Resistance to degradation of bonded restorations to simulated caries-affected primary dentin

MARCELA MARQUEZAN, DDS, MS, PHD, RAQUEL OSORIO, DDS, MS, PHD, ANA LIDIA CIAMPONI, DDS, MS, PHD & MANUEL TOLEDANO, DDS, MS, PHD

ABSTRACT: Purpose: To investigate the resistance to degradation of resin modified glass-ionomer cement (RMGIC) and adhesive/composite restorations in sound and simulated caries-affected dentin of primary teeth subjected to carious challenge using a pH-cycling model and load-cycling, by means of a microtensile test. **Methods:** Occlusal cavities were prepared in 60 sound exfoliated primary second molars. Half the specimens were submitted to pH-cycling to induce simulated caries lesion. The teeth were randomly restored with one of the two materials: (1) a RMGIC (Vitremer) and (2) a total-etch adhesive system (Adper Single Bond 2) followed by resin composite (Filtek Z100). After storage in distilled water at 37°C for 24 hours, control group specimens were subjected to test procedures while the specimens in the experimental groups were subjected to two different aging methods: load-cycling (50,000 cycles, 90N, 3Hz) or carious challenge (pH-cycling: alternately 8 hours in demineralizing and 16 hours in remineralizing solutions, for 10 days). Teeth were sectioned into 1 mm^2 beams and tested to failure under tension. ANOVA and multiple-comparisons tests were used ($P< 0.05$). **Results:** Vitremer bond strength was not altered by the condition of dentin. Conversely, Adper Single Bond 2 showed significantly lower bond strength values when bonded to simulated caries-affected dentin. Load-cycling did not influence bond strength for any of the tested materials, while carious challenge resulted in a significant decrease in microtensile bond strengths of Adper Single Bond 2, but not of Vitremer restorations. (*Am J Dent* 2010;23:47-52).

CLINICAL SIGNIFICANCE: The use of Vitremer (RMGIC) is encouraged for pediatric patients with caries activity, since it satisfactorily bonded to simulated caries-affected dentin and resisted caries challenge.

✉: Dr. Raquel Osorio, Avda. de las Fuerzas Armadas 1, 1B, 18014 Granada, Spain. E-✉: toledano@ugr.es

Introduction

The immediate bonding effectiveness of contemporary adhesive restorations is favorable, but some limited durability *in vivo* and *in vitro* has been detected for some two-step etch and rinse systems,[1,2] mainly because biomaterial-tooth interfaces are subject to hydrolytic degradation.[3] There have been attempts to simulate restoration aging by means of water storage,[4-7] NaOCl immersion,[8,9] thermal- and load-cycling,[8] and pH-cycling[10,11] in order to reproduce the caries challenge to which teeth and restorations are encountered in the oral environment.

Bond strength values to permanent caries-affected dentin are lower than those to sound dentin[12] and this substrate is more prone to degradation.[13] Primary dentin provides lower bond strength[14,15] probably due to the formation of a thicker layer of demineralization after etching, which is not adequately infiltrated by resin monomers.[16] This zone is also prone to degradation[17] and consequently, the use of weaker acids[16] or reduced etching time[18] have been proposed for primary teeth.

Glass-ionomer cements (GIC) self-adhere to tooth tissue because of its chemical bonding through the ionic interaction of the carboxyl groups of the polyalkenoic acid with calcium ions of hydroxyapatite, which remain attached to the collagen fibrils.[19] This chemical adhesion may be beneficial in terms of resistance to hydrolytic degradation.[2] The lack of adhesion between the collagen and the adhesive materials may lead to the destruction of proteins, which requires a combination of non infiltration/demineralization and activation of dentin matrix metalloproteinases.[6]

This study investigated the resistance to degradation of RMGIC and adhesive/composite restorations in sound and caries-affected dentin of primary teeth subjected to load-cycling aging, and caries challenge using pH-cycling, by means of the microtensile test. The null hypotheses tested were that bond strengths of the adhesive/composite and the RMGIC do not differ between simulated caries-affected dentin and sound dentin, and both are resistant to load- and pH- cycling.

Material and Methods

Sixty sound exfoliated primary second molars were used in this study. The human primary molars were obtained after the institutional informed consent from all donors. The research was approved by the Commission of Ethics in Research. Teeth were cleaned with pumice/water slurry, rinsed and stored in distilled water in a refrigerator (4°C) until use. The pulp chambers of 60 crowns were sealed with composite resin and their cups flattened with 220-grit abrasive paper. Occlusal Class I cavities (7 mm x 5 mm x 2 mm deep) were prepared using a high-speed handpiece with a cylindrical medium-grit (100 µm) diamond bur (#842[a]) under water irrigation. Each diamond bur was replaced after every five preparations.

Half of the specimens were subjected to an artificial caries induction process.

Artificial caries induction - The entire surface of each specimen, except for the internal surfaces of the cavity, was painted with two layers of an acid-resistant varnish. Simulated dentin carious lesions were created by a pH-cycling procedure, according to the protocol described in a previous report.[20] The demineralizing solution contained 2.2 mM $CaCl_2$, 2.2 mM NaH_2PO_4, and 50 mM acetic acid adjusted to a pH of 4.8. The remineralizing solution contained 1.5 mM $CaCl_2$, 0.9 mM NaH_2PO_4, and 0.15 M KCl adjusted to a pH of 7. Each speci-

Table 1. Materials used in the experimental groups.

Product	Components	Mode of application
Vitremer	Primer	Methacrylate functional copolymer of polyacrylic and polyitaconic acids, HEMA, ethanol and photoinitiators.
	Powder	Fluoroaluminosilicate glass, micro-encapsulated potassium persulfate, ascorbic acid, small amounts of pigments.
	Liquid	Aqueous solution of a polycarboxylic acid modified with pendant methacrylate groups, methacrylate functional copolymer of polyacrylic and polyitaconic acids, water, HEMA and photoinitiators.
		(1) Apply Primer for 30 seconds, using a light scrubbing motion. Mild air stream for 15 seconds. (2) Light-cure for 20 seconds. (3) Hand-mixed manipulation. (4) Insertion into a cavity using a syringe injector in a single increment. (5) Light-cure for 40 seconds. (6) Apply light-cure finishing gloss and light-cured for 20 seconds.
Adper Single Bond 2 (Adper Scotchbond 1 XT in Europe)		HEMA, water, ethanol, Bis-GMA, dimethacrylates, amines, methacrylate functional copolymer of polyacrylic and polyitaconic acids, 10% by weight of 5 nanometer-diameter spherical silica particles.
		(1) Etch for 15 seconds. (2) Rinse with water spray for 15 seconds, leaving tooth moist. (3) Apply two consecutive coats of the adhesive with a fully saturated brush tip. Dry gently for 2-5 seconds. (4) Light-cure for 10 seconds.

Abbreviations: HEMA: 2-hydroxyethilmethacrylate; Bis-GMA: bis-phenol A diglycidylmethacrylate.

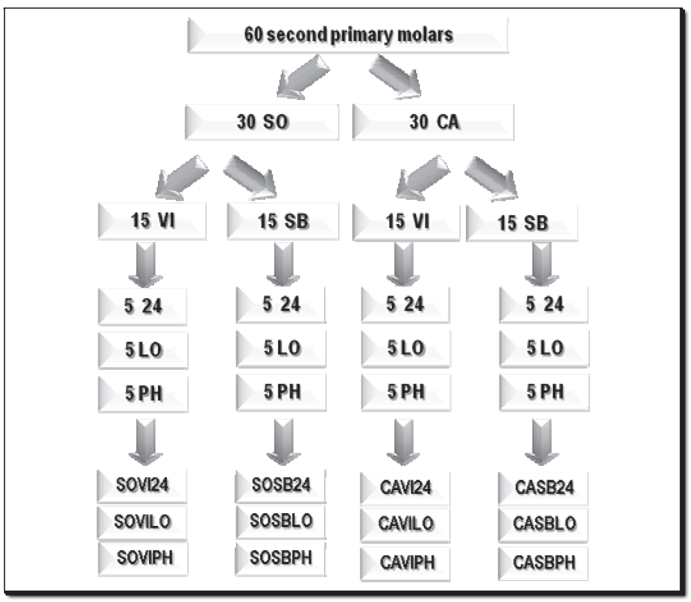

Fig. 1. Distribution of teeth into experimental groups (SO: sound dentin; CA: simulated caries-affected dentin; VI: Vitremer; SB: Adper Single Bond 2; 24: control= test in 24 hours; PH: pH cycling; LO: load cycling).

men was cycled for 8 hours in the demineralizing solution (10 ml) and 16 hours in the remineralizing solution (10 ml). This procedure was performed for 14 days with solutions renewed at each change, at 37ºC, and without shaking. The depth of dentin demineralization with this same method has been reported to be more than 100 µm depth.[21]

After the artificial caries lesion formation period, a diamond bur was used to clean the walls surrounding the cavities maintaining the demineralized dentin layer at the bottom of the cavities.

Restoration procedures - The prepared teeth were randomly divided into two groups (Fig. 1), according to the restorative materials: a RMGIC (Vitremer[b]) and a total-etch adhesive system (Adper Single Bond 2[b]) followed by a composite resin (Filtek Z100[b]). The methods of application of these materials were in accordance with the manufacturer's instructions (Table 1). Filtek Z100 was inserted using an incremental technique, and each layer was light-cured for 40 seconds with a Translux EC[c] halogen light-curing unit. The output intensity was monitored with a Demetron Curing Radiometer (Model 100[d]). A minimal output intensity of 600mW/cm^2 was employed for the experiments.

After storage in distilled water at 37°C for 24 hours, the occlusal surface of the restorations were ground in order to assure the exposure of the bonded interfaces to the bonded enamel-restorative material interfaces. Five restored teeth from each dentin condition and restorative material were subjected to one of the following procedures: (1) Tested immediately by the microtensile bond strength test (control 24 hours); or (2) mounted on plastic rings using acrylic resin for load-cycling (50,000 cycles, 90 N, 3 Hz) under water, with a compressive load applied to the center of the restoration using a 5 mm diameter spherical stainless steel plunger, attached to a cyclic loading machine (S-MMT-250NB[e]) before testing; or (3) submitted to the pH-cycling procedure, being alternately placed into containers with the demineralizing solution for 8 hours and the remineralizing for 16 hours. Solutions were the same as described above for carious lesion formation, but the cycling procedure was performed for 10 days.[10]

For the microtensile test, the teeth, five per group, were sequentially sectioned with a water-cooled diamond disc (Isomet 4000[f]), along the mesiodistal and buccolingual axis, in order to obtain beams with a square cross-sectional area of about 1 mm^2 for microtensile testing. Forty-five beams were obtained per group.

Each beam was attached to a modified Bencor Multi-T testing apparatus[g] with cyanoacrylate adhesive (Zapit[h]) and stressed to failure under tension in a universal testing machine (Instron 4411[i]), at a crosshead speed of 0.5 mm/minute. The fractured beams were removed from the testing apparatus and the cross-sectional area at the site of failure was measured to the nearest 0.01 mm with a pair of digital calipers.[j] Bond strength values were expressed in MPa and analyzed with a multiple ANOVA to examine the contributions of dentin condition (sound or simulated caries affected dentin), material (RMGIC or etch&rinse adhesive system) and challenge (mechanical or chemical). Interactions were included in the analy-

Table 2. Mean microtensile bond strengths (MPa) and standard deviation (SD) of the different restorative materials in sound and simulated caries-affected primary dentin after mechanical and chemical challenges. (n= 40-50).

	Vitremer		Adper Single Bond 2 + Filtek Z100	
	Sound dentin	Simulated caries-affected dentin	Sound dentin	Simulated caries-affected dentin
24 hours	8.86 (3.49) ^{Ba}	9.19 (3.87) ^{Ba}	26.95 (8.86) ^{Aa}	8.15 (5.25) ^{Ba}
Load-cycling	8.04 (3.36) ^{Ba}	9.14 (2.94) ^{Ba}	27.00 (8.22) ^{Aa}	9.05 (2.81) ^{Ba}
pH-cycling	7.79 (2.35) ^{Ba}	9.10 (2.58) ^{Ba}	21.88 (6.53) ^{Ab}	5.83 (2.44) ^{Cb}

For each horizontal row: values with identical upper case letters indicate no statistically significant difference (P> 0.05).
For each vertical column: values with identical lower case letters indicate no statistically significant difference among different challenging methods when the same material and dentin were maintained (P> 0.05).

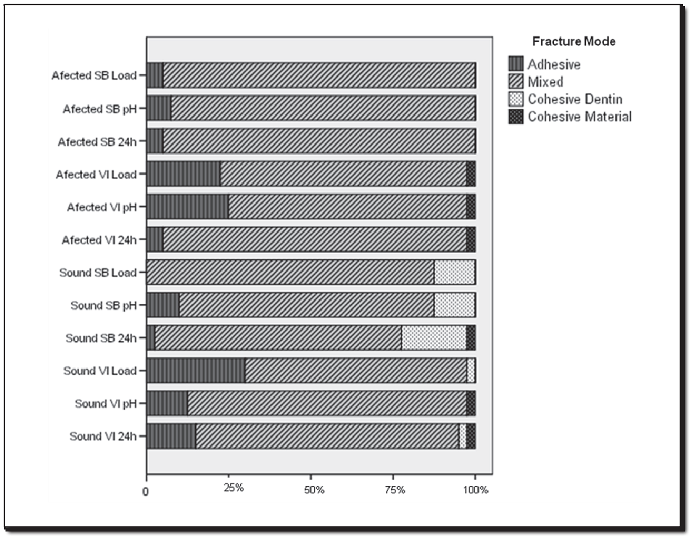

Fig. 2. Percentage distribution of failure mode of debonded specimens in each of the experimental groups.

sis. *Post hoc* multiple comparisons were conducted using Tukey's test (α= 0.05). The fractured specimens were examined under a stereomicroscope (Olympus SZ-CTV[k]) at x40 magnification to evaluate the fracture pattern. Failure modes were classified as adhesive, cohesive or mixed. Four representative debonded sticks from each group were prepared for scanning electron microscopy (SEM) analysis as follows: fixation in 2.5% glutaraldehyde for 12 hours at 4°C, washed with 20 ml of 0.2 M sodium cacodylate buffer (pH 7.4) for 1 hour, and washed in distilled water three times for 1 minute. For dehydration, the specimens were sequentially immersed in ethyl alcohol (25% for 20 minutes, 50% for 20 minutes, 75% for 20 minutes, and 95% for 20 minutes), then transferred to a critical-point dryer (HMDS) for 30 minutes. The prepared specimens were mounted on aluminum stubs, gold-sputtered at 10 mA for 1 minute (Unit E500[l]) and analyzed under SEM (1430 VP[m]).

Results

Dentin condition (F = 294.13; P< 0.001), material (F = 256.56; P< 0.001), and challenge (F = 8.79; P< 0.001) significantly influenced the microtensile bond strength. Interactions between dentin and material, and material/challenge were also significant (P< 0.001). Reliability was about 78%. Mean bond strength values and standard deviations obtained for the different groups are shown in Table 2. Premature failure was lower than 4% in all groups.

Vitremer bond strength was not influenced by the dentin condition. Conversely, Single Bond 2 presented significantly lower bond strength values when bonded to the simulated caries-affected dentin.

After load- or pH-cycling, Vitremer showed no changes when bonded to sound or simulated caries-affected dentin. Load-cycling did not influence bond strength of Adper Single Bond 2, although pH cycling resulted in a significant decrease in microtensile bond strengths when Adper Single Bond 2 was bonded to sound and simulated caries-affected dentin. Adper Single Bond 2 provided higher bond strength values in sound dentin in comparison to Vitremer. Materials presented similar bond strength values when bonded to simulated caries-affected dentin, except when Adper Single Bond 2 specimens were bonded to the simulated caries-affected dentin substrate and submitted to pH cycling; in this case, Adper Single Bond 2 showed lower bond strength than Vitremer.

Figure 2 presents the percentage failure modes of the debonded specimens according to the material type, the dentin substrate and the aging method. Mixed failure modes were the most frequently identified in all groups. Pure cohesive failures in dentin were associated with high bond strengths (*i.e*. Adper Single Bond 2 bonded to sound dentin). A small number of cohesive failures in material were observed in the Vitremer groups. In general, mixed failures in sound dentin groups were an association of an adhesive failure with a partial cohesive failure of the material, while mixed failures in caries-affected groups were a sum of an adhesive failure with partial dentin cohesive failure.

Fractographic analysis of the debonded dentin surfaces are shown in Figs. 3 and 4. When Vitremer was used (Fig. 3), both substrates, dentin and resin, are visible on the surface of the specimens, reflecting the more common mode of mixed failure. In both sound and simulated caries affected dentin, tubule entrances were partially filled with resin. An extensive cohesive failure and some porosity areas could be observed when samples were submitted to degradation. Adper Single Bond 2 (Fig. 4) frequently failed at the top of the hybrid layer in sound dentin. When this adhesive was applied on simulated caries-affected dentin, failures along the top, within or at the bottom of the hybrid layers were observed. Tubule entrances were partially filled with resin. The morphology of the simulated caries affected intertubular dentin was altered as a result of the loss of the incompletely infiltrated collagen fibrils. Figures 3 and 4 depict representative debonded specimens under SEM.

Discussion

Bond strength values of Adper Single Bond 2 bonded to simulated caries-affected dentin were significantly lower than they were when bonded to sound dentin. In sound primary den-

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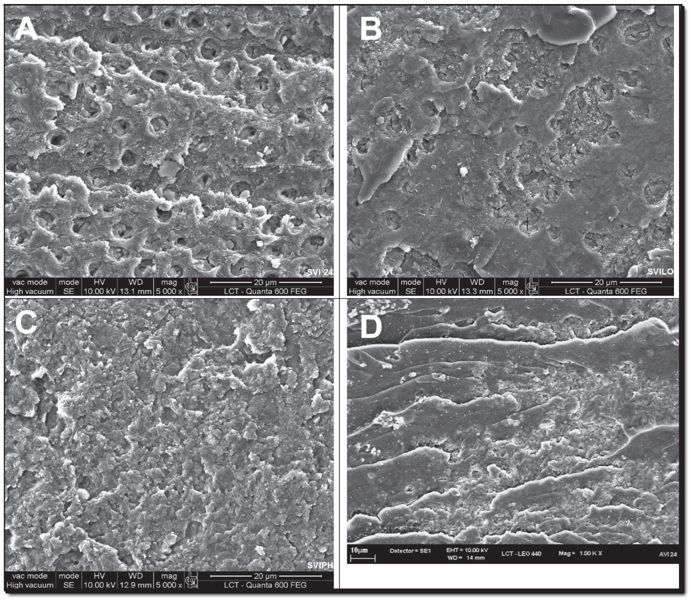

Fig. 3. SEM observations of the fractured primary dentin surface of specimens bonded with Vitremer. All presented failures at the interface with cement remnants over the surface characterizing mixed mode. A: (SOVI24) the effect of the Vitremer acidified primer can be seen by enlargement of tubule entrances. B (SOVILO) and C (SOVIPH): the close adaptation of RMGIC and the intertubular dentin after challenges. D (CAVI24): the same close adaptation pattern of RMGIC on simulated caries-affected dentin. Original magnification in the black bar.

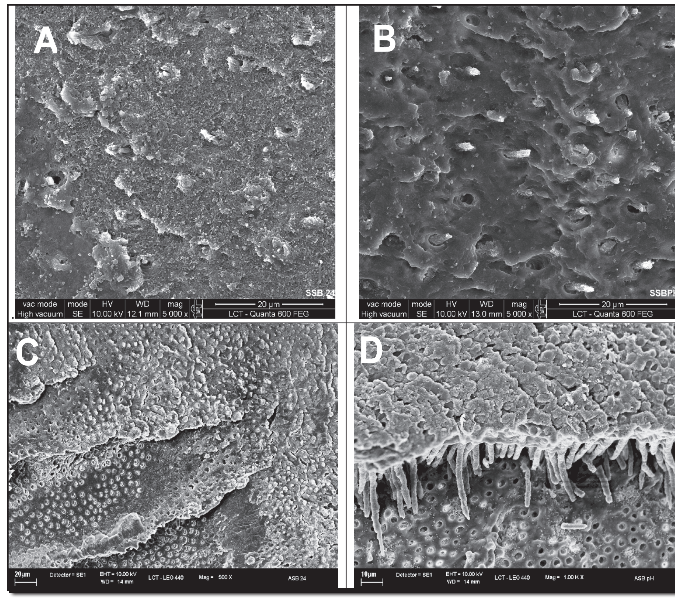

Fig. 4. SEM observations of the fractured primary dentin surface of specimens bonded with Adper Single Bond 2 that presented mixed failures at the interface. A (SOSB24): the effect of etching can be seen by intertubular dentin demineralization, collagen fibril exposure and enlargement of tubule entrances with resin tags inside; B (SOSBPH): resin tags within tubules. C (CASB24): simulated caries-affected dentin surface from which the adhesive layer was removed leaving some tubules filled with resin tags. D (CASBPH): it can be observed that the hybrid layer and resin tags were extracted from the caries-affected surface. Original magnification in the black bar.

tin, which is less mineralized than permanent dentin, thicker hybrid layer (25-30%), and the subsequent lack of complete penetration of adhesive resin into previously demineralized dentin, have been reported.[16] Caries-affected dentin Knoop hardness is only half that of normal dentin,[12,22] indicating that it has lost part of its mineral phase. The loss of mineral from intertubular dentin added to further mineral loss when it was treated with acidic bonding conditioners. This resulted in a deeper demineralized layer which, after penetration of the adhesive, allowed the formation of hybrid layers that are much thicker in caries-affected dentin when compared with normal dentin.[23-25] This increase in demineralized depth may contribute to the lower bond strengths to caries-affected dentin reported[13,23-25] since resin monomers may not penetrate as deeply as acid.[26,27] This might have been expressed in this study by the lower bond strengths recorded when using Adper Single Bond 2 in simulated caries-affected primary dentin, and by the great percentage of mixed failures with partial cohesive failure of the dentin. Fig. 4 depicts the simulated caries-affected surface of a debonded specimen in which the adhesive and hybrid layer were extracted from the underlying dentin. It should be highlighted that the breakdown of the dentin collagen cannot be reproduced by pH cycling, since *in vitro* evaluations cannot accomplish all factors involved in the carious process; but it provides a demineralized collagen-intact dentin, characteristic of the inner layer of caries-affected dentin.[28] Dentin tubules in caries-affected dentin are filled by mineral contents due to the dentin-pulp complex response (transparent layer).[29] This tubular occlusion may hamper adhesive infiltration preventing tag formation.[13,30] In the present study, however, numerous and lengthy resin tags in the simulated caries affected dentin groups were seen when the adhesive was applied (Fig. 4D), indicating that the *in vitro* model of pH cycling is capable of simulating chemical changes,[31] producing a demineralization layer, but cannot reproduce biological responses like tubular occlusion.

On the other hand, no significant differences in MTBS between simulated caries-affected and sound dentin were found when using Vitremer. Although, Choi *et al*[32] and Çehreli *et al*,[33] studying permanent and primary teeth respectively, found higher bond strength in sound than caries-affected dentin using RMGICs, Hosoya *et al*[34] found no significant difference when using a self etching primer adhesive system in caries-affected and sound primary tooth dentin. The use of weaker acids in self etching systems creates thinner hybrid layers,[35] providing plausible explanation for the prevention of an additional weakening of the already demineralized simulated caries-affected dentin.

This could explain why in the present study the RMGIC bond strength did not differ between simulated caries-affected and sound dentin. Figure 3 shows the close relationship between the RMGIC and both types of dentin. The Vitremer primer is composed of the methacrylate functional copolymer of polyacrylic and polyitaconic acids, HEMA, ethanol and photoinitiators. This primer is acidic in nature and its function is to modify the smear layer and adequately wet the tooth surfaces to facilitate adhesion of the glass-ionomer. This mechanism is similar to that of "mild" self etching adhesive systems; the basic difference is that glass-ionomers are self-etching through the use of a relatively high-molecular-weight (from 8,000-15,000) polycarboxylic acid-base polymer, while resin-based self etch adhesives make use of acidic low molecular-weight monomers.[2,36] This limits the GIC infiltration capacity, so that only shallow hybrid layers are formed.[2] The first null hypothesis tested was then partially rejected, since bond strength values of Single Bond 2 bonded to caries-affected dentin were significantly lower than they were when bonded to sound dentin, while there were no differences for Vitremer.

Mechanical stress by load-cycling did not affect bond strengths of either RMGIC or adhesive/composite restorations. No detrimental effect has been reported when using the first non-filled version of Single Bond and load cycling.[10] It may be that the internal porosities within RMGIC may have acted as ‘‘stress releasers’’, reducing stress transmission to the underlying dentin, similar to the situation reported for an adhesive system that presented internal voids within the adhesive layer.[37]

pH-cycling significantly influenced bond strength of adhesive/composite restorations to primary dentin, but did not affect RMGIC restorations. The *in vitro* model of pH cycling provides alternate periods of demineralization (DE) and remineralization (RE) simulating the caries process. It has been demonstrated that *in vitro* cariogenic challenge significantly reduces MTBS of adhesive/composite restorations.[10,11] This could be explained by the mineral loss at cavity margins that can reduce dentin strength and allow leakage along the biomaterial-tooth interface consequently weakening the union.[11] Fluoride-releasing restorative materials have been suggested to reduce demineralization around restorations preventing them from failing. Conventional and resin-modified GICs were shown to have the greatest ability to inhibit artificial caries lesions adjacent to restorations[38,39] due to their superior fluoride releasing capacity.[40] In the present study, the bond strength of RMGIC was not altered by the *in vitro* simulated caries challenge. Probably, the fluoride released by Vitremer inhibited enamel demineralization at the restoration margins, preventing bond degradation. Although RMGICs present a preventive effect against demineralization[34] and were shown to resist to caries challenge *in vitro*, it must be pointed out that secondary caries results from the continuity of the imbalance in the DE/RE process occurring in the oral environment. Thus, the second null hypothesis was partially rejected, because restorations were resistant to mechanical aging, but the adhesive/composite bond strength dropped after caries challenge.

Usually *in vitro* bond strength studies use flat surfaces to test the bonding effectiveness of adhesive dental materials. Clinically, however, restorative materials are inserted into cavities. Two major considerations should be taken into account when using cavities instead of flat surfaces: (1) tensions are created at the material/dentin interfaces due to high polymerization shrinkage stress. Such pre-stressed interfaces may be more susceptible to degradation;[41,42] and (2) the adjacent enamel bond may protect the biomaterial/dentin interface against degradation.[6,43] In the present study however, indirect exposure did not completely protect the adhesive-dentin interface against cariogenic challenge, corroborating other results[41] in which Single Bond presented a decrease in bond strength after 12 months of indirect exposure to water. Whereas, Vitremer bond strength was not influenced by pH cycling, which can be credited to the chemical adhesion to the surrounding walls of the cavity; also corroborating the findings of De Munck *et al*[41] that RMGIC bond strength was not altered after 12-month indirect exposure to water. Since caries-affected primary dentin substrates are difficult to gather because of the small dimensions and proximity of pulp, there is very limited information about the resistance to degradation and long-term bonding effectiveness to this clinically relevant substrate. Therefore, the artificial method for caries lesion formation allowed standardization and testing to be performed. Mechanical and cariogenic challenges enabled the *in vitro* evaluation of the durability of sound and caries-affected dentin bonds simulating an environment of caries activity within a relatively short term. Therefore, this study represents the first published attempt to evaluate the durability of bond to simulated primary caries-affected dentin after cariogenic challenge.

In the present study, the use of cavities, instead of flat dentin surfaces reduced the number of specimens expected for each tooth, which is already critical when testing primary teeth because of their reduced dimensions and the fragility of the substrate due to the thinner dentin thickness.[34,44] Despite this intrinsic fragility, pure cohesive failures in dentin were few and mainly found in groups with high bond strengths (*i.e*. Adper Single Bond 2 bonded to sound dentin). Mixed fracture modes were the most frequently identified in all groups, corroborating the literature.[4,10,11] A small number of cohesive failures in material were observed in Vitremer groups, differing with findings of Burrow *et al*[14] that found a higher number of cohesive failures within material when studying conventional and resin-modified versions of Fuji. Vitremer contains HEMA and it is a complex mixture of methacrylate copolymers, which may improve intrinsic material resistance and be responsible for the small number of cohesive failures in the present study. On the other hand, this increase in resin contents can impair the *in vitro* performance of Vitremer if compared with some other RMGICs.[45] An interesting aspect concerning failure patterns is that, in general, mixed failures in sound dentin groups were an association of an adhesive failure with a partial cohesive failure of the material, while mixed failures in simulated caries-affected groups were an association of an adhesive failure with a partial carious dentin cohesive failure. These results from morphologic evaluations and bond strength records suggest that the characteristics of the substrate directly impact the formed bond sites. The adhesion is possibly different when less mineral exists on the tooth surface.

In conclusion, the adhesive/composite bond strength decreases when bonded to simulated caries-affected dentin and when submitted to a cariogenic challenge. The RMGIC bond strength is not influenced by the dentin condition (sound or simulated caries-affected) either from mechanical stress or cariogenic challenge. The use of RMGIC is encouraged for pediatric patients with caries activity. Dentin matrix metalloproteinases are unlikely to be included in simulated, short-term aging studies. However, their role in the degradation process in bonded primary dentin should be further investigated.

a. Komet, Lemgo, Germany.
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Dr. Marquezan is a postgraduate student and Dr. Ciamponi is Professor, Department of Orthodontics and Pediatric Dentistry, University of São Paulo, USP, Brazil. Dr. Osorio and Dr. Toledano are Professors, Department of Dental Materials, School of Dentistry, University of Granada, Granada, Spain.

References

1. Van Meerbeek B, Perdigão J, Lambrechts P, Vanherle G. The clinical performance of adhesives. *J Dent* 1998;26:1-20.
2. De Munck J, Van Landuyt K, Peumans M, Poitevin A, Lambrechts P, Braem M, Van Meerbeek B. A critical review of the durability of adhesion to tooth tissue: Methods and results. *J Dent Res* 2005;84:118-132.
3. Hashimoto M, Ohno H, Kaga M, Endo K, Sano H, Oguchi H. *In vivo* degradation of resin-dentin bonds in humans over 1 to 3 years. *J Dent Res* 2000;79:1385-1391.
4. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Suzuki K, Lambrechts P. Four-year water degradation of a resin-modified glass-ionomer adhesive bonded to dentin. *Eur J Oral Sci* 2004;112:73–83.
5. Osorio R, Toledano M, Osorio E, Tay FR. Longevity of bonds made by composite and polyacid-modified resins to dentin using a dual-cured adhesive system. *Am J Dent* 2005;18:19-22.
6. Toledano M, Osorio R, Osorio E, Aguilera FS, Yamauti M, Pashley DH, Tay F. Durability of resin-dentin bonds: Effects of direct/indirect exposure and storage media. *Dent Mater* 2007;23:885-892.
7. Reis A, Grande RH, Oliveira GM, Lopes GC, Loguercio AD. A 2-year evaluation of moisture on microtensile bond strength and nanoleakage. *Dent Mater* 2007;23:862-870.
8. Osorio R, Toledano M, Osorio E, Aguilera FS, Tay FR. Effect of load cycling and *in vitro* degradation on resin-dentin bonds using a self-etching primer. *J Biomed Mater Res A* 2005;72:399-408.
9. Toledano M, Osorio R, Albaladejo A, Aguilera FS, Osorio E. Differential effect of *in vitro* degradation on resin-dentin bonds produced by self-etch *versus* total-etch adhesives. *J Biomed Mater Res A* 2006;77:128-135.
10. Rocha RO, Soares FZMS, Rodrigues Filho LE, Rodrigues CRMD. Influence of aging treatments on microtensile bond strength of adhesive systems to primary dentin. *J Dent Child* 2007;74:109-112.
11. Peris AR, Mitsui FH, Lobo MM, Bedran-Russo AK, Marchi GM. Adhesive systems and secondary caries formation: Assessment of dentin bond strength, caries lesions depth and fluoride release. *Dent Mater* 2007;23:308-316.
12. Ceballos L, Camejo DG, Victoria Fuentes M, Osorio R, Toledano M, Carvalho RM, Pashley DH. Microtensile bond strength of total-etch and self-etching adhesives to caries-affected dentine. *J Dent* 2003;31:469-477.
13. Erhardt MC, Toledano M, Osorio R, Pimenta LA. Histomorphologic characterization and bond strength evaluation of caries-affected dentin/ resin interfaces: Effects of long-term water exposure. *Dent Mater* 2008;24:786-798.
14. Burrow MF, Nopnakeepong U, Phrukkanon S. A comparison of microtensile bond strengths of several dentin bonding systems to primary and permanent dentin. *Dent Mater* 2002;18:239-245.
15. Uekusa S, Yamaguchi K, Miyazaki M, Tsubota K, Kurokawa H, Hosoya Y. Bonding efficacy of single-step self-etch systems to sound primary and permanent tooth dentin. *Oper Dent* 2006;31:569-576.
16. Nör JE, Feigal RJ, Dennison JB, Edwards CA. Dentin bonding: SEM comparison of the resin-dentin interface in primary and permanent teeth. *J Dent Res* 1996;75:1396-1403.
17. Hashimoto M, Ohno H, Endo K, Kaga M, Sano H, Oguchi H. The effect of hybrid layer thickness on bond strength: Demineralized dentin zone of the hybrid layer. *Dent Mater* 2000;16:406-411.
18. Sardella TN, de Castro FL, Sanabe ME, Hebling J. Shortening of primary dentin etching time and its implication on bond strength. *J Dent* 2005;33:355-362.
19. Yoshida Y, Van Meerbeek B, Nakayama Y, Snauwaert J, Hellemans L, Lambrechts P, Vanherle G, Wakasa K. Evidence of chemical bonding at biomaterial-hard tissue interfaces. *J Dent Res* 2000;79:709-714.
20. Mendes FM, Nicolau J. Utilization of laser fluorescence to monitor caries lesions development in primary teeth. *J Dent Child* 2004;71:139-142.
21. Erhardt MC, Rodrigues JA, Valentino TA, Ritter AV, Pimenta LA. *In vitro* μTBS of one-bottle adhesive systems: Sound *versus* artificially-created caries-affected dentin. *J Biomed Mater Res B Appl Biomater* 2007; 86B:181-187.
22. Nakajima M, Sano H, Zheng L, Tagami J, Pashley DH. Effect of moist *vs*. dry bonding to normal *vs*. caries-affected dentin with Scotchbond Multi-Purpose Plus. *J Dent Res* 1999;78:1298-1303.
23. Nakajima M, Ogata M, Okuda M, Tagami J, Sano H, Pashley DH. Bonding to caries-affected dentin using self-etching primers. *Am J Dent* 1999;12:309-314.
24. Yoshiyama M, Tay FR, Torii Y, Nishitani Y, Doi J, Itou K, Ciucchi B, Pashley DH. Resin adhesion to carious dentin. *Am J Dent* 2003;16:47-52.
25. Nakajima M, Kitasako Y, Okuda M, Foxton RM, Tagami J. Elemental distributions and microtensile bond strength of the adhesive interface to normal and caries-affected dentin. *J Biomed Mater Res B Appl Biomater* 2005;72:268-275.
26. Spencer P, Swafford JR. Unprotected protein at the dentin-adhesive interface. *Quintessence Int* 1999;30:501-507.
27. Sattabanasuk V, Shimada Y, Tagami J. Bonding of resin to artificially carious dentin. *J Adhes Dent* 2005;7:183-192.
28. Nakornchai S, Atsawasuwan P, Kitamura E, Surarit R, Yamauchi M. Partial biochemical characterisation of collagen in carious dentin of human primary teeth. *Arch Oral Biol* 2004;49:267-273.
29. Ogawa K, Yamashita Y, Ichijo T, Fusayama T. The ultrastructure and hardness of the transparent layer of human carious dentin. *J Dent Res* 1983;62:7-10.
30. Yoshiyama M, Tay FR, Doi J, Nishitani Y, Yamada T, Itou K, Carvalho RM, Nakajima M, Pashley DH. Bonding of self-etch and total-etch adhesives to carious dentin. *J Dent Res* 2002;81:556-560.
31. White DJ, Faller RV, Bowman WD. Demineralization and remineralization evaluation techniques. Added considerations. *J Dent Res* 1992;71:929-933.
32. Choi K, Oshida Y, Platt JA, Cochran MA, Matis BA, Yi K. Microtensile bond strength of glass ionomer cements to artificially created carious dentin. *Oper Dent* 2006;31:590-597.
33. Çehreli ZC, Akca T, Altay N. Bond strengths of polyacid-modified resin composites and a resin-modified glass-ionomer cement to primary dentin. *Am J Dent* 2003;16:47A-50A.
34. Hosoya Y, Kawada E, Ushigome T, Oda Y, Garcia-Godoy F. Micro-tensile bond strength of sound and caries-affected primary tooth dentin measured with original designed jig. *J Biomed Mater Res B Appl Biomater* 2006;77:241-248.
35. Osorio R, Toledano M, de Leonardi G, Tay F. Microleakage and interfacial morphology of self-etching adhesives in class V resin composite restorations. *J Biomed Mater Res B Appl Biomater* 2003;66:399-409.
36. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, Van Landuyt K, Lambrechts P, Vanherle G. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. *Oper Dent* 2003;28:215-235.
37. Monticelli F, Osorio R, Pisani-Proenca J, Toledano M. Resistance to degradation of resin-dentin bonds using a one-step HEMA-free adhesive. *J Dent* 2007;35:181-186.
38. Smales RJ, Gao W. *In vitro* caries inhibition at the enamel margins of glass ionomer restoratives developed for the ART approach. *J Dent* 2000;28:249-256.
39. Takeuti M, Marquezan M, Rocha RO, Rodrigues-Filho LE, Rodrigues CRMD. Inhibition of demineralization adjacent to tooth colored restorations in primary teeth after two *in vitro* challenges. *J Dent Child* 2007;74:209-214.
40. Sidhu SK, Watson TF. Resin-modified glass ionomer materials. A status report for the American Journal of Dentistry. *Am J Dent* 1995;8:59-67.
41. De Munck J, Shirai K, Yoshida Y, Inoue S, Van Landuyt K, Lambrechts P, Suzuki K, Shintani H, Van Meerbeek B. Effect of water storage on the bonding effectiveness of 6 adhesives to Class I cavity dentin. *Oper Dent* 2006;31:456-465.
42. Shirai K, De Munck J, Yoshida Y, Inoue S, Lambrechts P, Suzuki K, Shintani H, Van Meerbeek B. Effect of cavity configuration and aging on the bonding effectiveness of six adhesives to dentin. *Dent Mater* 2005;21:110-124.
43. De Munck J, Van Meerbeek B, Yoshida Y, Inoue S, Vargas M, Suzuki K, Lambrechts P, Vanherle G. Four-year water degradation of total-etch adhesives bonded to dentin. *J Dent Res* 2003;82:136-140.
44. Ramires-Romito AC, Reis A, Loguercio AD, de Goes MF, Grande RH. Micro-tensile bond strength of adhesive systems applied on occlusal primary enamel. *J Clin Pediatr Dent* 2004;28:333-338.
45. Toledano M, Osorio R, Osorio E, Fuentes V, Prati C, García-Godoy F. Sorption and solubility of resin-based restorative dental materials. *J Dent* 2003;31: 43-50.