©Operative Dentistry, 2013, **38-4,** E103-E127

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

A Reis • M Carrilho • L Breschi
AD Loguercio

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.

*Alessandra Reis, DDS, PhD, adjunct professor, State University of Ponta Grossa, Restorative Dentistry, Ponta Grossa, Brazil

Marcela Rocha Carrilho, DDS, PhD, professor, GEO/Bandeirante University of São Paulo and School of Dentistry, Schulich School of Medicine & Dentistry, Western University, London, ON, Canada

Lorenzo Breschi, DDS, PhD, professor, University of Trieste, Dental Sciences and Biomaterials, Trieste, Italy

Alessandro D. Loguercio, DDS, MS, PhD, adjunct professor, State University of Ponta Grossa, Restorative Dentistry, Ponta Grossa, Brazil

*Corresponding author: Rua Carlos Cavalcanti, 4748, Bloco M, Sala 64A – Uvaranas, Ponta Grossa, PR 84030-900, Brazil; e-mail: reis_ale@hotmail.com

DOI: 10.2341/12-258-LIT

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 interfaces[15,16] 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 others[6] 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.[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.[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.[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.[23] Previous laboratory investigators have detected that most of the commercial simplified adhesive formulations are highly prone to water sorption to a potentially damaging extent,[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.[24,28,29]

However, it is not only the hydrophilicity of the material that is responsible for water sorption.[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.[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%.[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.[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.[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%.[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.[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.[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[36] as well as the resin-dentin BS.[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.[42] As partially polymerized adhesives are more permeable to fluid movement,[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.[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,[6,9] which in turn, reduces the ultimate tensile strength[44] and elastic modulus[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.[15]

The more hydrophilic the material, the higher is its water sorption rate and the resultant hydrolytic

degradation.[15,24,28,45] As reported by Breschi and others,[15] 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.[46-48]

This is why the adhesive interfaces produced with simplified adhesives are considered semipermeable membranes.[2,49,50] 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.[51] 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.[52] Similarly, chlorhexidine (CHX) can be slowly released from urethane dimethacrylate–triethyleneglycoldimethacrylate (TEGDMA) resins because it can permeate the resin matrix.[53]

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.[2,54,55] 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[54,56] (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.[46,48,54]

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.[50]

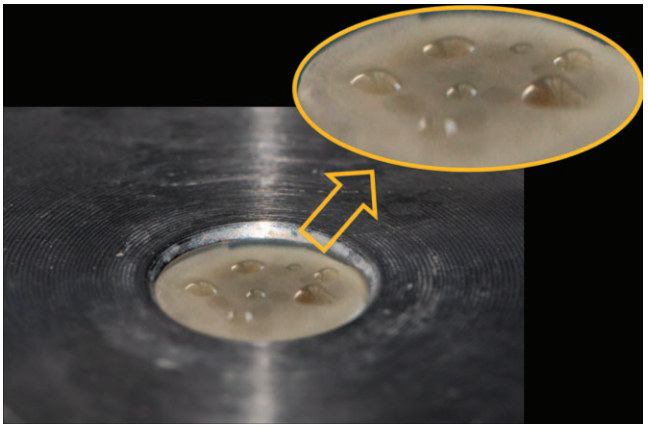

Figure 1. *Presence of small water blisters in a dentin surface under physiological pulpal pressure after adhesive application. These water blisters were observed five minutes after the light-curing step of a simplified SE system.*

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.[50] 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.[2,57] 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[17] 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

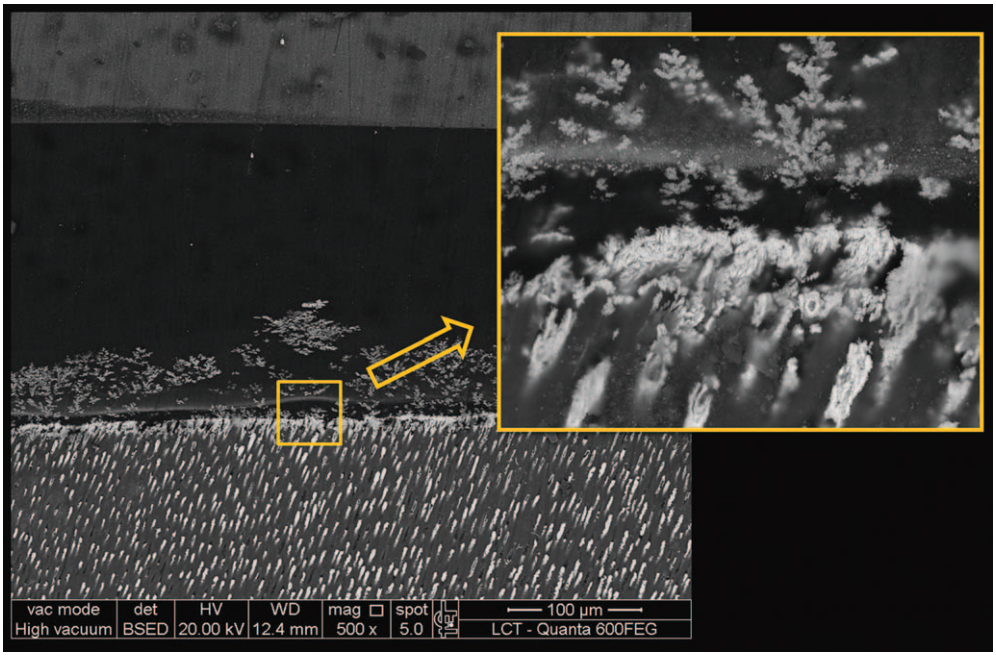

Figure 2. *SEM images of the adhesive interface produced by an SE system after five to six months of water storage. Observe the intense silver nitrate deposition and the formation of the water-tree channels (arrows).*

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.[58-61]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.[60,62]

Using micro-Raman spectroscopy,[59,63,64] 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.[59] 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,[65] as there was evidence of intense labeling of collagen fibrils by anti-type I collagen antibodies in the deepest part of the hybrid layer.[15,65]

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,[66] and the authors called it: nanoleakage or leakage inside the hybrid layer.

Resin elution from hydrolytically unstable polymeric hydrogels within hybrid layers[24,28] 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[67,68] and periodontal disease[69] have potential and relevant implications in the degradation of resin-dentin bonded interfaces.[70] By assaying the collagenolytic activity of mineralized dentin powder by using fluorescein-labeled type I collagen from bovine skin, Pashley and others[70] 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.[67,71,72]

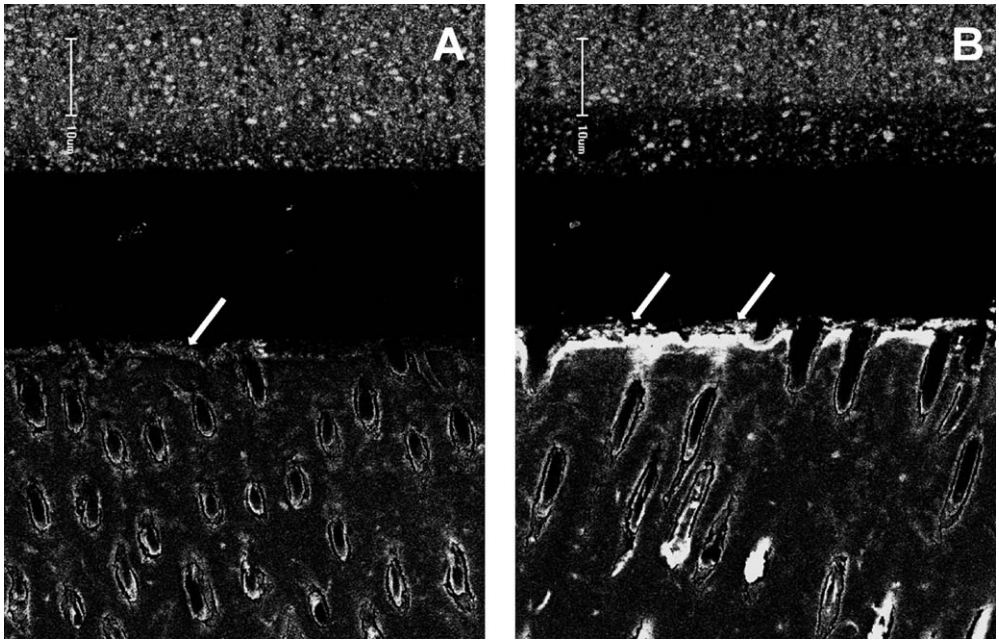

Figure 3. *SEM images of the adhesive interface produced by an ER adhesive immediately after bonding (A) and six months after water storage (B). Observe the increase in the silver nitrate (arrows) deposition over time.*

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.[73] Within the oral environment, considerable interest has been devoted to the detection, distribution, and function of host-derived MMPs.[74,75] 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),[76,77] collagenase (MMP-8),[78] and gelatinases A and B (MMP-2 and MMP-9, respectively).[79,80]

Description of the involvement of MMPs in the degradation of demineralized dentin matrix was first mentioned in a study by Tjäderhane and others.[67] 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.[67] As previously mentioned, more recently Pashley and others[70] 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[70, 81-83] 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.[4]

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).[84,85] 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.[86] Of 15 CPs genes detected, 10 were expressed in native pulp tissue and 11 in odontoblasts.[84]

In studies by Tersariol and others[84] and Nascimento and others,[85] 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 adhesives[81] and SE systems[82] 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 adhesives[87,88] 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.

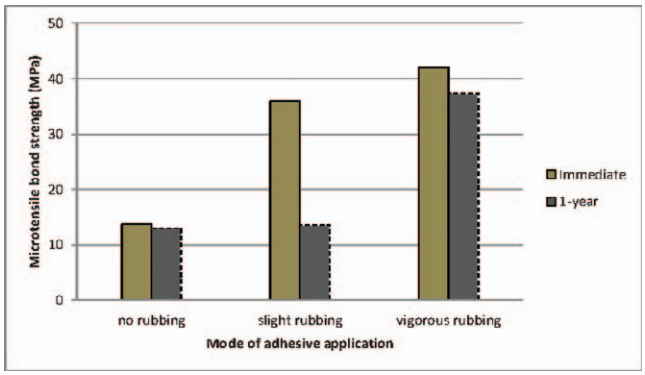

Figure 4. *Resin-dentin BS of two-step ER ethanol-based adhesive applied by different application modes at the immediate and one-year periods. No significant difference in the immediate and one-year resin-dentin BS was observed when the adhesive was vigorously rubbed onto the dentin surfaces. Adapted from Reis and others.[93]*

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 dentin[92,93] (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 adhesives[96] (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.

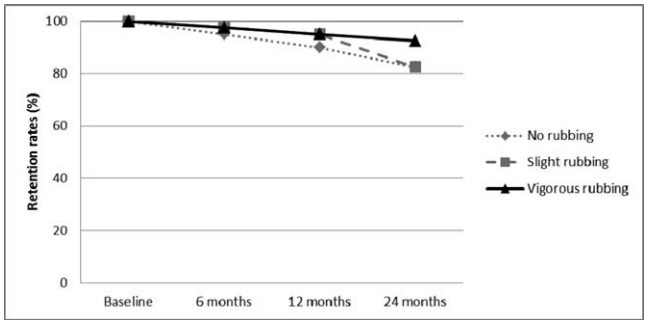

Figure 5. *Retention rates of Prime&Bond NT (Dentsply) applied in noncarious cervical lesions over 24 months of clinical service under different application methods. After 24 months, vigorous application method led to increased retention rates. Adapted from Loguercio and others.*[96]

The vigorous clinical approach was also shown to increase the durability of adhesive interfaces produced with SE adhesives when applied to dentin[98,99] (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.[99-101] 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.[102-104]

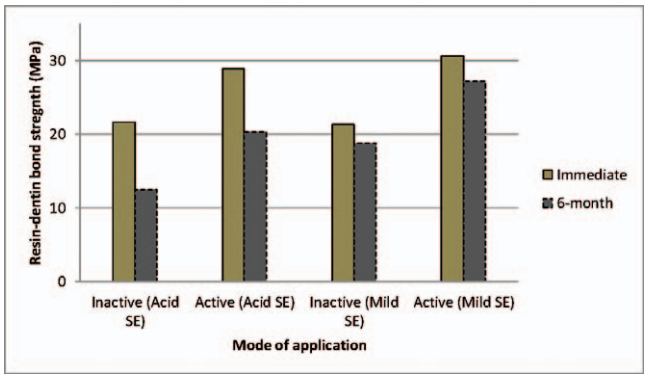

Figure 6. *Resin-dentin BS of a one-step SE applied by different application modes at the immediate and six-month periods. Active application improves the initial BS and decreases the degradation of resin-dentin BS. Adapted from do Amaral and others.*[99]

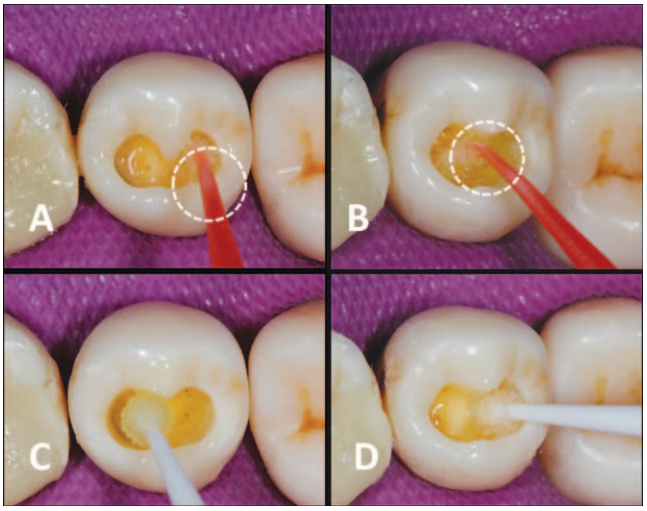

Figure 7. *The use of a flexible microbrush (A) is inadequate for the vigorous application mode, because it deforms as force is applied (B). This technique should be performed with a rigid microbrush (B) that does not deform under manual pressure.*

Another alternative is the application of multiple adhesive coats. Hashimoto and others[105] 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[106] 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[107] (Figure 8). The clinical benefits of this

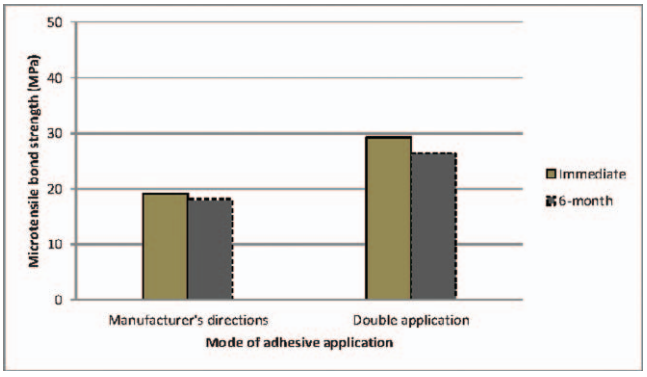

Figure 8. *Immediate and six-month resin-dentin BS of a one-step SE adhesive applied according to the manufacturer's directions and after duplicating the number of adhesive coats. Higher immediate and six-month BS values were observed by duplicating the number of adhesive layers. Adapted from Reis and others.*[107]

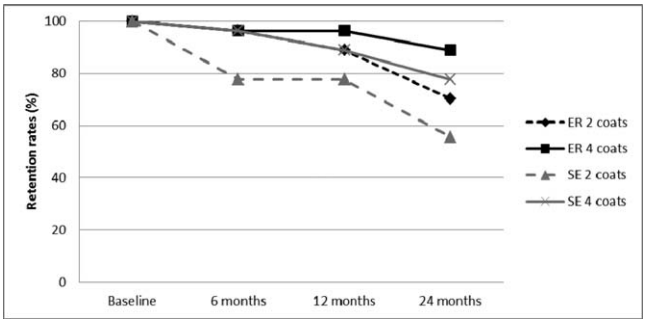

Figure 9. *Retention rates of a simplified ER adhesive (One Step, Bisco Inc) and a simplified SE adhesive (Tyrian, Bisco Inc) applied in two or four adhesive coats in noncarious cervical lesions over 24 months of clinical service. The use of four coats led to increased retention rates. Adapted from Loguercio and Reis.*[108]

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.

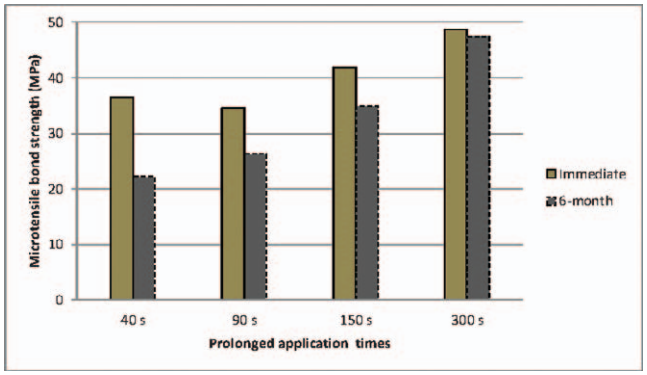

Figure 10. *Resin-dentin BS of two-step ER ethanol-based adhesive applied according to the manufacturer's directions (40 seconds) and after delayed polymerization. High immediate and stable six-month BS values were observed for the 300-second group. Adapted from Reis and others.*[110]

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 resinon 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

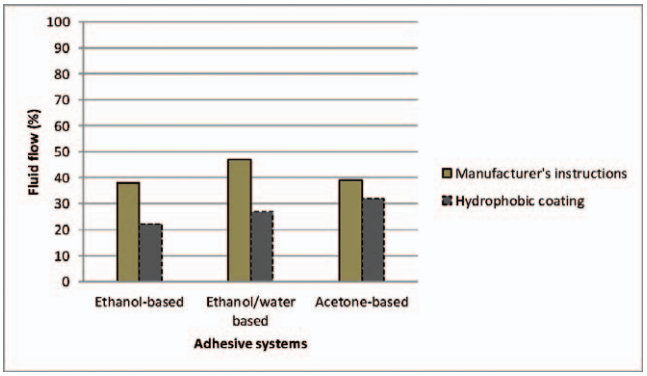

Figure 11. *Fluid flow (%) across resin-dentin interfaces after two-step ER adhesive application according to the manufacturer's directions or with an extra hydrophobic resin layer coating. Statistically lower fluid flow was observed with the extra coat of hydrophobic resin for all adhesives. Adapted from de Andrade e Silva and others.*[119]

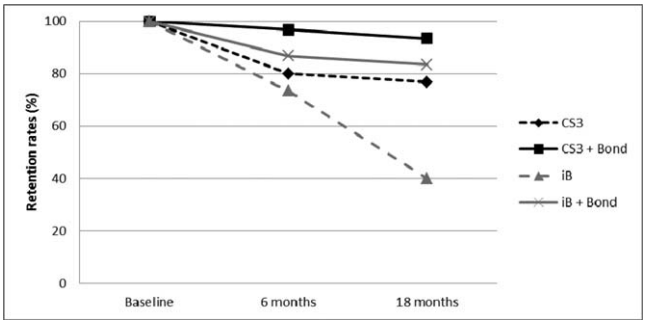

Figure 12. *Retention rates of two simplified SE adhesives (CS3 = Clearfil S3 Bond, Kuraray; iB = iBond Gluma Inside, Heraus Kulzer) applied in noncarious cervical lesions over 18 months of clinical service. The adhesives were applied according to the manufacturer's directions (CS3 and iB) or associated 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.*[120]

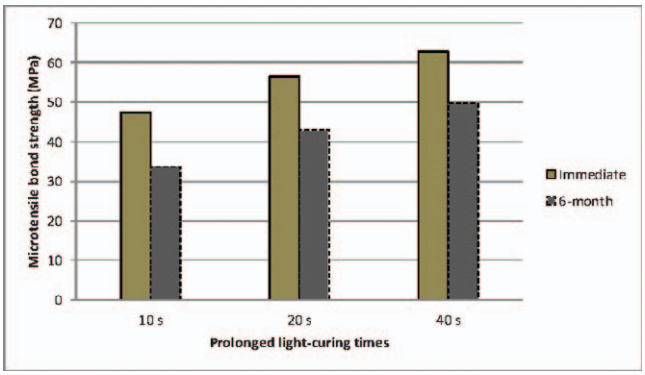

Figure 13. *Resin-dentin BS of two-step ER ethanol-based adhesive light-cured according to the manufacturer's directions (10 seconds) and after prolonged exposure times. The six-month resin-dentin BS obtained after a 40-second light exposure is similar to the immediate 10-second resin dentin BS. Adapted from Reis and others.*[124]

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.[115,116,117] 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.[42,118]

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.[15] Consequently, a more stable resin-dentin interface can be produced over time.[107] 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).[119]

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 in noncarious cervical lesions, after 18 months of clinical service, were 77.3 % and 40.0 %, respectively (Figure 12). After the application of a hydrophobic, non-solvated resin layer, these figures increased to 93.4% and 83.4%, respectively, thereby showing clinical evidence of the success of this clinical approach[120] (Figure 12). For the ER adhesive All Bond 3 (Bisco Inc, Schaumburg, IL, USA), the retention rates after 24 months of clinical service were 90.0% and 97% when the materials were used in a two-step and three-step process, respectively.[121] These results are in agreement with a recent clinical study published by Perdigão and others.[122] Once again, one cannot deny that the main disadvantage of this clinical protocol is that its application is more time consuming.

As resin permeability and monomer elution are both related to suboptimally polymerized bonding systems, two recent studies[42,118] 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.[123-124] Higher energy density (produced by the longer exposure times) enhances the formation of free radicals, which initiates polymerization.[125] Moreover, the unavoidable heat produced by light curing units[126,127] mainly during prolonged exposure time, is likely to increase solvent evaporation rates.[124] This may provide room for the formation of a high-molecular-weight and cross-linked polymer.[124]

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[124] and one-step SE materials[128] (Figure 13). By prolonging the exposure time during

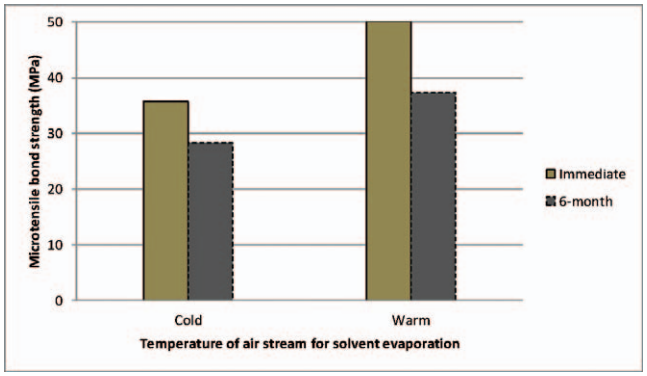

Figure 14. *Resin-dentin BS of two-step ER ethanol-based adhesive after solvent evaporation with a cold (20°C) and warm (60°C) air stream. Although degradation of the resin-dentin BS occurred for both groups, one can observe that the six-month resin-dentin BS in the warm group was similar to the immediate resin-dentin BS in the cold group, which shows that the degradation was less pronounced. Adapted from Reis and others.*[130]

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 adhesives[130] (Figure 14), probably because of increased solvent evaporation[129] (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

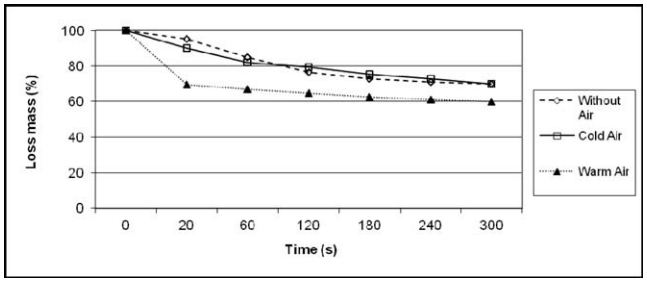

Figure 15. *Loss of mass (%) as a result of different solvent evaporation methods used for 300 seconds in two-step ER ethanol-based adhesive. The use of a warm-air stream favors solvent evaporation. Adapted from Klein-Júnior and others.*[129]

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[137] and SE systems.[138] Probably, heating the adhesive may alter the stability of the acidic monomers in such 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 units[140,141] 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 water-saturated 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

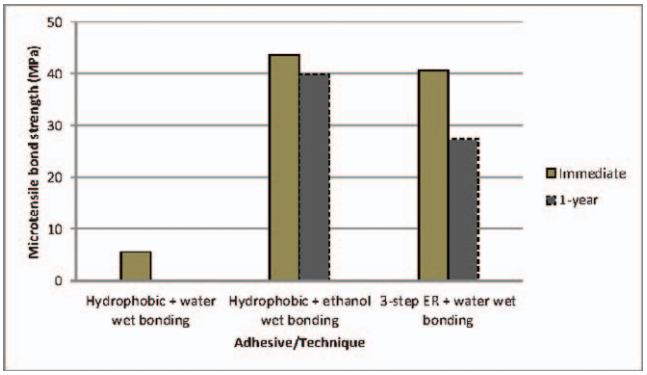

Figure 16. *Resin-dentin BS of an experimental hydrophobic Bis-GMA/TEGDMA adhesive applied on water- and ethanol-saturated dentin versus the resin-dentin BS of a commercial three-step ER ethanol-based adhesive (three-step ER) applied on wet-saturated dentin. Stable bonds were only achieved with the use of the hydrophobic adhesive by means of the ethanol-wet bonding technique. Adapted from Sadek and others.*[146]

from dentin.[143] The application of hydrophobic resins to ethanol-wet dentin, may provide resin interfaces with a five-fold reduction in water sorption rates.[28,44]

Resin-dentin interfaces with higher long-term stability were produced with the ethanol-wet bonding technique.[145,146] 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.[146] 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,[143,145,147] the protocol is time consuming and technique sensitive.[148] 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.[146] 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,[16] 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,[70] and probably also by cysteine cathepsins.[84,85] 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*.[7,12,149]

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.[150,151] There are several synthetic nonspecific MMPs inhibitors, such as CHX, ethylenediamine tetraaceticacid (EDTA),[70,81]tetracyclines and derivatives,[152] or specific MMPs inhibitors, such as SB 3CT[87,88] and galardin.[153] Some dental materials, such as zinc oxide cements,[154] 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.[155]

It was already shown that CHX has desirable MMP-inhibitory properties, even at very low concentrations.[156] Complete inhibition of MMP-2 and MMP-9 gelatinase activities occurred at CHX concentrations as low as 0.03%.[156] Carrilho and others[157] 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*.[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[158]; however, fortunately, its cytotoxic effect is dependent on the thickness of remaining dentin and occurs at a very low dentin depth.[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).[160,161] probably because of the high substantivity of CHX to mineralized and demineralized dentin substrates.[162] These short application times and low CHX concentrations convey a distinct advantage, in that it is not toxic to human periodontal cells[163] and odontoblast-like cells,[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.[164, 165] In addition, Tezvergil-Mutluay and others[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.[87,88,154] Among these, galardin, which is a synthetic MMP inhibitor, has been shown to reduce the degradation rate of hybrid layers[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.[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.[167]

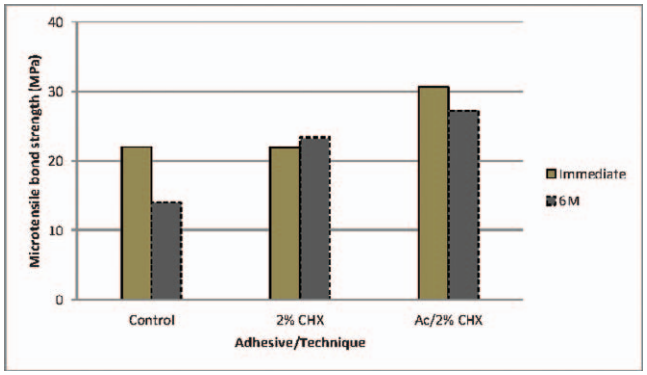

Figure 17. *Immediate and six-month (6M) resin-dentin BS of two-step ER acetone-based adhesive used according to the manufacturer's directions (control), after application of 2% CHX for 60 seconds after acid etching and after using a 2% CHX containing acid (ac/2% CHX). Stable BS was obtained in the 2%CHX and ac/2% CHX groups. Adapted from Stanislawczuk and others.*[164]

Despite the controversy regarding the role of SE adhesives in the activation of MMPs,[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.[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,[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.[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.[173-177]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.[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], carbodiimides,

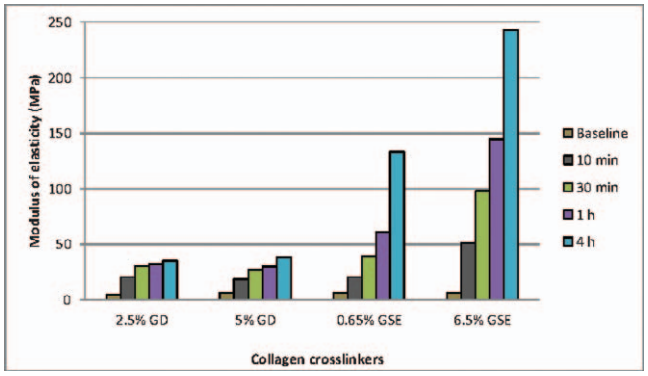

Figure 18. *Changes on the modulus of elasticity (MPa) of human demineralized dentin matrix over time in different concentrations of GD and grape-seed extract (GSE). Both the application time and concentration increase the modulus of elasticity of dentin. Adapted from Bedran-Russo and others.*[174]

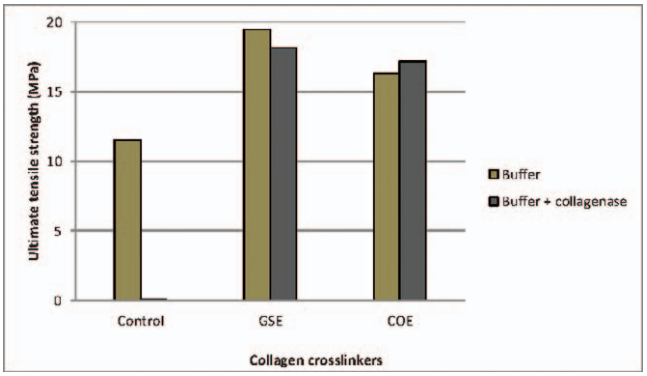

Figure 19. *In vitro human dentin matrix resistance against enzymatic degradation after application of two different cross-linker agents for one hour. The values report the ultimate tensile strength means of the demineralized dentin after immersion in a buffer, or a buffer + collagenase media for 24 hours. Adapted from Castellan and others.*[175]

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 eightto 40 times[174-176] (Figure 18). Previous studies have reported that these collagen cross-linkers have the ability to improve the short-term mechanical properties of dentin[174]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 adhesives[184] (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

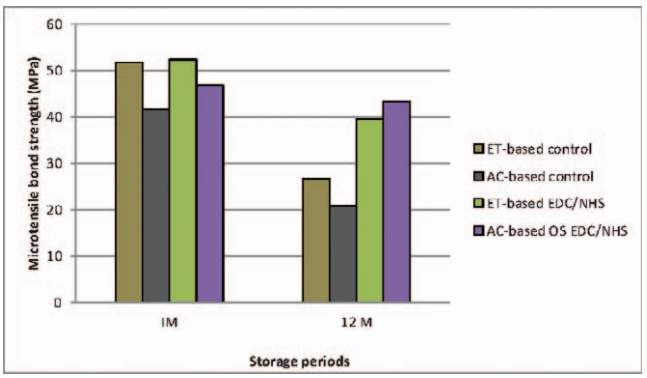

Figure 20. *Immediate (IM) and 12-month (12M) resin-dentin BS of adhesive interfaces produced with two-step ER ethanol-based adhesive (ET-based) and two-step ER acetone-based adhesive (AC-based) used according to the manufacturer's directions (control) after treatment of the demineralized dentin with a cross-linker agent (EDC/NHS) for 1 hour. The use of EDC/NHS increased stability of dentin-resin interfaces. Adapted from Bedran-Russo and others.*[184]

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[187-191]and the incompatibility between simplified ER adhesives and chemically/dual cured resin cements.[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[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.[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[198] or the use of hydrophilic photoinitiators such as QTX [3-(3,4-dimethyl-9-oxo-9Hthioxanthen-2-yloxy)-2-hydroxypropyl] trimethyl ammonium chloride][199] or water-soluble photoinitiators such as TPO [(diphenyl(2,4,6-trimethylbenzoyl)-phosphine oxide)][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[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.[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.[60,62]

Biomimetic remineralization provides a means for remineralizing incompletely infiltrated resin-dentin interfaces created by ER[202] and SE adhesives[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.[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.[16] The addition of bioactive glasses to the adhesive formulation has shown promising results, but it still requires further evaluations.[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*

Technique	Benefits	Rationale	Disadvantages
Vigorous rubbing application	1. Increases the resin-dentin BS	1. Increases solvent evaporation	1. Is difficult to standardize
	2. Decreases the degradation rate	2. Improves penetration of the resin monomers into the collagen fibrils	2. Requires rigid microbrushes
	3. Improves the clinical performance (ER and SE)		
Multiple coats	1. Improves the resin-dentin BS	1. Improves penetration of the resin monomers into the collagen fibrils	1. Lacks consensus regarding the number of coats
	2. Decreases the degradation rate (SE)	2. Decreases the continuous exposure of collagen fibrils	2. Is time consuming
	3. Decreases adhesive permeability 4. Improves the clinical performance (ER and SE)		3. Aging studies need to be performed for ER.
Delayed light-curing	1. Improves the resin-dentin BS durability (ER)	1. Increases solvent evaporation	1. Is time consuming
		2. Improves penetration of the resin monomers into the collagen fibrils	2. Aging studies need to be performed for SE 3. Requires clinical testing
Additional layer of hydrophobic resin coating	1. Improves the resin-dentin BS durability	1. Increases the hydrophobicity of the adhesive resins	1. Requires an additional material (hydrophobic adhesive)
	2. Decreases the degradation rate	2. Increases the degree of conversion of the adhesive layer	2. Is time consuming as it adds an extra step
	3. Decreases the adhesive permeability	3. Decreases the plasticization of the polymer network after water sorption	
	4. Improves the clinical performance (ER and SE)	4. Decreases the continuous exposure of collagen fibrils	
Prolonging the exposure time during light curing	1. Improves the resin-dentin BS durability	1. Increases the degree of conversion	1. Is time consuming
	2. Decreases the degradation rate (ER and SE)	2. Increases solvent evaporation	2. Biological studies need to be performed
	3. Decreases the adhesive permeability	3. Decreases the continuous exposure of collagen fibrils	3. Requires clinical testing
Warm air-stream for solvent evaporation	1. Improves the resin-dentin BS durability	1. Increases the degree of conversion	1. Requires special devices
	2. Decreases the degradation rate	2. Increases solvent evaporation	2. Biological studies need to be performed 3. Requires clinical testing

Table 1: Continued.

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

Abbreviations: ER, etch-and-rinse; SE, self-etch; BS, bond strength; MMP, matrix metalloproteinases.

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

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.

(Accepted 23 August 2012)

REFERENCES

1. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, & Vanherle G (2003) Buonocore memorial lecture. Adhesion to enamel and dentin: Current status and future challenges *Operative Dentistry* **28(3)** 215-235.
2. Tay FR, & Pashley DH (2003) Have dentin adhesives become too hydrophilic? *Journal of Canadian Dental Association* **69(11)** 726-731.
3. Armstrong SR, Vargas MA, Chung I, Pashley DH, Campbell JA, Laffoon JE, & Qian F (2004) Resin-dentin interfacial ultrastructure and microtensile dentin bond strength after five-year water storage *Operative Dentistry* **29(6)** 705-712.
4. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, Lambrechts P, & Vanherle G (2003) Four-year water degradation of total-etch adhesives bonded to dentin *Journal of Dental Research* **82(2)** 136-140.
5. Hashimoto M, Fujita S, Nagano F, Ohno H, & Endo K (2010) Ten-years degradation of resin-dentin bonds *European Journal of Oral Science* **118(4)** 404-410.
6. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, & Oguchi H (2000) In vivo degradation of resin-dentin bonds in humans over 1 to 3 years *Journal of Dental Research* **79(6)** 1385-1391.
7. Hebling J, Pashley DH, Tjaderhane L, & Tay FR (2005) Chlorhexidine arrests subclinical degradation of dentin hybrid layers in vivo *Journal of Dental Research* **84(8)** 741-746.
8. Reis A, Grande RH, Oliveira GM, Lopes GC, & Loguercio AD (2007) A 2-year evaluation of moisture on microtensile bond strength and nanoleakage *Dental Materials* **23(7)** 862-870.
9. Sano H, Yoshikawa T, Pereira PN, Kanemura N, Morigami M, Tagami J, & Pashley DH (1999) Long-term durability of dentin bonds made with a self-etching primer, in vivo *Journal of Dental Research* **78(4)** 906-911.
10. Abdalla AI (2010) Effect of long-term water aging on microtensile bond strength of self-etch adhesives to dentin *American Journal of Dentistry* **23(1)** 29-33.
11. Garcia-Godoy F, Kramer N, Feilzer AJ, & Frankenberger R (2010) Long-term degradation of enamel and dentin bonds: 6-year results in vitro vs. in vivo *Dental Materials* **26(11)** 1113-1118.
12. Carrilho MR, Geraldeli S, Tay F, de Goes MF, Carvalho RM, Tjaderhane L, Reis AF, Hebling J, Mazzoni A, Breschi L, & Pashley D (2007) In vivo preservation of the hybrid layer by chlorhexidine *Journal of Dental Research* **86(6)** 529-533.
13. Heintze SD, Ruffieux C, & Rousson V (2010) Clinical performance of cervical restorations—A meta-analysis *Dental Materials* **26(10)** 993-1000.
14. Peumans M, Kanumilli P, De Munck J, Van Landuyt K, Lambrechts P, & Van Meerbeek B (2005) Clinical effectiveness of contemporary adhesives: Asystematic review of current clinical trials *Dental Materials* **21(9)** 864-881.
15. Breschi L, Mazzoni A, Ruggeri A, Cadenaro M, Di Lenarda R, & De Stefano Dorigo E (2008) Dental adhesion review: Aging and stability of the bonded interface *Dental Materials* **24(1)** 90-101.
16. Liu Y, Tjaderhane L, Breschi L, Mazzoni A, Li N, Mao J, Pashley DH, & Tay FR (2011) Limitations in bonding to dentin and experimental strategies to prevent bond degradation *Journal of Dental Research* **90(8)** 953-968.
17. Nakabayashi N, Ashizawa M, & Nakamura M (1992) Identification of a resin-dentin hybrid layer in vital human dentin created in vivo: Durable bonding to vital dentin *Quintessence International* **23(2)** 135-141.
18. Sano H (2006) Microtensile testing, nanoleakage, and biodegradation of resin-dentin bonds *Journal of Dental Research* **85(1)** 11-14.
19. Vaidyanathan TK, & Vaidyanathan J (2009) Recent advances in the theory and mechanism of adhesive resin bonding to dentin: Acritical review *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **88(2)** 558-578.
20. Hashimoto M (2010) A review—Micromorphological evidence of degradation in resin-dentin bonds and potential preventional solutions *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **92(1)** 268-280.
21. Spencer P, Ye Q, Park J, Topp EM, Misra A, Marangos O, Wang Y, Bohaty BS, Singh V, Sene F, Eslick J, Camarda K, & Katz JL (2010) Adhesive/dentin interface: The weak link in the composite restoration *Annals of Biomedical Engineering* **38(6)** 1989-2003.
22. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, Coutinho E, Suzuki K, Lambrechts P, & Van Meerbeek B (2007) Systematic review of the chemical composition of contemporary dental adhesives *Biomaterials* **28(26)** 3757-3785.
23. Ferracane JL (2006) Hygroscopic and hydrolytic effects in dental polymer networks *Dental Materials* **22(3)** 211-222.
24. Malacarne J, Carvalho RM, de Goes MF, Svizero N, Pashley DH, Tay FR, Yiu CK, & Carrilho MR (2006) Water sorption/solubility of dental adhesive resins *Dental Materials* **22(10)** 973-980.
25. Reis AF, Giannini M, & Pereira PN (2007) Influence of water-storage time on the sorption and solubility behavior of current adhesives and primer/adhesive mixtures *Operative Dentistry* **32(1)** 53-59.

26. Fabre HS, Fabre S, Cefaly DF, de Oliveira Carrilho MR, Garcia FC, & Wang L (2007) Water sorption and solubility of dentin bonding agents light-cured with different light sources *Journal of Dentistry* **35(3)** 253-258.
27. Ito S, Hoshino T, Iijima M, Tsukamoto N, Pashley DH, & Saito T (2010) Water sorption/solubility of self-etching dentin bonding agents *Dental Materials* **26(7)** 617-626.
28. Ito S, Hashimoto M, Wadgaonkar B, Svizero N, Carvalho RM, Yiu C, Rueggeberg FA, Foulger S, Saito T, Nishitani Y, Yoshiyama M, Tay FR, & Pashley DH (2005) Effects of resin hydrophilicity on water sorption and changes in modulus of elasticity *Biomaterials* **26(33)** 6449-6459.
29. Malacarne-Zanon J, Pashley DH, Agee KA, Foulger S, Alves MC, Breschi L, Cadenaro M, Garcia FP, & Carrilho MR (2009) Effects of ethanol addition on the water sorption/solubility and percent conversion of comonomers in model dental adhesives *Dental Materials* **25(10)** 1275-1284.
30. Yiu CK, Pashley EL, Hiraishi N, King NM, Goracci C, Ferrari M, Carvalho RM, Pashley DH, & Tay FR (2005) Solvent and water retention in dental adhesive blends after evaporation *Biomaterials* **26(34)** 6863-6872.
31. Yiu CK, King NM, Carrilho MR, Sauro S, Rueggeberg FA, Prati C, Carvalho RM, Pashley DH, & Tay FR (2006) Effect of resin hydrophilicity and temperature on water sorption of dental adhesive resins *Biomaterials* **27(9)** 1695-1703.
32. Cho BH, & Dickens SH (2004) Effects of the acetone content of single solution dentin bonding agents on the adhesive layer thickness and the microtensile bond strength *Dental Materials* **20(2)** 107-115.
33. Jacobsen T, & Soderholm KJ (1995) Some effects of water on dentin bonding *Dental Materials* **11(2)** 132-136.
34. Carvalho RM, Mendonca JS, Santiago SL, Silveira RR, Garcia FC, Tay FR, & Pashley DH (2003) Effects of HEMA/solvent combinations on bond strength to dentin *Journal of Dental Research* **82(8)** 597-601.
35. Pashley EL, Zhang Y, Lockwood PE, Rueggeberg FA, & Pashley DH (1998) Effects of HEMA on water evaporation from water-HEMA mixtures *Dental Materials* **14(1)** 6-10.
36. Loguercio AD, Loeblein F, Cherobin T, Ogliari F, Piva E, & Reis A (2009) Effect of solvent removal on adhesive properties of simplified etch-and-rinse systems and on bond strengths to dry and wet dentin *Journal of Adhesive Dentistry* **11(3)** 213-219.
37. Paul SJ, Leach M, Rueggeberg FA, & Pashley DH (1999) Effect of water content on the physical properties of model dentine primer and bonding resins *Journal of Dentistry* **27(3)** 209-214.
38. Ye Q, Spencer P, Wang Y, & Misra A (2007) Relationship of solvent to the photopolymerization process, properties, and structure in model dentin adhesives *Journal of Biomedical Materials Research Part A* **80(2)** 342-350.
39. Hass V, Folkuenig MS, Reis A, & Loguercio AD (2011) Influence of adhesive properties on resin-dentin bond strength of one-step self-etching adhesives *Journal of Adhesive Dentistry* **13(5)** 417-424.
40. Reis A, Grandi V, Carlotto L, Bortoli G, Patzlaff R, Rodrigues Accorinte Mde L, & Dourado Loguercio A (2005) Effect of smear layer thickness and acidity of self-etching solutions on early and long-term bond strength to dentin *Journal of Dentistry* **33(7)** 549-559.
41. Takahashi A, Sato Y, Uno S, Pereira PN, & Sano H (2002) Effects of mechanical properties of adhesive resins on bond strength to dentin *Dental Materials* **18(3)** 263-268.
42. Cadenaro M, Antoniolli F, Sauro S, Tay FR, Di Lenarda R, Prati C, Biasotto M, Contardo L, & Breschi L (2005) Degree of conversion and permeability of dental adhesives *European Journal of Oral Science* **113(6)** 525-530.
43. Santerre JP, Shajii L, & Leung BW (2001) Relation of dental composite formulations to their degradation and the release of hydrolyzed polymeric-resin-derived products *Critical Reviews in Oral Biology & Medicine* **12(2)** 136-151.
44. Yiu CK, King NM, Pashley DH, Suh BI, Carvalho RM, Carrilho MR, & Tay FR (2004) Effect of resin hydrophilicity and water storage on resin strength *Biomaterials* **25(26)** 5789-5796.
45. Tanaka J, Ishikawa K, Yatani H, Yamashita A, & Suzuki K (1999) Correlation of dentin bond durability with water absorption of bonding layer *Dental Materials Journal* **18(1)** 11-18.
46. Chersoni S, Suppa P, Breschi L, Ferrari M, Tay FR, Pashley DH, & Prati C (2004) Water movement in the hybrid layer after different dentin treatments *Dental Materials* **20(9)** 796-803.
47. Hashimoto M, Ito S, Tay FR, Svizero NR, Sano H, Kaga M, & Pashley DH (2004) Fluid movement across the resin-dentin interface during and after bonding *Journal of Dental Research* **83(11)** 843-848.
48. Chersoni S, Suppa P, Grandini S, Goracci C, Monticelli F, Yiu C, Huang C, Prati C, Breschi L, Ferrari M, Pashley DH, & Tay FR (2004) In vivo and in vitro permeability of one-step self-etch adhesives *Journal of Dental Research* **83(6)** 459-464.
49. Tay FR, King NM, Chan KM, & Pashley DH (2002) How can nanoleakage occur in self-etching adhesive systems that demineralize and infiltrate simultaneously? *Journal of Adhesive Dentistry* **4(4)** 255-269.
50. Tay FR, & Pashley DH (2003) Water treeing—A potential mechanism for degradation of dentin adhesives *American Journal of Dentistry* **16(1)** 6-12.
51. Carrilho MR, Tay FR, Donnelly AM, Agee KA, Carvalho RM, Hosaka K, Reis A, Loguercio AD, & Pashley DH (2009) Membrane permeability properties of dental adhesive films *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **88(2)** 312-320.
52. Nakabo S, Torii Y, Itota T, Ishikawa K, Miyazaki K, Suzuki K, & Yoshiyama M (2002) Effects of fluoride release from bis-GMA/TEGDMA resin regulated by gamma-methacryloxypropyltrimethoxysilane on demineralization of bovine enamel *Biomaterials* **23(16)** 3503-3508.

53. Hiraishi N, Yiu CK, King NM, Tay FR, & Pashley DH (2008) Chlorhexidine release and water sorption characteristics of chlorhexidine-incorporated hydrophobic/hydrophilic resins *Dental Materials* **24(10)** 1391-1399.

54. Tay FR, Frankenberger R, Krejci I, Bouillaguet S, Pashley DH, Carvalho RM, & Lai CN (2004) Single-bottle adhesives behave as permeable membranes after polymerization. I. In vivo evidence *Journal of Dentistry* **32(8)** 611-621.

55. Tay FR, Pashley DH, Suh BI, Hiraishi N, & Yiu CK (2005) Water treeing in simplified dentin adhesives—Deja vu? *Operative Dentistry* **30(5)** 561-579.

56. Chersoni S, Acquaviva GL, Prati C, Ferrari M, Grandini S, Pashley DH, & Tay FR (2005) In vivo fluid movement through dentin adhesives in endodontically treated teeth *Journal of Dental Research* **84(3)** 223-227.

57. Carvalho RM, Pegoraro TA, Tay FR, Pegoraro LF, Silva NR, & Pashley DH (2004) Adhesive permeability affects coupling of resin cements that utilise self-etching primers to dentine *Journal of Dentistry* **32(1)** 55-65.

58. Santini A, & Miletic V (2008) Quantitative micro-Raman assessment of dentine demineralization, adhesive penetration, and degree of conversion of three dentine bonding systems *European Journal of Oral Science* **116(2)** 177-183.

59. Wang Y, & Spencer P (2003) Hybridization efficiency of the adhesive/dentin interface with wet bonding *Journal of Dental Research* **82(2)** 141-145.

60. Wang Y, & Spencer P (2005) Continuing etching of an all-in-one adhesive in wet dentin tubules *Journal of Dental Research* **84(4)** 350-354.

61. Wang Y, Spencer P, & Yao X (2006) Micro-Raman imaging analysis of monomer/mineral distribution in intertubular region of adhesive/dentin interfaces *Journal of Biomedical Optics* **11(2)** 024005.

62. Carvalho RM, Chersoni S, Frankenberger R, Pashley DH, Prati C, & Tay FR (2005) A challenge to the conventional wisdom that simultaneous etching and resin infiltration always occurs in self-etch adhesives *Biomaterials* **26(9)** 1035-1042.

63. Miyazaki M, Onose H, Iida N, & Kazama H (2003) Determination of residual double bonds in resin-dentin interface by Raman spectroscopy *Dental Materials* **19(3)** 245-251.

64. Miyazaki M, Sato H, Onose H, Moore BK, & Platt JA (2003) Analysis of the enamel/adhesive resin interface with laser Raman microscopy *Operative Dentistry* **28(2)** 136-142.

65. Breschi L, Prati C, Gobbi P, Pashley D, Mazzotti G, Teti G, & Perdigão J (2004) Immunohistochemical analysis of collagen fibrils within the hybrid layer: AFEISEM study *Operative Dentistry* **29(5)** 538-546.

66. Sano H, Takatsu T, Ciucchi B, Horner JA, Matthews WG, & Pashley DH (1995) Nanoleakage: Leakage within the hybrid layer *Operative Dentistry* **20(1)** 18-25.

67. Tjäderhane L, Larjava H, Sorsa T, Uitto VJ, Larmas M, & Salo T (1998) The activation and function of host matrix metalloproteinases in dentin matrix breakdown in caries lesions *Journal of Dental Research* **77(8)** 1622-1629.

68. van Strijp AJ, Jansen DC, DeGroot J, ten Cate JM, & Everts V (2003) Host-derived proteinases and degradation of dentine collagen in situ *Caries Research* **37(1)** 58-65.

69. Lee W, Aitken S, Sodek J, & McCulloch CA (1995) Evidence of a direct relationship between neutrophil collagenase activity and periodontal tissue destruction in vivo: Role of active enzyme in human periodontitis *Journal of Periodontal Research* **30(1)** 23-33.

70. Pashley DH, Tay FR, Yiu C, Hashimoto M, Breschi L, Carvalho RM, & Ito S (2004) Collagen degradation by host-derived enzymes during aging *Journal of Dental Research* **83(3)** 216-221.

71. Sulkala M, Larmas M, Sorsa T, Salo T, & Tjäderhane L (2002) The localization of matrix metalloproteinase-20 (MMP-20, enamelysin) in mature human teeth *Journal of Dental Research* **81(9)** 603-607.

72. Sulkala M, Wahlgren J, Larmas M, Sorsa T, Teronen O, Salo T, & Tjäderhane L (2001) The effects of MMP inhibitors on human salivary MMP activity and caries progression in rats *Journal of Dental Research* **80(6)** 1545-1549.

73. Visse R, & Nagase H (2003) Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry *Circulation Research* **92(8)** 827-839.

74. Hannas AR, Pereira JC, Granjeiro JM, & Tjäderhane L (2007) The role of matrix metalloproteinases in the oral environment *Acta Odontologica Scandinavica* **65(1)** 1-13.

75. Sorsa T, Tjäderhane L, & Salo T (2004) Matrix metalloproteinases (MMPs) in oral diseases *Oral Disease* **10(6)** 311-318.

76. Boukpessi T, Menashi S, Camoin L, Tencate JM, Goldberg M, & Chaussain-Miller C (2008) The effect of stromelysin-1 (MMP-3) on non-collagenous extracellular matrix proteins of demineralized dentin and the adhesive properties of restorative resins *Biomaterials* **29(33)** 4367-4373.

77. Mazzoni A, Papa V, Nato F, Carrilho M, Tjäderhane L, Ruggeri A Jr., Gobbi P, Mazzotti G, Tay FR, Pashley DH, & Breschi L (2011) Immunohistochemical and biochemical assay of MMP-3 in human dentine *Journal of Dentistry* **39(3)** 231-237.

78. Sulkala M, Tervahartiala T, Sorsa T, Larmas M, Salo T, & Tjäderhane L (2007) Matrix metalloproteinase-8 (MMP-8) is the major collagenase in human dentin *Archives of Oral Biology* **52(2)** 121-127.

79. Martin-De Las Heras S, Valenzuela A, & Overall CM (2000) The matrix metalloproteinase gelatinase A in human dentine *Archives of Oral Biology* **45(9)** 757-765.

80. Mazzoni A, Mannello F, Tay FR, Tonti GA, Papa S, Mazzotti G, Di Lenarda R, Pashley DH, & Breschi L (2007) Zymographic analysis and characterization of MMP-2 and −9 forms in human sound dentin *Journal of Dental Research* **86(5)** 436-440.

81. Mazzoni A, Pashley DH, Nishitani Y, Breschi L, Mannello F, Tjäderhane L, Toledano M, Pashley EL, & Tay FR (2006) Reactivation of inactivated endogenous proteolytic activities in phosphoric acid-etched dentine by etch-and-rinse adhesives *Biomaterials* **27(25)** 4470-4476.
82. Nishitani Y, Yoshiyama M, Wadgaonkar B, Breschi L, Mannello F, Mazzoni A, Carvalho RM, Tjäderhane L, Tay FR, & Pashley DH (2006) Activation of gelatinolytic/collagenolytic activity in dentin by self-etching adhesives *European Journal of Oral Science* **114(2)** 160-166.
83. Tay FR, Pashley DH, Loushine RJ, Weller RN, Monticelli F, & Osorio R (2006) Self-etching adhesives increase collagenolytic activity in radicular dentin *Journal Endodontics* **32(9)** 862-868.
84. Tersariol IL, Geraldeli S, Minciotti CL, Nascimento FD, Paakkonen V, Martins MT, Carrilho MR, Pashley DH, Tay FR, Salo T, & Tjäderhane L (2010) Cysteine cathepsins in human dentin-pulp complex *Journal Endodontics* **36(3)** 475-481.
85. Nascimento FD, Minciotti CL, Geraldeli S, Carrilho MR, Pashley DH, Tay FR, Nader HB, Salo T, Tjäderhane L, & Tersariol IL (2011) Cysteine cathepsins in human carious dentin *Journal of Dental Research* **90(4)** 506-511.
86. Dickinson DP (2002) Cysteine peptidases of mammals: Their biological roles and potential effects in the oral cavity and other tissues in health and disease *Critical Reviews in Oral Biology & Medicine* **13(3)** 238-275.
87. De Munck J, Mine A, Van den Steen PE, Van Landuyt KL, Poitevin A, Opdenakker G, & Van Meerbeek B (2010) Enzymatic degradation of adhesive-dentin interfaces produced by mild self-etch adhesives *European Journal of Oral Science* **118(5)** 494-501.
88. De Munck J, Van den Steen PE, Mine A, Van Landuyt KL, Poitevin A, Opdenakker G, & Van Meerbeek B (2009) Inhibition of enzymatic degradation of adhesive-dentin interfaces *Journal of Dental Research* **88(12)** 1101-1106.
89. Carvalho RV, Ogliari FA, de Souza AP, Silva AF, Petzhold CL, Line SR, Piva E, & Etges A (2009) 2-hydroxyethyl methacrylate as an inhibitor of matrix metalloproteinase-2 *European Journal of Oral Science* **117(1)** 64-67.
90. de Carvalho RV, Ogliari FA, Marques MR, de Souza AP, Petzhold CL, Line SR, Piva E, & Etges A (2011) Inhibition of the activity of matrix metalloproteinase 2 by triethylene glycol dimethacrylate *Clinical Oral Investigation* **15(5)** 643-648.
91. Tezvergil-Mutluay A, Agee KA, Uchiyama T, Imazato S, Mutluay MM, Cadenaro M, Breschi L, Nishitani Y, Tay FR, & Pashley DH (2011) The inhibitory effects of quaternary ammonium methacrylates on soluble and matrix-bound MMPs *Journal of Dental Research* **90(4)** 535-540.
92. Dal-Bianco K, Pellizzaro A, Patzlaft R, de Oliveira Bauer JR, Loguercio AD, & Reis A (2006) Effects of moisture degree and rubbing action on the immediate resin-dentin bond strength *Dental Materials* **22(12)** 1150-1156.
93. Reis A, Pellizzaro A, Dal-Bianco K, Gones OM, Patzlaff R, & Loguercio AD (2007) Impact of adhesive application to wet and dry dentin on long-term resin-dentin bond strengths *Operative Dentistry* **32(4)** 380-387.
94. Hashimoto M, Ohno H, Kaga M, Sano H, Endo K, & Oguchi H (2002) The extent to which resin can infiltrate dentin by acetone-based adhesives *Journal of Dental Research* **81(1)** 74-78.
95. Jacobsen T, & Soderholm KJ (1998) Effect of primer solvent, primer agitation, and dentin dryness on shear bond strength to dentin *American Journal of Dentistry* **11(5)** 225-228.
96. Loguercio AD, Raffo J, Bassani F, Balestrini H, Santo D, do Amaral RC, & Reis A (2011) 24-month clinical evaluation in non-carious cervical lesions of a two-step etch-and-rinse adhesive applied using a rubbing motion *Clinical Oral Investigation* **15(4)** 589-596.
97. Zander-Grande C, Ferreira SQ, da Costa TR, Loguercio AD, & Reis A (2011) Application of etch-and-rinse adhesives on dry and rewet dentin under rubbing action: a 24-month clinical evaluation *Journal of American Dental Association* **142(7)** 828-835.
98. Loguercio AD, Stanislawczuk R, Mena-Serrano A, & Reis A (2011) Effect of 3-year water storage on the performance of one-step self-etch adhesives applied actively on dentine *Journal of Dentistry* **39(8)** 578-587.
99. do Amaral RC, Stanislawczuk R, Zander-Grande C, Michel MD, Reis A, & Loguercio AD (2009) Active application improves the bonding performance of self-etch adhesives to dentin *Journal of Dentistry* **37(1)** 82-90.
100. do Amaral RC, Stanislawczuk R, Zander-Grande C, Gagler D, Reis A, & Loguercio AD (2010) Bond strength and quality of the hybrid layer of one-step self-etch adhesives applied with agitation on dentin *Operative Dentistry* **35(2)** 211-219.
101. Miyazaki M, Platt JA, Onose H, & Moore BK (1996) Influence of dentin primer application methods on dentin bond strength *Operative Dentistry* **21(4)** 167-172.
102. Ando S, Watanabe T, Tsubota K, Yoshida T, Irokawa A, Takamizawa T, Kurokawa H, & Miyazaki M (2008) Effect of adhesive application methods on bond strength to bovine enamel *Journal of Oral Science* **50(2)** 181-186.
103. Torres CR, Barcellos DC, Pucci CR, Lima Gde M, Rodrigues CM, & Siviero M (2009) Influence of methods of application of self-etching adhesive systems on adhesive bond strength to enamel *Journal of Adhesive Dentistry* **11(4)** 279-286.
104. Miyazaki M, Hinoura K, Honjo G, & Onose H (2002) Effect of self-etching primer application method on enamel bond strength *American Journal of Dentistry* **15(6)** 412-416.
105. Hashimoto M, De Munck J, Ito S, Sano H, Kaga M, Oguchi H, Van Meerbeek B, & Pashley DH (2004) In vitro effect of nanoleakage expression on resin-dentin bond strengths analyzed by microtensile bond test, SEM/EDX and TEM *Biomaterials* **25(25)** 5565-5574.
106. Ito S, Tay FR, Hashimoto M, Yoshiyama M, Saito T, Brackett WW, Waller JL, & Pashley DH (2005) Effects of

multiple coatings of two all-in-one adhesives on dentin bonding *Journal of Adhesive Dentistry* **7(2)** 133-141.

107. Reis A, Albuquerque M, Pegoraro M, Mattei G, Bauer JR, Grande RH, Klein-Júnior CA, Baumhardt-Neto R, & Loguercio AD (2008) Can the durability of one-step self-etch adhesives be improved by double application or by an extra layer of hydrophobic resin? *Journal of Dentistry* **36(5)** 309-315.

108. Loguercio AD, & Reis A (2008) Application of a dental adhesive using the self-etch and etch-and-rinse approaches: an 18-month clinical evaluation *Journal of American Dental Association* **139(1)** 53-61.

109. Camps J, & Pashley DH (2000) Buffering action of human dentin in vitro *Journal of Adhesive Dentistry* **2(1)** 39-50.

110. Reis A, de Carvalho Cardoso P, Vieira LC, Baratieri LN, Grande RH, & Loguercio AD (2008) Effect of prolonged application times on the durability of resin-dentin bonds *Dental Materials* **24(5)** 639-644.

111. Cardoso Pde C, Loguercio AD, Vieira LC, Baratieri LN, & Reis A (2005) Effect of prolonged application times on resin-dentin bond strengths *Journal of Adhesive Dentistry* **7(2)** 143-149.

112. Hashimoto M, Tay FR, Sano H, Kaga M, & Pashley DH (2006) Diffusion-induced water movement within resin-dentin bonds during bonding *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **79(2)** 453-458.

113. Toledano M, Proenca JP, Erhardt MC, Osorio E, Aguilera FS, Osorio R, & Tay FR (2007) Increases in dentin-bond strength if doubling application time of an acetone-containing one-step adhesive *Operative Dentistry* **32(2)** 133-137.

114. Erhardt MC, Osorio R, Pisani-Proenca J, Aguilera FS, Osorio E, Breschi L, & Toledano M (2009) Effect of double layering and prolonged application time on MTBS of water/ethanol-based self-etch adhesives to dentin *Operative Dentistry* **34(5)** 571-577.

115. King NM, Tay FR, Pashley DH, Hashimoto M, Ito S, Brackett WW, Garcia-Godoy F, & Sunico M (2005) Conversion of one-step to two-step self-etch adhesives for improved efficacy and extended application *American Journal of Dentistry* **18(2)** 126-134.

116. Brackett WW, Ito S, Tay FR, Haisch LD, & Pashley DH (2005) Microtensile dentin bond strength of self-etching resins: Effect of a hydrophobic layer *Operative Dentistry* **30(6)** 733-738.

117. Van Landuyt KL, Peumans M, De Munck J, Lambrechts P, & Van Meerbeek B (2006) Extension of a one-step self-etch adhesive into a multi-step adhesive *Dental Materials* **22(6)** 533-544.

118. Breschi L, Cadenaro M, Antoniolli F, Sauro S, Biasotto M, Prati C, Tay FR, & Di Lenarda R (2007) Polymerization kinetics of dental adhesives cured with LED: Correlation between extent of conversion and permeability *Dental Materials* **23(9)** 1066-1072.

119. de Andrade e Silva SM, Carrilho MR, Marquezini Junior L, Garcia FC, Manso AP, Alves MC, & de Carvalho RM (2009) Effect of an additional hydrophilic versus hydrophobic coat on the quality of dentinal sealing provided by two-step etch-and-rinse adhesives *Journal of Applied Oral Science* **17(3)** 184-189.

120. Reis A, Leite TM, Matte K, Michels R, Amaral RC, Geraldeli S, & Loguercio AD (2009) Improving clinical retention of one-step self-etching adhesive systems with an additional hydrophobic adhesive layer *Journal of American Dental Association* **140(7)** 877-885.

121. Reis A, Manica D, Ferneda F, Amaral R, Stanislawczuk R, Manso A, De Carvalho RM, & Loguercio AD (2010) A 24-month randomized clinical trial of a two- and three-step etch-and-rinse technique *American Journal of Dentistry* **23(4)** 231-236.

122. Perdigão J, Dutra-Correa M, Saraceni CH, Ciaramicoli MT, Kiyan VH, & Queiroz CS (2012) Randomized clinical trial of four adhesion strategies: 18-month results *Operative Dentistry* **37(1)** 3-11.

123. Hinoura K, Miyazaki M, & Onose H (1991) Effect of irradiation time to light-cured resin composite on dentin bond strength *American Journal of Dentistry* **4(6)** 273-276.

124. Reis A, Ferreira SQ, Costa TR, Klein-Júnior CA, Meier MM.& Loguercio AD (2010) Effects of increased exposure times of simplified etch-and-rinse adhesives on the degradation of resin-dentin bonds and quality of the polymer network *European Journal of Oral Science* **118(5)** 502-509.

125. Peutzfeldt A (1997) Resin composites in dentistry: The monomer systems *European Journal of Oral Science* **105(2)** 97-116.

126. Bouillaguet S, Caillot G, Forchelet J, Cattani-Lorente M, Wataha JC, & Krejci I (2005) Thermal risks from LED- and high-intensity QTH-curing units during polymerization of dental resins *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **72(2)** 260-267.

127. Bagis B, Bagis Y, Ertas E, & Ustaomer S (2008) Comparison of the heat generation of light curing units *Journal of Contemporay Dental Practice* **9(2)** 65-72.

128. Hass V, Luque-Martinez I, Sabino NB, Loguercio AD, & Reis A (2012) Prolonged exposure times of one-step self-etch adhesives on adhesive properties and durability of dentine bonds *Journal of Dentistry* **40(12)** 1090-1102.

129. Klein-Júnior CA, Zander-Grande C, Amaral R, Stanislawczuk R, Garcia EJ, Baumhardt-Neto R, Meier MM, Loguercio AD, & Reis A (2008) Evaporating solvents with a warm air-stream: Effects on adhesive layer properties and resin-dentin bond strengths *Journal of Dentistry* **36(8)** 618-625.

130. Reis A, Klein-Júnior CA, de Souza FH, Stanislawczuk R, & Loguercio AD (2010) The use of warm air stream for solvent evaporation: Effects on the durability of resin-dentin bonds *Operative Dentistry* **35(1)** 29-36.

131. Garcia FC, Almeida JC, Osorio R, Carvalho RM, & Toledano M (2009) Influence of drying time and temperature on bond strength of contemporary adhesives to dentine *Journal of Dentistry* **37(4)** 315-320.

132. Lovell LG, Newman SM, & Bowman CN (1999) The effects of light intensity, temperature, and comonomer composition on the polymerization behavior of dimetha-

crylate dental resins *Journal of Dental Research* **78(8)** 1469-1476.

133. Daronch M, Rueggeberg FA, & De Goes MF (2005) Monomer conversion of pre-heated composite *Journal of Dental Research* **84(7)** 663-667.
134. Froes-Salgado NR, Silva LM, Kawano Y, Francci C, Reis A, & Loguercio AD (2010) Composite pre-heating: effects on marginal adaptation, degree of conversion and mechanical properties *Dental Materials* **26(9)** 908-914.
135. Sadr A, Ghasemi A, Shimada Y, & Tagami J (2007) Effects of storage time and temperature on the properties of two self-etching systems *Journal of Dentistry* **35(3)** 218-225.
136. Ma S, Nakajima KF, & Nishiyama N (2009) Effects of storage temperature on the shelf life of one-step and two-step self-etch adhesives *Operative Dentistry* **34(4)** 472-480.
137. Reis A, Klein-Júnior CA, Accorinte Mde L, Grande RH, dos Santos CB, & Loguercio AD (2009) Effects of adhesive temperature on the early and 6-month dentin bonding *Journal of Dentistry* **37(10)** 791-798.
138. Shibuya-Chiba Y, Iwasa M, Tsubota K, Miyazaki M, Hirose H, & Platt JA (2010) Influence of storage conditions of adhesive vials on dentin bond strength *Operative Dentistry* **35(5)** 508-514.
139. Brannstrom M, Linden LA, & Astrom A (1967) The hydrodynamics of the dental tubule and of pulp fluid. A discussion of its significance in relation to dentinal sensitivity *Caries Research* **1(4)** 310-317.
140. Durey K, Santini A, & Miletic V (2008) Pulp chamber temperature rise during curing of resin-based composites with different light-curing units *Primary Dental Care* **15(1)** 33-38.
141. Santini A, Watterson C, & Miletic V (2008) Temperature rise within the pulp chamber during composite resin polymerisation using three different light sources *The Open Dentistry Journal* **2** 137-141.
142. de Souza PP, Hebling J, Scalon MG, Aranha AM, & Costa CA (2009) Effects of intrapulpal temperature change induced by visible light units on the metabolism of odontoblast-like cells *American Journal of Dentistry* **22**(3) 151-156.
143. Sadek FT, Pashley DH, Nishitani Y, Carrilho MR, Donnelly A, Ferrari M, & Tay FR (2008) Application of hydrophobic resin adhesives to acid-etched dentin with an alternative wet bonding technique *Journal of Biomedical Materials Research Part A* **84(1)** 19-29.
144. Tay FR, Pashley DH, Kapur RR, Carrilho MR, Hur YB, Garrett LV, & Tay KC (2007) Bonding BisGMA to dentin—A proof of concept for hydrophobic dentin bonding *Journal of Dental Research* **86(11)** 1034-1039.
145. Sadek FT, Braga RR, Muench A, Liu Y, Pashley DH, & Tay FR (2010) Ethanol wet-bonding challenges current anti-degradation strategy *Journal of Dental Research* **89(12)** 1499-1504.
146. Sadek FT, Castellan CS, Braga RR, Mai S, Tjäderhane L, Pashley DH, & Tay FR (2010) One-year stability of resin-dentin bonds created with a hydrophobic ethanol-wet bonding technique *Dental Materials* **26(4)** 380-386.
147. Hosaka K, Nishitani Y, Tagami J, Yoshiyama M, Brackett WW, Agee KA, Tay FR, & Pashley DH (2009) Durability of resin-dentin bonds to water- vs. ethanol-saturated dentin *Journal of Dental Research* **88(2)** 146-151.
148. Osorio E, Toledano M, Aguilera FS, Tay FR, & Osorio R (2010) Ethanol wet-bonding technique sensitivity assessed by AFM *Journal of Dental Research* **89(11)** 1264-1269.
149. Brackett WW, Tay FR, Brackett MG, Dib A, Sword RJ, & Pashley DH (2007) The effect of chlorhexidine on dentin hybrid layers in vivo *Operative Dentistry* **32(2)** 107-111.
150. Tezvergil-Mutluay A, Agee KA, Hoshika T, Carrilho M, Breschi L, Tjäderhane L, Nishitani Y, Carvalho RM, Looney S, Tay FR, & Pashley DH (2010) The requirement of zinc and calcium ions for functional MMP activity in demineralized dentin matrices *Dental Materials* **26(11)** 1059-1067.
151. Osorio R, Yamauti M, Osorio E, Ruiz-Requena ME, Pashley DH, Tay FR, & Toledano M (2011) Zinc reduces collagen degradation in demineralized human dentin explants *Journal of Dentistry* **39(2)** 148-153.
152. Golub LM, Ramamurthy NS, McNamara TF, Greenwald RA, & Rifkin BR (1991) Tetracyclines inhibit connective tissue breakdown: New therapeutic implications for an old family of drugs *Critical Reviews in Oral Biology and Medicine* **2(3)** 297-321.
153. Breschi L, Martin P, Mazzoni A, Nato F, Carrilho M, Tjäderhane L, Visintini E, Cadenaro M, Tay FR, De Stefano Dorigo E, & Pashley DH (2010) Use of a specific MMP-inhibitor (galardin) for preservation of hybrid layer *Dental Materials* **26(6)** 571-578.
154. Santos MC, de Souza AP, Gerlach RF, Trevilatto PC, Scarel-Caminaga RM, & Line SR (2004) Inhibition of human pulpal gelatinases (MMP-2 and MMP-9) by zinc oxide cements *Journal of Oral Rehabilitation* **31(7)** 660-664.
155. Perdigão J, Reis A, & Loguercio AD (2013) Dentin adhesion and MMPs: a comprehensive review *Journal of Esthetic & Restorative Dentistry*, Prepublished: Feb 19, 2013. doi: 10.1111/jerd.12016
156. Gendron R, Grenier D, Sorsa T, & Mayrand D (1999) Inhibition of the activities of matrix metalloproteinases 2, 8, and 9 by chlorhexidine *Clinical Diagnostic Laboratory Immunology* **6(3)** 437-439.
157. Carrilho MR, Tay FR, Donnelly AM, Agee KA, Tjäderhane L, Mazzoni A, Breschi L, Foulger S, & Pashley DH (2009) Host-derived loss of dentin matrix stiffness associated with solubilization of collagen *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **90(1)** 373-380.
158. de Souza LB, de Aquino SG, de Souza PP, Hebling J, & Costa CA (2007) Cytotoxic effects of different concentrations of chlorhexidine *American Journal of Dentistry* **20(6)** 400-404.

159. Lessa FC, Nogueira I, Huck C, Hebling J, & Costa CA (2010) Transdentinal cytotoxic effects of different concentrations of chlorhexidine gel applied on acid-conditioned dentin substrate *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **92(1)** 40-47.

160. Loguercio AD, Stanislawczuk R, Polli LG, Costa JA, Michel MD, & Reis A (2009) Influence of chlorhexidine digluconate concentration and application time on resin-dentin bond strength durability *European Journal of Oral Science* **117(5)** 587-596.

161. Breschi L, Mazzoni A, Nato F, Carrilho M, Visintini E, Tjäderhane L, Ruggeri A Jr., Tay FR, Dorigo Ede S, & Pashley DH (2010) Chlorhexidine stabilizes the adhesive interface: A 2-year in vitro study *Dental Materials* **26(4)** 320-325.

162. Carrilho MR, Carvalho RM, Sousa EN, Nicolau J, Breschi L, Mazzoni A, Tjäderhane L, Tay FR, Agee K, & Pashley DH (2010) Substantivity of chlorhexidine to human dentin *Dental Materials* **26(8)** 779-785.

163. Chang YC, Huang FM, Tai KW, & Chou MY (2001) The effect of sodium hypochlorite and chlorhexidine on cultured human periodontal ligament cells *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology and Endodontology* **92(4)** 446-450.

164. Stanislawczuk R, Amaral RC, Zander-Grande C, Gagler D, Reis A, & Loguercio AD (2009) Chlorhexidine-containing acid conditioner preserves the longevity of resin-dentin bonds *Operative Dentistry* **34(4)** 481-490.

165. Stanislawczuk R, Reis A, & Loguercio AD (2011) A 2-year in vitro evaluation of a chlorhexidine-containing acid on the durability of resin-dentin interfaces *Journal of Dentistry* **39(1)** 40-47.

166. Tezvergil-Mutluay A, Mutluay MM, Gu LS, Zhang K, Agee KA, Carvalho RM, Manso A, Carrilho M, Tay FR, Breschi L, Suh BI, & Pashley DH (2011) The anti-MMP activity of benzalkonium chloride *Journal of Dentistry* **39(1)** 57-64.

167. Scaffa PM, Vidal CM, Barros N, Gesteira TF, Carmona AK, Breschi L, Pashley DH, Tjäderhane L, Tersariol IL, Nascimento FD, & Carrilho MR (2012) Chlorhexidine inhibits the activity of dental cysteine cathepsins *Journal of Dental Research* **91(4)** 420-425.

168. Lehmann N, Debret R, Romeas A, Magloire H, Degrange M, Bleicher F, Sommer P, & Seux D (2009) Self-etching increases matrix metalloproteinase expression in the dentin-pulp complex *Journal of Dental Research* **88(1)** 77-82.

169. Soares CJ, Castro CG, Santos Filho PC, & da Mota AS (2007) Effect of previous treatments on bond strength of two self-etching adhesive systems to dental substrate *Journal of Adhesive Dentistry* **9(3)** 291-296.

170. Jacques P, & Hebling J (2005) Effect of dentin conditioners on the microtensile bond strength of a conventional and a self-etching primer adhesive system *Dental Materials* **21(2)** 103-109.

171. Campos EA, Correr GM, Leonardi DP, Barato-Filho F, Gonzaga CC, & Zielak JC (2009) Chlorhexidine diminishes the loss of bond strength over time under simulated pulpal pressure and thermo-mechanical stressing *Journal of Dentistry* **37(2)** 108-114.

172. Zhou J, Tan J, Chen L, Li D, & Tan Y (2009) The incorporation of chlorhexidine in a two-step self-etching adhesive preserves dentin bond in vitro *Journal of Dentistry* **37(10)** 807-812.

173. Al-Ammar A, Drummond JL, & Bedran-Russo AK (2009) The use of collagen cross-linking agents to enhance dentin bond strength *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **91(1)** 419-424.

174. Bedran-Russo AK, Pashley DH, Agee K, Drummond JL, & Miescke KJ (2008) Changes in stiffness of demineralized dentin following application of collagen crosslinkers *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **86(2)** 330-334.

175. Castellan CS, Pereira PN, Grande RH, & Bedran-Russo AK (2010) Mechanical characterization of proanthocyanidin-dentin matrix interaction *Dental Materials* **26(10)** 968-973.

176. Castellan CS, Pereira PN, Viana G, Chen SN, Pauli GF, & Bedran-Russo AK (2010) Solubility study of phytochemical cross-linking agents on dentin stiffness *Journal of Dentistry* **38(5)** 431-436.

177. Bedran-Russo AK, Yoo KJ, Ema KC, & Pashley DH (2009) Mechanical properties of tannic-acid-treated dentin matrix *Journal of Dental Research* **88(9)** 807-811.

178. Embery G, Hall R, Waddington R, Septier D, & Goldberg M (2001) Proteoglycans in dentinogenesis *Critical Reviews in Oral Biology & Medicine* **12(4)** 331-349.

179. Bedran-Russo AK, Pereira PN, Duarte WR, Drummond JL, & Yamauchi M (2007) Application of crosslinkers to dentin collagen enhances the ultimate tensile strength *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **80(1)** 268-272.

180. Han B, Jaurequi J, Tang BW, & Nimni ME (2003) Proanthocyanidin: A natural crosslinking reagent for stabilizing collagen matrices *Journal of Biomedical Materials Research Part A* **65(1)** 118-124.

181. Sung HW, Chang WH, Ma CY, & Lee MH (2003) Crosslinking of biological tissues using genipin and/or carbodiimide *Journal of Biomedical Materials Research Part A* **64(3)** 427-438.

182. Cheung DT, Tong D, Perelman N, Ertl D, & Nimni ME (1990) Mechanism of crosslinking of proteins by glutaraldehyde. IV: In vitro and in vivo stability of a crosslinked collagen matrix *Connective Tissue Research* **25(1)** 27-34.

183. de Pascual-Teresa S, Santos-Buelga C, & Rivas-Gonzalo JC (2000) Quantitative analysis of flavan-3-ols in Spanish foodstuffs and beverages *Journal of Agricultural and Food Chemistry* **48(11)** 5331-5337.

184. Bedran-Russo AK, Vidal CM, Dos Santos PH, & Castellan CS (2010) Long-term effect of carbodiimide on dentin matrix and resin-dentin bonds *Journal of Biomedical Materials Research Part B: Applied Biomaterials* **94(1)** 250-255.

185. Pashley DH, Tay FR, Breschi L, Tjäderhane L, Carvalho RM, Carrilho M, & Tezvergil-Mutluay A (2011) State of

the art etch-and-rinse adhesives *Dental Materials* **27(1)** 1-16.

186. Tezvergil-Mutluay A, Mutluay MM, Agee KA, Seseogullari-Dirihan R, Hoshika T, Cadenaro M, Breschi L, Vallittu P, Tay FR, & Pashley DH (2012) Carbodiimide cross-linking inactivates soluble and matrix-bound MMPs, in vitro *Journal of Dental Research* **91(2)** 192-196.
187. SM, De Andrade e Silva Malacarne-Zanon J, Carvalho RM, Alves MC, De Goes MF, Anido-Anido A, & Carrilho MR (2010) Effect of oxalate desensitizer on the durability of resin-bonded interfaces *Operative Dentistry* **35(6)** 610-617.
188. Oberg C, Pochapski MT, Farago PV, Granado CJ, Pilatti GL, & Santos FA (2009) Evaluation of desensitizing agents on dentin permeability and dentinal tubule occlusion: An in vitro study *General Dentistry* **57**(5) 496-501; quiz 502-493, 535-496.
189. Sadek FT, Pashley DH, Ferrari M, & Tay FR (2007) Tubular occlusion optimizes bonding of hydrophobic resins to dentin *Journal of Dental Research* **86(6)** 524-528.
190. Sauro S, Watson TF, Mannocci F, Tay FR, & Pashley DH (2009) Prevention of water contamination of ethanol-saturated dentin and hydrophobic hybrid layers *Journal of Adhesive Dentistry* **11(4)** 271-278.
191. Yiu CK, Hiraishi N, Chersoni S, Breschi L, Ferrari M, Prati C, King NN, Pashley DH, & Tay FR (2006) Single-bottle adhesives behave as permeable membranes after polymerisation. II. Differential permeability reduction with an oxalate desensitiser *Journal of Dentistry* **34**(2) 106-116.
192. Garcia EJ, Reis A, Arana-Correa BE, Sepulveda-Navarro WF, Higashi C, Gomes JC, & Loguercio AD (2010) Reducing the incompatibility between two-step adhesives and resin composite luting cements *Journal of Adhesive Dentistry* **12(5)** 373-379.
193. Vachiramon V, Vargas MA, Pashley DH, Tay FR, Geraldeli S, Qian F, & Armstrong SR (2008) Effects of oxalate on dentin bond after 3-month simulated pulpal pressure *Journal of Dentistry* **36(3)** 178-185.
194. Breschi L, Mazzoni A, Pashley DH, Pasquantonio G, Ruggeri A, Suppa P, Mazzotti G, Di Lenarda R, & Tay FR (2006) Electric-current-assisted application of self-etch adhesives to dentin *Journal of Dental Research* **85(12)** 1092-1096.
195. Mazzoni A, Visintini E, Vita F, Pasquantonio G, Saboia VP, Ruggeri A Jr., Di Lenarda R, Dorigo E, & Breschi L (2009) ElectroBond improves immediate dentin microtensile bond strength of two etch-and-rinse adhesives *Journal of Adhesive Dentistry* **11(1)** 27-33.
196. Pasquantonio G, Tay FR, Mazzoni A, Suppa P, Ruggeri A Jr., Falconi M, Di Lenarda R, & Breschi L (2007) Electric device improves bonds of simplified etch-and-rinse adhesives *Dental Materials* **23(4)** 513-518.
197. Visintini E, Mazzoni A, Vita F, Pasquantonio G, Cadenaro M, Di Lenarda R, & Breschi L (2008) Effects of thermocycling and use of ElectroBond on microtensile strength and nanoleakage using commercial one-step self-etch adhesives *European Journal of Oral Science* **116(6)** 564-570.
198. Erhardt MC, Osorio R, Viseras C, & Toledano M (2011) Adjunctive use of an anti-oxidant agent to improve resistance of hybrid layers to degradation *Journal of Dentistry* **39(1)** 80-87.
199. Ye Q, Park J, Topp E, & Spencer P (2009) Effect of photoinitiators on the in vitro performance of a dentin adhesive exposed to simulated oral environment *Dental Materials* **25(4)** 452-458.
200. Cadenaro M, Antoniolli F, Codan B, Agee K, Tay FR, Dorigo Ede S, Pashley DH, & Breschi L (2010) Influence of different initiators on the degree of conversion of experimental adhesive blends in relation to their hydrophilicity and solvent content *Dental Materials* **26(4)** 288-294.
201. Tay FR, & Pashley DH (2008) Guided tissue remineralisation of partially demineralised human dentine *Biomaterials* **29(8)** 1127-1137.
202. Tay FR, & Pashley DH (2009) Biomimetic remineralization of resin-bonded acid-etched dentin *Journal of Dental Research* **88(8)** 719-724.
203. Kim J, Vaughn RM, Gu L, Rockman RA, Arola DD, Schafer TE, Choi KK, Pashley DH, & Tay FR (2010) Imperfect hybrid layers created by an aggressive one-step self-etch adhesive in primary dentin are amendable to biomimetic remineralization in vitro *Journal of Biomedical Materials Research Part A* **93(4)** 1225-1234.
204. Kim YK, Mai S, Mazzoni A, Liu Y, Tezvergil-Mutluay A, Takahashi K, Zhang K, Pashley DH, & Tay FR (2010) Biomimetic remineralization as a progressive dehydration mechanism of collagen matrices—implications in the aging of resin-dentin bonds *Acta Biomaterialia* **6(9)** 3729-3739.
205. Sauro S, Osorio R, Watson TF, & Toledano M (2012) Therapeutic effects of novel resin bonding systems containing bioactive glasses on mineral-depleted areas within the bonded-dentine interface *Journal of Materials Science: Materials in Medicine* **23(6)** 1521-1532.