Overview of Clinical Alternatives to Minimize the Degradation of the Resin-dentin Bonds

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Clinical Relevance
The use of several clinical approaches to improve resin impregnation and the strength of polymer formed by the adhesives as well as to reduce the activation of host-derived proteases can improve the longevity of the resin-dentin bonds.

SUMMARY
The incorporation of hydrophilic and acidic resin monomers substantially improved the initial bonding of contemporary etch-and-rinse (ER) and self-etch (SE) adhesives to intrinsically wet dental substrates, providing quite favorable immediate results, regardless of the bonding approach used. However, in the long term, the bonding effectiveness of most simplified ER and SE adhesives drop dramatically. This review examines the fundamental processes that are responsible for the aging mechanisms involved in the degradation of the resin-bonded interfaces and some possible clinical approaches that have been effective in minimizing or even preventing the degradation of the adhesive interfaces produced with simplified adhesives. The incorporation of some of the feasible approaches - described in this review - may improve the quality of the adhesive restorations performed in clinical practice, while manufacturers develop bonding materials that are less susceptible to the aging mechanisms present in the oral environment.

INTRODUCTION
Contemporary adhesives interact with the dental substrates using one of two different bonding strategies: 1) the etch-and-rinse (ER) approach, which requires smear layer removal before the use of dentin adhesive, and 2) the self-etch (SE)
approach, in which the smear layer is maintained as a substrate for bonding. In addition, these two bonding strategies may be classified according to the number of application steps (ie, one to three) required to couple the resin composites to dental substrates.1

ER adhesives require a separate etching step. Thus, an inorganic acid (mostly 30%-40% phosphoric acid) is applied to dental substrates and then rinsed off. This step is followed by a priming treatment, wherein amphiphilic functional resin monomers (eg, 2-hydroxyethyl methacrylate [HEMA]) are applied to dental substrates to make them prone to receiving a mixture of relatively more hydrophobic resin monomers that will complete the bonding procedure. This sequence of events exemplifies a three-step application procedure. Simplified two-step ER adhesives combine the primer and adhesive resin into a single application step. On the other hand, SE adhesives no longer require a separate etching step. This approach requires the use of non-rinse acidic monomers that simultaneously etch and prime dentin. The bonding procedure with SE adhesives can be achieved using either two- or one-step systems, depending on whether the etching/primer agent is separated from the adhesive resin or combined with it to allow a single application step.

Early generations of dental adhesives were relatively hydrophobic, and dry substrates were required for bonding. Some (or most) of these adhesives were placed on smear layer-covered dentin, but their formulations did not contain acidic functional monomers, so that they could not penetrate the smear layer to couple with the underlying mineralized dentin. As a consequence, the resulting bond strengths (BSs) were very low.2 Modifications of adhesive formulations by the inclusion of more hydrophilic monomers and acidic resin monomers, along with solvents, made the adhesive solutions more compatible with the moist dentin surface, which, in turn, yielded significant improvements in the immediate bonding effectiveness of most current adhesive systems.2

Although the incorporation of hydrophilic and acidic resin monomers has substantially improved the initial bonding of contemporary ER and SE adhesives to intrinsically wet dental substrates, potential problems associated with these hydrophilic formulations have been increasingly reported. Several laboratory and ex vivo/in vivo studies have related significant drops of resin-dentin BS values after short- and long-term investigations.3-12 Dramatic loss of effectiveness of some adhesive systems, in terms of reduction in mechanical and/or dentinal sealing properties, has also been shown when they were clinically evaluated in non carious cervical lesions.13,14

This problem has shifted the focus of researchers’ investigations to evaluating the aging mechanisms involved in the degradation of the resin-bonded interfaces15,16 as well as some possible clinical approaches to minimize or even preventing the degradation of the adhesive interfaces produced with simplified adhesives. Thus, the objective of this review is to discuss some of the factors involved with the degradation of the hybrid layer and eventually discuss some clinical alternatives presented in the literature to improve the resistance of the bonded interface to the degradation phenomena. Some considerations as regards modifications to the adhesive composition will also be addressed.

**FACTORS INVOLVED IN THE AGING OF BONDING INTERFACES**

Bonding interfaces are created by impregnation of the dentin substrate by blends of resin co-monomers. Thus, the hybrid layer is a mixture of dentin organic matrix, residual hydroxyapatite crystallites, resin monomers, and solvents.17 Based on this, it seems quite natural for one to hypothesize that stability of the resin-bonded interface relies on the proper impregnation and conversion of resin monomers into mineralized/demineralized dentin substrate to provide adequate retention and dentinal sealing. Moreover, it is clear that the stability of hybrid layers ultimately depends on the intrinsic resistance of their individual components to the degradation phenomena.1,15,18

One of the first in vivo articles on the long-term durability of resin/dentin bonds was published in 1999.9 This study demonstrated that resinous materials were extracted from the resin-dentin interface over time, and that the hybrid layer became more porous in vivo. Later on, Hashimoto and others6 recovered resin composite restorations placed in primary teeth after exfoliation and evaluated them by scanning electron microscopy (SEM). The authors also observed that much of the hybrid layer had disappeared over one to three years of clinical service. In addition, they detected loss of resinous material and degradation of collagen fibrils within the hybrid layer. Meanwhile, several laboratory investigations reported reductions in resin-dentin BS when specimens were stored in distilled water or artificial saliva for periods ranging from three months to 10 years.3-5,8,10,11.
The exact mechanism responsible for hybrid layer degradation is not completely understood yet.\textsuperscript{15,19,20} However, it seems that the first stage of biodegradation involves extraction of the resins that had infiltrated into the dentin matrix via water-filled nanometer-sized voids within the hybrid layer and enzymatic attack of the exposed collagen fibrils, leading to their depletion.\textsuperscript{18,21}

**Degradation of the Polymer Network**

An examination of the structure of the most popular dental monomers reveals that they are heteroatom polymers, having carbon and oxygen or nitrogen in their backbones.\textsuperscript{22} In addition, their structure shows the presence of hydrolytically susceptible groups, such as ester and urethane, as well as hydroxyl, carboxyl, and phosphate groups.\textsuperscript{23} Previous laboratory investigations have detected that most of the commercial simplified adhesive formulations are highly prone to water sorption to a potentially damaging extent.\textsuperscript{24-27} The extent and rate of water uptake is dependent upon the density of the polymer network and the potential for hydrogen bonding and polar interactions. In other words, it depends on to the hydrophilicity of the adhesive.\textsuperscript{24,28,29}

However, it is not only the hydrophilicity of the material that is responsible for water sorption.\textsuperscript{28,30,31} The retention of solvents and/or water during polymerization compromises the integrity of the adhesive interface. A recent investigation prepared experimental dental adhesive formulations by mixing neat co-monomer blends of different hydrophilicities with fixed quantities of solvent and water.\textsuperscript{30} The percentage of solvent retained in acetone-based and ethanol-based adhesive mixtures increased significantly with increasing hydrophilicity of resin blends, varying from 4.9\% to 13.2\%.\textsuperscript{30} This situation worsened when water (simulating the water required for the wet bonding technique, or the inherent wetness of dentin) was added to these co-monomer–solvent mixtures. This resulted in a large increase in solvent/water retention in these solvated adhesive mixtures. Solvent retention varied from 26.4\% to 41.6\%, even using evaporation times three to 12 times longer than those usually recommended by the manufacturers of adhesive systems.\textsuperscript{30}

Retained organic solvent and water within the adhesive resin can severely compromise the structural integrity of the hybrid layer. Increasing the concentration of acetone in single-bottle adhesives led to a decrease in the microtensile BS, with morphologic manifestations of cracks and interfacial gaps appearing along the bonded interfaces.\textsuperscript{32} The presence of remnant water during polymerization may also interfere with polymerization of the resin system and leave residual, unreacted monomers that are more susceptible to leaching in these particular regions. It has been demonstrated that adding 0.2 mL of water or more per milliliter of co-monomer decreases the degree of conversion of the bonding resin by 25\% to 53.5\%.\textsuperscript{33}

Although most dental adhesive manufacturers recommend protocols for solvent evaporation, it is known that complete solvent elimination is critical, if not impossible, especially when it comes to highly hydrophilic adhesive blends.\textsuperscript{30,34,35} Then, residual volatile solvents diluted in the liquid adhesive may further prevent the approximation between reactive pendant species, making cross-linking reaction inside the hybrid layer more difficult.\textsuperscript{36-38} Thus, instead of achieving optimal macromolecular packing density, the polymer backbone may have had its free space greatly augmented to a level directly related to the amount of organic solvent present during polymerization. Consequently, this affects the ultimate tensile strength of the adhesive itself\textsuperscript{39} as well as the resin-dentin BS.\textsuperscript{39-41}

The problem continues. Incomplete polymerization maximizes the known adhesive permeability of simplified ER and SE adhesives, probably because of the relatively higher concentrations of hydrophilic monomers and solvents.\textsuperscript{42} As partially polymerized adhesives are more permeable to fluid movement,\textsuperscript{29} they may expedite water sorption and compromise the long-term integrity of the adhesive-composite bond.

The easy passage of water within the adhesive and hybrid layers softens the polymer by swelling the network and reducing the frictional forces between the polymer chains.\textsuperscript{23,43} After the relaxation process, unreacted monomers trapped in the polymer network are released into the surroundings, creating new channels for water penetration; through these channels, water diffuses even more in a self-perpetuating manner. The elution of residual and low-molecular-weight polymers also increases the porosity within the hybrid layer,\textsuperscript{6,9} which in turn, reduces the ultimate tensile strength\textsuperscript{44} and elastic modulus\textsuperscript{28} of the simplified adhesives even further. These are considered some of the main reasons for resin degradation within the hybrid layer, contributing to the reduction of resin-dentin BS created by dental adhesives over time.\textsuperscript{15}

The more hydrophilic the material, the higher is its water sorption rate and the resultant hydrolytic
As reported by Breschi and others, irrespective of the bonding strategy, by combining hydrophilic and ionic resin monomers into the bonding, such as in simplified adhesives (ie, two-step ER and one-step SE systems), the bonded interface lacks a non-solvated hydrophobic resin coating. This leads to the creation of hybrid layers that are permeable not only to water from oral environment but also to water fluxes coming from the dentinal tubules. 

This is why the adhesive interfaces produced with simplified adhesives are considered semipermeable membranes. In fact, a recent study has pointed that, depending on the degree of hydrophilicity (ie, the higher, the more prone), dental adhesives may not only allow the passage of water, but they can be also permeated by particles with a relatively high molecular weight. It is this permeability of methacrylate-based resins to solutes that explains the mobility and diffusion of fluoride out of resin matrices constituted of fluoride-containing compounds. Similarly, chlorhexidine (CHX) can be slowly released from urethane dimethacrylate–triethylene glycol dimethacrylate (TEGDMA) resins because it can permeate the resin matrix.

The increase in permeability of contemporary simplified adhesives to water is readily apparent when they are used for sealing crown preparations of vital deep dentin in vivo before impressions are taken for indirect restorations. After application of these adhesives to vital crown preparations and removal of the oxygen-inhibited layer, impressions of these “sealed” crown preparations were obtained with a low-viscosity polyvinyl siloxane impression material. The impressions were poured with epoxy resins to produce replicas of the crown preparations for SEM examination. The simplified adhesives did not provide a hermetic seal for vital deep dentin (unless they were immediately covered with light-polymerized resin composites), as evidenced by transudation of dentinal fluid across the polymerized adhesives to form fluid droplets along the surface of the adhesive (Figure 1). These water movements within the adhesive layer can be driven by osmotic pressure gradients due to high concentrations of dissolved inorganic ions and hydrophilic resin monomers in the adhesive layer, and they result in the formation of water blisters.

This passage of water was also revealed by studying the permeability of bonded interfaces by SEM, using a detectable tracer such as ammoniacal silver nitrate. This tracer stains water-filled diffusion pathways throughout the bonded interface, which are often manifested when creating the so-called “water-trees” (Figure 2). These are characteristic water channels at the surface of the hybrid layer that extend into the adhesive layer.

Thus, not only is the water from the oral environment responsible for the hydrolytic degradation of the resin-bonded interfaces but also the water drawn from the underlying mineralized dentin due to the osmotic gradient created by the dissolved ions within the oxygen inhibition layer of these polymerized adhesives. As a consequence, the absorbed water molecules, drawn through the adhesive, can become trapped at the adhesive-composite interface, thereby interfering with bonding to the resin composites. This contributes to the lack of stability of resin-bonded interfaces over time.

The following factors are responsible for the degradation of the polymer network:

- Hydrophilicity of the adhesive resins
- Entrapment of solvent/water within the polymerized adhesive, which prevents adequate cross-linking and degree of conversion
- Plasticization of the polymer by water sorption from the oral environment and the underlying dentin.

Degradation of the Collagen Fibrils

Nakabayashi and others defined the hybrid layer as the structure formed in hard dental tissues by the demineralization of the surface and subsurface, followed by infiltration of monomers and subsequent polymerization. Ideally, resin monomers should
penetrate the depth of demineralization produced by phosphoric acid when using ER systems. However, literature findings have demonstrated that in ER systems, adhesive penetration always occurs to a lesser extent than that of dentin demineralization. This may also occur in SE adhesives. An in vitro study challenged the general concept that SE adhesives etch and infiltrate into dentin to the same extent as this concept depends on the adhesive acidity.

Using micro-Raman spectroscopy, the extent of adhesive penetration into the adhesive interface was calculated. At the first micrometer of the resin-dentin interface, only ~68% of the concentration of bisphenylglycidyldimethacrylate (bis-GMA) in the original ER adhesive penetrated the demineralized dentin. This is thought to be due to the insolubility of bis-GMA in water-saturated dentin. By means of immunohistochemical analysis, other studies confirmed that more collagen fibrils at the bottom of the hybrid layer are not fully encapsulated by resin monomers, as there was evidence of intense labeling of collagen fibrils by anti-type I collagen antibodies in the deepest part of the hybrid layer.

This discrepancy between demineralization and monomer infiltration results in incompletely infiltrated zones along the bottom of the hybrid layer, which contain denuded collagen fibrils even immediately after bonding. This evidence was first demonstrated by Sano and others, and the authors called it: nanoleakage or leakage inside the hybrid layer.

Resin elution from hydrolytically unstable polymeric hydrogels within hybrid layers may continue to occur through the nanoleakage channels during aging, producing continuous collagen fibril exposure. This fact renders the previously resin-infiltrated collagen matrices more susceptible to attack by proteolytic enzymes, and this can be seen by the increase in silver nitrate uptake in adhesives interfaces over time (Figure 3).

Recently, it was demonstrated that the enzymes involved in the breakdown of the collagen matrices during the pathogenesis of dentin caries and periodontal disease have potential and relevant implications in the degradation of resin-dentin bonded interfaces. By assaying the collagenolytic activity of mineralized dentin powder by using fluorescein-labeled type I collagen from bovine skin, Pashley and others demonstrated an intrinsic collagenolytic activity in human mineralized dentin. These authors speculated that such proteolytic activity could be exerted by dentinal matrix metalloproteinases (MMPs), which on that occasion had already been shown to be potentially expressed in the dentin-pulp complex.
MMPs are a family of Zn\(^{2+}\)-dependent and Ca\(^{2+}\)-dependent enzymes, collectively called matrixins. These endogenous enzymes are important components in many biological and pathological processes because of their ability to degrade almost all extracellular matrix components. Within the oral environment, considerable interest has been devoted to the detection, distribution, and function of host-derived MMPs. Several MMPs have been identified within the dentin-pulp complex compartments. Dentin matrix has been shown to contain at least four MMPs: stromelysin-1 (MMP-3), collagenase (MMP-8), and gelatinases A and B (MMP-2 and MMP-9, respectively).

Description of the involvement of MMPs in the degradation of demineralized dentin matrix was first mentioned in a study by Tjäderhane and others. Human MMP-2, MMP-8, and MMP-9 were identified in demineralized dentinal lesions. The experiments conducted in the mentioned study provided critical evidence that bacterial acids are required for the removal of minerals in tooth decay and for the subsequent activation of host MMPs, but bacteria alone could not cause dentin matrix degradation. Therefore, it was assumed that after demineralization, activated host-MMPs would ultimately be responsible for destroying the dentin matrix in caries lesion progression. As previously mentioned, more recently Pashley and others began discussing the likely involvement of MMPs in the degradation of poorly resin-infiltrated hybrid layers. Nowadays it is thought that the release and the subsequent activation of these endogenous enzymes during dentin bonding procedures could be responsible for the almost complete disappearance of portions of hybrid layers from resin-dentin bonds that were aged in water for 4 years.

More recently, another group of proteases were identified in compartments of both sound and carious human dentin, and they formed part of the cysteine proteases (CPs). Human CPs are best known from the ubiquitously expressed lysosomal cathepsins B, H, and L, and dipeptidyl peptidase I, which until recently were thought to mediate primarily housekeeping functions in the cell. Of 15 CPs genes detected, 10 were expressed in native pulp tissue and 11 in odontoblasts.

In studies by Tersariol and others and Nascimento and others, the potential correlation between the MMP and cysteine cathepsin activities in intact or carious dentin, respectively, was also investigated. Results showed that MMP and cysteine cathepsin activities expressed highly significant correlations between intact and carious dentin, even though the activities in carious lesions were approximately 10 times higher than in intact dentin. In conclusion, these data indicate the collagenolytic/gelatinolytic activity of dentin may be due not only to
the presence of MMPs but also to cysteine cathepsin activities.

Although etching with phosphoric acid may inhibit the collagenolytic/gelatinolytic activity of dentin,70,81 the application of simplified ER adhesives81 and SE systems82 was shown to reactivate endogenous enzymatic activities in dentin that were previously inactivated by phosphoric acid etching. This even exceeds the original activity in untreated mineralized dentin.81

However, there seem to be some exceptions. Recent studies have reported that some mild SE adhesives87,88 and some monomers from simplified adhesive systems, such as TEGDMA, HEMA, and quaternary ammonium methacrylates, are capable of inhibiting MMPs activity.89-91

The following factors are responsible for the degradation of the collagen fibrils:

- Incomplete penetration of the resin monomers into the entire depth of demineralization produced by phosphoric acid or some SE adhesives
- Continuous exposure of collagen fibrils as a result of degradation of the polymer network
- Activation of host-derived proteases (MMPs and cathepsins), involved in degradation of the organic component of the hybrid layer, by adhesive systems

**HOW TO IMPROVE RESIN-DENTIN BOND STABILITY**

Based on the foregoing discussion, one may assume that the quality of the adhesive interface relies on the quality of the hybrid layer, that is, on proper impregnation of the dentin substrate in order to fully encapsulate the exposed collagen fibrils in a highly cross-linked polymer.

Several studies have focused their investigations on the effects of modified standard clinical protocols to obtain adhesive interfaces with higher resistance to degradation. All of these clinical approaches focus on: 1) improving resin impregnation into mineralized and demineralized dentin substrates, 2) improving the strength of the polymer formed by the adhesive systems, and 3) improving the resistance of collagen fibrils to enzymatic degradation. Obviously there are some clinical approaches that may improve bond durability by two or even three mechanisms; however, for didactic reasons they will be classified into one of these approaches.

**Improving Resin Impregnation Into Mineralized and Demineralized Dentin Substrates**

The use of a vigorous rubbing application has been claimed to increase BS durability of adhesive systems applied on either a wet or dry demineralized dentin92,93 (Figure 4). When using manufacturers’ protocols for simplified ER systems, resin monomers, particularly those with high molecular weight, have limited diffusion into the wet demineralized dentin.59,63,94 The mechanical pressure applied to the demineralized dentin surface during vigorous rubbing compresses the collapsed collagen network, and as the pressure is relieved, the compressed collagen expands and the adhesive solution may be drawn into the collapsed collagen mesh.93,95 Moreover, the vigorous rubbing application could probably increase solvent diffusion outward. Although this technique does not alter the hydrophilicity of the adhesive, one may expect changes in polymer chain topology by the reduction in the intrinsic fraction of nanopores, which makes room for increased polymer cross-linking. This indeed explains why some degree of degradation, although at a lower rate, was still observed for one two-step ER acetone-based adhesive systems after the vigorous application mode.93 Recent clinical evaluation indicated that this clinical approach was capable of increasing the retention of restorations placed in non carious cervical lesions using ER adhesives96 (Figure 5). The benefits of this clinical approach can also be seen in another clinical trial,97 which demonstrated that that similar retention rates in class V cavities could be achieved when ER adhesives were applied to either moist or air-dried demineralized dentin, as long as the adhesives were vigorously rubbed onto demineralized dentin.
The vigorous clinical approach was also shown to increase the durability of adhesive interfaces produced with SE adhesives when applied to dentin (Figure 6). In this case, the agitation of one-step SE systems is likely to carry fresh acidic resin monomers in to the basal part of the etched dentin, producing more aggressive demineralization and carrying more resin, which in turn promotes better interaction with the smear layer and underlying dentin. This technique, however, depends on the operator’s interpretation of the vigorous application mode and cannot be performed with bristle brush applicator. The operator should apply the adhesive using a rigid microbrush applicator (Figure 7) with as much pressure as can be manually applied on the dentin surface. Additional concerns about the fragility of the acid etched enamel prisms with this application method may be raised by some clinicians. However, the increased BS values in enamel after active application rule out the possibility that this technique may be detrimental to enamel.

Another alternative is the application of multiple adhesive coats. Hashimoto and others demonstrated that the immediate BS increased with each adhesive coating up to four coats. At the same time nanoleakage decreased with each coat, being almost absent after four or more coats for simplified ER adhesives. Similarly, Ito and others observed that by simply applying more coats of simplified SE adhesives, the strength and quality of dentin bonding could be improved. Although this clinical approach did not prevent reductions in resin-dentin BS, the degradation rates of simplified SE adhesives after six months of water storage were less pronounced than they were at interfaces where adhesives were applied according to the manufacturer’s directions (Figure 8). The clinical benefits of this
technique were already reported in a previous clinical study for both ER and SE adhesives \(^{108}\) (Figure 9).

The rationale behind the good performance of this technique is that as the first layers of the adhesive begin to etch the dentin substrate, it might become rapidly buffered by the hydroxyapatite \(^{109}\) so that the additional layers of unpolymerized acidic monomers may improve the etching ability of the adhesives by increasing their concentration. Simultaneous to this process, more impregnation of resin might occur by the additional supply of the adhesive resin. \(^{106}\) Unfortunately, the literature lacks information on the laboratory longevity of this technique for ER adhesives. The main disadvantage of this clinical protocol is that it goes against clinicians’ preference for faster application protocols.

As the impregnation of demineralized dentin and solvent evaporation seem to be time-driven processes, a delayed light-curing protocol can ensure better resin penetration and faster solvent evaporation. \(^{110,111}\) Reis and others \(^{110}\) applied two simplified ER systems on flat occlusal dentin surfaces, according to the manufacturer’s directions. The light curing procedure was performed immediately after application or after delayed periods that ranged from 90 to 300 seconds. The authors demonstrated that, in addition to the statistical increase in the immediate BS values, stable resin-dentin bonds were reached even after three years of water storage for the 300-second group (Figure 10). This is probably the result of better adhesive infiltration and consequent reduction in water permeability through the adhesive interface. \(^{112}\) For SE adhesives, this protocol was also shown to improve the immediate BS values, \(^{113,114}\) but there is still a lack of data on the durability of SE adhesive interfaces when using this protocol, which has not been evaluated in laboratory or clinical trials.

**Improving the Strength of the Polymer Formed by Adhesives Systems**

One way to counteract the hydrophilicity of simplified adhesives is to apply an additional coating of hydrophobic resin on the polymerized simplified adhesive. The use of a hydrophobic coating after the application of simplified SE and ER adhesive systems leads to a thicker and more uniform adhesive layer with low retained water and solvent concentration and significant reduction in the fluid flow rate. \(^{115,116}\) (Figure 11). Examples of hydrophobic resin coats are the adhesive (third step) of the Scotchbond Multi-Purpose Adhesive (3M ESPE, St. Paul, MN, USA) or the bond (second step) of the Clearfil SE Bond (Kuraray, Osaka, Japan). Sealants...
and flowable composites can also be used as hydrophobic resin coatings.

This technique is also capable of eliminating the incompatibility of simplified SE adhesives with self-polymerized composites. Dentin bonding systems that use separated non-solvated hydrophobic bonding resin (three-step ER and two-step SE) show a higher degree of polymerization and less permeability to water. Thus, by using this technique, one would be converting the simplified adhesive into a primer. This primer would be further concentrated with more hydrophobic monomers contained in the additional surface coating. Consequently, a more stable resin-dentin interface can be produced over time.

This protocol transforms a simple-layer adhesive into a multilayer one, but with the difference that the most hydrophilic simplified adhesive can be photoactivated before the application of the most hydrophobic, non-solvated bonding resin. More densely compacted hybrid layers are produced, probably by reducing the concentration of unreacted monomers between the primed and bonded layers (Figure 11).

Recently, two randomized clinical trials evaluated the effects of applying an additional hydrophobic resin coating on simplified adhesives. For the SE adhesives, the retention rates of Clearfil S3 Bond (Kuraray) and iBondGluMa Inside (Heraeus Kulzer, Hanau, Germany), applied according to the manufacturer’s directions (CS3 and IB) or associated with a coat of hydrophobic layer (CS3 + Bond and IB + Bond). The use of hydrophobic coating increased the retention rates of both adhesives. Adapted from Reis and others.20

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As resin permeability and monomer elution are both related to suboptimally polymerized bonding systems, two recent studies proposed to extend the exposure time during light curing. The study showed that extending the curing times of simplified adhesives beyond those recommended by the manufacturers resulted in adhesive interfaces with a high degree of conversion and reduced permeability, which appeared to be a possible means for improving the performance of these adhesives. Higher energy density (produced by the longer exposure times) enhances the formation of free radicals, which initiates polymerization. Moreover, the unavoidable heat produced by light curing units mainly during prolonged exposure time, is likely to increase solvent evaporation rates. This may provide room for the formation of a high-molecular-weight and cross-linked polymer.

Recent studies have demonstrated that although this clinical approach could not prevent the known degradation of adhesive interfaces, the extent of such degradation was significantly lower for two-step ER adhesives and one-step SE materials (Figure 13). By prolonging the exposure time during
light polymerization one may slow down the degradation rate of the adhesive interfaces.

Unfortunately, this approach needs to be clinically tested. Apart from this, there is no denying that it requires more clinical time to accomplish, and the intensity of the light curing device may also affect the outcomes of this technique.

Earlier, the role of solvent entrapment in the attainment of a high cross-linked polymer was extensively discussed. Based on these considerations, the use of a warm air stream (60°C) for solvent evaporation was recently investigated.129-131 This approach was shown to increase the immediate and the six-month resin-dentin BS of simplified ER adhesives130 (Figure 14), probably because of increased solvent evaporation129 (Figure 15) and consequently higher polymer cross-linking, as excess solvent would prevent the approximation of polymer chains. The benefits of this technique should be tested on the durability of the SE adhesives, and this protocol should be evaluated clinically. A clear disadvantage of this technique is that it requires special devices to produce a warm air stream.

Recently, several studies have focused on the influence of composite preheating on composite properties.132,133 Composite preheating is capable of enhancing monomer conversion, as it increases molecular mobility and collision frequency of reactive species, leading to higher polymer cross-linking.134

Considering that adhesive systems are mainly composed of monomers, an increase in the adhesive temperature might increase the resin–dentin BS. Based on this supposition, preheating of adhesive systems was recently investigated for ER and SE adhesive systems.135-138

However, contrary to previous expectations, this approach did not improve the immediate or long-term resin-dentin BS produced by ER systems.136 Probably, heating the adhesive may alter the stability of the acidic monomers in such a way that it jeopardizes their bonding effectiveness.135,136

An important issue that should be mentioned is the potential effect on pulp and dentinal fluid flow of high temperature produced by the use of warm air-drying, prolonged exposure times, or heated adhesive. The most widely accepted mechanism of dentin hypersensitivity is the hydrodynamic theory,139 whereby fluid flow within dentinal tubules is altered (increased or changed directionally) by thermal, tactile, or chemical stimuli near the exposed tubule surfaces. This alteration would lead to stimulation of A-δ fibers surrounding the odontoblasts and may cause dentin sensitivity. In addition, it has been demonstrated that the temperature rise produced by light-curing units140,141 negatively affects the metabolism of cultured pulp cells.142 Therefore, the use of the latter three approaches in superficial, medium, and deep cavities should be a matter of further investigations.

As mentioned earlier, a decreasing gradient of resin monomer diffusion within the acid-etched dentin results in incompletely infiltrated zones along the bottom of the hybrid layer, particularly for hydrophobic monomers such as Bis-GMA.59 This is thought to be due to Bis-GMA’s insolubility in watersaturated dentin. If ethanol replaces rinse-water from acid-etched matrices, relatively hydrophobic neat Bis-GMA/TEGDMA resins can infiltrate the demineralized dentin to create hydrophobic hybrid layers, in the so-called ethanol-wet bonding technique.143, 144 This technique, therefore, allows the use of hydrophobic resins that absorb little water
from dentin. The application of hydrophobic resins to ethanol-wet dentin, may provide resin interfaces with a five-fold reduction in water sorption rates. Resin-dentin interfaces with higher long-term stability were produced with the ethanol-wet bonding technique. The authors investigated the immediate and one-year resin-dentin BS of an experimental three-step hydrophobic adhesive using ethanol-wet bonding and water-wet bonding versus those obtained with a three-step hydrophilic ER adhesive using water-wet bonding (Figure 16). One can observe that the experimental hydrophobic resin layer could only obtain high immediate BS values when applied on an ethanol-saturated dentin. The immediate values of this approach were statistically similar to those obtained by a commercial three-step ER system using the water-wet technique; however, stable bonds after one year of water storage were observed only for the former.

Although dentin bonding with hydrophobic resins using the ethanol-wet bonding technique has shown encouraging results, the protocol is time consuming and technique sensitive. Usually, ethanol-saturation is achieved by using a series of ascending ethanol concentrations, a process that takes approximately three to four minutes for the whole process, which defies the principles of user-friendliness and technique simplification.

In view of this, other simplified dehydration protocols were evaluated, and some of them were shown to be as effective as the full protocol to produce stable bonds. Association of this protocol using a primer with CHX (see the next topic) may also show promising results.

The effects of dentin dehydration by means of ethanol on pulp cell viability, as well as the use of warm air drying and prolonged exposure times, should be carefully investigated before this technique is used in a clinical scenario. As reported by Liu and others, ethanol wet-bonding is generally conceived to be a bonding philosophy rather than a bonding technique because of its clinical impracticality.

**Improving Collagen Fibril Resistance to Enzymatic Degradation**

The other weak link in the adhesive interface is the susceptibility of collagen fibrils to enzymatic degradation by host-derived MMPs, and probably also by cysteine cathepsins. Thus, it has been claimed that the use of protease inhibitors, as a preliminary dentin treatment, reduces interfacial aging over time by inhibiting the activation of these endogenous dentin enzymes, which are alleged to be responsible for the degradation of collagen fibrils in the absence of bacterial contamination in vivo.

Specifically for MMPs, synthetic inhibitors are thought to act by nonspecific chelation of cations, such as zinc and calcium, which are required to maintain the enzymatic function of MMPs. There are several synthetic nonspecific MMPs inhibitors, such as CHX, ethylenediamine tetraacetic acid (EDTA), tetracyclines and derivatives, or specific MMPs inhibitors, such as SB 3CT and galardin. Some dental materials, such as zinc oxide cements, have also been shown to reduce MMP-2 and MMP-9 activities, probably because of the same mechanism of action. A recently published review describes several other methods that have been recently advocated to silence the activity of MMPs and cysteine cathepsins.

It was already shown that CHX has desirable MMP-inhibitory properties, even at very low concentrations. Complete inhibition of MMP-2 and MMP-9 gelatinase activities occurred at CHX concentrations as low as 0.03%. Carrilho and others showed that the collagenolytic activity of demineralized dentin was significantly reduced by pretreatment with 2% CHX in comparison with untreated specimens.

This indeed is the rationale behind the use of CHX as dentin pretreatment. Hybrid layers from CHX pretreated dentin exhibited normal structural integrity of the collagen network compared with the
progressive disintegration of the fibrilar collagen network detected in the control teeth in vivo.\textsuperscript{7, 12,149}

As far as the ethanol-wet bonding technique is concerned, the previous application of 2\% CHX for 60 seconds on demineralized dentin means that another bonding step needs to be accomplished during the restorative procedure, and this is contrary to the clinician's preference for simplification. Another disadvantage of this approach is that even low CHX concentrations cause mild trans-dentinal cytotoxic effects to odontoblast-like cells when in direct contact with cells\textsuperscript{158}; however, fortunately, its cytotoxic effect is dependent on the thickness of remaining dentin and occurs at a very low dentin depth.\textsuperscript{159}

The use of low CHX concentrations (0.2\% to 0.002\%) has also been shown effective in preventing the dentin interfaces from degradation even in shorter application times (15-30 seconds),\textsuperscript{160,161} probably because of the high substantivity of CHX to mineralized and demineralized dentin substrates.\textsuperscript{162} These short application times and low CHX concentrations convey a distinct advantage, in that it is not toxic to human periodontal cells\textsuperscript{163} and odontoblast-like cells,\textsuperscript{158} and it is less time-consuming.

The addition of 2\% CHX to the phosphoric acid conditioner (Figure 17) was also shown to be effective in reducing the degradation of resin-dentin bonds without including an extra step in the dentin bonding protocol.\textsuperscript{164, 165} In addition, Tezvergil-Mutluay and others\textsuperscript{166} showed that benzalkonium chloride, which is usually added to the acidic conditioner of Bisco Inc, has anti-aging properties against MMP. The effect of using this acid for the prevention and/or reduction of resin-dentin BS degradation, as regards ER adhesives, has shown good results (unpublished data).

The effectiveness of specific MMP-inhibitors on dentin bonding is being investigated as they normally exert inhibitory activity in extremely low concentrations.\textsuperscript{87,88,154} Among these, galardin, which is a synthetic MMP inhibitor, has been shown to reduce the degradation rate of hybrid layers\textsuperscript{154} produced with a two-step ER adhesive. Nevertheless, the use of CHX seems to be more advantageous than use of MMP-specific inhibitors, because it also inhibits the activity of cysteine cathepsins, the other class of proteases that have recently been detected in human dentin.\textsuperscript{84} Thus, not only does CHX seem to be a broad antimicrobial spectrum but it is also a broad antiproteolytic agent, inhibiting MMPs and cysteine cathepsins.\textsuperscript{167}

Despite the controversy regarding the role of SE adhesives in the activation of MMPs,\textsuperscript{82,87,88,168} several studies have been conducted to evaluate the effect of MMP inhibitors, such as CHX or EDTA applied on dentin before SE adhesives.\textsuperscript{169-171} None of these studies evaluated the resin-dentin BS after aging protocols, which prevents conclusions from being reached regarding the benefits of this approach. EDTA-treated surfaces showed increased immediate BS values,\textsuperscript{169,170} but this could well be attributed to the demineralizing effect of the EDTA on smear-layer covered dentin. This issue should be a matter of future investigations.

The inclusion of CHX in adhesives would be a way to use protease inhibitors without adding an extra step to the bonding protocol. Previous studies investigated the inclusion of CHX in a three-step ER and in two-step SE adhesives.\textsuperscript{87,88,172} Although opinions about the benefits of this approach are not unanimous, promising results have been observed and further studies should evaluate this inclusion in simplified ER and SE adhesives.

Another approach recently investigated by some authors is the reinforcement of collagen fibrils with the use of collagen cross-linkers.\textsuperscript{172-177} Fibrillar type I collagen accounts for 90\% of the dentin organic matrix, and the remaining 10\% consists of non-collagenous proteins such as phosphoproteins and proteoglycans.\textsuperscript{178} Lower biodegradation rates and high mechanical properties of collagen are desirable; thus the induction of exogenous collagen cross-linking has been proposed as a mechanism to improve the mechanical stability and reduce the biodegradation rates of the collagen network. Several synthetic (glutaraldehyde [GD], carboiimides,
such as 1-Ethyl-3-[3-dimethylaminopropyl] carbodiimide hydrochloride/ N-hydroxysuccinimide (EDC/NHS) and others) and naturally occurring (genipin, proanthocyanidin (PA)) from grape seed extract and others) agents can induce exogenous collagen cross-linking.173,175,179-181

GD is widely used as a biological tissue fixative and has been shown to affect the mechanical property of various biological tissues,182 while PA is a naturally occurring plant metabolite bioflavonoid. PAs are considered one of the most important classes of secondary metabolites in the plant kingdom and are found in fruits, vegetables, nut, seeds, flowers, and barks.180 It is well documented that cocoa and its products, along with grape seed, are among the richest sources of PAs.183

Changes in the mechanical properties of demineralized dentin matrix after treatment with some cross-linkers were both concentration and time dependent, with increases ranging from eight to 40 times174-176 (Figure 18). Previous studies have reported that these collagen cross-linkers have the ability to improve the short-term mechanical properties of dentin174 and its resistance to degradation by collagenases (Figure 19).176 Improved immediate resin-dentin BS values were also reported,173,175,177 in addition to more stable bonds after 12 months, for some adhesives184 (Figure 20).

As dentin collagen is already highly cross-linked, some researchers doubt whether the increase in resin-dentin bond longevity is explained only by an increase in cross-linking density.16 This is why some authors believe this increase could well be attributed to the MMPs inhibition properties of such cross-linkers,16,185 as has recently been shown and reported.186

A clear limitation of the use of collagen cross-linker agents is the long treatment time. Generally, more than one hour is required to improve the mechanical properties of the collagen network,173,174,176 which makes the technique clinically impractical. A possible approach to reducing the application time is by increasing the cross-linker concentration or using several cross-linkers simultaneously. Another concern is that this protocol cannot be used for SE adhesive systems as there is no dentin surface demineralization before SE adhesive application.

Future studies should focus on investigating the possibility of shorter application times and the use of controlled drug-delivery systems to recharge the...
collagen cross-linker at the interface over time and promote preservation and self-healing of the adhesive interface.

OTHER CLINICAL APPROACHES

As contemporary two-step ER adhesive systems are not able to perfectly seal exposed dentin surfaces, alternative treatments should avoid transudation of water from dentin to the adhesive layer. One alternative is the adjunctive use of oxalate desensitizers on acid-etched dentin before adhesive application. This approach was shown to reduce the hydraulic conductance of dentin\textsuperscript{187-191} and the incompatibility between simplified ER adhesives and chemically/dual cured resin cements.\textsuperscript{192} As the hydraulic conductance of dentin is reduced, the polymer within the hybrid layer is likely to become more protected against the deleterious effects of water from the underlying dentin, which may favor BS stability. However, recent publications\textsuperscript{187,193} have shown that the use of this approach reduced the stability of the interface after medium to long-term water storage (ie, three to 12 months of storage). One cannot rule out the fact that some incompatibility between the oxalate product and the adhesive could be the reason for the failure of this protocol, and this should not discourage further evaluations on this issue. As this protocol requires previous dentin demineralization, it would not be applicable to SE adhesive systems.

Other investigations have reported increases in the resin-dentin BS of simplified ER and SE adhesives with the use of an electrically assisted device.\textsuperscript{194-197} The device (ElectroBond, Seti, Rome, Italy) produces a particular electric signal that is claimed to enhance resin monomer infiltration into demineralized dentin collagen matrix by iontophoresis. Despite the improvements in the immediate resin BS, the literature lacks information on the effect of this approach on the stability of the dentin bonding over time.

FINAL REMARKS

There are other successful strategies to improve the durability of the bonding performance through modification of adhesive system compositions. For instance, the inclusion of ascorbic acid on ER adhesives\textsuperscript{198} or the use of hydrophilic photoinitiators such as QTX [3-(3,4-dimethyl-9-oxo-9Hthioxanthen-2-yl oxy)-2-hydroxypropyl] trimethyl ammonium chloride\textsuperscript{199} or water-soluble photoinitiators such as TPO ([diphenyl(2,4,6-trimethylbenzoyl)-phosphine oxide])\textsuperscript{200} may be ways to improve the stability of the resin dentin bonds. However, as this depends on the manufacturers, and it is not under clinical control, it is beyond the scope of this review to discuss this aspect. Recently, Tay and Pashley published two studies\textsuperscript{201,202} that used nanotechnology principles to mimic the events that occur in biomineralization. Unfortunately, it seems that contemporary ER adhesives are not capable of completely replacing water from the extrafibrillar and intrafibrillar collagen compartments with resin monomers.\textsuperscript{58,59,61,94} This may also occur with SE adhesives, challenging the general concept that SE adhesives etch and infiltrate the dentin substrate at the same depth.\textsuperscript{60,62}

Biomimetic remineralization provides a means for remineralizing incompletely infiltrated resin-dentin interfaces created by ER\textsuperscript{203} and SE adhesives\textsuperscript{203} by means of a dehydration process in which water from the intrafibrillar collagen fibril compartments are progressively replaced by apatites. As water is an important element that induces the degradation of resin-dentin bonds, this strategy may be capable of preserving the longevity of these bonds.\textsuperscript{204}

Unfortunately, remineralization was performed via a lateral diffusion mechanism by the immersion of specimen slabs in a remineralizing medium containing dissolved biomimetic analogs. Experiments are in the process of development for translating this proof-of-concept strategy into a clinically applicable technique.\textsuperscript{16} The addition of bioactive glasses to the adhesive formulation has shown promising results, but it still requires further evaluations.\textsuperscript{205}

CONCLUSIONS

Contemporary dentin bonding is not as durable as has previously been assumed. Although there is a trend toward adhesives with simplified application procedures, so far simplification appears to induce loss of effectiveness. In addition, the simplified adhesives are faster and easier to use clinically, but there is a rapid rise in the resultant technique sensitivity.

Thus, how can more durable adhesive interfaces be produced? Unfortunately, one should spend more time to enhance dentin impregnation and solvent evaporation to produce a highly cross-linked polymer capable of covering and protecting the exposed collagen fibrils from degradation.

A safe clinical protocol would etch the dentin substrate with a CHX-containing acid followed by application of multiple adhesive coats under vigor-
Table 1: Summary of the Clinical Alternative Presented in This Review

<table>
<thead>
<tr>
<th>Technique</th>
<th>Benefits</th>
<th>Rationale</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vigorous rubbing application</td>
<td>1. Increases the resin-dentin BS</td>
<td>1. Increases solvent evaporation</td>
<td>1. Is difficult to standardize</td>
</tr>
<tr>
<td></td>
<td>2. Decreases the degradation rate</td>
<td>2. Improves penetration of the resin monomers into the collagen fibrils</td>
<td>2. Requires rigid microbrushes</td>
</tr>
<tr>
<td></td>
<td>3. Improves the clinical performance (ER and SE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple coats</td>
<td>1. Improves the resin-dentin BS</td>
<td>1. Improves penetration of the resin monomers into the collagen fibrils</td>
<td>1. Lacks consensus regarding the number of coats</td>
</tr>
<tr>
<td></td>
<td>2. Decreases the degradation rate (SE)</td>
<td>2. Decreases the continuous exposure of collagen fibrils</td>
<td>2. Is time consuming</td>
</tr>
<tr>
<td></td>
<td>3. Decreases adhesive permeability</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Improves the clinical performance (ER and SE)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delayed light-curing</td>
<td>1. Improves the resin-dentin BS durability (ER)</td>
<td>1. Increases solvent evaporation</td>
<td>1. Is time consuming</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2. Improves penetration of the resin monomers into the collagen fibrils</td>
<td>2. Aging studies need to be performed for SE</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3. Requires clinical testing</td>
</tr>
<tr>
<td>Additional layer of hydrophobic resin coating</td>
<td>1. Improves the resin-dentin BS durability</td>
<td>1. Increases the hydrophobicity of the adhesive resins</td>
<td>1. Requires an additional material (hydrophobic adhesive)</td>
</tr>
<tr>
<td></td>
<td>2. Decreases the degradation rate</td>
<td>2. Increases the degree of conversion of the adhesive layer</td>
<td>2. Is time consuming as it adds an extra step</td>
</tr>
<tr>
<td></td>
<td>3. Decreases the adhesive permeability</td>
<td>3. Decreases the plasticization of the polymer network after water sorption</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. Improves the clinical performance (ER and SE)</td>
<td>4. Decreases the continuous exposure of collagen fibrils</td>
<td></td>
</tr>
<tr>
<td>Prolonging the exposure time during light curing</td>
<td>1. Improves the resin-dentin BS durability</td>
<td>1. Increases the degree of conversion</td>
<td>1. Is time consuming</td>
</tr>
<tr>
<td></td>
<td>2. Decreases the degradation rate (ER and SE)</td>
<td>2. Increases solvent evaporation</td>
<td>2. Biological studies need to be performed</td>
</tr>
<tr>
<td></td>
<td>3. Decreases the adhesive permeability</td>
<td>3. Decreases the continuous exposure of collagen fibrils</td>
<td>3. Requires clinical testing</td>
</tr>
<tr>
<td>Warm air-stream for solvent evaporation</td>
<td>1. Improves the resin-dentin BS durability</td>
<td>1. Increases the degree of conversion</td>
<td>1. Requires special devices</td>
</tr>
<tr>
<td></td>
<td>2. Decreases the degradation rate</td>
<td>2. Increases solvent evaporation</td>
<td>2. Biological studies need to be performed</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>3. Requires clinical testing</td>
</tr>
</tbody>
</table>
ous application mode. The adhesive coatings should be left undisturbed and not in contact with light for as much time as possible before light curing, and the latter procedure should be performed with at least twice the time recommended by the manufacturer. When possible, the placement of an additional coat of hydrophobic resin may also be beneficial.

With the recent and growing acceptance of minimally invasive dentistry, many bonding protocols will be performed in caries-affected dentin; therefore, we should start focusing on how to improve the immediate BS to this substrate and on alternatives to make this adhesive interface resistant to degradation over time.

Several strategies were explained to improve the longevity of the restorations. Although each strategy has its merits and limitations, it is probably the amalgamation and translation of several strategies into a single treatment approach (Table 1) that may

<table>
<thead>
<tr>
<th>Technique</th>
<th>Benefits</th>
<th>Rationale</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol bonding technique</td>
<td>1. Improves the resin-dentin BS durability</td>
<td>1. Increases the hydrophobicity of the adhesive interface</td>
<td>1. Is not clinically feasible</td>
</tr>
<tr>
<td></td>
<td>2. Decreases the degradation rate</td>
<td>2. Increases the degree of conversion of the polymer</td>
<td>2. Is a time-consuming and difficult technique</td>
</tr>
<tr>
<td></td>
<td>3. Facilitates solvent removal</td>
<td>3. Biological studies need to be performed</td>
<td>4. Requires clinical testing</td>
</tr>
<tr>
<td></td>
<td>4. Decreases the water sorption rate</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5. Improves the penetration of the resin monomers into the collagen fibrils</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of MMPs inhibitors</td>
<td>1. Improves the resin-dentin BS durability</td>
<td>1. Inhibits MMPs activity</td>
<td>1. Can be easily performed but can add an extra step if used apart from the conditioner and adhesive</td>
</tr>
<tr>
<td></td>
<td>2. Decreases the degradation rate</td>
<td>2. Protects non-protected collagen fibrils</td>
<td>2. More studies with SE need to be performed</td>
</tr>
<tr>
<td></td>
<td>3. Improves the clinical performance (ER)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reinforcement of the collagen fibrils by use of collagen cross-linkers</td>
<td>1. Improves the resin-dentin BS durability</td>
<td>1. Reinforces the demineralized collagen fibrils</td>
<td>1. Is not clinically feasible</td>
</tr>
<tr>
<td></td>
<td>2. Improves the resistance of collagen fibrils to collagenases</td>
<td>2. May produce MMPs inhibition</td>
<td>2. Is not applicable to SE adhesives</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3. Requires clinical testing</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: ER, etch-and-rinse; SE, self-etch; BS, bond strength; MMP, matrix metalloproteinases.
overcome the critical barriers currently encountered in bonding to dentin.

Conflict of Interest

The authors of this manuscript certify that they have 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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