# Dental Adhesion: Mechanism, Techniques and Durability

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*Contemporary dental adhesives show favorable immediate results in terms of bonding effectiveness. However, the durability of resin-dentin bonds is their major problem. It appears that simplification of adhesive techniques is rather detrimental to the long term stability of resin-tooth interface. The hydrostatic pulpal pressure, the dentinal fluid flow and the increased dentinal wetness in vital dentin can affect the intimate interaction of certain dentin adhesives with dentinal tissue. Bond degradation occurs via water sorption, hydrolysis of ester linkages of methacrylate resins, and activation of endogenous dentin matrix metalloproteinases. The three-step etch-and-rinse adhesives still remain the gold standard in terms of durability. This review discusses the fundamental process of adhesion to enamel and dentin with different adhesive techniques, factors affecting the long term bonding performance of modern adhesives and addresses the current perspectives for improving bond durability.*

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## INTRODUCTION

One of the greatest challenges in restorative dentistry is to obtain an effective seal of the tooth-restoration interface. Composite restorations rely on adhesive systems which form a micromechanical bond with the tooth structure. The original multicomponent bonding systems are gradually being replaced with simplified, consolidated adhesive systems that are more user-friendly. Despite significant improvements of adhesive systems, the bonded interface remains the weakest area of tooth-colored restorations. Although the incorporation of hydrophilic and acidic resin monomers has substantially improved the initial bonding of contemporary adhesives to intrinsically wet dental substrates, few manufacturers have recognized the potential problems associated with these increasingly hydrophilic adhesives. Most of the current dental adhesives reveal excellent immediate and short-term bonding effectiveness but the durability and stability of resin-bonded interfaces still remains questionable.[1-3] Hence, the objective of this review is to describe the fundamental process of adhesion to enamel and dentin, and to discuss the current trend of simplifying bonding in both the etch & rinse and self-etch adhesives along with the potential *in vivo* degradation processes involved and various strategies to optimize bonding effectiveness.

### Dentin as a Bonding Substrate

Unlike enamel bonding which is obtained with relative ease, bonding to dentin has continued to be a challenge. This is partly due to the biological characteristics of dentin, namely, its high organic content, its tubular structure with presence of odontoblastic processes, the continuous moist condition due to presence of dentinal fluid, intratubular pressure and permeability of the dentin.[4] Although enamel is a highly mineralized tissue composed of more than 90% (by vol) hydroxyapatite, dentin contains substantial proportion of water and organic material, primarily type 1 collagen.[5] Dentin is an intrinsically hydrated tissue, penetrated by a maze of 1 to 2.5µm diameter fluid-filled dentinal tubules and the intertubular dentin contains collagen fibrils with the characteristic collagen banding. There is continuous transudation of dentinal fluid due to intrapulpal pressure in vital dentin that has a magnitude of 25 to 30 mm Hg or 34 to 40 cm of water.[6,7] The number of tubules decreases from about 45000/mm$^2$ close to the pulp to about 20000/mm$^2$ near the DEJ.[8] The average tubule diameter ranges from 0.63 µm at the periphery to 2.37 µm near the pulp.[9] The tubules occupy an area of only 1% of total surface near the DEJ, whereas they occupy 22% of the surface close to the pulp.[10] That also means that the water content of superficial dentin is only 1% whereas it is 22% near the pulp.

Dentin permeability is an important factor affecting

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bonding and there are regional differences in permeability. The permeability of dentin is not uniform throughout teeth, because the number of tubules/mm$^2$ is not uniform. Superficial dentin is therefore very different from deep dentin. Hybrid layers in superficial dentin are almost free of resin tags whereas approximately 50% of the hybrid layers in deep dentin are composed of hybridized resin tags.[11] Dentin permeability can be quantitated by measuring hydraulic conductance of dentinal tubules which is related inversely to their length and directly related to the fourth power of their radius.[12,13] As dentin is made thinner during cavity preparation, the tubules become shorter and hyperconductive relative to thick dentin. Therefore in deep vital hyperconductive dentin, it is difficult to control the outward seepage of dentinal fluid that sometimes compromises resin bonding. Dentin near pulp horns is more permeable than dentin further away as the density and diameter of tubules are highest near pulp horns.[14] Axial dentin is more permeable than the pulpal floors of class II cavities.[13,15] The dentin beneath carious lesion (caries-affected or sclerotic dentin) is much less permeable than normal dentin.[16]

The smear layer formed after tooth preparation fills the orifices of dentin tubules, forming smear plugs and decreases dentin permeability by 86%.[17] The removal of the smear layer and smear plugs with acidic solutions results in an increase of the fluid flow onto the exposed dentin surface, which can again interfere with adhesion. Besides acid-etching, other factors also affect dentin permeability such as the vasoconstrictors in local anesthetics, which decrease pulpal pressure and fluid flow in the tubules, radius and length of the tubules, the viscosity of dentinal fluid, the pressure gradient, the molecular size of the substances dissolved in the tubular fluid, and the rate of removal of substances by the blood vessels in the pulp. All of these variables make dentin a dynamic substrate and consequently a difficult substrate for bonding.

### Current Classification System for Adhesives

The fundamental principle of adhesion to tooth substrate is based upon an exchange process by which inorganic tooth material is exchanged for synthetic resin.[18] This process involves two phases. One phase consists of removing calcium phosphates by which microporosities are exposed at both the enamel and dentin tooth surface. The other so called hybridization phase involves infiltration and subsequent in situ polymerization of resin within the created surface microporosities. Based upon the clinical approach, bonding to tooth hard tissues can be accomplished using one of the following adhesion strategies- the etch & rinse approach, self-etch approach and glass-ionomer approach.[19] Etch & rinse technique involves the use of 30-40% phosphoric acid etchant. This conditioning step is followed by a priming step and application of the adhesive resin, resulting in a three-step application procedure (three-step etch & rinse adhesives). Simplified two-step etch & rinse adhesives (single-bottle adhesives) combine the primer and adhesive resin into a single bottle for single application step.

The self-etch approach is based on the use of non-rinse acidic monomers that simultaneously condition and prime dentin and enamel. The self-etch systems have been classified as two-step and one-step (all-in-one) adhesives. Most common self-etch adhesives involve two application steps with the self-etch primer followed by an adhesive resin, resulting in two-step self-etch adhesives. Recently one-step self-etch systems (all-in-one) have been introduced that incorporate all the components of an adhesive system (etchant, primer and bonding resin) into a single solution and combine all the three bonding steps into a single-application. Self-etch adhesives have also been classified according to their etching aggressiveness: ultra-mild ($pH \geq 2.5$), mild ($pH \approx 2$), intermediate ($pH \approx 1.5$) and strong ($pH \leq 1$).[19,20]

### Adhesion Mechanism of Etch & Rinse Adhesives to Enamel and Dentin

In enamel, etch & rinse technique is still the most effective approach to achieve efficient and stable bonding and requires selective dissolution of hydroxyapatite crystals through etching.[19] Two types of resin tags interlock within the etch-pits. Macro-tags fill the space surrounding the enamel prisms, while numerous micro-tags result from resin infiltration and polymerization within the tiny etch-pits at the cores of the etched enamel prisms. The latter are especially thought to contribute the most with regard to retention to enamel.[19]

In dentin, phosphoric acid treatment removes the smear layer produced during cavity preparation and concurrently results in a 3-5 μm deep demineralization of the dentin surface.[21] This results in exposed collagen fibrils which are nearly completely uncovered from hydroxyapatite, and form a micro-retentive network for micro-mechanical interlocking of monomers. This interlock was first described by Nakabayashi, Kojima and Masuhara in 1982 and is commonly referred to as hybrid layer.[22] To achieve effective and durable bonding, this resin infiltration into the filigree of exposed collagen fibers should be as complete as possible. Collagen fiber network should be in a fully expanded state to facilitate resin penetration. Concurrent with hybridization, resin tags seal the unplugged dentin tubules and offer additional retention through hybridization of the tubule orifice wall. According to Van Meerbeek *et al*, resin tag necks at the top 5-10 μm of the tubule orifices are thought to contribute most to retention and sealing effectiveness while actual length of the tags may be considered as being of secondary importance.[18]

### Adhesion Mechanism of Self-Etch Adhesives to Enamel and Dentin

Self-etch adhesives use acidic monomers to condition tooth structure rather than traditional phosphoric acid, however they do not produce the same degree of porosity in enamel surfaces as that attained with phosphoric acid etching in etch & rinse systems.[23] Since enamel bonding is primarily based on micromechanical interlocking of a low viscosity resin into microporosities, the extent and depth of the etching pattern should logically influence the bonding performance of an adhesive. Scanning electron microscopic studies indicate that an enamel etching pattern caused by self-etch adhesive is not as deep and appears to be less retentive compared with the etching pattern resulting from phosphoric acid treatment and that the degree of enamel etching depends on the pH of the self-etch adhesive.[24-28]

The weak acidity of these self-etch adhesives raises the question of whether the adhesives are able to penetrate the enamel surface and yield durable bonding with the restored tooth. Self-etch adhesives create an irregular, non homogenous etch pattern, whereas phosphoric acid removes the enamel smear layer and leads to a honey-comb structure surface.[28,29] The demineralization depth of enamel surface is lower for self-etch adhesives compared to the etch & rinse approach (1.5-3.2μm vs. 6.9μm).[23] The shallower etching pattern on enamel and subsequent reduced micro-mechanical retention might jeopardize bonding. Thus bonding of self-etch systems to enamel still remains critical and is controversially discussed by various authors.[30,31]

In dentin, *strong* self-etch adhesives exhibit rather deep demineralization effects and lead to a bonding mechanism and ultra morphology similar to that produced by etch & rinse adhesives. Bonding mechanism of *mild* self-etch adhesives to dentin is also based on hybridization with the difference that only submicron hybrid layers are formed and resin tag formation is less pronounced. Within such submicron hybrid layers, collagen fibrils are not completely deprived from hydroxyapatite (in contrast to etch & rinse adhesives), leaving residual hydroxyapatite still attached to collagen, which may serve as a receptor for additional chemical bonding.[32] Carboxylic acid-based monomers like 4-MET (4-methacryloxyethyl trimellitic acid) and phosphate based monomers, such as phenyl-P (2-methacryloxyethyl phenyl hydrogen phosphate), and 10-MDP (10-methacryloxydecyl dihydrogen phosphate) present in self-etch adhesives have a chemical bonding potential to calcium of residual hydroxyapatite.[32] This chemical bonding may results in bonds that better resist hydrolytic degradation processes and thus may help keep the restoration margins sealed for longer periods.[18]

With an etch & rinse or *strong* self-etch approach, the transition of the exposed collagen fibril network to the underlying unaffected dentin is quite abrupt. While in *intermediary strong* self-etch adhesives, there is two-fold build-up of the dentinal hybrid layer with a completely demineralized top layer and a partially demineralized base.[19] Here the deepest region of the hybrid layer up to a maximum of 1 μm still contains hydroxyapatite, by which the transition of the hybrid layer to the underlying unaffected dentin is more gradual. These adhesives are more acidic than the *mild* self-etch adhesives, by which better micromechanical interlocking is achieved at enamel and dentin. The residual hydroxyapatite at the hybrid layer base may still allow for chemical intermolecular interaction, as with *mild* self-etch adhesives.

### Bonding Techniques in Etch & Rinse Adhesives

The collagen fibril network of demineralized dentin represents a soft, delicate bonding substrate that may contribute to the technique sensitivity of etch & rinse adhesives. In dentin, for proper formation of the hybrid layer, water is essentially required to prevent collapse of the collagen fibers. However, clinician should be able to balance over a short distance between wet and dry. Dehydration of the acid-conditioned dentin surface through air-drying is thought to induce surface tension stress, causing the exposed collagen network to collapse, shrink and form a compact coagulate that is impenetrable to resin.[33] On the other hand, if some water remains inside the interfibrillar spaces, the loose quality of the collagen matrix is maintained and the interfibrillar spaces are left open.[34] Therefore two clinical techniques have been suggested to achieve adequate hybridization i.e. dry bonding technique and wet bonding technique depending on the kind of solvent of the primer/adhesive used. In the etch & rinse adhesive systems, hydrophilic primer monomers are dissolved in volatile solvents, such as acetone and ethanol.[35] These solvents may aid in displacement of the remaining water as well as carrying the polymerizable monomers into the opened dentin tubules and through the nano-spaces of the collagen web.[36] The primer solvents are then evaporated by gently air-drying, leaving the active primer monomers behind.

In the dry bonding technique, the substrate field is air-dried and uses adhesive systems that provide water-based primers to re-hydrate and thus re-expand the air-dried and consequently collapsed collagen network on the dentin surface. This allows resin monomers to still interdiffuse efficiently.[37] The other alternative is to keep the acid-etched dentin surface moist and to rely on the water-chasing capacity of acetone-based primers. This clinical technique is commonly referred to as wet-bonding and was introduced by Kanca and Gwinnett in 1992.[34,38-40] With the etch & rinse systems, when an acetone-based adhesive is used, the highly technique-sensitive wet-bonding technique is mandatory.[36] Otherwise, gentle air-drying of acid-etched dentin following a dry-bonding technique still guarantees effective bonding when a water/ethanol-based adhesive is used.[37]

### Technique Sensitivity in Etch & Rinse Adhesives: How Wet Dentin Should be in Wet Bonding Technique?

Wet-bonding technique can only guarantee efficient resin interdiffusion if all the remaining water on the dentin surface is completely eliminated and replaced by monomers during the subsequent priming step. When the water inside the collagen network is not completely displaced, the polymerization of resin inside the hybrid layer may be affected or, at least, the remaining water will compete for space with resin inside the demineralized dentin.[41] Over-wetting may lead to dilution or deterioration of the monomers, reduced final degree of cure and cause phase separation of the hydrophobic and hydrophilic monomer components, resulting in blister and globule formation at the resin-dentin interface. However, the optimal amount of surface wetness necessary for wet bonding varies among marketed etch & rinse adhesive systems, which are acetone-based, ethanol-based or water-based.[42,43]

Also, it is impossible to simultaneously achieve uniform wetness on the axial, pulpal and gingival walls because of differences in hydraulic conductance between superficial and deep dentin[44,45] and the presence of caries affected or sclerotic dentin in which the dentinal tubules are partially or completely obliterated.[46,47] Thus, it is not uncommon to have over-wet regions and over-dry surfaces in the same preparation, which causes nonuniform resin bonding. The optimum level of moisture required to maintain the integrity of collagen without compromising bonding is difficult to ascertain clinically. Itthagarun and Tay proposed that if it is necessary to choose between over-drying or over-wetting of total-etched deep dentin, the former is to be preferred, since vital deep dentin is intrinsically wet after removal of the smear layer.[48]

## Self-Etch Adhesives - A Drive for Simplification but Not Without Limitations

An approach for decreasing the technique-sensitivity of wet bonding is to return to dry bonding to smear layers, but using much more acidic monomers dissolved in water–HEMA primers. Clinically, self-etch systems not only simplify the bonding process by eliminating steps, but also eliminate some of the technique-sensitivity associated with the use of etch & rinse systems.[18] Furthermore, as the moisture level of dentin is not a critical factor with these adhesives, the issue of wet bonding is not a concern. Moreover the risk of incomplete resin infiltration is eliminated by simultaneous infiltration of the exposed collagen fibril scaffold with resin up to the same depth of demineralization.[18] However, one-step self-etch adhesives are extremely hydrophilic as they contain high concentrations of both ionic and hydrophilic monomers.[49] Besides that, a high amount of water is added to these one-step adhesive solutions. Water is required to dissociate the weak acidic methacrylate monomers into ionized form for permeation into the smear layer and underlying mineralized dentin. It is difficult to evaporate water from these one-step self-etch adhesives, and, even if evaporation is successful, water will rapidly diffuse back from the bonded dentin into the adhesive resin. Such hydrophilicity renders these adhesives very permeable and denies their ability to hermetically seal dentin surfaces. This water sorption plasticizes polymers and lowers their mechanical properties.[50] Although hydrophobic dimethacrylates are added to all-in-one adhesives to produce stronger cross linked polymer networks, the hydrophilic monomers tend to cluster together before polymerization to create hydrophilic domains and microscopic water filled channels called water-trees.[51-54] These water-trees permit movement of water from the underlying dentin, through the hybrid and adhesive layers to the adhesive-composite interfaces.[55] It has been suggested that the osmotic gradient responsible for the induction of this type of water transport is derived from the dissolved ions within the oxygen inhibition layer of these polymerized adhesives.[56] These ions osmotically attract water, which diffuses in from the outside through the hydrophilic adhesive layer to create the water blisters. Moreover, the collection of water droplets on the surface of a polymerized adhesive can result in a mode of polymerization of the resin composites that is referred to in polymer chemistry as emulsion polymerization.[57] In such situations, the hydrophilic composite forms an emulsion in the presence of water, which results in the appearance of numerous resin beads along the interface instead of a continuous film of polymerized composite. Therefore, it can be stated that the simplification of bonding steps has not improved the quality or the durability of bonding to dental tissues. Water sorption by hydrophilic and ionic resin monomers within both the hybrid layer and the adhesive layer may contribute to the degradation of resin-dentin bond strength over time as the hydrophilicity and hydrolytic stability of resin monomers are generally antagonistic.

## Durability of the "Bond"

Durability of the bond between resin and tooth substrate is of significant importance for the clinical longevity of adhesive restorations. However, the long-term stability of the resin-bonded dentin is still questionable. There is a general consensus that resin-dentin bonds obtained with contemporary hydrophilic dentin adhesives deteriorate over time.[58,59] The incorporation of increasing concentrations of ionic and hydrophilic resin monomers in simplified adhesives arises from the need to bond to wet dentin substrates. However, such objectives are accomplished at the expense of creating potentially permeable, unstable resin matrices that are prone to water sorption, resin leaching and hydrolysis over time thereby decreasing their mechanical properties.[60,61] Thus the use of hydrophilic simplified adhesives may never achieve the goal of creating a durable coupling between the resin composites and resin-bonded dentin. The loss of bond strength has been primarily attributed to degradation of the hybrid layer at the dentin-adhesive interface. The deterioration of the hybrid layer is due to a variety of physical and chemical factors, including hydrolysis and enzymatic degradation of exposed collagen as well as adhesive resin. Discrepancy between demineralization depth and resin infiltration can result in exposed collagen at the base of hybrid layer which is not enveloped by bonding resin and is therefore, vulnerable to degradation.[62] Although bacterial enzymes may be involved in the degradation of the hybrid layer, host-derived matrix metalloproteinases (MMPs) play a pivotal role.[63] MMPs are a class of zinc and calcium dependent endopeptidases capable of degrading all extracellular matrix components. Human dentin contains at least MMP-2, MMP-8, MMP-9 and MMP-20.[64-66] Some of these MMPs (MMP-8) attack collagen while others (MMP-2 and MMP-9) attack gelatin. They are trapped within the mineralized dentin matrix during tooth development and play strategic roles in tooth development and dentinal caries.[67,68] The majority of MMPs are produced as latent zymogens (pro-MMPs). Mild acids have been shown to be able to activate

MMPs. Both etch & rinse and self-etch adhesives have mild acidity, therefore can release and activate endogenous MMPs during dentin bonding.[69-71] These activated MMPs can slowly hydrolyze unprotected collagen fibrils in incompletely infiltrated hybrid layers.[63] In addition to imperfect infiltration of the dentin substrate, water is another indispensable factor for the hydrolytic function of MMPs. As MMPs are hydrolases, the existence of water is necessary for them to hydrolyze peptide bonds in collagen resulting in the degradation of the resin-dentin interface.[72] Oliveira *et al* confirmed the important role of water in resin-dentin interface degradation, by showing no loss of dentin-adhesive bond strength with time when mineral oil was used as a storage medium instead of water.[73] Resin elution also occurs through nanoleakage channels over time from unstable polymeric hydro-gels within the hybrid layer.[74] This can uncover collagen fibrils so that they are susceptible to hydrolysis by proteolytic enzymes released from the dentin matrix and odontoblast secretion. De Munck *et al* suggested that this may be the reason for the almost total disappearance of parts of the hybrid layer in resin-dentin bonding after four years water storage.[75] The breakdown of collagen may increase the water content, one of the major causes of further collagen degradation at the bonded interface, inducing deterioration of long-term dentin bonding.

## Strategies to Optimize Bonding Effectiveness

Most simplified (one-step self-etch and two-step etch & rinse) adhesives have been shown to be the least durable, while three-step etch & rinse and two-step self-etch adhesives continue to show the highest performances.[76] It is well documented that simplification of adhesive techniques has not been able to improve durability of the bond at resin-tooth interface. Among the different factors responsible for degradation of the resin-bonded dentin interface, some play a pivotal role particularly when simplified adhesives are employed. Insufficient resin impregnation of dentin, high permeability of the bonded interface, sub-optimal polymerization, phase separation and activation of endogenous collagenolytic enzymes are some of the recently reported factors that reduce the longevity of the bonded interface.[76] As bond strength and durability seem to rely on the quality of the hybrid layer (i.e. on the proper impregnation of the dentin substrate) rather than on the thickness or morphology of the hybrid layer/resin tags, various altered application techniques are currently under research to improve the bonding efficacy of contemporary dental adhesives:

1. Application of MMP inhibitors during dentin bonding
2. Application of collagen cross-linkers to demineralized dentin surface
3. Hydrophobic resin layer application
4. Additional enamel etching in self-etch adhesives
5. Double application of the adhesive
6. Agitation for deeper penetration of adhesive
7. Extending dry time for enhanced evaporation of solvent
8. Deproteinization of acid conditioned dentin surface
9. Ethanol Wet Bonding
10. Biomimetic Remineralization

## MMP Inhibitors (MMPI) in Dentin Bonding

The low but persistent endogenous collagenolytic activity can be completely inhibited by the use of protease inhibitors which might help in long term preservation of the hybrid layer. Chlorhexidine (CHX) has been extensively used as an intracanal irrigant in endodontics or cavity cleansers in conservative dentistry. It has proven biocompatibility and good anti-bacterial action both in vivo and vitro. CHX has been found to have MMP inhibitory and antienzyme properties (against MMP-2, -8 and -9) even at low concentrations (0.02-0.0001%).[77] CHX applied to dentin prior to the use of etch & rinse adhesives has been shown to decrease the collagen degradation with time.[78-81] Brackett *et al* demonstrated in vivo that 2% chlorhexidine application after acid-etching greatly reduced the degradation of dentin hybrid layers at 12 months formed with Prime & Bond NT beneath class I resin composite restoration.[79] Zhou *et al* incorporated CHX in the primer of Clearfil SE Bond and reported preservation of dentin bonds in vitro when CHX concentration in the primer was higher than or equal to 0.1%.[82] At low concentrations, the inhibitory effect of CHX on MMPs is thought to be related to a cation-chelating mechanism, wherein the sequestration of metal ions, such as calcium and zinc, would hamper the activation of the catalytic domains within MMPs.[83,84] Nevertheless, the MMP inhibitory effect of CHX seems to be dose dependent and, at high concentrations, it may likely inactivate MMPs by enzyme denaturation rather than by chelation of cations. [77,85]

Various other MMPIs have been recently investigated like Marimastat, CT 1166, Ilomastat (also called galardin), zoledronate-a third generation bisphosphonate, Green tea polyphenols, especially epigallocate-chin gallate (EGCG).[72,86] Tetracyclines are commonly used as antibiotics in the treatment of periodontitis. It has been shown both in vitro and in vivo that tetracyclines and their semi-synthetic forms, doxicycline and minocycline, have the ability to inhibit MMP-1, MMP-2 and MMP-12.[87] Another kind of effective and safe MMP inhibitor is non-antimicrobial chemically modified tetracyclines, which inhibit both the release and activity of MMPs through Ca2+ chelation.[88]

## Collagen Cross-linkers in Dentin Bonding

While most of the developments in adhesive dentistry have focused on the improvement of bonding agents and techniques, limited investigation has explored the contributions of collagen structure/stability to bond strength. The stabilization of dentin collagen with biocompatible cross-linking agents to increase mechanical properties and decrease enzymatic degradation may be of clinical importance to improve dentin bond strength. Collagen in biological tissue is strengthened by the formation of native cross-links, which provides the fibrillar resistance against enzymatic degradation as well as greater tensile properties. Among all types of collagen, type I collagen is the predominant genetic product, and it is an essential molecule to provide tissues and organs with tensile strength, form, and cohesiveness.[89] Type-I collagen is present in tissues as fibrils that are stabilized by lysyloxidase-mediated covalent intermolecular cross-linking.[90] Chemical cross-linkers have been reported to further stabilize collagen fibrils in several connective tissues.[91-93] Glutaraldehyde, a widely used synthetic cross-linker, can enhance collagen stability; however, it presents some drawbacks, such as toxicity.[91]

Proanthocyanidins (PA) are a class of bioflavonoids that are naturally occurring plant metabolites available in fruits, vegetables, nuts, seeds, flowers and barks.[92,94,95] Recently, PA have gained popularity in the fields of nutrition, health and medicine due to their physiological activities such as anti-oxidant, anti-microbial, anti-inflammatory properties, effects on cardiovascular diseases, anti-allergic and enzyme inhibitory activity against phospholipase A2, cyclooxygenase and lipooxygenase.[96-99] PA lack toxicity and are known to stabilize and increase the cross-linkage of type-1 collagen fibrils. Cocoa beans and grape seeds are well known sources of PA. Grape seed extract has been reported to induce exogenous cross-links.[92] The proposed mechanisms for interaction between PA and proteins include covalent, ionic, hydrogen bonding, and hydrophobic interactions.[92]

Bedran-Russo *et al* reported that the application of two naturally occurring cross-linkers, i.e., PA and genipin, to demineralized dentin collagen significantly improved ultimate tensile strength, indicating its potential value in restorative dentistry.[93] Al-Ammar *et al* also depicted increase in dentin bond strength with pre-treatment of acid-etched dentin surface with different collagen cross-linkers including grape seed extract.[100] They suggested that the application of selective collagen cross-linkers during adhesive restorative procedures may be a new approach to improve dentin bond strength properties. Castellan *et al* demonstrated that the elastic modulus of demineralized dentin was significantly increased by the PA treatment regardless of the exposure time.[101] Green *et al* using scanning electron microscope demonstrated the presence of intact collagen fibrils in the hybrid layer of acid-etched dentin specimens bonded with adhesive containing 5% PA after exposure to collagenase solution.[102] They concluded that the presence of grape seed extract PA in dental adhesives may inhibit the biodegradation of unprotected collagen fibrils within the hybrid layer.

### Additional Hydrophobic Resin Layer Application

By executing the rationale behind the use of two-step self-etch systems, the performance of one-step self-etch adhesives may also be improved by treating them as a primer and covering it with a hydrophobic resin coating such as those employed in conventional three-step etch & rinse adhesives. Brackett et al, Reis et al and Albuquerque et al reported increase in resin-dentin bond strength of one-step self-etch adhesives after additional application of hydrophobic resin layer.[103-105] Several mechanisms have been proposed for the better performance of adhesives after hydrophobic layer application. Firstly, the additional application of hydrophobic resin layer increases the concentration of hydrophobic monomers, reducing the relative concentration of solvents and hydrophilic monomers within the adhesive interface.[105] Secondly, the application of a hydrophobic coat also seems to limit the diffusion of water through the hybrid layer to the interface between the adhesive and resin composite which could have otherwise occurred rapidly.[106] That could in turn inhibit polymerization and thereby weaken the adhesive-resin composite interface. Thirdly, the additional layer of hydrophobic adhesive increased the thickness of the adhesive layer that is known to reduce polymerization stresses.[107] However, the effect of this alternative technique seems to vary with the brand of adhesive tested and requires further investigation. Lodovici *et al* demonstrated that the application of an extra coat of hydrophobic solvent-free bonding resin was not able to minimize the damage caused by thermal/mechanical load cycling to the adhesive interfaces obtained with flat dentin surfaces.[108] However their study was done using a three-step etch & rinse and two-step self-etch system while most of the current literature regarding the application of an additional hydrophobic resin layer has demonstrated the effect to be beneficial for simplified one-step self-etch adhesives bonded to dentin.

### Additional Enamel Etching in Self-etch Adhesives

Different studies have indicated the potential benefit of additional enamel etching with phosphoric acid prior to the use of self-etch adhesives.[109-114] Khosravi *et al* showed that simplified all-in-one adhesive systems need pre-etching of the enamel margins with phosphoric acid for an effective seal.[114] Brackett *et al* reported a significant decrease in leakage of self-etch adhesives at enamel margins of class V cavities when prior enamel-etching with phosphoric acid was done.[109] Luhrs *et al* showed that additional phosphoric acid etching significantly increased the shear bond strength to enamel of all the examined self-etch adhesives.[112] Van Meerbeek *et al* also reported more marginal defects at the enamel margins of class V composite restorations when prior phosphoric acid etching was not done with mild two-step self-etch adhesives, although they recorded no difference in clinical performance.[111] Ermis *et al* evaluated over a three year period, the clinical performance of class III composite restorations bonded with a mild two-step self-etch adhesive with and without additional enamel etching.[113] Although a retention rate of 100% was reported for both the non-etch and the etch groups, the non-etched restorations revealed more marginal defects and superficial marginal discoloration than the etched restorations. Contrary to these studies, Watanabe *et al* demonstrated that 2 of the 5 single-step self-etch adhesives tested showed no significant increase in bond strength due to prior acid etching. They suggested that not only the depth of enamel etching, but also the composition and mechanical properties of the adhesives might play important roles in bonding.[31]

### Double Application of Adhesive

In conventional three-step systems, the primer should assure efficient wetting of the exposed collagen fibrils, displace any residual surface moisture, and sufficiently carry monomers into the interfibrillar channels. The adhesive resin should fill up the remaining pores between the collagen fibrils, form resin tags that seal the opened dentinal tubules, initiate and advance the polymerization reaction, stabilize the formed hybrid layer and resin tags and provide sufficient methacrylate double bonds for co-polymerization with the successively applied restorative resin.[18] In simplified (two-step etch & rinse and one-step self-etch adhesives) one-bottle systems, the functions of the primer and the adhesive resin should be perfectly combined. Therefore, these one-bottle systems are associated with higher technique sensitivity.[1] As these combined primer/adhesive resin solutions have a higher solvent-to monomer ratio, there are chances that such adhesives are applied in a very thin layer.[18] However, for efficient bonding, the one-bottle solution needs to be abundantly applied. Monomers should be sufficiently supplied to saturate the exposed collagen fibril network, as well as to establish a satisfactorily thick resin layer on top of the hybrid layer. This distinct resin layer is supposed to act as a flexible, intermediate shock-absorber. According to the elastic bonding concept, this shock-absorber may help to protect the adhesive joint against early failure caused by the shrinking composite cured on top. Therefore, while using one-bottle adhesives, it is recommended to apply multiple layers to ensure a sufficiently thick resin film on top of the hybrid layer.[18]

Albuquerque *et al* reported that double application resulted in increased resin dentin bond strength for Adper Prompt L-Pop but not for Xeno III and G-Bond.[105] They stated that other factors like filler content and solvent type may affect the results. Elkassas *et al* reported that doubling the adhesive layer applications significantly improved the bond strength of the two self-etch adhesives (Xeno IV and G-Bond); however, it had a negative effect on the bond strength of the total-etch adhesive (Prime & bond NT). [115]

### Agitation for Deeper Penetration of Adhesive

The application with agitation of one-step self-etch systems is likely to carry fresh acidic resin monomers to the basal part of the etched dentin, producing a more aggressive demineralization, facilitating diffusion of the monomers and promoting a better interaction with the smear layer and underlying dentin. Reis *et al* stated that rubbing action can improve the kinetics and allow for better monomer diffusion inward, while solvents are diffusing outward.[116] Tewari & Goel concluded that primer agitation and primer drying time both affect dentin shear bond strength in vivo.[117] Amaral *et al* also demonstrated that application of one-step self-etch adhesives with agitation on the dentin surface is an excellent clinical tool capable of improving the resin-dentin bond strength.[118]

### Extending Dry Time for Enhanced Evaporation of Solvent

Major characteristics of simplified adhesive systems include hydroplilicity and a relatively high concentration of solvents in solution. A general consensus exists that solvents have to be evaporated from resin-infiltrated dentin matrix, as remaining solvent can adversely affect polymerization of adhesives and consequent quality and durability of the bonds. Following the application of adhesives, air-drying is required in order to remove solvent from the dentin surface. Residual solvent can be clinically avoided by allowing an evaporation time between application and curing of the adhesive system. The air-drying time allowed to evaporate solvent can have a significant effect on bond strength, depending upon the type of solvent, tooth-syringe distance, air blowing step and the temperature used to evaporate it from the surface. Garcia *et al* reported that the duration and air-temperature of drying step affected the bond strength of the tested adhesives. They concluded that use of warm air-dry stream seems to be a clinical tool to improve bonding of self-etch adhesives.[119] Chiba *et al* also suggested that air-drying time of singe-step self-etch adhesives was a significant factor influencing dentin bond strength.[120]

### Deproteinization of Acid Conditioned Dentin Surface

Deproteinization of the etched dentin would eliminate the problem of handling the delicate collagen network exposed by the acid, which contributes to the technique sensitivity of etch and rinse adhesives. Removal of the organic collagen layer following acid conditioning and subsequent bonding directly to the partially demineralized dentin layer may produce a more durable adhesion to the hydroxyapatite component of the dentin substrate. Sodium hypochlorite (NaOCl) is a non-specific proteolytic agent that effectively removes organic compounds at room temperature and has been frequently applied for collagen removal with controversial results. Improvement in adhesion to demineralized and deproteinized dentin has been reported, but no interference and decrease in adhesion have also been reported.[121-124] This may be because, demineralization and deproteinization (with NaOCl) of dentin could be influenced by various factors present in different studies, such as adhesive system composition, depth (superficial vs deep dentin), type of dental substrate (vital vs non-vital dentin), cavity configuration, sensitivity of the adhesive systems to the oxidizing effect of NaOCl and variation in the concentration and duration of application of NaOCl. Nagpal *et al* reported that NaOCl treatment of acid conditioned dentin surface resulted in deeper resin tag penetration into dentinal tubules when observed under scanning electron microscope.[124]

### Ethanol Wet Bonding

Although the incorporation of hydrophilic resin monomers has considerably improved the ability of contemporary adhesive systems to bond to wet dentin substrates, it has also resulted in increased water sorption and rapid deterioration of their mechanical properties, compromising the longevity of resin-dentin bonds. Recent studies have shown that it is possible to bond hydrophobic resin monomers to acid-etched dentin *ex vivo* with an experimental ethanol-wet bonding technique.[125-128] Ethanol wet bonding is an *in vitro* technique developed for the application of etch & rinse adhesives, that embraces the important concept of slowly replacing water from interfibrillar and intrafibrillar spaces within the demineralized collagen matrix with ascending concentrations of ethanol. In this technique, ethanol, a polar solvent with less hydrogen bonding capacity than water is used for chemical dehydration of the demineralized collagen network. It produces shrinkage of the collagen fibrils enlarging the interfibrillar spaces, for hydrophobic monomers to infiltrate the matrix more optimally.[129] The ethanol saturated collagen matrix is rendered less hydrophilic and is more compatible with hydrophobic resin monomers and prevents phase separation of ethanol-soluble hydrophobic resin monomers.[125] Hosaka *et al* reported that ethanol wet bonding increased the durability of resin-dentin bonds due to higher resin uptake and better resin sealing of the collagen matrix, thereby minimizing endogenous collagenolytic avtivities.[130] Ethanol wet bonding technique is analogous to the technique used in electron microscopy for tissue embedding, where water present in biological tissues is gradually replaced by stepwise immersion in an ascending series of solvents. Therefore, use of five ascending ethanol concentrations each with 30 seconds application time has been recommended for the ethanol wet bonding technique, with absolute ethanol applied three times. However this procedure takes approximately three to four minutes which defies the principle of user-friendliness and technique simplification. Therefore a more simplified dehydration protocol is required as one developed by Sadek *et al*, reducing the application time from 30 sec to 15 sec.[127]

### Biomimetic Remineralization of Resin-bonded Acid-etched Dentin

The most compelling problem associated with resin-dentin bonds is their limited durability, caused partially by water-sorption-induced hydrolysis of the hydrophilic resin components present in these adhesives and partially by degeneration of collagen fibrils via endogenous MMPs derived from demineralized dentin. Although collagen degradation in imperfect hybrid layers may be postponed by the application of chlorhexidine as a MMP inhibitor, a zone of resin-sparse demineralized dentin inadvertently remains that is potentially susceptible to creep or cyclic fatigue rupture during function. It is also doubtful whether the relatively short substantivity of chlorhexidine permits it to be retained permanently within the denuded collagen matrix. Biomimetic remineralization of denuded collagen fibrils appears to be an alternative strategy for revival of resin-dentin bonds. The biomimetic remineralization protocol developed by Tay and Pashley, involves the binding of two biomimetic analogs to dentin collagen so that the doped collagen can guide the scale and distribution of apatite remineralization.[131] The remineralization medium consists of a set Portland cement/simulated body fluid system containing polyacrylic acid (PAA) and polyvinylphosphosphonic acid (PVPA) as biomimetic analogs for amorphous calcium phosphate dimension regulation and collagen targeting. PAA is used to mimic the stabilization function of dentin matrix protein 1 (DMP 1) on amorphous calcium phosphate precursors.[132] The PAA reduces these precursors released by interaction of set Portland cement with the simulated body fluid to a nanoscale. While PVPA is a polyanion that mimics the negative charges of phosphoproteins such as DMP 1, phosphophoryn, or bone sialoprotein and their roles in organizing aspects of mineralization.[133]

Although the biomimetic remineralization model provides a proof-of-concept for extending the longevity of resin-dentin bonds, however, in the current model, each resin-dentin slab is placed sideways on top of a set Portland cement block in the remineralization solution.[134,135] This strategy is not possible clinically and delivery systems that incorporate set Portland cement particles in either the adhesive or restorative material, have to be considered for remineralization to proceed from the top of the adhesive-bonded dentin to the mineralized dentin base via diffusion of the amorphous calcium phosphate nanoprecursors.[134] Additional strategies need to be developed for incorporating the biomimmetic analogs as part of the delivery systems.

## CONCLUSION

The current trend in the development of adhesive systems, attempts to simplify bonding steps and make them more user-friendly. However, optimizing speed and efficiency should be accomplished without major tradeoffs in the quality or durability of tooth resin bonds. It is a proven fact that any kind of simplification in the clinical application procedures results in loss of bonding effectiveness. There are many hurdles in successful bonding of contemporary adhesives like: incomplete resin infiltration, excess solvent and very thin adhesive layer in simplified adhesives, water sorption and hydrolytic breakdown, incomplete polymerization, insufficient enamel etching ability of self-etch adhesives and collagen degradation by host-derived enzymes. The three-step etch & rinse adhesives still remain the gold standard in terms of durability. Only the two-step self-etch adhesives approach the gold standard and do have some additional clinical benefits. Following factors have been attributed for poor bonding performance of all-in-one self-etch adhesives in dentin: 1) presence of highly hydrophilic monomers that are sensitive to water sorption from the underlying dentin, increasing hybrid layer permeability and nanoleakage, 2) high concentration of protic solvents and dissolved molecular oxygen within the adhesive layer as a result of poor evaporation, 3) the limited thickness of the adhesive layer, which may also magnify the oxygen inhibition effect on adhesive polymerization, 4) during solvent evaporation, the monomer/water ratio may change and subsequently result in phase separations and blistering. Furthermore, an adverse acid-base reaction and adhesive permeability may contribute to the incompatibility between some simplified adhesives to resin composite.

Different approaches have been under research to improve the quality and stability of resin-tooth interface but their effect seems to depend on interplay of various factors and varies for different adhesives. Although various studies have investigated the role of MMPI and collagen cross-linkers in dentin bonding, still gaps remain in our knowledge regarding the optimal concentration, duration of application and their effect on specific adhesive systems based on different solvents. New bonding systems should provide durable MMP-inhibitory functionality to preserve the integrity of the hybrid layer and to improve dentin bonding durability of adhesive restorations. Further studies are clearly warranted to evaluate the effects of collagen cross-linking agents on bond strength of different adhesive systems to dentin. Although ethanol-wet-bonding looks promising, it involves an extra step of replacing rinsing water with 100% ethanol. Thus, more evidence is needed to justify this extra step. Bonding potential to enamel remains the weakest property of mild self-etch adhesives. Therefore, developing monomers with stronger chemical bonding potential to hydroxyapatite may also help to further improve their bonding performance in enamel.

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