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Buonocore Lecture

Dentin Bonding: Can We Make it Last?

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Clinical Relevance

Bond strength to dentin decreases with time because of the hydrolytic degradation of the hybrid layer components dentin collagen and adhesive resin. Inhibition of enzymes responsible for the collagen degradation may improve the bond strength durability.

SUMMARY

In dentin bonding, contemporary dental adhesive systems rely on formation of the hybrid layer, a biocomposite containing dentin collagen and polymerized resin adhesive. They are usually able to create at least reasonable integrity of the hybrid layer with high immediate bond strength. However, loss of dentin-bonded interface integrity and bond strength is commonly seen after aging both *in vitro* and *in vivo*. This is due to endogenous collagenolytic enzymes, matrix metalloproteinases, and cysteine cathepsins, responsible for the time-dependent loss of hybrid layer collagen. In addition, the hydrophilic nature of adhesive systems creates problems that lead to suboptimal hybrid layers. These problems include, for example, insufficient resin impregnation of dentin, phase separation, and a low rate of polymerization, all of which may reduce the longevity of the bonded interface.

Preservation of the collagen matrix integrity by inhibition of endogenous dentin proteases is key to improving dentin bonding durability. Several approaches to retain the integrity of the hybrid layer and to improve the long-term dentin bond strength have been tested. These include the use of enzyme inhibitors, either separately or as incorporated into the adhesive resins; increase of collagen resistance to enzymatic degradation; and elimination of water from the interface to slow down or eliminate hydrolytic loss of the hybrid layer components. This review looks at the principles, current status, and future of the different techniques designed to prevent the loss of hybrid layer and bond strength.

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INTRODUCTION

Dentin bonding is a form of tissue engineering, in which mineral is replaced with resin monomers to form a biocomposite comprising dentin collagen and cured resin. The adhesive-dentin interface is expected to form a tight and permanent connection between dentin and composite resins.

Dentin bonding can be accomplished with etch-and-rinse (ER) or self-etch (SE) adhesives. The common issue for both is to create a route for adhesive resin infiltration into collagenous matrix. In ER bonding systems this pathway is created with acid, which dissolves the minerals to the depth of 5-10 μm and leaves the highly porous dentin collagen network suspended in water. Then the collagen network is infiltrated with resin monomers.[1] SE systems contain acidic resin monomers that simultaneously etch and prime the dental substrate.[2] Adhesives contain solvents (water, ethanol, or acetone) to dissolve the monomers, maintain the expanded state of the collagen network, and allow the monomers to fill the spaces within and around the collagen fibrils. Chemical polymerization of these monomers, activated by the curing light, results in a polymer-collagen biocomposite, commonly called the hybrid layer.[1,2]

The hybrid layer is a highly organic interface that is relatively hydrophobic, acid resistant, and tough. However, regardless of the system or material used, the creation of the hybrid layer is not perfect.[1,2] The morphological, physiological, and pathological heterogeneity of dentin[3,4] (Figure 1); limited time available for the procedure; water required to keep the collagen network open for resin penetration[5]; and limited degree of conversion (rate of polymerization) are the major obstacles to repeatedly achieving uniform dentin bonds. Additionally, hydrolytic (water-related) degradation of both components of the hybrid layer, the collagen matrix and the adhesive resin, seriously compromises the long-term integrity of the adhesive interface and the durability of the bond strength.[1,6] Biodegradation of resin-dentin interfaces also increases bacterial microleakage,[7] leading to undetected secondary caries.

Contemporary Adhesive Systems

Traditional three-step ER adhesives use primers containing hydrophilic monomers and solvents. They aim to displace water and prepare the collagen scaffold for the infiltration of the solvent-free, hydrophobic bonding resin.[1,8,9] Simplified two-step ER systems combine the hydrophilic primer and the hydrophobic resin into one solution. In addition, SE adhesives are subdivided into two-step and one-step categories.[2] Although the simplified two-step ER and one-step SE adhesives may be more user-friendly, it is widely acknowledged that three-step ER and two-step SE systems produce more favorable results.[1,2,8-11]

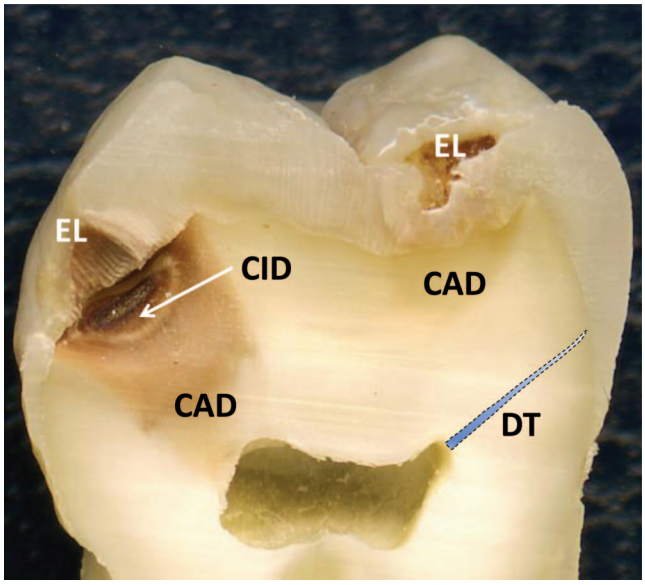

Figure 1. *The common phenomena in dentin, all of which affect the dentin as the substrate for bonding. Mineral content and the structure and composition of dentin in caries-affected dentin (CAD) and especially in caries-infected dentin (CID) is different from intact dentin, to various degrees, depending on the location. Dentinal tubules (DTs) are wide at the dentin-pulp border and gradually narrow toward the dentin-enamel junction, with the increasing amounts of highly mineralized peritubular dentin. As the tubule diameter decreases, the intrinsic wetness and dentinal fluid flow into the bonded surface decrease. EL, enamel lesion; CID, caries-infected dentin; CAD, caries-affected dentin; DT, dentinal tubules.*

DENTIN AS A SUBSTRATE FOR BONDING

To understand the problems involved in dentin bonding, we need to provide a brief overview of the dentin structure and composition to understand the complexity of the tissue in relation to the adhesion and adhesive performance. Approximately 50 vol% of dentin is mineral, the rest being type I collagen and noncollagenous proteins (30 vol%) and water (approximately 20 vol%).[3] Intertubular dentin contains well-organized mineralized collagenous organic matrix. Dentinal tubules have an inverted-cone shape, narrowing from the dentin-pulp border toward the dentin-enamel junction (DEJ) (Figure 1). Each tubule contains highly mineralized peritubular dentin, the amount of which increases toward the DEJ. Therefore, in cavities, the relative tubular and intertubular dentin areas vary depending on the depth and location of the cavity. Since dentin permeability depends on the size and patency of dentinal tubules, regional variations in dentin permeability and intrinsic wetness (due to dentinal fluid) depend strongly on the location of the cavity.[12] These variations in dentin structure and permeability directly affect bonding.[1,9,10,13] Higher surface moisture results in lower bond strengths in deep

compared to superficial dentin, as immediate bond strengths in deep dentin are generally 30-50% lower than in superficial dentin.[9,10] The relationship between morphology and permeability and how they affect adhesion has been thoroughly discussed in recent reviews.[9,10] We are lacking studies that compare the bond strength durabilities between deep and shallow cavities. However, it may be safe to speculate that lower immediate bond strength and increased risk for hydrolytic degradation of hybrid layer components result with faster and/or more pronounced loss of bond strength with time.

Intact vs Carious Dentin

The minimally invasive dentistry concept aims to limit the cavity preparation to caries removal, although where the limit should be set may be debatable. In any case, most caries excavation methods leave caries-affected and even caries-infected dentin to serve as the bonding substrate.[14]

The immediate bond strengths to caries-affected dentin are commonly 20-50% lower than to sound dentin, and even lower with caries-infected dentin.[9-11,14-17] Caries progression reduces the mineral content, increases porosity, and causes changes in the dentin collagen structure and distribution and noncollagenous protein content.[4] These changes can significantly reduce dentin mechanical properties, such as hardness, stiffness, tensile strength, modulus of elasticity, and shrinkage during drying,[18-21] which make the dentin in and under the hybrid layer more prone to cohesive failures under occlusal forces (Figure 2). Caries demineralization also increases dentin wetness.[19] Lower mineral content allows phosphoric acid or acidic monomers to demineralize the matrix more deeply than in normal dentin, resulting in even more residual water in exposed collagen matrix.[19] The hybrid layers tend to be much thicker and poorly infiltrated, regardless of the bonding system used.[9,10] Together these changes directly affect the strength and durability of the dentin-resin interface.

LOSS OF BOND STRENGTH

In principle, the loss of dentin bond strength can be caused by the hydrolytic degradation of one or both components of the hybrid layer, dentinal collagen and adhesive. Water is needed to maintain dentin collagen scaffold expansion for the resin monomer infiltration.[5] However, excess moisture may cause a phase separation between hydrophobic and hydrophilic monomers, resulting in irregular resin infiltration and forming blisters and voids at the interface. In addition, excess water or other solvents reduce monomer conversion.[8] The consequences of poor resin infiltration and conversion are the reduced durability of the interface, increased enzymatic degradation of the exposed collagen, and the hydrolysis of the poorly polymerized adhesive.[6,9,22,23]

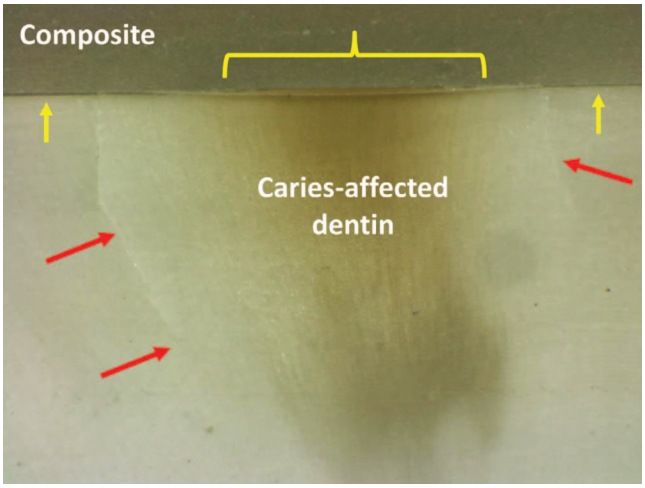

Figure 2. *Composite-dentin interface in caries-affected dentin. At the site of caries-affected dentin, a gap in the restoration-dentin interface is observed (yellow bracket), while the adhesive layer over intact dentin appears normal (yellow arrows). In addition, fracture lines (red arrows) appear surrounding the caries-affected dentin, presumably caused by polymerization shrinkage and stress, impacted by the lower mechanical properties of carious dentin. Stereomicroscope image with reflected light, 32x magnification.*

Hydrolytic Degradation of Adhesives

Dentin wet bonding requires primers or primer/adhesives to contain hydrophilic and ionic monomers to ensure proper hybridization of exposed collagen matrix. 2-Hydroxyethyl methacrylate (HEMA) has many advantages that make it the most commonly used hydrophilic adhesive monomer: it is a small monomer, well solvable in water, ethanol, and/or acetone; it acts as a solvent for hydrophopic monomers; and is relatively biocompatible in polymerized form. An extensive review[22] listed 62 commercially available adhesives, out of which 48 (77%) contained HEMA. Hydrophilicity, however, is a double-sided sword, as, for example, HEMA absorbs water both in the cured and uncured states, which may inhibit polymerization, reduce mechanical properties, and lead to the hydrolytic degradation of polymerized adhesive.[22]

Hydrolytic Degradation of Collagen

Loss of collagen in the hybrid layer was identified about 15 years ago in studies of aged resin-dentin bonds *in vitro*[24] and *in vivo*.[24-27] Ever since, a vast

Table 1: *Matrix Metalloproteinases (MMPs) and Cysteine Cathepsins Detected to Date in Human Dentin. The Proposed Substrates and Function in the Hybrid Layer Are Also Presented for Each Enzyme. It Is Essential to Remember that the Substrates and Functions Are Only Suggestive and Are Based Mainly on Studies with Tissues Other than Dentin. As Studies with Enzyme-specific Inhibitors Are Lacking, the Exact Role and Relative Importance of Each Enzyme Are Not Well Known*

Enzyme	Other Names	Substrates	Function	Reference Nos.
MMP-2	Gelatinase A	Native and partially degraded collagen	Collagen degradation	28-35, 38
MMP-9	Gelatinase B	Native and partially degraded collagen	Collagen degradation	29, 31, 33-35, 38
MMP-8	Collagenase-2	Type I collagen	Collagen degradation	28, 29, 38
MMP-3	Stromelysin-1	Proteoglycans; other noncollagenous proteins	Removal of PGs and PPs	36, 37
MMP-20	Enamelysin	Amelogenin, DSPP	Unknown	38, 42
Cathepsin B	—	Collagen	Collagen degradation	35, 39, 53
Cathepsin K	—	Type I collagen	Collagen degradation	35

Abbreviations: DSPP, dentin sialophosphoprotein; PGs, proteoglycans; PPs, phosphoproteins.

number of morphological and bond strength studies have confirmed time-related loss of collagen in the hybrid layer. Human dentin contains several enzymes that together can degrade dentin collagen matrix proteins, including type I collagen. These enzymes belong to matrix metalloproteinases (MMPs) or cysteine cathepsins (Table 1).[23]

To date, intact human dentin has been shown to contain MMP-8 (collagenase-2),[28,29] MMP-2 and -9 (gelatinases),[28-35] MMP-3 (stromelysin-1),[36,37] MMP-20,[38] cysteine cathepsin B,[35,39] and cysteine cathepsin K.[35] Intense gelatinolytic activity is present in dentinal tubules,[40,41] and MMP-20 is found in dentinal fluid.[42] The physiological roles of these enzymes in dentin are not known, but they have been suggested to participate in peritubular and tertiary dentin formation and in the release of dentinal growth factors during caries, which in turn would regulate pulp defensive reactions.[23,43-47]

Both MMPs and cysteine cathepsins have also been indicated to participate in dentinal caries pathogenesis.[4,35,48-53] MMP-2, -9, and -8 are present in carious dentin,[32,38,45,48] and their activation in pH fluctuations relevant to caries lesions and the role of MMPs in the degradation of dentin collagen have been demonstrated.[48,50] The acidity of the ER[54] and SE[55] adhesives also activates dentinal enzymes. Cysteine cathepsins have also been identified in carious dentin.[35,53] It is important to note that cathepsin K and B and MMP-2 and -9 are dramatically increased in caries-affected dentin compared to intact dentin,[35] and several MMPs increase in dentinal tubules of carious teeth.[42,51,56] MMP inhibitors eliminate human salivary MMP activities[50,57] and reduce caries progression in dentin in animal experiments.[49,50]

METHODS TO IMPROVE DURABILITY

Removal of Caries-infected Dentin

Partial caries removal significantly reduces the risk of pulp exposure and postoperative pulpal symptoms.[58] At the same time it should be recognized that bond strength to carious dentin is weaker and likely more prone to degradation than is bond strength to normal dentin. Bond strength is inversely proportional to caries progression, caries-infected dentin showing the lowest bond strength.[15,16,59,60] Elimination of caries-infected dentin[61] and adequate marginal sealing over sound dental structure[62] are also considered a prerequisite for arresting the caries progression under the restoration and can therefore be seen as minimum requirements for dentin bonding.

Chemical Bonding

Chemical bonding of functional adhesive monomers to hydroxyapatite calcium ions has been suggested to maintain the hybrid layer collagen integrity. These functional monomers, such as 10-methacryloyloxidecyldihydrogen phosphate (10-MDP), 4-methacryloyloxyethyl trimellitic acid (4-MET), or N,N-diethanol p-toluidine (phenyl-P), are usually included in SE adhesives.[2] Mild SE adhesives (with a pH of approximately 2 or higher) leave some hydroxyapatite around the collagen fibrils, believed to keep the resident collagenolytic enzymes inactive and prevent collagen degradation. However, even with 10-MDP (the monomer with the most stable chemical bond) the bond strengths show time-dependent reduction both *in vitro* and *in vivo*.[63-67] Apparently the adhesive penetration and/or polymerization are not sufficient to protect the deepest part of the hybrid layer, which seems to be the weakest area.[63,67,68] If

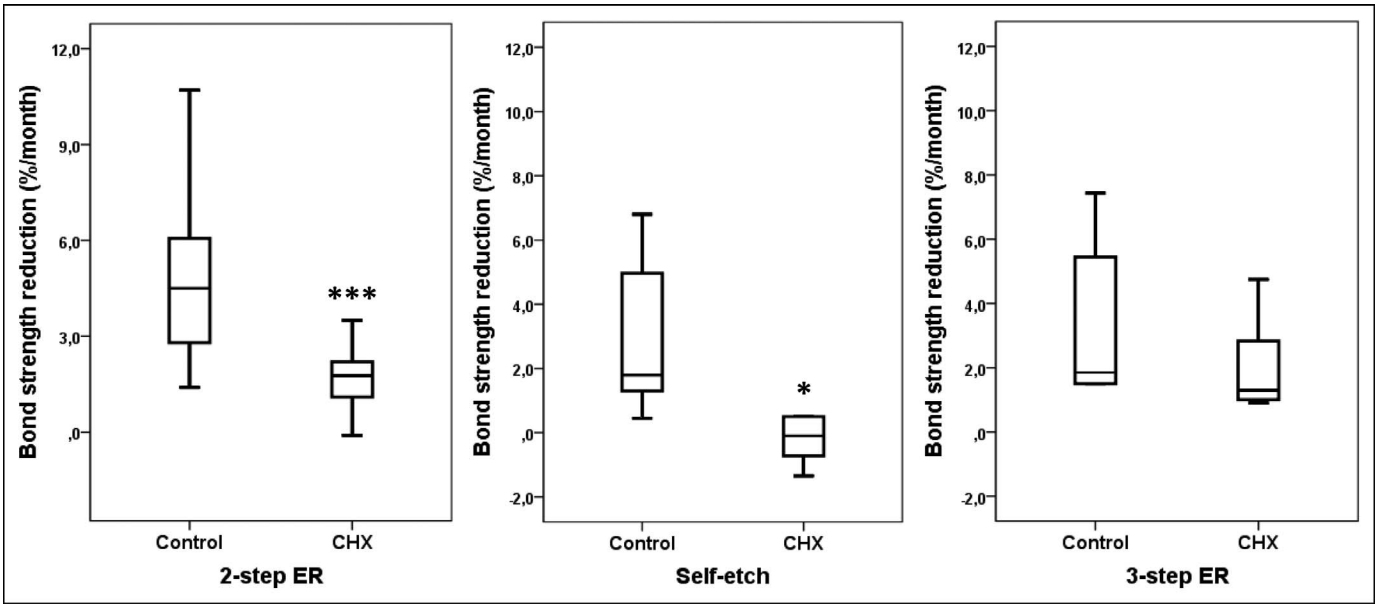

Figure 3. *The comparison of the bond strength reduction (percentage per month) between control and chlorhexidine-treated samples. All microtensile and shear bond strength studies with at least 0.2% chlorhexidine and at least six-month* in vitro *or* in vivo *duration that reported immediate (24 hour to one week) and aged samples bond strengths were included in the analysis. The data included 25 articles, of which 23 involved two-step ER adhesives (77 groups altogether), six studied SE adhesives (15 groups), and five studied three-step ER adhesives (10 groups). ***, significantly different from controls,* $p < 0.001$; *, *significantly different from control,* $p < 0.05$.

collagen degradation is not completely eliminated, enzyme inhibition is still needed even with the mild SE adhesives containing functional monomers[6] and may improve bond durability.[66,69]

Enzyme Inhibitors

Numerous approaches based on enzyme inhibition have been tested to slow down or eliminate collagen degradation to preserve bond strength, chlorhexidine being the most popular compound.[6] Chlorhexidine inhibits collagenases and gelatinases present in dentin.[70-73] Several *in vitro*[74-83] and *in vivo*[84-87] studies have demonstrated that chlorhexidine preserves resin-dentin bonds by eliminating or at least delaying collagen degradation in the hybrid layer (Figure 3).

One clinical drawback of the clinical use of chlorhexidine has been the need for a separate priming of dentin before the application of adhesive. In *in vivo* experiments, 15-60 second application of, typically, 0.2-2.0% chlorhexidine on acid-etched dentin before primer/adhesive application has been shown to result in preservation of the hybrid layer and bond strength.[84-87] Incorporation of chlorhexidine into the adhesives is not always a simple task. Chlorhexidine may have a concentration- and material-dependent effect on resin water sorption and solubility,[88,89] degree of conversion,[89,90] and mechanical properties.[89] However, the potential of incorporating chlorhexidine in ethanol-solvated hydrophobic resins has been recognized.[88] In spite of these problems, recent *in vitro* studies have been promising, at least with relatively low chlorhexidine concentrations.[90] Two-step SE adhesive with chlorhexidine added into the primer is effective in inhibiting dentinal MMPs[69] and preventing bond strength loss *in vitro*,[66] and adding chlorhexidine into simplified ER adhesive reduces nanoleakage and time-related loss of bond strength[83] without affecting mechanical properties.[83,91] The first commercially available adhesive system containing 0.2% chlorhexidine (Peak Universal Bond, Ultradent Products Inc, South Jordan, UT, USA) was introduced recently. Whether it is effective in preventing the hybrid layer degradation and time-related loss of bond strength remains to be demonstrated.

Chlorhexidine can also be released from the adhesive in a concentration- and adhesive-dependent manner,[91] which may prove to be beneficial for the controlled enzyme inhibition. Another clinically appealing approach could involve addition of chlorhexidine into the etching acid, as it has been shown to significantly reduce nanoleakage and loss of bond strength for up to two years.[77,79]

Very few studies have addressed the question of the ability of enzyme inhibition to improve long-term bond strength in caries-affected dentin. Komori and others[16] demonstrated with two-step ER adhesive that 2% chlorhexidine significantly improved the six-month bond strength to caries-affected dentin when compared to control treatment: the bond strengths were comparable to the immediate values both with and without caries.[16] Another study[92] demonstrated similar findings with Clearfil SE Bond (Kuraray Co, Ltd, Tokyo, Japan) after two years of aging under simulated pulp pressure. Chlorhexidine has also shown significantly better bond strengths *in vivo* in primary molars for up to 18-20 months when compared to nontreated controls.[93] However, the same study demonstrated time-related loss of bond strength in chlorhexidine-treated teeth as well.[93]

Other MMP Inhibitors—In spite of chlorhexidine's high affinity (substantivity) and stable binding both to mineralized and demineralized dentin,[94,95] the inhibition effect may be lost with time. Because the chlorhexidine molecule is large and water soluble, it may leach out of the hybrid layer, thus limiting its long-term antiproteolytic effect.[93] This reality has driven researchers to look for better alternatives with which to preserve the hybrid layer. Several compounds known to inhibit MMPs have been tested.

Benzalkonium chloride (BAC) is a quaternary ammonium surface-acting agent that has been used as a cavity disinfectant and desensitizer. Commercially available BAC-containing etchants can be used without affecting immediate bond strength to enamel or dentin.[96] BAC also inhibits dentinal MMPs[97-99] and may also improve the durability of dentin bonding when incorporated either into the etchant or the adhesive.[99]

Tetracyclines and their antimicrobially inactive analogs inhibit MMP with their cationic chelating properties and have been shown to inhibit salivary and dentinal MMPs as well.[49,50,57,100] From this group, only minocycline has so far been shown to be potentially useful in adhesive dentistry.[101] Bisphosphonates, such as batimastat, galardin, and zoledronate, also inhibit MMPs by chelating active-site zinc, and zoledronate inhibits carious dentin MMPs.[50] Galardin (0.2 mM) reduces the bond strength loss as effectively as chlorhexidine.[102] Other studies with galardin, batimastat, or SB-3CT (selective gelatinase MMP inhibitor) have failed to confirm the results,[103-105] but in those studies significantly (1:20) lower concentrations were used. This indicates that for effective enzyme inhibition in dentin, higher concentrations are needed than are required with purified recombinant enzymes.

Even though zinc is essential for the activity of MMPs, high zinc concentrations greatly reduce MMP-mediated dentin collagen degradation.[106,107] Zinc-containing adhesives may improve hybrid layer durability and preserve bond strength and may even induce dentin remineralization at the bonded interface.[108-110] The effect is believed to occur via zinc binding to collagen, which protects the cleavage sites of MMPs.[100] However, excessive presence of zinc ions may jeopardize the bonding performance of MDP-containing SE adhesives.[111]

Interestingly, along with tin, zinc has been consistently found under amalgam fillings in dentin considered to represent caries-affected dentin[112,113] and also in artificially demineralized dentin exposed to amalgam.[112] It is tempting to speculate that zinc MMP-inhibiting and/or remineralizing effects may contribute to the resistance of amalgam to secondary caries.

MMP-inhibiting Monomers or Solvents—Another approach with significant clinical interest involves using adhesive monomers that would inhibit collagenolytic enzymes. Polymerizable quaternary ammonium methacrylates (QAMs) are cationic, like chlorhexidine. 12-Methacryloyloxydodecylpyridinium bromide (MDPB) belongs to the QAMs and has been incorporated into SE primer (Clearfil Protect Bond: Kuraray) because of its antimicrobial properties and its ability to copolymerize with adhesive monomers. QAMs inhibit MMPs and demineralized dentin collagen degradation almost completely, MDPB being one of the most effective.[114] Collectively, the *in vitro* and *in vivo* data indicate that MDPB-containing adhesives may be superior in the prevention of bond strength loss, when compared to other SE adhesives.[6,60,64,67,104]

Recently, dimethyl sulfoxide (DMSO) has been identified as a potential solvent with MMP-inhibiting effect.[115] DMSO is a polyfunctional molecule with hydrophilic and hydrophobic groups and an excellent penetration enhancer for medical purposes.[116] It is fully miscible in all solvents and capable of solving many, if not all, monomers used in adhesive dentistry. Because DMSO breaks down the self-associative tendency of water[117] it may reduce water entrapped between the adhesive polymeric chains and enhance the wetting of collagen,[118] allowing better penetration of adhesive into the collagen matrix. These properties may be behind its improved immediate and long-term bond strength; part of the

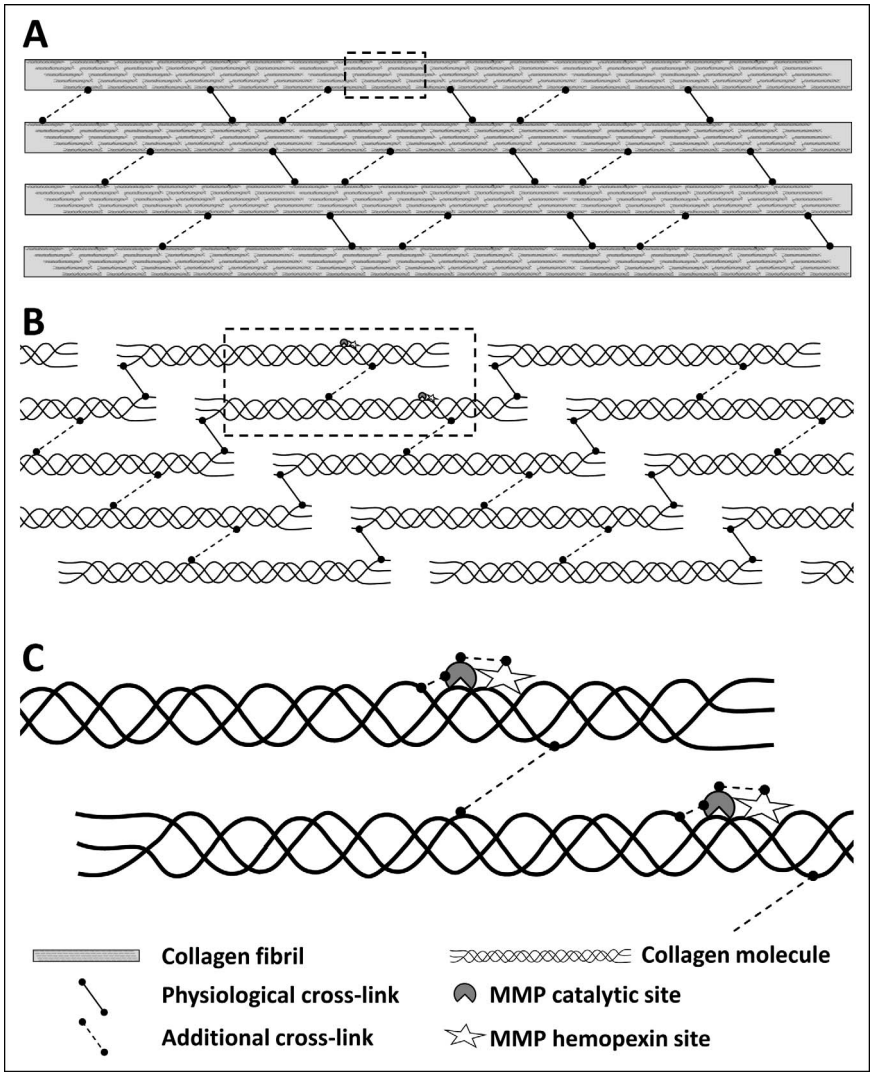

Figure 4. *The proposed mechanism of the effects of an increase in cross-links in dentin matrix (the model adapted from Liu and others[119]). (A) Additional cross-links between collagen microfibrils increase the collagen matrix stiffness and may improve the hydrophobic resin infiltration without the risk of matrix collapse due to drying. (B) More detailed view from the area marked with dashed line in A, demonstrating individual collagen molecules within microfibril. The additional intermolecular cross-links contribute to the increased mechanical properties and increase the matrix resistance against collagen-degrading enzymes. (C) Individual collagen molecules from the marked area in B. Cross-linking may cause conformational changes in the active site, catalytic domain of the protein, and/or binding site of collagenolytic enzymes: all of these may eliminate the enzymes' collagen-degrading capacity.*

effect may be related to its MMP-inhibiting action.[115] Good compatibility with both tissue and adhesive components make DMSO an attractive alternative, as it may be incorporated into the adhesive systems, but more work is needed to confirm the initial promising results.

IMPROVING THE RESISTANCE OF HYBRID LAYER COMPONENTS BY BIOMODIFICATION

Since the pulpal cells cannot remodel or repair lost dental tissue, current restorative therapy aims to replace decayed tissue to restore tooth morphology and function. However, development of biomodification strategies, such as increased collagen cross-linking and biomimetic remineralization, to improve the tissue properties and stability by chemically modifying the tissue offer an interesting approach for adhesive dentistry.[6,21,119,120]

Use of Cross-linkers

The intermolecular and intermicrofibrillar cross-linking is the basis for the stability, strength, and viscoelasticity of dentin collagen matrix. The quantity and type of cross-linking also determines collagen thermal stability and ability to resist biodegradation. Increasing cross-linking of the exposed dentin collagen aims to improve hybrid layer matrix stability[119,120] and may also offer a means by which to increase the use of more hydrophobic adhesives without the risk of collagen matrix collapse during adhesive application (Figure 4).

Studies of cross-linkers have primarily focused on their ability to enhance the mechanical properties of demineralized dentin.[120] In addition to stiffening, cross-linking can also affect enzymatic degradation by allosteric silencing of collagenolytic enzymes (Figure 4) or by altering the enzyme binding site in

the collagen molecule.[119-124] The cross-linkers tested include synthetic cross-linkers such as carbodiimide and glutaraldehyde, physical (photo-oxidative) cross-linking with riboflavin, and naturally occurring compounds such as genipin and proanthocyanidins.[120] Here we will concentrate on the studies dealing with the preservation of the hybrid layer; for detailed information about cross-linkers in dentin biomodification, the reader is referred to a recent extensive review.[120]

Glutaraldehyde is a widely known cross-linker that has been used in dental products, but its cytotoxicity seriously limits its clinical use.[6,120] Carbodiimide hydrochloride has very low cytotoxicity but may have limited cross-linking capacity.[120] It has been demonstrated to eliminate collagen degradation and preserve bond strength *in vitro*,[125] even though the time needed for the effect may still be too long for clinical practice.[6] Dentin treatment with carbodiimide is effective in inhibiting dentinal MMP activity alone or mixed with HEMA, which indicates that it could be added to adhesive primers.[124] Proanthocyanidins are also effective.[126,127] An increase in immediate dentin bond strength may be achievable even with shorter treatment times,[128,129] and improved durability of long-term bond strength has also been indicated.[81,130] Riboflavin has also been successfully tested, but the need for ultraviolet light or separate cross-linker light curing[131,132] reduces its clinical acceptability.

Biomimetic Remineralization

Biological mineralization of all hard tissues is a progressive dehydration process; with the increasing mineral content the water content of the collagen matrix decreases correspondingly to maintain a constant volume.[21,119] In dentin bonding, resin adhesive is incapable of dehydrating the collagen matrix sufficiently,[1,5,6,133,134] leaving behind water that will allow hydrolysis of the hybrid layer components. Biomimetic remineralization mimics the progressive dehydration of natural biomineralization by replacing matrix water with apatite crystallites (for a comprehensive review, see Niu and others[21]). In the hybrid layer, replacing water with minerals would increase mechanical properties and inhibit water-related hydrolysis.

In biomimetic remineralization of the hybrid layer, polyanions (eg, polyacrylic acid or polyaspartic acid) bind to collagen and serve as analogs of dentin phosphoproteins that regulate physiological mineralization, allowing calcium binding and promoting apatite nucleation. The hybrid layer is covered with a "therapeutic" composite containing amorphous calcium phosphate as a source for apatite. *In vitro* studies indicate that biomimetic remineralization has great potential for remineralizing hybrid layers or caries-like dentin.[21] These studies have also demonstrated the preservation of the mechanical properties of the hybrid layer[135] and bond strength[136] with time.

Even if biomimetic remineralization strategy has great potential—and should perhaps be the ultimate goal of research—in preventing the loss of hybrid layer integrity, it must be realized that to date the strategy is still at the proof-of-concept stage. Development of clinically applicable materials that would contain and release the critical components of the process (at least calcium and phosphate source and biomimetic analogs) involves considerable challenges.[6,21,119]

ELIMINATION OF HYDROPHILIC ADHESIVES

In attempts to reduce the hydrolytic degradation of adhesive, less hydrophilic HEMA-free adhesives have been created.[8] However, the solvation effect of HEMA is also lost. When other solvents, such as acetone or ethanol, evaporate, water tends to separate adhesive components, making these adhesives prone to phase separation.[137,138] The resulting water blisters may lower the immediate bond strength. The studies examining the durability of bond strength with HEMA-free adhesives are limited and the results are conflicting, but generally, loss of bond strength seems to occur.[139-141] Extended evaporation (air drying) of the adhesive layer may reduce the blister formation.[137,138,142]

Ethanol-wet Bonding

Preventing hybrid layer degradation with the use of MMP-inhibitors or MMP-inhibitor–conjugated resin monomers will continue to be the predominant method for extending the longevity of resin-dentin bonds until a more proactive solution becomes clinically available. However, a water-rich zone rich in polymerized or unpolymerized hydrophilic monomers along the resin-dentin interface continues to be a problem. Even if collagen matrix can be preserved, loss of integrity through the degradation of adhesive component will remain.[143] This may be the reason for the slow loss of bond strength even when enzyme inhibition has been successful.

Ethanol-wet bonding aims to use ethanol to dehydrate demineralized dentin matrices and to assist the infiltration of more hydrophobic monomers

into dentin.[1,5,6] Infiltration of hydrophobic monomers decreases water sorption and solubility and resin plasticization and may prevent or at least reduce enzymatic hydrolysis of collagen.[144-147] Together, these would lead to improved bond durability.[1,6] Currently, however, technique sensitivity and generally long treatment times prohibit ethanol-wet bonding in clinical settings, and more user-friendly and reproducible techniques or materials need to be developed for everyday use.[6,9]

CAN DENTIN BONDING BE MADE TO LAST?—CONCLUSIONS

The overall clinical success of composite restorations is multifactorial and therefore is unlikely to be predicted by even a battery of *in vitro* test methods. Only limited evidence exists to correlate marginal quality and bond strength in the laboratory with the clinical performance of bonded dental composites.[148] However, it is clear that rapid deterioration of dentin bonding does not improve the restoration longevity. It is equally clear that it is possible to prevent or at least significantly slow down hybrid layer degradation.

As discussed above, carious dentin is not necessarily an ideal substrate for strong and durable hybrid layer formation. Therefore, careful removal of at least caries-infected (soft) dentin and removal of carious dentin close to the cavity margins are minimum requirements.

Despite the development of faster and more simple adhesives, conventional three-step ER and two-step SE adhesives are still considered the most reliable alternatives and the benchmark for dental adhesion in clinical practice.[1,2,8,9,133,149] Addition of a separate hydrophobic adhesive resin after the hydrophilic primer makes the interface more hydrophobic and seals it more effectively, resulting in a more durable bond than is obtained with the simplified two-step ER or one-step SE adhesives. Although enamel-bonded restoration margins cannot prevent the degradation of resin-dentin hybrid layer *in vivo*,[84,85] a proper sealing of enamel margins should result in a more favorable clinical outcome.[134,149] Since milder SE adhesives in particular do not necessarily provide sufficient enamel etching, separate acid-etching of enamel margins is recommended with SE adhesives.[2,150]

Inhibition of collagenolytic enzymes with chlorhexidine has the best *in vitro* and *in vivo* evidence in terms of clinical use as well. Although chlorhexidine may not be perfect, and although long-term clinical performance studies are still lacking, no adverse effects have been reported either. Some manufacturers already recommend application of chlorhexidine as an optional step after acid-etching or before application of SE primer. It is safe to conclude that until other approaches have been proven safe and at least equally effective, chlorhexidine (either via separate application or as incorporated into adhesive system) can and should be used.[6,133]

The techniques and materials that allow the use of hydrophobic adhesives offer another attractive alternative, but this approach may require new monomers with different chemistry. Combining cross-linkers with ethanol-wet bonding, DMSO, or a corresponding agent may offer an easier approach. If cross-linkers can be used to stiffen the exposed collagen matrix sufficiently to prevent shrinkage during rapid and complete removal of water, it should be possible to infiltrate the matrix with hydrophobic monomers. Increased resistance and enzyme inhibition with cross-linkers, ethanol, and DMSO together with complete encapsulation of collagen should efficiently prevent collagen matrix degradation, and an absence or minimal amount of hydrophilic monomers would help to preserve the adhesive component of the hybrid layer. Biomimetic remineralization—returning the hybrid layer collagen to, or close to, its original mineralized state—should, however, remain the ultimate goal.

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Conflict of Interest

The author has no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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