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Degradation of the resin–dentin bonds after simulated and inhibited cariogenic challenge in an *in situ* model

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Abstract: The aim of this study was to evaluate the resin–dentin bonds of two simplified etch-and-rinse adhesive after simulated cariogenic and inhibited cariogenic challenge *in situ*. Dental cavities (4 mm wide, 4 mm long, and 1.5 mm deep) were prepared in 60 bovine teeth with enamel margins. Restorations were bonded with either adhesive Adper Single Bond 2 (3MESPE) or Optibond Solo Plus (Kerr). Forty restorations were included in an intra-oral palatal appliance that was used for 10 adult volunteers while the remaining 20 dental blocks were not submitted to any cariogenic challenge [NC group] and tested immediately. For the simulated cariogenic challenge [C+DA], each volunteer dropped 20% sucrose solution onto all blocks four times a day during 14 days and distilled water twice a day. In the inhibited cariogenic challenge group [C + FA], the same procedure was done, but slurry of fluoride dentifrice (1.100 ppm) was applied instead of water. The restored bovine blocks were sectioned to obtain a slice for cross-sectional Vickers microhardness evaluation and resin–dentin bonded sticks (0.8 mm^2) for resin–dentin microtensile evaluation. Data were evaluated by two-way ANOVA and Tukey's tests ($\alpha = 0.05$). Statistically lower microhardness values and degradation of the resin–dentin bonds were only found in the C + DW group for both adhesives. The *in situ* model seems to be a suitable short-term methodology to investigate the degradation of the resin–dentin bonds under a more realistic condition. © 2012 Wiley Periodicals, Inc. J Biomed Mater Res Part B: Appl Biomater 100B: 1466–1471, 2012.

Key Words: adhesive system, microtensile bond strength, degradation, *in situ* model, microhardness

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INTRODUCTION

The incorporation of hydrophilic and acidic resin monomers has substantially improved the immediate bonding of contemporary etch-and-rinse adhesives to intrinsically wet dental substrate.[1] However, potential problems associated with these increasingly hydrophilic formulations, as their limited long-term durability was raised. This shifted the focus of researchers' investigations for the evaluation of aging mechanisms involved in degradation of the resin-bonded interfaces.[2,3]

Most of the knowledge we have about the longevity of dentin bonds are based on *in vitro* studies, which showed significant reduction on resin–dentin bond strengths values after short-term and long-term immersion in water.[4–10]

The immersion of specimens in other solutions[11–15] and the use of pH,[16–19] thermal,[20–24] and mechanical loading cycling[22,24–26] as well as their combinations[17,22,24] are other attempts to simulate some of the complex interactions that these restorations are prone to in an oral environment.

The degradation phenomena have sporadically been studied *in vivo*[4,27–29] as they depend on approval by a local Ethics Committee, it requires much more time to gather important information and a higher cost is involved in the procedure, which makes clinical research more difficult than laboratory evaluations.[30] Moreover, by the time the results are published there is a great probability that the products are no longer on the market and new versions are already available.

In situ models have been used to evaluate the cariogenic and anticariogenic properties of several materials.[31] This kind of experiment may be considered as an intermediate stage between *in vivo and in vitro* studies, allowing the control of clinical relevant conditions that may be related to the degradation of bonded interfaces such as the cariogenic challenge in a relatively short period of time. To the extent of the author's knowledge, this model has never been used to observe the degradation of the resin–dentin interfaces and may offer a possibility to investigate interface-degradation issues under more realistic conditions.

Therefore, the objective of this study was to evaluate the degradation pattern of two simplified etch-and-rinse

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TABLE I. Adhesive Systems: Composition, Groups, and Application Mode

Adhesive Systems	Composition	Application Mode
Optibond Solo (Kerr Corp, Orange, CA, USA)	1. Kerr Gel Etchant: 37.5% H3PO4 2. Adhesive: Bis-GMA, HEMA, GDMA, GPDM, ethanol, canforquinone, ODMAB, BHT, filler (fumed SiO2, barium aluminoborosilicate, Na2SiF6), coupling factor A174	A. Acid-etch (15 s) B. Rinse (15 s) C. Air-dry (30 s) D. Dentin rewetted with water E. First coat of adhesive with slightly agitation (15 s) F. Air-dry (5 s) G. Second coat of adhesive with slightly agitation (15 s) H. Air-dry (5 s) I. Light-cure (10 s–600 mW/cm^2)
Adper Single Bond 2 (3MESPE, St. Paul, MN, USA)	1. Scotchbond Etchant: 35% phosphoric acid 2. Adhesive – Bis-GMA, HEMA, dimethacrylates, polyalquenoic acid copolymer, initiators, water, and ethanol	A. Acid-etch (15 s) B. Rinse (15 s) C. Air-dry (30 s) D. Dentin rewetted with water E. One coat of adhesive with slightly agitation (10 s) F. Air-dry (5 s) G. Second coat of adhesive with slightly agitation (15 s) H. Air-dry (5 s) I. Light-cure (10 s–600 mW/cm^2)

Abbreviations: Bis-GMA: bisphenol A diglycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate; GDMA: glycerol dimethacrylate; GPDM: glycerol phosphate dimethacrylate; ODMAB: 2-(ethylhexyl)-4-(dimethylamino)benzoate (co-initiator); BHT: butylhydroxytoluene or butylated hydroxytoluene or 2,6-di-(*tert*-butyl)-4-methylphenol (inhibitor).

adhesives after simulated and inhibited cariogenic challenge *in situ*. The null hypothesis of this study is that no significant degradation of the resin–dentin bonds will occur for both adhesives under simulated or inhibited *in situ* cariogenic challenge.

METHODS AND MATERIALS

Ethical aspects

This study was approved by the local ethical committee in research for animal and human from the local university under protocol number 07734/08. Ten adult volunteers took part in this study after signing an informed and written consent.

Study design

The study involved a factorial 2 × 2 split-mouth design of caries induction by plaque accumulation and sucrose use, performed in one phase of 14 days. The factors under evaluation were adhesive systems at two levels (Adper Single Bond, 3M ESPE, St. Paul, MN, USA, and Optibond Solo, Kerr, Danburry, CT, USA) and condition at three levels (immediate, non-cariogenic [NC]; aged under cariogenic challenge + distilled water [C + DW]; aged under cariogenic challenge + fluoride application [C + FA]). The NC group was designed to obtain the bond strength value of the non-aged bonded interfaces at the immediate period. The C + DW and C + FA were designed to represent a simulated and inhibited caries progression, respectively. A total of six experimental conditions were then tested.

Teeth preparation and restoration

Sixty bovine mandibular incisors were obtained. Teeth free from cracks or any other kinds of structural defects were selected. The teeth were sterilized by storage in 10% buffered formalin solution, pH 7, for 7 days[32] and stored in distilled water for up 2 months after extraction. A flat and superficial enamel surface was exposed on each tooth after wet grinding the occlusal enamel on # 180-grit SiC paper. On each tooth, one standardized rectangular cavity was prepared in the buccal surfaces (4 mm wide, 4 mm long, and 1.5 mm deep) with a carbide bur (# 330, KG Sorensen Ind. & Com. Ltda., Barueri, SP, Brazil), so that the axial wall was located in dentin and thickness of enamel border ranged from 0.3 to 0.5 mm. In case these two conditions were not satisfied, the enamel surface was ground flat again and the cavity was deepened.

The two etch-and-rinse adhesive systems described earlier were then applied in the cavities (Table I). The light curing step was performed by the recommended time (10 s) using a quartz–tungsten–halogen curing device (VIP, Bisco, Schaumburg, IL, USA; 600 mW/cm^2). The cavities were incrementally filled with the microhybrid composite Opallis in three increments (FGM Prod. Odont. Ltda, Joinville, SC, Brazil). Each increment was individually light activated for 40 s. All bonding procedures were carried out by a single operator at 24°C and 50% relative humidity.

Bovine dental blocks containing the restorations (6 × 6 × 3 mm) were taken from the buccal surface using a diamond saw under water cooling. All borders of the dental blocks were coated with an acid resistant nail varnish (Colorama, São Paulo, SP, Brazil), except from the top surface. These blocks were stored in a moist environment, at 37°C for 24 h.[33,34] After that, 40 specimens were placed in palatal appliances for *in situ* challenge ($n = 20$ for each adhesive),

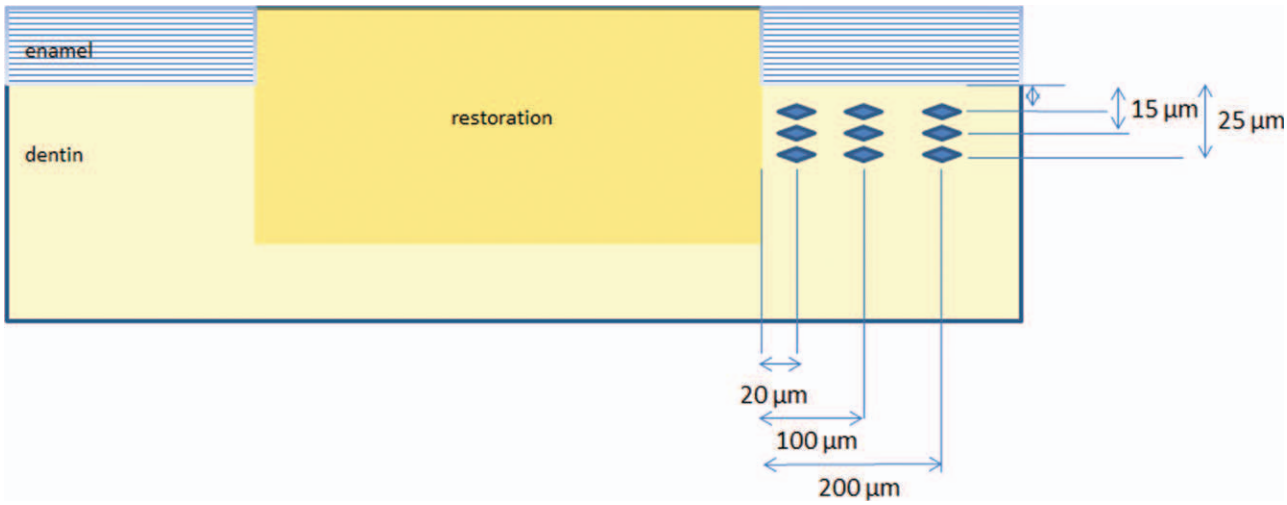

FIGURE 1. Schematic representation of the cross-sectional microhardness (CSMH). [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

while the other half was tested immediately ($n = 10$ for each adhesive), as described later on.

Palatal device preparation

For each volunteer, acrylic custom-made palatal devices were made with four sites (6.5 × 6.5 × 4 mm), in which the dental slabs were positioned and fixed with wax. To allow plaque accumulation and to protect it from mechanical disturbance, a plastic mesh was fixed to the acrylic resin, leaving a 1-mm space from the surface of the specimen.[34,35] Within each side of the palatal device, the positions of the specimens were randomly determined.

Intra-oral phase

During a 1-week lead-in period, and throughout the entire experimental phase, the volunteers brushed their teeth with a non-fluoride silica-based dentifrice formulation (Fleming Manipulação, Ponta Grossa, PR, Brasil) prepared for this study. To provide a cariogenic challenge in all four specimens, the volunteers were instructed to remove the device and to drip 20% sucrose solution (Fleming Manipulação, Ponta Grossa, PR, Brasil) onto all blocks four times a day (8.00 and 11.00 am and 3.30 and 7.00 pm) during 14 days.[36] Five minutes later, the device was re-inserted in the mouth.

Twice a day (8.30 and 12.30 am), the volunteers were instructed to remove again the palatal device to apply a fluoride [C + FA group] or distilled water [C + DW group] onto the exposed surfaces. The device was removed and slurries (1:3 w/v) of a fluoride solution (1100 ppm; Fleming Manipulação, Ponta Grossa, PR, Brasil) were applied in half of the specimens of each adhesive. The other half received the application of distilled water instead. The solutions should be kept into position for 5 min. After that, the device was washed in tap water and re-inserted in the mouth.

All volunteers consumed fluoridated water (0.6–0.8 mg F/l) and they were instructed to wear the intraoral devices the whole time for 14 consecutive days, removing them only for teeth and appliance hygiene and during the meals. The appliances were extra-orally brushed, except the restorations, and volunteers (26.9 mean age, range 22–34) were asked to brush carefully over palatal area, to avoid disturbing the biofilm covering the mesh. They were asked to brush their teeth and the appliance for up to 5 min.

On day 15 of the intraoral phase, around 12 h after the last application of the sucrose solution, the volunteers stopped wearing the intraoral device. The restoration was then removed, washed in tap water and longitudinally, so that a thin slice of the restoration was used for cross-sectional microhardness (CSMH) measurements and the remaining for resin–dentin microtensile bond strength evaluation (μTBS). The same procedure was performed for the restorations not placed in the palatal appliance [NC group].

Cross-sectional microhardness

The thin restoration slab was embedded in acrylic resin, the cut surface being exposed, for subsequent flattening and polishing with 1000, 1500, 2000, and 2500-grit SiC paper and 1 and 0.25 μm diamond paste (Erios Prod. Odont., São Paulo, SP, Brazil) using a polish cloth.

After ultrasonic cleaning, cross-sectional microhardness measurements were made in dentin with a microhardness tester (HMV-2, Shimadzu, Tokyo, Japan) equipped with a Vickers indenter (VHN) under a 15 g load for 5 s. Three lines of 03 indentations each were made, one lane being 20 μm distant from the restoration margin and the other, 100 and 200 μm distant. The indentations were made at the following depths from the enamel–dentin junction: 5, 15, and 25 μm (Figure 1).

Microtensile bond strength evaluation

The remaining restoration was submitted to the μTBS test. Each restoration was longitudinally sectioned in both "*x*" and "*y*" directions across the pulpal bonded interface with a diamond saw in a Labcut 1010 machine (Extec Corp.,

TABLE II. Means and Standard Deviations for Vickers Microhardness (VHN) and Statistical Significance*

	Condition		
Adhesive Systems	NC	C + DW	C + FA
Optibond Solo	70.2 ± 27.4 A	56.8 ± 15.5 B	65.7 ± 32.1 A
Adper Single Bond 2	75.9 ± 28.5 A	51.8 ± 10.8 B	71.2 ± 36.4 A

NC: immediate, non-cariogenic group; C + DW: simulated cariogenic challenge + distilled water; C + FA: inhibited cariogenic challenge + fluoride application.

* Different upper case letters indicated means statistically significant ($p < 0.05$).

Enfield, CT, USA), under water cooling at 300 rpm to obtain bonded sticks with a cross-sectional area of approximately 0.8 mm^2. The number of premature failures per tooth during specimen preparation was recorded. The cross-sectional area of each stick was measured with the digital caliper to the nearest 0.01 mm and recorded for subsequent calculation of the microtensile bond strength (Absolute Digimatic, Mitutoyo, Tokyo, Japan). Each dental block could provide approximately 4–6 resin–dentin specimens with a remaining dentin thickness ranging from 0.8 to 1.2 mm.

Each bonded stick was attached to a modified device (Odeme Prod. Med. Odont., Joaçaba, SC, Brazil) for microtensile testing with cyanoacrylate resin (Super Bonder Gel, Loctite, SP, Brazil) and subjected to a tensile force in a universal machine (Emic, São José dos Pinhais, PR, Brazil) at 0.5 mm/min in their respective storage period. The failure modes were evaluated at 400X (HMV-2, Shimadzu, Tokyo, Japan) and classified as cohesive (failure exclusive within dentin or composite, C), adhesive (failure at resin/dentin interface – A), or adhesive/mixed (failure at resin/dentin interface that included cohesive failure of the neighboring substrates, A/M).

Statistical analysis

To have an overall assessment of the caries lesion formation, all microhardness values taken from each tooth were averaged, and just one value per tooth were used in the statistical analysis. The same procedure was done for the μTBS. The bond strength of all sticks from the same tooth was averaged for statistical purposes. The premature failures were not included in the tooth mean. The data from both tests were then analyzed by a two-way ANOVA (Adhesive system *vs.* Condition) and Tukey's *post hoc* test at $\alpha = 0.05$.

RESULTS

Cross-sectional microhardness

The statistical analysis revealed that only the main factor Condition was statistically significant ($p < 0.05$). Statistically lower microhardness values were observed only for specimens submitted to simulated cariogenic challenge [C + DW] under *in situ* condition (Table II).

Microtensile bond strength

The mean cross-sectional area ranged from 0.81 to 1.12 mm^2 and no difference among groups was detected ($p > 0.05$). The distribution of specimens according to the failure pattern is depicted in Table III. No premature failure occurred during sample preparation for any of the bonding conditions.

The overall μTBS values for SB and OS under the experimental conditions are shown in Table IV. Only the main factor Condition was statistically significant ($p < 0.05$). Significant lower μTBS values were observed only under simulated cariogenic challenge in the *in situ* condition.

DISCUSSION

The fast and frequent development of new materials and restorative techniques requires also quick assessments for the estimation of their clinical performance. *In vivo* studies are ideally suited to evaluate both the performance and the longevity of restorative materials, but their feasibility is complicated or even precluded by the associated high costs, bureaucratic requirements, and long durations. Laboratory studies, on the other hand,[37] offer the advantages of lower costs, shorter duration, greater standardization due to the possibility of isolation of variables and have been widely used to predict the performance and longevity of adhesive materials.[30,38]

However, a clear disadvantage of laboratory studies is that they do not resemble all the challenges a bonded restorations are prone to under clinical service for prolonged periods of time. The use of aging methods such as water storage usually requires 6 months to detect similar drops on the μTBS values,[30] and although this period of time may be shorter when daily water exchange is performed[39] or other solutions such as ethanol[11,12] or NaOCl[13–15] are used, they all share the disadvantage of not resembling a clinically relevant condition.

Thermal,[20–24] mechanical,[22,24,26] and pH cycling[16,17,19] attempt to simulate important clinical conditions; however,

TABLE III. Percentage of Specimens According to Pattern Failure of Microtensile Bond Strength for All Experimental Groups

		Pattern Failure			
Adhesive Systems	Condition	Adhesive	Mixed	Cohesive (Dentine or Resin)	Premature Failure
Optibond Solo	NC	80	20	00	00
	C + DW	100	00	00	00
	C + FA	100	00	00	00
Adper Single Bond 2	NC	100	00	00	00
	C + DW	100	00	00	00
	C + FA	100	00	00	00

NC: immediate, non-cariogenic group; C + DW: simulated cariogenic challenge + distilled water; C + FA: inhibited cariogenic challenge + fluoride application.

TABLE IV. Means and Standard Deviations for Microtensile Bond Strength (MPa) and Statistical Analysis*

	Condition		
Adhesive Systems	NC	C + DW	C + FA
Optibond Solo	20.5 ± 5.8 A	15.7 ± 3.2 B	19.3 ± 4.1 A
Adper Single Bond 2	20.2 ± 3.2 A	12.4 ± 1.4 B	18.5 ± 2.7 A

NC: immediate, non-cariogenic group; C + DW: simulated cariogenic challenge + distilled water; C + FA: inhibited cariogenic challenge + fluoride application.

* Different upper case letters indicated means statistically significant ($p < 0.05$).

they lack standardization in the number of cycles, temperature, dwell time, immersion time, load and load frequency and this may hinder comparison of study results and lead to contradictory findings.[38] Standardization of these *in vitro* protocols is required to allow comparison between studies and to determine the number of cycles and regimens from which the adhesive interface begins to degrade.

The results of this study demonstrated that the *in situ* model may be used as a useful method to age adhesive restorations with the advantage of resembling most of the challenging conditions restorations are submitted in the oral environment, except from mechanical loading. Compared to the immediate results where no aging method was performed, significant reductions of μTBS values were observed after a 14-day simulated cariogenic challenge.

Although the exact mechanism responsible for the degradation of the hybrid layer has not been completely understood yet, it seems that first stage of degradation involves the elution of the hydrophilic resins that had infiltrated the dentin by water sorption and solubility phenomena.[40] Water sorption reduces the frictional forces between the polymer chains, which decrease the mechanical properties of the polymeric material[41] due to polymer swelling.

Besides that, the non-infiltrated demineralized dentin and the exposed collagen fibrils due to resin elution can be enzymatically attacked by host-derived metalloproteinases [MMPs].[28,29,42] MMPs are a class of zinc- and calcium-dependent endopeptidases[43,44] that are trapped within the mineralized dentine matrix during tooth development. These host-derived MMPs were shown to be activated by the etch-and-rinse adhesive systems[45] and their release following activation during dentine bonding[28,29,42] are thought to be responsible for the *in vivo* manifestation of thinning and disappearance of collagen fibrils from hybrid layers in aged, bonded dentine.[4,29,42]

This mechanism of bonding degradation has been reported as the main reason for the drops of the μTBS after short- and long-term water storage and may also explain the degradation of the resin–dentin bonds observed in this study. However, it does not explain why drops in the resin–dentin bond strength occurred only after a short-term 14-day simulated cariogenic challenge period and not under inhibited cariogenic challenge.

One can observe from the finding of this study that in the simulated cariogenic challenge group, loss of enamel minerals at the margin of restorations was observed, a situation not expected when specimens are stored for varied periods of time in water or in the inhibited cariogenic challenge group. This demineralization on the enamel margins may have enhanced gap formation at the interface and increased the flow of fluids and bacteria through the adhesive interface, thus leading to faster undesirable consequences on the bond strength of adhesive systems.

Other biological aspects may have worked synergistically to produce degradation of the resin–dentin bonds in the C + DW group. It is known that in tooth caries formation, bacterial acids are required for the removal of minerals and for the subsequent activation of host MMPs, since bacteria alone cannot cause dentin matrix degradation.[46] Thus, the dentin matrix-bounded MMPs from specimens submitted to *in situ* cariogenic challenge were likely activated by two distinct mechanisms, i.e., by bacterial acids[46] presented in the *in situ* model and also by the application of the etch-and-rinse adhesives.[45] This dual activation mechanism of the host-derived MMPs may have resulted in a more intense degradation rate of the collagen, being therefore responsible for the fast drops in the resin–dentin bond strength after simulated cariogenic challenge. However, this hypothesis needs further investigations.

Two different etch-and-rinse adhesives were employed in this study. Although they share the same bonding strategy, a clear difference among them is that only Optibond Solo contains fluoride. Although this material was shown to be able to release significant amount of fluoride, it was not able to inhibit the development of root caries.[47] This earlier finding was confirmed in the present investigation since no significant difference among the materials was observed in the present *in situ* study.

Unfortunately the findings of the present investigation cannot be compared to other studies since to the best our knowledge this study was the first one that employed the *in situ* model to investigate the degradation of resin–dentin bonds that occurs with simplified etch-and-rinse adhesives.

CONCLUSIONS

The *in situ* model seems to be a suitable short-term methodology to investigate the degradation of the resin–dentin bonds. Degradation of the resin–dentin bonds after a 14-day cariogenic challenge is only observed if no fluoride is applied onto the dental blocks surfaces.

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