycline and doxycycline as a preliminary step after acid-etching on the immediate bonding performance was recently investigated,[134] the use of TCs and their analogues in dentin bonding as a way to prolong resin–dentin bonds is still scarce in the literature and should be the focus of further investigation because of the potential role of TCs on MMP inhibition.

Quaternary Ammonium Salts

Quaternary ammonium compounds are water-soluble molecules; therefore, they may leach out of adhesive interfaces. These substances possess antimicrobial properties and have been incorporated into dental resins.[135–137] The quaternary ammonium molecules still have significant bacteriostatic activity when they become immobilized in the resin matrix.[137]

One of the advantages of using quaternary ammonium methacrylates, such as 12-methacryloyloxydodecylpyridinium bromide (MDPB),[135,136,138] is that they can copolymerize with adhesive monomers. Clearfil Protect Bond (Kuraray Noritake Dental Inc., Osaka, Japan) was the first commercial dentin adhesive to incorporate MDPB in its composition.

Similarly to CHX, quaternary ammonium methacrylates are cationic. A recent study showed that quaternary ammonium methacrylate resin monomers have various degrees of inhibitory activity of MMPs.[139] Whether or not this monomer retains its inhibitory effect against MMPs after becoming copolymerized with other monomers in an adhesive formulation is an issue that deserves further investigations. However, when degradation of resin–dentin bonds was evaluated using Clearfil Protect Bond or Clearfil SE Bond (adhesive with very similar composition, but without MDPB), the results showed that the degradation pattern (decreasing of the bond strength and more pronounced water trees) was observed for Clearfil SE Bond, but not for Clearfil Protect Bond (currently available as Clearfil SE Protect), indicating better preservation of hybrid layer when adhesive containing MDPB was used.[140]

Benzalkonium chloride (BAC) is also one antimicrobial substance containing a quaternary ammonium group in its molecule. This substance has been included in an acid phosphoric gel (i.e., ETCH-37 w/BAC and ETCH-10 w/BAC, Bisco Inc., Schaumburg, IL, USA) for several years. The use of these BAC-containing phosphoric acid gels did not affect the immediate bond strength to enamel and dentin.[141] Recently, the anti-MMPs properties of BAC were tested against MMP-2, -8, and -9. The results showed potential for this substance to inhibit MMP-2, -8, and -9.[142] However, one advantage compared with CHX-containing phosphoric acid gel is that when the

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etching gel contains BAC, this molecule binds strongly to demineralized dentin even after rinsing.[142]

Ethylenediaminetetraacetic Acid

Ethylenediaminetetraacetic acid (EDTA) is one of the most commonly used agents for irrigation during mechanical instrumentation of the root canal system. It has been also used as an adjunct to mechanical root periodontal therapy.[143,144] EDTA decalcifies smear layer-covered dentin superficially, but its action is self-limiting. EDTA acts as a chelating agent, reacting with calcium ions from dentin hydroxyapatite and forms soluble calcium salts.[143] As EDTA is an effective Zn^{2+} and Ca^{2+} chelator,[145] it might inhibit MMP activity. In fact, EDTA has inhibitory effect against human dentin MMP-2 and MMP-9 when applied for 1 to 5 minutes.[146,147]

As a consequence, the use of EDTA has been suggested as a dentin pretreatment for dentin adhesives.[148,149] The results showed an increase in dentin bond strength, as compared with phosphoric acid and other types of dentin-conditioning agents.[148–150] EDTA also preserved the dentin-adhesive interface in longevity tests.[150]

One drawback is that EDTA is removed from dentin by extensive rinsing with water. There may be no residual EDTA left to inhibit the activity of MMPs.[146,147] It has also been showed that collagen in EDTA-demineralized dentin was as susceptible to MMP degradation as collagen in dentin etched with phosphoric acid.[66] Therefore, it is not clear if the preservation of hybrid layer from EDTA is a result of a shallow dentin demineralization or from MMP inhibition. Future studies are needed to test this hypothesis.

METHODS TO INCREASE THE DURABILITY OF DENTIN BONDS

Cross-linking Agents

Cross-linking is considered a potential method for improving the stability and resistance of collagen degradation within the demineralized dentin matrix.[151,152] An increase in the extent of cross-linking of the collagen fibrils prior to adhesive application may result in increased bonding durability. Apart from increasing the strength of the collagen to degradation by proteases, some cross-linking agents were shown to have anti-MMP properties.[32,153] However, one of the major disadvantages of using cross-linking agents to inactivate MMPs and cysteine cathepsins is that the application/activation time needed to achieve the desirable therapeutic effect may not be within clinical feasibility. Another potential disadvantage is that a water-rich collagen matrix with poor mechanical properties may be retained within the hybrid layer.

Several methods have been used to increase collagen cross-linking. Chemically induced cross-linking has been tried in dentin adhesion since the 1980s by applying *glutaraldehyde* as a component of a priming solution.[154] Various other chemical cross-linking reagents, which include epoxy compounds, carbodiimide, proanthocyanidin, and genepin have been used,[151,153,155–158] but some have drawbacks such as toxicity, difficult to control cross-linking rates, and instability.[156]

More recently, physical methods, specifically ultraviolet radiation, have been tried in dentistry and in ophthalmology.[159–161] Ultraviolet (UVA)-activated *riboflavin* has been shown to increase bond strength, stabilize the adhesive interface, and inhibit dentin MMPs.[159] Riboflavin has potential in adhesive dentistry because it is activated with a UVA blue light and is easy to apply, besides being biocompatible.[160] UVA-activated riboflavin increased the mechanical properties, mechanical stability, and resistance to the degradation of dentin collagen.[160] Collagen fibers also became more resistant to collagenolytic digestion upon treatment with UVA-activated riboflavin.[160]

As UVA-activated riboflavin was found to stimulate the cross-linking of the dentin collagen matrix, this technique may have potential applications in adhesive dentistry as a dentin pretreatment, or as a modifier for etch-and-rinse and/or self-etch adhesives.[160]

Proanthocyanidins are part of a naturally occurring group of polyphenolic compounds that belong to

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the category known as condensed tannins. Proanthocyanidin is the plant flavonoid prevalent in pine bark, elm tree, some nuts, flowers, and grape seeds,[156,162,163] being known as a potent antioxidant and cross-linking agent with low toxicity.

Grape seed extract is one of the most used sources of proanthocyanidin.[162] The use of grape seed extracts improved the ultimate tensile strength, stiffness,[151,157] and long-term stability[155,164] of dentin collagen. In addition to its cross-linking effect, proanthocyanidin has also been shown to inhibit the synthesis of several MMPs from macrophages and inhibit the catalytic activity of MMP-1 and MMP-9.[156,165]

The effect of proanthocyanidin on dentin bonds is still not well understood. Although the incorporation of 2% proanthocyanidin into experimental dental adhesives had no adverse effect on dentin bond strength, a concentration of 3% proanthocyanidin significantly decreased bond strengths.[166]

Carbodiimides have been used as alternative cross-linking agents to glutaraldehyde because they do not contain toxic components.[153] Carbodiimides increase the mechanical properties of the dentin collagen matrix, but it takes from 1 to 4 hours for carbodiimides to be effective,[167] which makes the procedure clinically intolerable. They cross-link all proteins nonspecifically by activating the carboxylic acid groups.

Carbodiimides can inactivate dentin MMP-9 and other dentin proteases with only 1-minute application time,[153] which may open the door for future research on using carbodiimides to stabilize the dentin bonding interface. However, it is still unknown whether carbodiimides, as with some other potential MMP inhibitors, will trigger any negative reaction in the pulp tissue.

Biomimetic Remineralization

The concept of dentin remineralization after acid dissolution of hydroxyapatite crystals has been known for some time. Dr. Fusayama and coworkers characterized dentin in carious lesions as consisting of a superficial layer and a deeper layer.[168–170] Whereas the outer layer was deemed as physiologically unrecalcifiable containing degenerated collagen fibers with virtual disappearance of cross-links, the inner layer was depicted as intermediately decalcified, with sound collagen fibers, and apatite crystals bound to the fiber, therefore physiologically recalcifiable.

Recently, the concept of biomimetic remineralization applied to dentin bonding has been extensively studied.[171–173] When dentin hydroxyapatite is dissolved by acidic agents included in etch-and-rinse and self-etch adhesives, water immediately fills in the spaces previously occupied by hydroxyapatite. The resin monomers included in current dentin adhesives are unable to replace the collagen extrafibrillar and intrafibrillar water. This phenomenon is indirectly depicted as silver ions (nanoleakage) penetrating the empty nanovoids within the hybrid layer structure (Figures 6 and 7).

The use of CHX as an MMP inhibitor does not preclude the accumulation of water at the resin–dentin interface after 12 months on in vivo aging.[174] By remineralizing the water-embedded denuded collagen fibrils within the hybrid layer, proteolytic degradation may be prevented through immobilization of MMPs, increasing the longevity of resin–dentin bonds.[174]

Hybrid layers created by etch-and-rinse adhesives[175,176] and self-etch adhesives[109,173] have been shown to be remineralizable with a biomimetic mineralization approach. The concept of biomimetic mineralization mimics what occurs in biomineralization.[171] Ideally, the water in the resin-poor spaces within the hybrid layer would be replaced with apatite crystallites that are small enough to occupy the extrafibrillar and intrafibrillar areas of the collagen matrix, and has been adopted for remineralization of resin–dentin bonds.[172] For etch-and-rinse adhesives, apatite crystallites were observed in both extrafibrillar and intrafibrillar spaces after biomimetic mineralization.[32]

One of the obstacles for biomimetic remineralization of the poorly infiltrated hybrid layer is that there may be no residual hydroxyapatite crystallites in the

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demineralized collagen network to serve as nucleation site for further remineralization. Unfortunately, remineralization was performed via a lateral diffusion mechanism by the immersion of specimen slabs in a remineralizing medium containing dissolved biomimetic analogues. This technique is not clinically applicable in the present form.[32]

Ethanol Wet-bonding

Acid-etching activates dentin MMPs.[20,73] MMPs may remain active even after resin infiltration into the collagen interfibrillar spaces.[41,73,177] MMPs split collagen peptide chains into fragments by adding water across collagen peptide bonds. As MMPs become inactive in the absence of water, the replacement of water with an organic solvent, such as ethanol, in the demineralized collagen matrix, might improve the longevity of the bonds.

The water that fills the intrafibrillar and interfibrillar spaces, after chemical dissolution of hydroxyapatite by acids, should be totally displaced by resin monomers during bonding.[178] Recent in vitro research has tested the replacement of water with ethanol in etched dentin, a technique known as "ethanol wet-bonding."[179–181] This in vitro technique was developed for the application of etch-and-rinse adhesives into an ideal water-free demineralized dentin matrix.[179] Ethanol is used to chemically dehydrate the collagen matrix,[50] creating wider interfibrillar spaces that allow the permeation of hydrophobic resins to infiltrate the matrix more thoroughly (Figure 9) as a potential mechanism for extending the longevity of resin–dentin bonds.[180,181]

When acid-etched dentin is saturated with 100% ethanol instead of water, the bond strengths of both hydrophilic and hydrophobic resins increase significantly. Although ethanol wet-bonding appears promising, it involves an extra step of replacing rinsing water with 100% ethanol. The time needed to replace water with ethanol in etched dentin would make the technique difficult to implement in a clinical environment. Recent methods to simplify the ethanol wet-bonding technique, without compromising bond strengths and nanoleakage, have not been successful.[182] Additionally, the presence of ethanol in the hybrid layer may preclude the process of remineralization.[183]

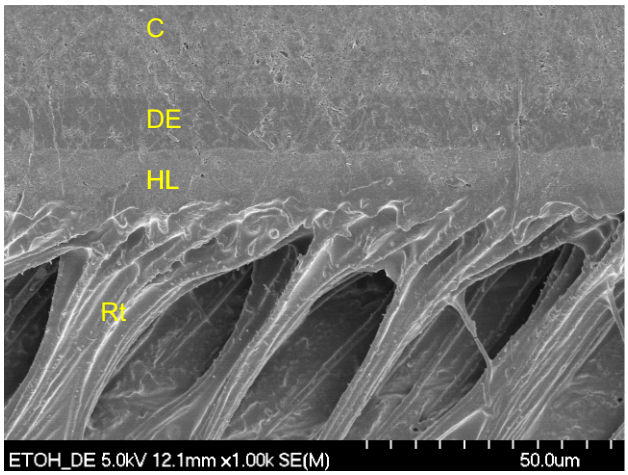

FIGURE 9. Scanning electron microscopy (SEM) micrograph of resin–dentin interface formed by a hydrophobic bonding resin. Water was replaced with 100% ethanol in etched dentin prior to applying and curing the hydrophobic resin. Specimen was decalcified in 6N HCl for 30 seconds, followed by deproteinization in 2% NaOCl for 10 minutes. C = resin composite; DE = hydrophobic resin; HL = hybrid layer; Rt = resin tag.

Because ethanol has been shown to cause collapse and shrinkage of collagen fibrils, ethanol wet-bonding may end up resulting in reduced adhesive infiltration. Another point of concern is the fact that, ethanol is not effective in inhibiting MMPs.[184] More research is needed in this area.

FUTURE RESEARCH

Research on exogenous MMP inhibitors for dental use is still in the early stages (Table 2). MMPs are potentially involved at several phases of cancer progression including tumor initiation, vascularization, invasion, and metastasis.[188,189] The roles of MMPs in cancer reflect their physiologic ability, through limited proteolysis, to restructure the tissue microenvironment and to convey extracellular signaling. In the healthy organism, MMPs are involved in several tissue

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TABLE 2. Potential MMP inhibitors, clinical availability, and important clinical remarks for longevity of resin–dentin bonds

MMP inhibitor	Clinically available for dental use?	How can it be incorporated into the bonding technique?	Available commercial products	Longevity studies	Important clinical remarks
Chlorhexidine (CHX)	Yes	1. Added to the phosphoric acid etchant 2. Used as an aqueous solution after acid-etching (etch-and-rinse adhesives) 3. Incorporated into the adhesive system (etch-and-rinse or self-etch adhesive)	1. Discontinued 2. Consepsis and Consepsis V (Ultradent Products, South Jordan, UT, USA) 3. The adhesive Peak Universal Bond (Ultradent Products) contains 0.2% CHX	1. The use of CHX-containing acid preserved the hybrid layer from degradation after in vitro storage for 2 years[107] 2. The application of an aqueous CHX solution after acid-etching resulted in stable resin–dentin bonds after approximately 14 months of clinical service[78] 3. Not available	One report suggested that CHX be used in low concentration (< 0.04%) because of the risk of pulp cytotoxicity[185]
Ethylenediaminetetraacetic Acid (EDTA)	Yes	Used as an aqueous solution of 2% EDTA on smear layer-covered dentin (self-etch adhesives); or on demineralized dentin after acid-etching (etch-and-rinse adhesives)	Available as an adjuvant to NaOCl to remove the smear layer in endodontics, in a concentration of 17% Dentists would need to dilute it to 2%—EDTA Plus (Essential Dental Systems, Hackensack, NJ, USA); EDTA Solution 17% (Pulpdent Co., Watertown, MA, USA); EDTA Solution (Vista Dental Products, Racine, WI, USA); CanalPro EDTA 17% (Coltene, Altstätten, Switzerland); SmearClear (SybronEndo, Orange, CA, USA); and many others	Not available	Longer exposure may result in excessive removal of both peritubular and peritubular dentin[186] EDTA lacks an antibacterial effect[187]
Galardin	No	Still in early stages of testing	No	1. One in vitro study showed that galardin preserved hybrid layer from degradation[80]	Expensive. More studies are needed, including biocompatibility studies with the pulp tissue
Quaternary ammonium salts	Yes	1. Added to the phosphoric acid etchant 2. Incorporated into the adhesive systems	1. HV Etch, Etch-37, and Uni-Etch (Bisco, Inc., Schaumburg, IL, USA)—they all contain BAC 2. Clearfil SE Protect and Clearfil S^3 Bond Plus (Kuraray America, Houston, TX, USA)—both contain MDPB	1. Not available 2. Clearfil Protect Bond (also known as Clearfil SE Protect) showed good in vitro results; however, it is not clear if these results were related to the quaternary ammonium monomer	More studies are needed, including long-term durability studies with BAC
Tetracycline (TC) and analogues	Yes	Use as an aqueous solution after acid-etching (etch-and-rinse adhesives)	As antibiotics	Not available	Unstable. Longevity studies are needed.
Epigallocatechin-3-gallate (EGCG)	No	Still in early stages of testing	None for clinical use	Not available	Longevity studies are needed
Glutaraldehyde	Yes	1. Incorporated into the adhesive system 2. Use as a desensitizer	1. Gluma Comfort Bond & Desensitizer (Heraeus Kulzer, South Bend, IN, USA); iBOND Total-etch (Heraeus Kulzer) 2. Calm-it (Dentsply Caulk, Millford, DE, USA); G5 (Clinician's Choice Dental Products, Inc., New Milford, CT, USA); Gluma Desensitizer and Gluma Desensitizer PowerGel (Heraeus Kulzer); Glu/Sense (Centrix, Shelton, CT, USA); MicroPrime G (Danville, San Ramon, CA, USA); Telio CS Desensitizer (Ivoclar Vivadent, Amherst, NY, USA).	The original Gluma (Bayer Dental) showed controversial results; it is not clear whether they were related to the presence of glutaraldehyde	More studies are needed

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TABLE 2. Continued

MMP inhibitor	Clinically available for dental use?	How can it be incorporated into the bonding technique?	Available commercial products	Longevity studies	Important clinical remarks
Collagen cross-linkers (carbodiimide, proanthocyanidin, and genepin)	No	Still in early stages of testing	None for clinical use	Not available	Longevity studies are needed A clinically feasible technique must be developed
Ultraviolet-activated riboflavin	No	Still in early stages of testing	Still experimental	One study with promising in vitro results[159]	More studies are needed, including biocompatibility studies
Biomimetic remineralization	No	Still in early stages of testing	Still experimental	Promising in vitro results[174–176]	A clinically feasible technique must be developed

BAC = Benzalkonium chloride; MDPB = 12-methacryloyloxydodecylpyridinium bromide; MMP = matrix metalloproteinase.

remodeling processes including embryo implantation, bone development, mammary gland development, and wound healing.[121,123,190,191] The role of MMPs in dentin bonding is not completely understood. There are several issues that still need further clarification:

1 The immunoreactivity of MMP-2 is localized primarily in predentin and near the dentin-enamel junction (DEJ) in teeth from subjects age 12 to 30 years[192]
2 MMP-2 and MMP-9 are gelatinases and are unable to digest collagen fibrils directly, so the initial step has to be performed by another mechanism[193]
3 MMPs may not inhibit the degradation of bonded interfaces formed by self-etch adhesives[193]
4 The preservation of the hybrid layer may happen in the absence of MMP inhibitors[174,194]
5 Although the immediate bond strengths were increased when the MMP inhibitors were added to Optibond FL and G-Bond, after 3 months there was no difference in bond strengths[110]
6 CHX does not eliminate water from the bonding interface and does not prevent hydrolysis from the layer of adhesive on the top of the water-rich surface of the hybrid layer[174]
7 CHX may leach from the adhesive/dentin interface,[139] which will decrease the overall concentration of CHX with time. Although it has been shown that there is a reduced therapeutic efficacy of CHX below a concentration of 0.2%,[195] a bond strength study showed that stable bonds were maintained for up to 6 months even when 0.002% CHX was used for 15 seconds.[81]

The long-term bond strength of MMPs' effects was not evaluated. Future studies must be carried out using MMPs, such as CHX, in controlled-release mechanisms, as used in endodontic, periodontal, and implant therapy.[196–198]

Several exogenous (natural and synthetic) inhibitors of MMPs are available in medicine. Some have shown efficacy in animal models of cancer and arthritis, but clinical trials have yet to show a strong benefit from treatments with MMP inhibitors. Tetracycline derivatives, such as doxycycline, when used as MMP inhibitors, have shown potential to treat abdominal aortic aneurysms, a chronic degenerative condition associated with a life-threatening risk of rupture.[199] An alternative approach to selective MMP inhibition in dentin bonding would be to take advantage of the effect of several exogenous MMP inhibitors by combining them in a highly specific primer solution.

By remineralizing the water-rich naked collagen fibrils within the hybrid layer, molecular immobilization of MMPs may be accomplished in a manner that is analogous to what occurs during mineralization of hard tissues. The use of other methods to remove residual

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water from hybrid layers may be another area of future research.

Research on new water-friendly resin monomers and photoinitators is underway. Increasing the monomer conversion rate even in humid oral environments would be advantageous for the preservation of resin–dentin bonds. Antibacterial adhesive components, such as quaternary ammonium methacrylates, are now added to commercial adhesives. These molecules have potential as MMP inhibitors.

Researchers have recently tried to target the Zn^{2+} and Ca^{2+} sites on the MMP molecule.[200–202] More research is needed on these new avenues for inhibiting the collagenolytic activity at the resin–dentin interface.

DISCLOSURE

The authors have no financial interest in any of the companies whose products are mentioned in this paper.

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