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Durability of bonds and clinical success of adhesive restorations

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Abstract

Resin-dentin bond strength durability testing has been extensively used to evaluate the effectiveness of adhesive systems and the applicability of new strategies to improve that property. Clinical effectiveness is determined by the survival rates of restorations placed in non-carious cervical lesions (NCCL). While there is evidence that the bond strength data generated in laboratory studies somehow correlates with the clinical outcome of NCCL restorations, it is questionable whether the knowledge of bonding mechanisms obtained from laboratory testing can be used to justify clinical performance of resin-dentin bonds. There are significant morphological and structural differences between the bonding substrate used in *in vitro* testing versus the substrate encountered in NCCL. These differences qualify NCCL as a hostile substrate for bonding, yielding bond strengths that are usually lower than those obtained in normal dentin. However, clinical survival time of NCCL restorations often surpass the durability of normal dentin tested in the laboratory. Likewise, clinical reports on the long-term survival rates of posterior composite restorations defy the relatively rapid rate of degradation of adhesive interfaces reported in laboratory studies. This article critically analyzes how the effectiveness of adhesive systems is currently measured, to identify gaps in knowledge where new research could be encouraged. The morphological and chemical analysis of bonded interfaces of resin composite restorations in teeth that had been in clinical service for many years, but were extracted for periodontal reasons, could be a useful tool to observe the ultrastructural characteristics of restorations that are regarded as clinically acceptable. This could help determine how much degradation is acceptable for clinical success.

Keywords

Dentin; Adhesives; Durability; Clinical Outcome

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Introduction

Attempts to determine the effectiveness of adhesive systems must include durability testing. The pioneer work of Buonocore evaluated the quality of adhesion by determining the “survival” time of bonds of acrylic resin made to enamel and dentin [1, 2]. As stated by Buonocore (1955), *“At this time we feel that because evidence of this nature has not been previously reported, the reasons for the increased adhesion are less important than the finding that the adhesive bond attained on treated (ie. acid-treated) as compared to untreated (ie. control), surfaces survived oral conditions for relatively long periods of time”*, it became clear that the value of the newly developed technique was because the improved adhesion was more durable than previous adhesion techniques.

The issue of bond durability has dominated most current research in both resin-enamel and resin-dentin bonding. Because bonds made to enamel are regarded as reliable and durable [3], most of the attention has been devoted to understand why bonding to dentin does not match the durability of its neighboring hard tissue. Several reasons have been given to explain why bonding to dentin is still a challenge, despite of the improvements in dental adhesive technology and advances in bonding knowledge. These include the heterogeneity of the structure and composition of dentin, the dentin surface characteristics after bur cutting and chemical treatments; and bond strategy and physicochemical properties of the adhesives, among other variables [3-8]. Most of the current knowledge of bonded interfaces originated from laboratory studies. The question as to whether these laboratory outcomes are somehow related or can be predictive of clinical performance remains dubious. Except for a few weak relationships [7], most of the attempts to correlate laboratory and clinical data are inconclusive [7, 9].

While it is widely recognized that the characteristics of the bonding substrate plays a major role on the quality of adhesion [5], and that clinically-relevant substrates include caries-affected, caries-infected, sclerotic, deep, and bur cut dentin, major new insights in bonding mechanisms are often generated from laboratory studies using sound, freshly cut and sand-paper abraded dentin as the testing substrate. Clinical effectiveness of adhesives is assessed from the performance of restorations placed in Class V, non-carious cervical lesions [10]. This approach provides direct evidence of the ability of the adhesive to effectively bond, because the restorations fail by loss of retention. However, the type of sclerotic substrate encountered in such lesions is rather unique [11, 12] and may not reflect how adhesives bond to other clinically available surfaces for bonding. Class II composite restorations fail frequently because of marginal leakage that leads to secondary caries [13, 14]. The breakdown of interfacial sealing poses a challenge to the longevity of restoration [15, 16]. If longevity of these restorations are mainly affected by leakage of oral fluids and bacteria along the interface [7], studies on this phenomenon should be more clinically relevant to better predict the clinical performance of adhesive restorations [7, 17, 18]. Instead, bond strength data are generally used for such predictive analysis, even though no correlation seems to exist between bond strength and marginal leakage [19]. All this may account for the difficulties in establishing a reliable and predictive relationship between durability of bonds measured in the laboratory and clinical success of adhesive restorations. Several clinically possible adjunctive procedures have been suggested to improve short-, and perhaps long-term adhesion to dentin. These include ethanol wet-bonding [20, 21], extended adhesive application time [22-24] use of warm air to accelerate solvent evaporation [24], use of protease enzyme inhibitors [25-29], use of collagen cross-linkers [30-32], and rubbing action during the adhesive application [33, 34]. While these strategies have been proved quite effective under laboratory and short-term *in vivo* conditions [26, 31, 35-38], only a few have been translated to a controlled clinical testing [39-41].

While durability testing in the laboratory has consistently demonstrated bond degradation within a relatively short period of time [42], clinical data indicate that resin-dentin bonds last much longer [7, 43-45]. This suggests that the mechanisms involved in the degradation of bonds observed in laboratory may not apply at the same rate clinically, or the effects of the degradation of the bonds have a secondary role in the clinical success of restorations.

This article will not provide an exhaustive review the topic on durability of bonds and the respective clinical outcome. Several excellent review articles have been published within the last 2 years that cover the current knowledge on that topic in detail [3, 5, 7]. Rather, this review intends to critically analyze some of the approaches used to evaluate the effectiveness of adhesive systems and, perhaps, stimulate new approaches to this topic.

The Class V Non-Carious Cervical Lesions (NCCL). A clinical effectiveness paradigm

Clinical effectiveness of adhesive systems is ideally conducted in Class V non-carious cervical lesions (NCCL) as recommended by the ADA [46]. Such lesions are preferred because [10]: (a) cervical lesions do not provide any macromechanical retention, therefore ineffective bonding will result in loss of the restoration; (b) the restoration contains both enamel and dentin margins; (c) they are usually located on the buccal aspect of anterior and premolar teeth, thus offering good access for operative procedures and subsequent evaluation by direct visualization or replication; (d) restorative procedures are relatively easy and minimal, thus reducing the operator variability; (e) lesions are widely available and are seen in multiple teeth, thus facilitating patient selection and study design; and (f) the mechanical properties of the composite resin is less important to the outcome than the actual performance of the adhesive. [7].

The development of non-carious cervical lesions involves multifactorial etiologies. They usually form due to a combination of erosion, abrasion and abfraction [47-49]. They are characterized by the presence of sclerotic dentin that has been physiologically and pathologically altered, resulting in partial or complete obliteration of the dentinal tubules by the presence of sclerotic casts. Patency of tubules is found in sensitive areas, which are usually sparsely distributed among occluded tubules [50]. The ultrastructural analysis of NCCL revealed specific features that make this a unique bonding substrate, probably not found anywhere else in the mouth [11, 51, 52]. These lesions present a complex structure with high variability of tubule occlusion. The surface is characterized by the presence of a hypermineralized layer of varied thickness (Fig. 1), which is invariably associated with the presence of bacteria (Fig. 2a,b). Denatured collagen fibrils have been found underneath the hypermineralized layer, probably serving as a scaffold for mineral deposition. Apparently sound, cross-banded collagen could only be observed at the base of the hypermineralized layer where it transitions to underlying sclerotic dentin. The thickness and composition of the structure also varies depending on the location in a NCCL (Fig. 1). Thicker and more bacterially-contaminated layers are found in the deepest regions of the lesion. Along the occlusal and gingival walls, the hypermineralized layers are thinner, and the gingival wall may be devoid of bacteria. The dynamic model by which these lesions are formed and develop results in a unique multilayer structure that is in constant change both locally and over time [11]. Because of such characteristics, bond strengths to naturally formed NCCL have systematically being reported as being 20%-50% lower than bonds made to sound dentin. This reduced bonding efficacy is a result of the obstacles present in NCCL that prevent the “optimal” interaction of adhesive resins with this substrate [11, 51-53], at least in a manner similar to what has been demonstrated with sound dentin. As thoroughly demonstrated by Tay & Pashley [11], the hybrid layer morphology after self-etching or wet bonding with etch-and-rinse adhesives in natural, intact sclerotic dentin is substantially

different from that observed in sound, abraded dentin created with the same C-factor (Fig. 1). It is remarkable, however, that this reduced bonding efficacy is capable of retaining NCCL restorations in clinical service for periods much longer [3, 7, 10] than laboratory studies take to demonstrate significant degradation of bonds made to sound dentin [4, 54, 55].

Regarding effectiveness, the current knowledge about the resin-enamel and resin-dentin bonding mechanisms was mostly generated from laboratory studies generally using, sound enamel and dentin from extracted human third molars [5, 7]. In contrast, the current knowledge about how adhesive systems perform clinically was originated from clinical trials using Non Carious Cervical Lesions (NCCL) [3, 7]. As seen above, NCCLs contain a unique dentin bonding substrate that differs from that of mid-coronal third molars. It is widely accepted that the chemical and structural characteristics of bonding substrates highly influence the bonding mechanisms and the consequent bond strength outcome of adhesive systems [5]. Without challenging this premise, one could not readily apply the knowledge in bonding mechanisms originated from mid-crown third molar dentin to justify the clinical outcome in NCCL. Only a few studies are available that have explored the mechanisms through which adhesives interact with the unique NCCL substrate [11, 51-53, 56, 57]. These studies were conducted approximately 7 - 15 years ago. It is unfortunate that more recent studies are not available using current adhesive systems. It would be desirable, for instance, to know whether the A-D bonding concept [8] also applies to the dentin substrate encountered in NCCL. This concept explains how and why chemical bonding with hydroxyapatite through functional monomers, such as 10-MDP and 4-MET, of mild self-etch adhesives and the polyalkenoic acid of glass-ionomer cements enhances durability of bonds made with these adhesives to sound dentin. Based on the survival rates of restorations placed on NCCL using Clearfil SE Bond (Kuraray Inc. Tokyo, Japan) [8, 56, 58], a mild-self etch adhesive that contains 10-MDP as the functional monomer, it is plausible to assume that chemical bonding not only occurs, but it is possibly improved in NCCL due to its hypermineralized surface characteristics. The 8-years clinical survival of NCCL restorations placed with Clearfil SE Bond [58] largely surpasses the durability of bonds made with this adhesive to sound dentin as reported from laboratory studies [3, 8]. Glass-ionomer cements that bond to both enamel and dentin largely by chemical interaction of functional polymers with hydroxyapatite, are known to outperform resin adhesives in clinical trials using Class V NCCL [7, 8, 45, 59, 60]. Recent long-term clinical trials indicate that bonds made to NCCL can provide up to 13 years in service [44, 45, 58, 59]. Unfortunately, no long-term laboratory studies on the interfacial morphology and bond strength of adhesive resins to NCCL are available. Such studies could help focus on the actual mechanisms supporting the clinical success of these restorations. If laboratory studies can reproduce the clinical stability of the bonds and the actual bond strength can be measured over time, then one can have a better estimate of how much bond strength is necessary to retain a Class V NCCL restoration.

The degradation of adhesive interfaces due the action of endogenous dentin proteolytic enzymes acting on exposed collagen fibrils is recognized as a mechanism that significantly compromises the durability of bond strengths and the integrity of hybrid layers made to dentin [54, 61, 62]. This knowledge has also been mostly generated from coronal, sound dentin from molar and premolar teeth. To the best of the authors' knowledge, there are no studies available that had investigated the presence, activity or the role of endogenous dentin proteases on resin-dentin bonds made to NCCLs. Enzymatic degradation of bonded interfaces are mainly observed with etch-and-rinse adhesives [61-63] because they have thicker demineralized zones that pose a higher risk of forming incompletely resin-infiltrated hybrid layers, thus leaving collagen fibrils exposed. Hybrid layer formation of about 5 μ m (Fig. 1) was observed along the occlusal and gingival walls of phosphoric acid-etched

sclerotic NCCL, whose morphology was similar to that observed in acid-etched sound dentin [11]. Conversely, hybrid layer morphology on the deepest parts of NCCL was erratic in appearance and eccentric in shape that did not resemble that seen in sound dentin (Fig. 1). When the hypermineralized layer is present in NCCL, it usually covers a bed of denature collagen fibrils [11]. If the hypermineralized layer is so thick that acid-etching does not dissolve it [11], then the collagen fibrils will remain within the bonded interface. This hypermineralized layer may keep the matrix proteases covered with mineral crystallites and inactive. Although hybrid layer morphology, as we are accustomed to see in sound dentin, may be observed in NCCL bonded interfaces, no information is available as to how well-infiltrated are these hybrid layers. If we assume that hybridization in NCCL suffers from the same obstacles for resin infiltration as described for sound dentin [6, 64], the longer durability of NCCL-resin bonds may be due to the fact that the matrix proteases remain mineralized and inactive. Indeed, support for this speculation can be found in a recent study by Kim et al. (2010) [65]. In that study, resin-dentin bonds were created on mid-coronal extracted third molars using the etch-and-rinse, One-Step (Bisco) or Single Bond (3M-ESPE). The bonded teeth were reduced to $0.9 \times 0.9 \times 6$ mm sticks and incubated in 37°C water for accelerated aging. A second group of bonded sticks was incubated in a biomimetic remineralizing solution. Specimens were removed from both groups at regular intervals for up to 1 year to permit measurement of microtensile bond strength and transmission electron microscopy of the resin-dentin bonded interface. In the control groups, the resin-dentin bond strengths fell from 37-40 MPa at baseline to 21-23 MPa after 1 yr. In the remineralizing group, the bond strength at time zero were 39-42 MPa and did not fall below 38-39 MPa, a nonsignificant decrease over 1 year and *in vitro* incubation. TEMs showed that the control hybrid layers degraded, while the remineralized hybrid layers did not [65]. Thus, remineralization of resin-spare water-rich hybrid layers prevented endogenous protease-induced degradation. Enzymatic degradation of collagen at NCCL bonded interfaces, is rarely considered as a cause of failures in clinical trials. Rather, clinical failures are generally attributed to the hydrolysis of the adhesive [3, 7, 44, 58].

Clearly, more basic laboratory studies are needed to further explore the bonding mechanisms to NCCL. There is much to learn from the unmatched durability of these bonds that could translate to improved effectiveness of adhesive systems.

Effectiveness of Adhesives in Supporting Longevity of Posterior Composite Restorations

The quality of resin composite restorations in posterior teeth is usually evaluated by a system of clinical parameters referred to as Modified USPHS Criteria or USPHS/CDA Criteria [66, 67]. Retention of posterior composite restorations is not exclusively determined by the ability of adhesives to bond to the cavity walls. Except in cases where minimal intervention is required and the resultant preparation is shallow with divergent walls, retention in composite resin restorations is largely determined by the self-retentive cavity configuration and friction to opposing cavity walls. In contrast to restorations of NCCL, one would not expect Class I or Class II resin-composite restorations to fall off their restored surfaces, even in total absence of adhesion. Because of that, the quality of adhesion in posterior resin-composite restorations is indirectly evaluated by parameters such as marginal integrity (presence of ditching and/or gaps), marginal staining or discoloration, and ultimately, the presence of caries contiguous with the margin of the restoration, usually referred as secondary caries [66-68]. Advanced ditching and staining and the presence of secondary caries are determinants of clinical failure and replacement of the restoration [67, 69]. Secondary caries has consistently been identified as one of the major deterrents of longevity in posterior resin composite restorations [70-72]. Secondary caries has therefore

been regarded as a clinical failure resulting from leakage of oral fluids along the interface between the restorative material and dental hard tissues [72-75]. Leakage, in turn, is a result of the inability of the adhesive to seal the interface. *In vitro* studies have shown a clear relationship between marginal defects and microleakage with secondary caries [74, 76, 77]. *In vivo* studies, however, have failed to demonstrate that leakage or marginal gaps smaller than 250 to 400 microns are determinants of demineralization beneath restorations [78, 79]. The relationship between marginal gap size and geometry and development of secondary caries have been extensively investigated in cariology using microcosm biofilm models [77, 80-82]. Such models could be used in resin-dentin bonds studies to investigate how biofilm affects the durability of bonds [83, 84].

Although it has been shown that there is no correlation or agreement between marginal leakage and bond strength testing [19], the latter has been systematically employed as the preferred method to evaluate bond effectiveness of adhesive systems and infer associations with clinical performance of composite restorations [3, 7]. It is noteworthy, however, that associations between bond strength data and clinical performance of resin composite restorations, established valid parameters to qualify the effectiveness of several available marketed adhesive systems [7]. For instance, consistently lower bond strengths of single-step self-etch adhesives, was associated with poorer clinical performance in NCCL. Conversely, superior performance of the so-called gold standard adhesive systems (ca. three-step etch & rinse Optibond FL, from Kerr Inc; and the two-step self-etch Clearfil Bond SE, from Kuraray Inc.) was found in both laboratory bond strength and clinical trials [3, 7].

In contrast to NCCL (see above), *in vitro* bond strength data produced in extracted normal molar and premolar teeth encounter more similarities with the substrate available in posterior resin composite restorations. Laboratory studies aiming to evaluate effectiveness of adhesives through durability tests have consistently demonstrated significant reductions in bond strength within relatively short periods of time (ca. 6-12 m) after immersion in water or artificial saliva [42]. Because of that, it has been inferred that such degradation of the bonds may lead to premature clinical failures of adhesive restorations. This causal relationship, however, does not obtain support from the clinical literature available on the performance of resin composite restorations in posterior teeth. If resin-dentin bond strengths decrease irreversibly over time, then one would expect that secondary caries would be responsible for an increasing rate of clinical failures. This, however, has not been the case in clinical trials with the longest evaluation periods [43, 72, 85]. Secondary caries is indeed one of the major causes of restoration replacement, but it is largely associated with patients considered at high risk for caries [72, 85]. This implies that the clinical effectiveness of the adhesive system does not exclusively depend on its ability to maintain reliable bond strengths over time. The fact that adhesives with high bond strength in laboratory studies also presented superior clinical performance in NCCL has created the equivocal concept that high and durable bond strengths are required for long-lasting restorations. Adhesives with high and stable bond strengths over time are lacking. Apart from a recent study that has demonstrate stable bonds for two adhesives over a period of 10 years of water storage [86], others have only been able to demonstrated stable bonds for up to 2 years when strategies for increasing the durability of the bonds are used [42]. Even the so-called gold standard adhesives have shown inconsistent results in different studies, including some from the same laboratory [55, 63, 87]. Adhesives with initial low and/or unstable bond strengths over time may be considered ineffective under the parameters of a laboratory study. However, this is not direct evidence that such adhesive will perform poorly clinically. Clinical trials with the longest evaluation period have shown excellent results with adhesives that were available at the time the study commenced, which means 12-22 years ago. For instance, the recently published 22-year clinical evaluation of posterior resin composite restorations used Scotchbond 2 (3M ESPE) and XR Bond (Kerr) to bond P-50 (3M ESPE) and Herculite XR

(Kerr), respectively. The reported shear or tensile bond strengths to dentin for these adhesives were in the range of 4 to 15 MPa [88-90], which despite of limitations in direct comparison, are rather lower values than one could expect from current systems. Another 12-year clinical trial reported superior performance of resin composites over amalgam restorations in low-risk caries patients [85]. The adhesive system used in 93% of the composite restorations in that study was PhotoBond/SA primer, a three-step etch&rinse system (Kuraray Inc., Tokyo, Japan). All these adhesives are no longer on the market and have been replaced by improved versions. Although there are no direct comparisons of their laboratory bond strength with more recent and current systems, it is probable that they would give lower bond strengths than contemporary adhesives.

It is clear that laboratory and clinical effectiveness of adhesive systems are judged by different criteria. While an effective adhesive system as measured by *in vitro* bond strength testing is more likely to offer improved performance in clinical service, it is striking that deceptive laboratory results may still provide effective clinical performance in well-motivated, low risk patients [43, 85]. The combination of strategies to improve bond durability of adhesive systems [42] with improved oral health care and patient motivation is the key for success of adhesive restorations (Fig 3).

The Enigma of the Protective Enamel Rim

It has been widely accepted that resin-enamel bonds are reliable and durable [3, 91-93]. Indeed, restorations whose clinical success relies mostly on enamel bonds (eg. pit-and-fissure sealants, laminate veneers) may be found in excellent clinical condition after many years of service (Fig. 4). Because of this, there is a common belief that the presence of enamel at the margins of a cavity offers the opportunity for a perfect sealing against the ingress of oral fluids and bacteria, thus protecting the more vulnerable bonds to adjacent dentin [55, 94-96]. In other words, when cavity margins are bonded to enamel, bonds made to dentin are more durable. Indeed, this has been demonstrated *in vitro* when bonds are made to flat dentin surfaces further prepared with fine grit sand paper [55, 94]. In those studies, human third molars were transversally sectioned to expose flat dentin surfaces, all surrounded by enamel. The entire surface was bonded with different adhesive systems using different bonding strategies. Bonded teeth were then either stored as whole teeth or after being longitudinally sectioned in two halves to expose bonded interface with dentin along the crown diameter. In that way, one group had only the enamel bond exposed to the water storage medium and the other had one surface of the dentin bond exposed to the medium. One study also used oil to replace water as an experimental storage medium for the teeth with exposed resin-dentin interfaces [94]. Bonded specimens were stored for 1 year [94] or up to 4 years [55] before being sectioned into beams and tested in microtensile bond strength. Control specimens were tested 24 hours after being bonded. General results of both *in vitro* studies indicated that the presence of resin-enamel bond offers protection to the adjacent resin-dentin bonds. Conversely, when resin-dentin bonded interfaces were exposed to water during storage, significant reductions in bond strength were observed for most of the adhesives tested. General explanations for the results focused on the effects of the water sorption from the media on the mechanical properties of the adhesives, which are known to cause reductions in of interfacial strengths [6, 97]. Presumably, without the protective peripheral seal of enamel bond, water more easily reached resin-dentin interfaces and promoted degradation of the structure of the adhesives, thus resulting in reduced bond strengths with time. Not surprisingly, bonds made with the more hydrophobic adhesives, i.e., the three-step etch & rinse systems that use solvent-free adhesives, were more resistant to degradation even when dentin interfaces were exposed [55]. The fact that the bond strength of specimens with resin-dentin interfaces exposed were either maintained or increased after storage in oil [94] reinforces the concept that the effects of water on the

adhesives was the leading cause of bonds degradation over time. Indeed, methacrylate-based adhesives are susceptible to the attack by chemicals and enzymes present in oral fluids and are biodegradable [98-104]. However, both Armstrong et al. (2006) [105] and Toledano et al. (2007) [106] incubated resin-dentin sticks in *Clostridium* collagenase and cholesterol esterase or in bacterial collagenase, respectively for 12 week or 1 year, to determine if enzymes could lower resin-dentin bond strengths. While Armstrong et al. (2006) found a slight decrease in bond strength after 12 weeks incubation in cholesterol esterase, collagenase had no more effect than did storage in artificial saliva. Toledano et al. (2007) also found no effect of collagenase. They concluded that these exogenous collagenase enzymes were too large to diffuse into the bonded interface. However, these enzymes are known to attack the surface of resin-composites [99, 102].

For an elegant description of events that leads to the degradation of adhesives, please refer to Spencer et al. (2010) [6]. These results were interpreted as indicating that resin composite restorations with enamel margins are more likely to survive, because the more reliable and durable surrounding enamel bond protects the bond to dentin. Unfortunately, the presence of an enamel bonded rim did not seem to produce the same protection when bonds were made in Class I cavities cut in extracted teeth with diamond burs [87]. In a high C-factor cavity configuration, with high polymerization contraction stresses, pooling of the adhesive resulting in thicker layers that compromise solvent evaporation, and the presence of a thick smear layer due to bur cutting, all seem to summate to reduce the durability of the bonds to dentin, regardless of the presence of a resin-enamel sealed margins [87, 107-110]. The concept that a bonded enamel margin increases the durability of bonds to dentin assumes that most, if not all factors that cause bond degradation are external and have to break the marginal seal and diffuse along the resin dentin interface to affect the bond. In light of current knowledge, this assumption does not apply. The activity of host-derived enzymes (ca. MMPs and cysteine-cathepsins) in the degradation of exposed collagen fibrils due to incomplete hybridization has been extensively demonstrated to occur in resin-dentin bonds, even when there is a so-called protective bonded enamel rim [25-27, 111, 112]. A common feature in these studies is the experimental design. All employed a Class I cavity prepared in occlusal surfaces using burs to expose middle to deep dentin, on which bonding was performed. Because of this experimental design, all preparations included a sound acid-etched enamel cavosurface angle at the margins of the restorations. By assuming that marginal enamel bonding offered adequate sealing against ingress of external fluids, the results suggested that the observed degradation of hybrid layers and consequent loss of adhesion strength were more due to elements confined in dentin rather than those originated from oral fluids or storage media. In other words, it suggests that the action of endogenous dentin proteases attacking unprotected collagen played a major role on the degradation of the bonds made to dentin. Moreover, significant decreases in bond strength and extensive degradation of collagen fibrils in those studies could be observed within relatively short periods of time (ca. 6 to 14 months).

The apparent controversy regarding the protective effect of enamel bond adjacent to dentin bond deserves further analysis. Dentin proteases are activated by the bonding procedures (ca. demineralization) [54, 113] and will degrade collagen whenever it is left exposed, unprotected by the infiltrating adhesive resins. Long-term durability of bond strength to dentin appears attainable when there is an adequate rim of enamel bond, the surface is sound, prepared in vitro, flat and by fine grit sand paper (ca. 600 grit SiC or finer), and a relatively hydrophobic adhesive (ca. with an additional coat of hydrophobic resin) [114-119] is used and properly applied to ensure optimal infiltration, and the storage media is water, sometimes containing antibacterial agents [55]. When this scenario is present, even a simplified, relatively more hydrophilic adhesive may survive, mostly because of the protective effect of bonded enamel against the diffusion of water across the bonded interface

[55]. In the ideal scenario above, demineralized collagen fibrils are less likely to be left exposed, thus diminishing the overall effects of dentin proteases-induced degradation on the stability of the interface. A flat surface eliminates challenging stresses of curing contraction, allows for a uniform adhesive layer and improved solvent evaporation, and water storage may underestimate enzymatic activity [120]. Additionally, the enamel rim is always sound and because it is also prepared by fine grit, wet sand paper, surface damages and cracks that could facilitate the ingress of fluids are less likely to occur [87]. Conversely, this ideal is far from being attainable in most clinical situations. As described above, in cavities prepared by burs the adhesive effectiveness will be challenged by contraction stresses and will have to deal with thicker smear layers. Although this may not be a problem for etch-and-rinse adhesives, it has been demonstrated that the collagen of the smear layer is not removed by acid etching [121, 122]. Instead, this disorganized collagen is denatured by shear stresses associated with bur cutting and forms a gelatinous coat that compromises adhesive infiltration. The pooling of the adhesive in internal line angles results in thicker layers, from which solvent evaporation is more difficult. Excess residual solvent compromises adhesive polymerization and makes them more susceptible to water sorption and its negative consequences on mechanical properties [6]. These unfavorable influences summate even more when dealing with bur-created cavities in vital teeth. Variations in dentin wetness [123] affect sensitivity of adhesives to dentin depth [5]. As cavity preparations are made deeper in dentin, external fluids have a longer path to diffuse along the interface, but more fluid from shorter dentinal tubules can reach the adhesive interface [124]. The presence of a vital pulp offers the opportunity for pulpal MMPs to diffuse to the adhesive interface via dentinal fluid and perhaps enhance local collagenolytic and gelatinolytic activity [125], thus leading to a faster degradation of the unprotected collagen fibrils. These events probably explain why bonds made in vivo in Class I cavities presented a rapid rate of degradation as measured by reduced bond strength and disappearance of the hybrid layers regardless the presence of bonded enamel margins [26, 27, 111].

In a study designed to evaluate the effect of gap geometry on secondary caries wall lesion development, Nassar and González-Cabezas (2011) [80] mounted prepared tooth specimens in a custom-made gap stage that created 4 different sized gaps in 4 experimental groups: a 30-micron gap throughout both enamel and dentin (group 1); a 30-micron enamel gap and 530-micron dentinal gap (group 2); a 525-micron gap in both enamel and dentin (group 3); or a 525-micron enamel gap and 1,025-micron gap in dentin (group 4). Secondary caries was induced by a cycling microbial caries model and the outcomes of enamel and dentin wall-lesions evaluated by confocal microscopy. They concluded that the presence of additional space at the dentinal cavity wall area did not affect the development of secondary caries as long as the enamel gap was small (group 2). However, when the enamel gap increased to approximately 500 microns, the presence of additional space at the dentinal wall (group 4) resulted in the development of dentinal wall lesions in the deeper parts of the cavity model. When gaps were uniform along the enamel and dentinal interfaces (groups 1 and 3), the size of the dentinal walls lesions was positively correlated with the size of the gaps. Unfortunately, studies on simulation of a secondary caries model contribute little to discussions on how the presence of a bonded interface would affect the outcome of secondary caries progression [77, 80, 82]. Rather, they employ gaps that were purposely created in different sizes and geometry, but the exposed enamel and dentin walls along the gaps had not been previously bonded with an adhesive system. Considering that marginal gaps are likely to occur in bonded interfaces due to polymerization contraction stresses [126], and increase in extension and size due to functional stresses [127], it would be desirable to combine simulated secondary caries models with simulated functional stresses to further investigate how enamel and dentin bonds affect the overall progression of interfacial lesions.

It seems that the presence of enamel-bonded margins of restorations cannot *per se* protect the long-term integrity of adjacent resin-dentin bonds. It is important, however, to bear in mind that the degradation of bonds observed in the *in vivo* studies discussed above, were associated with clinically acceptable restorations, with no signs of marginal failure or post-operative sensitivity. When restoring a cavity with enamel margins, clinicians should expect a more favorable outcome and predict improved durability of the treatment. The existence of a peripheral resin-enamel seal seems to retard the ingress of external fluids and oral bacteria that would certainly accelerate the rate of degradation of the resin-dentin interface. The most favorable prognosis of a restoration with enamel margins could also be viewed from the perspective that cavities with enamel margins are indicative of their smaller size and, therefore, tend to present a higher survival rate [43]. Additionally, smaller size cavities indicate that the patient sought treatment at an early stage of caries development, which is suggestive that the patient is concerned about his/her oral health, and probably better motivated for oral hygiene habits. All this works in concert to protect adhesive bonds.

Assessment of *In Vivo* Bonded Interfaces. Retrieval and Analysis of Clinically Aged Resin-Enamel and Resin-Dentin Interfaces

Scientists around the world have long shown how human history has been unfolded by retrieving aged entombed bodies, sunken ships, fossils, deep layers of earth crust, etc., and evaluating them using current technologies and knowledge. Their meticulous observations as well as their retrieved data collection from those entities brought us to what we believe it is our background and are the basis for establishing the development of our knowledge regarding our existence. Interestingly, the two main items from which data can be retrieved over millenia are teeth and bone. In laboratory durability testing, teeth are bonded and stored in some aging media. After pre-determined periods of time, tooth specimens or part of the specimens are retrieved from the media and prepared for bond strength tests and morphological analysis of the bonded interface. This approach has been used in many studies to evaluate the status of resin-dentin interfaces over time, and form the basis of our knowledge on how such interfaces degrade with time [7]. It would be highly desirable if the same approach could be applied clinically. The ability to follow up the morphological, chemical and mechanical changes that an adhesive joint undergoes when in function in the mouth would certainly provide definitive evidence on their behavior and permit improvements where needed. The intra-oral imaging technology currently available does not allow for such microscopic evaluations required, and, obviously, extraction and/or destructive methods are unacceptable. In real-life, however, it is not uncommon that teeth are extracted for periodontal, prosthetic or other justifiable clinical reasons. Some of these teeth include resin composite restorations that, regardless of their clinical judgment as satisfactory or not, were in service until being extracted. These extracted teeth probably carry important information on how the bonded interface performed in real life service and function. If appropriate records of what adhesive, bonding strategy, resin composite, etc., can also be retrieved, the relevance of the information increases significantly.

Failures can provide a tremendous wealth of information for changes in the quality of bonded interfaces over time, particularly when one analyzes in depth the reasons for their occurrence [128]. Retrieved primary molars offer, for instance, a suitable *in situ* test model for macro and microscopic investigations of the restorations after several years in the oral environment [129]. A retrieval study model was used to help answer the highly relevant clinical question as to whether bacteria can grow underneath sealed carious pit and fissure lesions [130]. A similar approach has been used to evaluate the clinical performance of ceramic crowns. Failed, fractured ceramic crowns were taken for optical and SEM evaluation to produce images of the fragments that were further fractographically analyzed

to determine the causes of failures [131]. From the studies above and few others that have used the retrieval approach to investigate clinically serviced restorations [27, 132-134] it is clear the potential benefits of this approach.

A few examples on how the retrieval method can bring important information about the clinical performance of adhesive interfaces can be seen in Figs. 5 and 6 after 10 plus years of clinical service. Although no records were available to identify the bonding strategy and the type of adhesive used, the SEM images suggest a gradual dissolution of the adhesive with partial disruption of the joint in several locations along the interface. The adhesive layer presents multiple small porosities, and so does the adjacent restorative resin. It seems that the silane coupling of the resin matrix with the filler particles has disappeared. Similar loss of nanofillers have recently been reported [135, 136]. The images presented here were obtained using SEM only. Once the retrieved tooth is available and its records audited, SEM, TEM and microtensile bond strength test can be applied to the interface. Enzymatic activity could be investigated immunohistochemically and/or by *in situ* zymography [137, 138]. New, laser-induced breakdown spectroscopy could also be used to analyze the mineral content of the interface in a non-destructive way [139]. The clinical status of the marginal integrity could be directly evaluated in comparison with the conditions of the interface.

Another interesting approach is the use of nanoDynamic Mechanical Analysis of aged bonded interfaces [140]. This technique scans the mechanical properties across sectioned bonded interfaces to provide images of the complex, storage and loss moduli of interfacial structures as color-coded images. Thus, any loss of hybrid layers and replacement with water is identified in such images as a very low complex modulus zone. These new techniques may provide a better understanding of how bond degradation relates to clinical quality of the restoration.

As mentioned before, thousands of studies have been done in laboratory settings searching for evidence on how resin-dentin interfaces could behave clinically in bonded restorations. However, there are important limitations to directly apply what is learned from laboratory-studied sound dentin to what is encountered in the clinical setting. That way, retrieval of information from aged, extracted restored teeth has a great potential for shedding light on how laboratory data is actually representative of the clinical performance of restorative dental materials.

Concluding remarks

Recent reviews on the topic of durability of resin-dentin bonds and the respective clinical outcome of adhesive restorations have gathered a wealth of information that reflects the advanced status of the current knowledge of adhesive systems and how improvements may be made. Bond strength testing in combination with micromorphological analysis of the interface have been used to measure the effectiveness of adhesives and of strategies to increase the durability of resin-dentin bonds. The clinical effectiveness of resin-dentin bonds is measured by the retention rate of NCCL restorations. There are tremendous differences between the morphology of the interfaces produced on dentin from sound extracted teeth versus that produced on natural NCCL, which suggests that their bonding mechanisms may be different. This shortcoming has to be taken into account when using laboratory data to justify clinical outcomes. Laboratory durability studies have suggested that resin-dentin bonds degrade at a much faster rate than clinical restorations take to fail. This implies that the durability of the bonded interface may play only a secondary role on the clinical survival of restorations. While achieving high bond strength is a requirement for optimal performance in laboratory studies, much lower bond strength might be required to retain a NCCL restoration in function and to seal interfaces in posterior composite restorations. The

analysis of *in vivo* interfaces of retrieved restored teeth may reveal important features of the degraded interface that will help understand which aspects are determinants of failure and which are not. It is hoped that the issues raised in this article will stimulate future research in the field.

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Fig 1. Schematic of potential deterrents to resin-infiltration following total-etching or self-etching in sound and sclerotic dentin of NCCL.

Fig 2.

A: This demineralized TEM micrograph showed a hypermineralized layer (HM) within the deepest part of a wedge-shaped lesion that was about 14 μm thick. Bacteria colonies were trapped inside this layer (hollow arrow) by a thin hypermineralized layer (pointer). Another species of bacteria (arrowhead) accumulated along the surface of the hypermineralized layer. Dentinal tubules were not occluded with sclerotic casts and were also filled with bacteria (solid arrow). **B:** Demineralized TEM micrograph of an “erratic” hybrid layer from the apex of a wedge-shaped lesion that was etched with 40% phosphoric acid and bonded using Clearfil Liner Bond 2V. The thickness of the hybrid layer varied from being absent (arrow) where a hypermineralized layer (HM) was present, to 5 μm (Hd) where the latter was thin and was eroded by bacteria (B). A: adhesive; SD: sclerotic dentin.

Fig 3.

Clinical aspect of resin composite restorations after several years in function, placed by the same operator. They illustrate the satisfactory clinical outcome of earlier adhesives, composite resins and bond techniques in well-motivated, low risk patients.

A, B and **C** correspond to a mesio-occluso-distal resin composite restoration in the first upper left pre-molar after 18 years of clinical service. Materials used were ScotchBond Multi Purpose (3M ESPE) adhesive and Herculite XR (Kerr) resin composite. Generalized wear (**B**) and marginal staining on the distal, cervical dentin margin (**C**) can be observed. There were no clinical or radiographic signs of secondary caries; **D** and **E** correspond to a mesio-occlusal-buccal resin composite restoration in the first left lower molar after 14 years of clinical service. Materials used were Prime&Bond 2.1 (Dentsply) adhesive and resin composite Charisma (Heraeus-Kulzer). Although significant wear with marginal exposure on the occlusal aspect, no signs of secondary caries were detected both radiographically (**D**) and clinically (**E**); **F** and **G** are a mesio-occlusal restoration in the first right lower molar and an occlusal restoration in the second right lower molar, both after 7 years of clinical service. Materials applied were Single Bond (3M ESPE) adhesive and resin composite P60 (3M

ESPE). No signs of failure due to bond degradation, both radiographically (**F**) and clinically (**G**).

Fig 4. Radiograph, close and wide view of a Class IV transversal fracture restored with UV-cured Nuval-Fil (Dentsply, Caulk) resin composite and using Adaptic ARM (Johnson & Johnson) bonding agent. The photographs and X-Ray were taken on June 2011. The restoration was in service for 35 years. The presence of acid-etched bonded enamel rim ensured retention and marginal integrity of the restoration for many years. (Case treated by Dr. Aymar Pavarini, DDS, PhD, retired Professor of Pediatric Dentistry at Bauru School of Dentistry, University of São Paulo, Bauru, SP, Brazil).

Fig 5.

SEM micrographs of laboratory polished/ acid demineralized resin-enamel interface of a retrieved restored tooth after 10 plus years of clinical service. The adhesive joint (AJ) presents a reasonable quality even after such a long period of service in the mouth. Note the loss of silica nanofillers above the adhesive joint detachment in the resin-based composite (RBC) leaving porosities in the adhesive joint; open arrows = filler particle loss.

Fig 6.

SEM micrographs of laboratory polished/ acid demineralized adhesive dentin joint of a retrieved restored tooth after 10 plus years of clinical service. A: Adhesive-dentin interface shows good overall morphology with relative small, debonded interface at the bottom of the AJ. It is possible that such defects are artifacts of specimen preparation. Infiltration of the adhesive into dentinal tubules is seen by the present of resin tags both at the adhesive interface and dentinal tubules below it. In this specimen, the tubules can parallel to the dentin surface. B: SEM micrograph of another tooth taken at higher magnification showing large interfacial voids at the middle of the adhesive joint layer but leaving part of the resin adhesive material attached to the underlying sound dentin. This may explain why even in the presence of subclinical interfacial failures there is no dentin sensitivity because the tubules remain sealed with adhesive. The void may be in part due to a thick adhesive joint formed or poor operator performance while applying the adhesive system.