Assessment of In Vitro Methods Used to Promote Adhesive Interface Degradation: A Critical Review

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ABSTRACT

One factor that has a great influence on clinical performance of dental restorations is their resistance to degradation. Morphological changes in the structure of tooth-restoration interface aged in the oral environment have been reported. However, even though the in vivo performance is the ultimate testing environment for predicting the behavior of restorations because of the complexity of intraoral conditions, in vitro models such as thermocycling, mechanical loading, pH cycling, and aging of materials in distilled water, NaOCl, and food-simulating solutions may provide important information about the fundamental mechanisms involved in resin-tooth interface degradation. Most recently, the effect of host-derived enzymes and the storage in deproteinizing solutions (such as aqueous NaOCl) on the degradation of resin-dentin bonds has also been described. This review considers the importance of these in vitro methods on bond durability interface in an attempt to understand the behavior of restoratives over time. The first section is focused on the mechanism of in vivo biodegradation, whereas the second looks at studies that have described the influence of water storage, NaOCl storage, host-derived matrix metalloproteinases, thermocycling, mechanical loading, pH cycling, and food-simulating solutions on the degradation of the adhesive interface. It is obvious that these methodologies do not occur separately in the oral cavity, but that each one has a specific importance in the mechanisms of bond degradation.

CLINICAL SIGNIFICANCE

The in vitro methods used to simulate bond degradation may describe important points related to the clinical performance of restorations. This article evaluates the mechanism of the in vivo biodegradation of adhesive interfaces as well as the influences that various testing methods have on these bonds.

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INTRODUCTION

The durability of bonds between the adhesive system and dentin is of critical importance for the longevity of restorations because the degradation of this interface can weaken adhesion[1] and lead to gap formation between the tooth and the restorative material.[2] It seems that deficient resin infiltration facilitates the permeation of biological fluids that ultimately compromise the quality of the adhesive interface.[1] Clinically, microleakage and marginal deterioration have been described as the major factors involved in the degradation and longevity of restorations,[3–6] as they are associated with undesirable effects including postoperative sensitivity, marginal staining, and secondary caries.[2,3,7]

Most studies that evaluate resin-dentin bond strength have been performed over short periods of time, generally 24-hour periods.[4,8,9] However, changes in pH, temperature, chewing loads, and chemical attacks commonly occur in the oral cavity and challenge the resin-tooth interface over months to years.[10,11]

Although the mouth is the ultimate testing environment for predicting the behavior of restorations[12] because of the complexity and diversity of intraoral conditions, in vitro models, such as thermal loading or pH cycling, and even food-simulating solutions, may be more important in providing information about the fundamental mechanisms of resin-tooth bond degradation. Thus, it is possible to simulate the aging of restorations and predict, as close as possible to clinical conditions, the complex undergoing within the oral cavity and their interactions with the durability of resin-tooth bonds over time. In this way, future research might determine how degradation occurs and allow prediction of the long-term clinical durability of resin-dentin bonding.

The goal of this study was to evaluate in vitro tests used to simulate restoration degradation.

MECHANISMS OF BOND DEGRADATION, IN VIVO

One factor that has great influence on the satisfactory clinical performance of dental restorations is their resistance to biodegradation. Biodegradation of restorative materials has been associated with undesirable effects on the surface and subsurface,[12] including the resin matrix, the filler content, and the matrix-filler interface.[13] Morphological changes in the bond structure of tooth-restoration interface aged in the oral environment for long periods have been reported.[11,14]

The biomaterial interface is mainly subjected to chemical and mechanical degradation.[15] Chemically, the tooth-material interface is exposed to water and human/bacterial enzymes present in saliva[16] and released from the dentin matrix,[17] which might lead to hydrolysis and plasticizing of the resin components,[18] with their subsequent leaching and degradation.[15] Morphologically, the exposed demineralized dentin zone at the base of the hybrid layer resulting from incomplete resin impregnation within the collagen network may be the major site of bond susceptibility to hydrolytic degradation.[14] This susceptibility may be increased by the degradation of the exposed collagen fibrils by the proteolytic enzymes[19] that are released from leucocytes, salivary glands, and plaque bacteria,[14,20] or by the dentin itself.[17]

In addition, not only the collagen fibrils but also the filler-matrix interface is affected by hydrolysis, which leads to the detachment of filler particles and gap formation. In this respect, water sorption reduces the frictional forces between the polymer chains, which decrease the mechanical properties of the resin, and makes the polymer swell.[18]

Concerning mechanical stresses in the oral environment, the resin-tooth interface is loaded during each chewing cycle.[21,22] At some sites, stress concentration may exceed the interfacial fracture strength, which leads to crack

initiation. This may cause crack propagation that affects the structural integrity of the material.[15] In addition to occlusal stresses, intraoral temperature changes may also induce repetitive contraction/expansion stresses at the tooth-adhesive interface,[23] thus increasing the effects of water sorption.

However, it is difficult to discriminate the specific factors involved in resin-tooth bonding degradation mainly because of the complexity of intraoral conditions. So, in vitro models that simulate these conditions have been employed in an attempt to develop an efficient laboratory model that is able to promote bond degradation, thus helping to predict restoration behavior over time.

IN VITRO METHODS FOR ADHESIVE INTERFACE DEGRADATION

Aging by Storage

Immersion in water has been the most common artificial technique to predict the behavior of resin-based restorative materials[1,24,25] because the presence of water is crucial for their deterioration,[26] and its effect is very pronounced when *etch-and-rinse* adhesive systems are used. The ideal situation in which this adhesive system completely penetrates demineralized dentin is rarely achieved.[27,28] Incomplete resin impregnation and imperfect polymerization of adhesive resin can create bond defects resulting from uninfiltrated demineralized zones and/or discrepancy between the depth of acid etching and resin infiltration.[24,29–31] These zones have been described as porous regions with exposed and unstable demineralized collagen network surrounded by nanometer-sized, interfibrilar spaces filled with water.[30] This porous zone can be penetrated by solutions, such as silver nitrate, even in gap-free restorations, which has been termed nanoleakage.[29,30] Nanoleakage may increase over time because of water sorption, thereby accelerating hybrid layer degradation.[32]

Within etch-and-rinse adhesive systems, two patterns of adhesive interface degradation after water storage have been reported: (1) the disorganization of the organic part of the hybrid layer[24,33] by the disintegration and disappearance of collagen fibrils from aged, bonded, or unbonded dentin[24,34–36] resulted from hydrolytic attack;[24,33] and (2) the degradation of the resin part that occurs with the extraction and loss of resin material from the hybrid layer[24,37,38] that leaves microspaces at the bonded interface and allow penetration of oral fluid, enzymes, or bacteria over time.[37] These morphological changes in collagen and resin by hydrolysis may be responsible for the degradation that results in bond-strength reduction.[24] Also, sorption/desorption cycles may cause microcracks and damage the polymer network. In the long term, the resin itself can chemically decompose, thus affecting resin-dentin bonding,[1,18,22] even after relatively short water-storage periods,[1,10,33,39–44] which shows that some resin adhesives may severely degrade after long-term water storage.[11,20,43–45]

S*elf-etching* adhesive systems were developed to promote the dissolution of the inorganic phase of dentin using acidic monomer, with simultaneous infiltration of adhesive monomer around the collagen network that results in fewer exposed collagen fibrils.[27] This full encapsulation of collagen fibrils by the bonding resin is thought to protect the resin-dentin interface against degradation. Also, the potential benefit of the additional chemical interaction between the functional monomer (as part of "mild" self-etch adhesives) and residual hydroxyapatite has regained attention.[35] However, incomplete resin infiltration was also observed as nanoleakage within such hybrid layers[30,46,47] despite the ability of these adhesives to etch and prime simultaneously. This has been attributed to the incomplete removal of water that is associated with the hydrophilic resin monomers via hydrogen bonding.[17,46] Also, water trees have been found in these adhesive layers after 1 year of aging.[48] These water

trees can be formed by slow water sorption through the adhesives that expedited the leaching of adhesive components, resulting in an increase of porosity along the hybrid layer of aged specimens.[11] Despite these morphological findings, stable bond strength with self-etch adhesives has been found over 1 year of water storage,[48] and it is clearly dependent on the particular adhesive tested.[49]

Regarding resin-modified glass ionomer cements, it has been reported that bond degradation after water immersion may occur within the mixed resin/polyalkenoic matrix[20,50] as well as in the fluoride-releasing glass particles.[51] Furthermore, the creation of an absorption layer resulting from water diffusion through the resin components results in the local swelling of material,[52] which may increase water flow toward the glass ionomer-dentin interface, thus accelerating the aging process.[50]

Studies examining the durability of bonding require a large number of factors to be carefully controlled. One of them is bacterial growth, which needs to be inhibited in order to maintain pH stability over time.[33] As an antibacterial agent, sodium azide has been added to storage solutions, and it has been described as a simple, reproducible, and low-cost means of storing teeth for a bond durability study.[33] Also, 0.5% Chloramine T, a disinfectant used in water purification, can be used in long-term storage studies.[39] Chloramine T is recommended by a technical report for guidance on testing of adhesion to tooth structure by the International Organization for Standardization (ISO).[53] However, the effects of these agents on bond strength and adhesive interface degradation are still unknown. It has been stated that chloramines did not produce significant changes in dentin shear bond strength when compared with freshly extracted teeth, unlike irradiation, thymol, methanol, and glutaraldehyde.[54] On the other hand, Armstrong and colleagues[39] reported that although the collagen is encapsulated by hydroxyapaptite in a tooth's natural state, it is possible that alterations to the organic content of dentin may occur because of the chloramine storage media, which may affect the durability of the adhesive joint. It seems that more work is needed to clarify the effect of storage media on bond strength and durability.

Other factors concerning storage protocol are important in bond durability studies, such as the period of immersion in solutions, which has been ranging from a few months[1,32,39,40,55–57] to years,[33,45,57] and the frequency that this solution is changed. With this respect, it has been suggested that changing the solution routinely may also accelerate hydrolysis at the interface between dentin and the hybrid layer, and also between the hybrid layer and the resin cement.[32,41] Also, the durability of resin-dentin bonds has been found to depend on the specific test used. Sectioning the specimens into sticks before storage might be an option for speeding up the process,[37,39] because reducing the cross-sectional area of specimens inevitably shortens the time required for water diffusion through the exposed regions of the resin-dentin interface.[39]

The lack of bond degradation in specimens stored in oil versus the relatively rapid degradation in water demonstrates the importance of water storage of restoratives on the durability of bonds in vitro. Nevertheless, even though the longevity of adhesion depends on the hydrolytic stability of the resin components, degradation cannot be completely attributed to the effects of water. Degradation is a complex process that includes the disintegration and dissolution of materials in saliva and other types of chemical/physical events caused by occlusal loading and thermal stress as well as enzymatic attacks and pH effects.[1,26] Moreover, the intrinsic mechanical properties of composite resins and adhesive systems must be considered[1] because each resin/tooth bonding component is thought to contribute to ultimate bond strength.

Storage in NaOCl Solution

A recently introduced method to assess bond durability is to expose the adhesive interface to an aqueous sodium hypoclorite solution.[35,58,59] The fractographic analysis of in vivo degraded resin-dentin bonds has shown that collagen fibrils that are incompletely infiltrated by adhesive resin are deproteinized. The 10% NaOCl solution, which can act as a deproteinizing agent, has been described as a rapid method to simulate the degradation that occurs in vivo[58,59] by the removal of organic components from resin-bonded teeth that are not completely enveloped.[59,60] It acts by forming superoxide radicals in the aqueous solution, thus inducing oxidation phenomena that fragment the peptide chain.[35,58,61]

Yamauti and colleagues,[58] one of the pioneers in describing this methodology, demonstrated that the use of a 10% NaOCl solution was able to dissolve the hybrid layer for both etch-and-rinse and self-etch adhesive systems, and thus, a decrease in the microtensile bond-strength values was observed after a 5-hour period of storage. Similar results were obtained by Monticelli and colleagues.[61] It seems that this decrease is strongly related to the storage period because storage in NaOCl solution for 1 hour was not sufficient to completely dissolve the hybrid layer. Also, De Munck and colleagues[59] reported that a 1-hour period of storage in a 10% NaOCl solution could not affect the bond-strength values for enamel. It has been argued that the NaOCl solution is responsible for only one part of the degradation process, that is, the chemical degradation of the organic content, and it has no significant effect on the degradation process of their resin components, in which the water uptake seems to be more important.[59] On the other hand, Toledano and colleagues[60] demonstrated that immersion of specimens in a 10% NaOCl aqueous solution for 5 hours could promote resin dissolution at the hybrid layer, and its effect is dependent on the adhesive system used.

Even though further studies are necessary to determine the effects of the NaOCl solution on bond degradation, it has been speculated that, depending on the period of storage, the NaOCl solution only accelerates the deterioration of the organic adhesive interface part,[59,62] in which the hybrid layer and the immediate underlying dentin substrate is expected to be chemically altered, whereas the degradation of the resin part is expected to be not affected by it.[59] This is thought to occur mainly because the resin components degrade, as described before, by the plasticization phenomena resulting from water uptake from the environment and by the chemical decomposition of the resin in the long term. Consequently, this test cannot be used to predict the overall clinical performance of the adhesive, as it only focuses on one kind of interface degradation process.[59] However, if further studies associate this methodology with long-term water storage, valuable information can be obtained regarding the stability of the components of the hybrid layer.[59] Also, as NaOCl is about half NaOH, it must be determined how much of the degradation process occurs as a result of high pH versus the oxidizing effects of hypoclorite ions. Therefore, studies that run pH 14 controls using 5% NaOH would be of interest.

Collagen Degradation by Host-Derived Enzymes

The degradation of collagen fibrils has not been totally attributed to storage of specimens in water during aging, but also to the breakdown of acid-demineralized collagen matrices by endogenous enzymatic activities, such as host-derived matrix metalloproteinases, the MMPs.[17,62] MMPs are a class of zinc- and calcium-dependent endopeptidases[63,64] that are capable of degrading the dentin organic matrix after demineralization.[16]

There has been evidence that simple etch-and-rinse adhesive systems may react to these endogenous enzymes present in dentin that were previously inactivated by

phosphoric acid-etching.[65] The effect of self-etching systems on the activation of dentin proteolytic enzymes has recently been reported as proportional to their acidity.[66]

In the context of dentin bonding, an in vitro method has been recently used[17,65] in attempting to confirm the paradigm that endogenous collagenolytic and gelatinolytic activities derived from dentin result in the degradation of the hybrid layer. It consists of the preparation of a mineralized dentin powder that is treated with a chelating agent (Ethylene Diaminetetraacetic Acid) or an acid-etching (usually phosphoric acid) to simulate the procedure of partial dentin demineralization. Then, the adhesive system is added to the wet, nondesiccated, demineralized dentin powder and the proteolytic activity is measured.

Using this methodology, Pashley and colleagues[17] demonstrated that the MMPs are present in the dentin structure in the absence of the contribution from bacterial or salivary MMPs. This low, but persistent, endogenous collagen activity was completely inhibited by the use of protease inhibitors, which preserved the structural integrity of the collagen fibrils and could arrest the degradation of the hybrid layer. The use of chlorexidine after acid-etching as a potential inhibitor of dentin hybrid layer degradation was also confirmed in an in vivo study.[62]

The effect of host-derived enzymes is of crucial importance to the knowledge of the intrinsic mechanism of resin-dentin interface degradation, and it cannot be separated from the effects of hydrolysis during aging because MMPs chemically add water across collagen bonds. They are not active in the absence of water. The incorporation of an in vitro methodology that demonstrates the residual collagenolytic activity in dentin would be helpful in predicting the durability of bonds.

According to Yang and colleagues,[67] the organic portion of the hybrid layer seems to be the most affected by degradation because the artificial aging of the interface via thermocycling regimens leads to structural changes of collagen fibrils. These authors observed with a scanning electronic microscope (SEM) that the top of the hybrid layer contains disorganized collagen fibrils from the original smear layer, which are degraded over time. In addition, the presence of intact collagen fibrils that were not denaturized during acid conditioning and remain beneath the smear layer was shown by an atomic force microscope analysis. However, even though these fibrils are intact, they may be structurally unstable because of poor resin infiltration or loss or resin protection over time, resulting in the deterioration of the adhesive interface and, consequently, lower bond-strength durability.

Aging by Thermocycling

Thermocycling is a commonly used thermal fatigue method to evaluate bond durability,[67–69] simulating the thermal changes that occur in the oral cavity caused by eating, drinking, and breathing.[23]

This type of test induces repetitive contraction/expansion stresses at the tooth-material interface resulting from the high thermal contraction/expansion coefficient of composites.[15] This may result in crack propagation along the bonded interface and gap formation. Gaps of different dimensions are created, allowing the passage of fluids in and out of the interface.[23] Therefore, adhesive failures may be found between the bonding resin and dentin after thermocycling, showing that this method has an influence on bond-strength values.[67]

Water absorption during thermocycling may compensate for resin polymerization shrinkage, thus minimizing the occurrence of stresses that could induce adhesive failure.[70] Thus, the influence of thermocycling on resin-tooth interface durability cannot be separated from hydrolysis effects.[68] Additionally, thermocycling effects may be ascribed to water heating, which probably accelerates the effects of

hydrolysis on interface.[15] Thus, water uptake is facilitated, and, consequently, degraded by-products are extracted from the interface at elevated temperatures.[14,71]

The thermocycling regimens used in reported studies differ with respect to the number of cycles, temperature, and dwell time (immersion of specimens in hot and cold fluids). Cycling number ranges from 100,[72] 500,[5] 1,000,[43] 1,500,[5] 2,000,[73,74] 2,500,[75,76] 3,000,[68,71] 5,000,[77,78] 10,000,[79] 15,000,[67] 30,000,[71] and up to 50,000[80] cycles. The number of cycles is usually arbitrarily set, which makes it difficult to compare published results. It is estimated that approximately 10,000 thermal cycles correspond to 1 year of clinical function.[23] This estimate is based on the hypothesis that such cycles might occur 20 to 50 times a day,[23] which makes the 500-cycle regimen proposed by the ISO standard (ISO TR 11450)[53] insufficient to simulate the long-term challenging of bond durability.[23,71,73] Many reports that used ISO protocol concluded that thermocycling did not affect the bond strength and microleakage of adhesive systems.[5,23,69,73] On the other hand, Miyazaki and colleagues[71] observed that a regimen of 30,000 cycles was able to decrease bond strength. This suggests that thermocycling has a negative effect on the restorative interface after a large number of cycles,[68,81,82] indicating that the major factor that accelerates the aging process during thermocycling may be the deleterious effect of water.[68]

The literature shows that there is a wide range in temperature extremes in thermocycling baths, such as 4 and 60°C,[83,84] 5 and 55°C,[5,10,50,73,74] 15 and 45°C,[85] 5 and 45°C,[5] and 5 and 60°C.[71] Under normal drinking conditions, it seems that temperatures at tooth surface range from 15 to 45°C.[85] However, refrigerated food may be kept at about 4°C.[5] Palmer and colleagues,[86] observing volunteers drinking hot and cold liquids, concluded that temperatures between 0 and 67°C are appropriate for dental material thermocycling, whereas Ernst and colleagues[87] demonstrated that most of the alternating temperature stresses (usually limited between 5 and 55°C) cover the temperature range that actually occurs in the oral cavity.

The time of immersion of specimens in hot and cold solutions (dwell time) is usually 15 seconds,[5] 30 seconds,[10,67] and 60 seconds.[68,69,80] Even though the ISO standard[53] suggests the immersion of dental materials for at least 20 seconds in each bath,[78] it has been pointed out that patients would not tolerate direct contact of a vital tooth with extremely hot or cold substances for a long time. Therefore, a short dwell time (no longer than 15 seconds) would be recommendable to simulate the clinical situation.[5,88,89]

To mimic the expected intraoral timings, three variations of temperature per cycle have been suggested,[77] with a longer dwell time used with an intermediated temperature of 37°C, and a shorter dwell time for the temperature extremes.[23] Intervals between baths have varied, from 3 seconds[69] to 15 seconds.[5] It has been suggested that shorter intervals may simulate more faithfully the abrupt changes of temperature that occur in the oral cavity.[87]

Thermocycling seems to be a valid in vitro method to accelerate the aging of restorative materials. However, reasoning for the choice of temperature and timing conditions is rarely given.[23] The varied number of cycles, temperatures, dwell time, and intervals between baths hinder comparison of the study results. Consequently, results obtained from thermocycling are contradictory.[10,69,75,78] Furthermore, the relative contribution of thermocycling to bond-strength degradation depends on the specific test setup, number of cycles,[77] and adhesive systems[10,78] and their functional monomers,[80] as well as on the C-factor,[78] substrate, cavity depth, surface preparation, storage time, and characteristics of the smear layer.[73]

Thus, in an attempt to understand the phenomena involved in the degradation of resin-based restorative materials in vitro, a standard thermocycling regimen is required to allow the comparison of materials and procedures between reports.

Aging by Mechanical Loading

Teeth are continually subjected to stresses during chewing, swallowing, and parafunctional habits such as bruxism. Vertical loading introduced by food bolus between opposing teeth can be evenly distributed over the entire occlusal surface, and stresses will be disseminated throughout its surface.[90] These occlusal stresses may challenge the long-term survival of bonds, resulting in the mechanical degradation of the adhesive interface.[11,60]

Mechanical loading tests have been used to predict the influence of mechanical factors involved in the oral environment [73,74,90] and to provide a better understanding of the in vivo behavior of dental adhesion.[76] Furthermore, this mechanical simulation leads to a decrease in bond strength because loading may cause fatigue at the adhesive interface resulting from the presence of preexisting water channels within adhesive systems, which probably enhance water sorption when restorations are under functional stress.[76] In addition, loading and unloading of teeth with filled cavities result in transitory or permanents gaps.[91] Thus, water uptake in the resin-dentin interface is facilitated, decreasing the durability of resin-dentin bond over time.[76]

When load is applied, compressive stress at the middle and tensile stress on both ends of the tooth can be expected along the bonded interface.[90] This is proposed as the situation during masticatory movement when occlusal or occlusoproximal restorations are loaded directly by the opposing teeth.[90]

Studies that used mechanical testing differ with respect to the number of load cycles, varying from 1,000 to 8,000,[l,60] 50,000,[76] up to 100,000.[74,92] When evaluating the adhesion between the composite resin and tooth, it has been observed that 100,000 cycles are insufficient to affect bond strength when applied alone.[74] In most reports, load is 50 to 90 N on the average,[60,74,90] even though axial loads up to 150 N have been observed during chewing and swallowing.[93] The frequency of 0.5 Hz during fatigue tests seems to be close to that of reported chewing cycles in vivo.[94]

Previous leakage reports[74,90] combined with load cycling have provided divergent results. Although it is clearly assumed that the amount of nanoleakage (i.e., interfacial disruption) should increase with the number of cycles, Frankenberger and Tay[76] found that nanoleakage decreased as stress increased. This may be attributed to the application of cyclic compressive stress to a beam that was secured directly in the middle and top of a metal stub. Thus, when compressive stresses were applied, the preexisting water channels may have been squeezed out of the interface over time, resulting in the decrease of nanoleakage. In addition, such variations[74,76,90,92,95] may be ascribed to the difference between materials, load direction and value, number of cycles, cavity size and type, characteristics of smear layer, and operators.[73,90]

This suggests that the effect of mechanical loading to bonded interface is still unclear. Therefore, some investigations have combined thermal and mechanical cycling to explain how degradation occurs and to give more details about the performance of adhesive systems.[73,74,92,95] Recently, Bedran De Castro and colleagues[74] reported that when thermal and mechanical load cycling were performed concomitantly, a significant decrease in microtensile bond strength of a total-etch adhesive to dentin was observed, in comparison with specimens that were thermocycled or submitted to mechanical loading alone. Probably, the effect of loading is accelerated by thermocycling.

Aging by pH Cycling

An important issue to understand the phenomena involved in the biodegradation of resin-based restorative materials and to predict the behavior of a restorative material is the use of pH cycling regimens.

In 1986, Featherstone and colleagues[96] proposed an in vitro pH cycling model to simulate the clinical situation more closely. This pH cycling model consisted of immerging specimens into an acid solution (pH 4.3, for 6 hours at 37°C), which is a reasonable estimate for subjects who consume sugar frequently, and storage in artificial saliva for 17 hours at 37°C. At the end of the week, the samples were maintained only in artificial saliva. This model for evaluating cariogenic challenges proved to be qualitatively and quantitatively similar to in vivo studies. Since then, variations from this model have been developed, such as the dynamic pH cycling model modified by Serra and Cury[97] in 1992 and the Featherstone[98] model for caries inhibition.

Acid-challenged resin-based restoratives have been reported to undergo greater micromorphological damages than after storage in distilled water or artificial saliva.[12] However, little information has been reported about the influence of pH cycling on tooth-resin bond degradation and bond-strength values, as well as the way a chemical attack can influence its durability, as most reports use thermal cycling, mechanical loading, or long-term storage to evaluate bond degradation. It seems that water droplets increase significantly after lactic acid challenge, pronunciating the effects of hydrolysis,[99] which has been described as the main factor involved in matrix decomposition. Matrix expansion causes pore formation inside the material, from which organic substances can be released, decreasing the longevity of a restoration.[24] SEM studies have shown the deterioration and erosion of an adhesive surface after acid challenge, accompanied by the bulk degradation of adhesives.[99]

The adverse effect of pH on the adhesive interface may also be ascribed to the loss of enamel minerals at the margin of restorations.[100] These factors may enhance gap formation and increase the flow of fluids and bacteria through the adhesive interface, thus leading to undesirable consequences on the bond strength of adhesive systems. However, further studies should be performed to predict the effect of acid challenge on bond durability to better understand its chemical influence on tooth-resin interface and to establish a specific in vitro protocol for assessing the degradation of the adhesive interface and bond durability.

Food-Simulating Solutions

Food-simulating liquids have been frequently used to evaluate in vitro the mechanism of the degradation of bonds and the mechanical properties of composite resins.[101–107]

The liquids used to simulate foods are usually 10%,[107] 50%,[106] and 75% ethanol.[102–104] These solutions simulate aqueous, acidic, and low-alcohol foods. Glyceryl triprylate coprate,[107] which simulates fatty foods, is also employed, although less frequently.

The solutions within the oral environment may affect the shear bond strength[102,104] of composites, dentin surface, and its chemical composition. The use of lower concentrations of ethanol as a food-simulating solution demonstrated no effect on the roughness[106] and surface staining[107] of composite resins. However, materials conditioned with higher percentages of ethanol (e.g., 75%) exhibit both dissolution and thinning after 30 days, and the uptake of this solution seems to occur through the resin matrix,[104] which may be weakened and separated,[107] thus facilitating crack propagation after a 30-day immersion.[103] Morphologically, conditioning with a 75% ethanol solution yielded a partial loss of smear layer and plugs, as well as possible collagen degradation.[104]

A decrease in fracture strength after 1 year of ethanol exposure was attributed mainly to the softening and expansion of the resin matrix and the cracking within the resin at the filler/matrix interface.[101] Moreover, high ethanol diffusion may occur in HEMA-containing (2-hydroxyethyl methacrylate) adhesives.[104]

ROLE OF ENAMEL ON BOND DURABILITY

Effective adhesion of etch-and-rinse adhesive systems to enamel has been achieved and proven to be a durable clinical procedure for routine applications in adhesive restorative dentistry. Preserving adjacent enamel as much as possible is one of the most important guidelines when preparing cavities for adhesive restorations.[27]

Considering these adhesive systems, it has been reported that an enamel border leads to stability of bond strength over time. This stability can be attributed to the protective role of the surrounding resin-enamel bond against degradation.[45,108] The enamel borders can work as a retarding obstacle for water diffusion; therefore, water needs to cross a longer diffusion path in restorations with an enamel margin. In a clinical situation, dentin bonding using etch-and-rinse adhesive systems may be more durable if all cavity margins are located in enamel. Also, it has been demonstrated that within 6 months of water storage, the diffusion occurs slowly from the periphery to the inner region, making the outer surface more susceptible to water degradation. However, De Munck and colleagues[45] found no significant difference in bond strength at the outer enamel sample area (closer to the water source) and at the central area after 4 years of water degradation. It is expected that 4 years is long enough to promote water diffusion throughout the entire sample.

Concern is often raised regarding the bonding effectiveness of self-etch adhesives to enamel. Numerous studies provide diverse data that suggest equal,[109] reduced[10,110,111] bonding effectiveness as compared with conventional phosphoric acid-etching, depending on the adhesive system used. Current long-term in vitro results have shown that two-step self-etch adhesive systems exhibit stable bond strength to enamel[112,113] without causing decalcification or damage to the enamel surface.[112] This may be related to their micromechanical interlocking through hybrid layer formation and to additional chemical interaction between the functional monomer and residual hydroxyapatite. Clinical studies have shown that the effectiveness of the two-step self-etching adhesive system remained excellent after 3[114] and 5[115] years of function, and that an additional etching of the enamel cavity margins resulted in an improved marginal adaptation on the enamel side.[114,115] Also, Koshiro and colleagues[116] found that the bonding interface of self-etch adhesive systems was excellent over 1 year of in vivo degradation. This suggests that the bond strength of this adhesive system may be initially low but becomes stable over time.[116] However, the one-step adhesives are commonly associated with lower bonding effectiveness, which must be attributed in part to the dissolution of hydrophilic and hydrophobic monomers in a relatively highly concentrated solvent.[117] Because of its high hydrophilicity, one-step self-etch adhesives have been reported to behave as semipermeable membranes, allowing fluids to pass through and seriously impairing bond durability.[118] Therefore, future long-term research is necessary, mainly for the so-called one-step self-etching adhesive systems, to provide valuable information about the bonding durability of this adhesive system to enamel.

CONCLUSIONS

1. Long-term water storage has been the most used in vitro methodology to simulate the degradation of an adhesive interface over time, and, most recently, the storage in an NaOCl solution may be associated with providing additional information about the degrada-

tion of the organic part of the hybrid layer.
2. Also, the effects of water storage cannot be separated from the collagen degradation by host-derived enzymes. In vitro methodologies that demonstrate the effect of these enzymes on bond degradation are helpful in determining the mechanism adhesive interface degradation for all available adhesive systems.
3. Thermocycling and mechanical load are important factors concerning the mechanical stresses that affect bond durability, but standardization of these in vitro protocols is required to allow comparison between studies and to determine the number of cycles from which the adhesive interface begins to degrade.
4. Further research is required to clarify the influence of pH cycling on adhesive interface durability.
5. Food-simulating solutions in high concentrations, such as 75% ethanol, may affect the bond strength of composites to dental substrates as well as the mechanical properties of dental materials.
6. Even though these in vitro protocols do not occur separately in an intraoral condition, they are important in providing information about the mechanism of biodegradation in in vitro studies.
7. For etch-and-rinse adhesive systems, enamel plays an important role in protecting the bond against degradation. However, future research is required to determine its role on the bond durability of self-etch adhesives, mainly for the "one-step" systems.

DISCLOSURE

The authors do not have any financial interest in the companies whose materials are included in this article.

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