Microtensile bond strength of etch-and-rinse and self-etch adhesives to artificially created carious dentin

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This article evaluates a pH-cycling model for simulation of caries-affected and caries-infected dentin (CAD and CID, respectively) surfaces, by comparing the bond strength of an etch-and-rinse and a self-etch adhesive system. For both adhesives, bonding to sound dentin (SD) showed that the microtensile bond strength (μTBS) values of SD, CAD, and CID were SD > CAD > CID ($P < 0.05$). Knoop microhardness number mean values followed the same trend. Adhesive systems were not able to totally penetrate into CAD and CID, forming more irregular resin-dentin interdiffusion zones and atypical resin tags than SD. The tested in vitro pH-cycling caries model allowed the evaluation of specific dentin substrate alterations in response to μTBS. The type of dentin and its histological structure played an important role in etch-and-rinse and self-etch bonding, as lower μTBS values were attained in CAD and CID.

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Carious dentin consists of a soft, outer layer of caries-infected dentin (CID), in which caries is actively in progress, and a relatively harder inner layer of intact, bacteria-free remineralizable caries-affected dentin (CAD).[1,2] In light of the minimally invasive strategy for remineralization and dentin permeability reduction, the primary aim in the excavation of carious dentin is to remove only the outer layer of highly infected, denatured CID.[3,4]

However, the inherent subjectivity in detection of the excavation boundary can result in clinically significant differences in the quality and quantity of dentin removed by different operators.[5] In operative treatment of carious lesions in dentin, the surface left at the end of cavity preparation will play a significant role in the bonding of the adhesive restorative materials.[6,7] Since it is very difficult for clinicians to verify the real dentin condition before placing a restoration, it is possible that adhesive procedures are being erroneously executed to substrates that are composed of sound dentin (SD), CAD, and CID in different parts of the same cavity.[8]

Despite significant enhancements in dentin bonding technology, a deeper knowledge of resin adhesion to the different dentin substrates would be helpful in developing more reliable and clinically long-lasting restorations. Therefore, it would also be useful to simulate CAD and CID conditions in vitro for evaluating bond strengths, since it might create a standardized substrate, permitting better comparisons among materials and techniques.[9,10]

This in vitro study aimed to evaluate the microtensile bond strength (μTBS) of an etch-and-rinse and a self-etching bonding agent to artificially created CAD and CID surfaces.

Materials and methods

Thirty-six bovine incisors had their buccal surfaces ground flat (180-600 grit) under running water to provide uniform dentin surfaces. Flattened teeth were randomly divided into 3 groups (n = 12). The teeth in Group 1 (SD) were immersed in artificial saliva at 37° C during the experimental period, the teeth in Group 2 were submitted to an artificial induction of CAD surfaces with a pH-cycling regimen, and the teeth in Group 3 were submitted to an artificial induction of CID surfaces with a pH-cycling regimen. Both CAD and CID pH regimens were developed modifying the models used by Shinkai et al and Wefel et al in preliminary pilot/experimental studies.[11,12] The experimental specimens (Groups 2 and 3) received 2 coats of an acid-resistant, fast-drying nail varnish and a layer of sticky wax, except for a 25 mm² window on the buccal dentin surface.

CAD specimens were submitted to 8 demineralization/hyper-remineralization cycles at 37° C. Each cycle included a 3-hour immersion in a demineralizing solution (156.25 mL/tooth, pH = 4.5) followed by a 45-hour immersion in a hyper-remineralizing solution (78.125 mL/tooth, pH = 7) that contained 10 ppm of fluoride. The demineralizing solution was renewed prior to the beginning of the fifth cycle, and the hyper-remineralizing solution was renewed prior to the beginning of each new cycle.[11,12] CID specimens were submitted to 4 demineralization/remineralization cycles at 37° C. Each cycle included a 2-hour immersion in a demineralizing solution (156.25 mL/tooth, pH = 4.5) followed by a 22-hour immersion in a remineralizing solution (78.125 mL/tooth, pH = 7). Both solutions were renewed prior to the beginning of each new cycle. The compositions of the pH solutions are listed in Table 1.

Teeth from each dentin substrate (SD, CAD, and CID) were rinsed and randomly re-assigned to 2 subgroups according to the adhesive system used (n = 6). Table 2 displays mode of application, components, and manufacturers of each adhesive system. A total-etch self-priming adhesive system, Single Bond (SB), and a self-etching adhesive, Clearfil SE Bond (SEB), were applied following manufacturers' instructions. Resin build-ups, each 6 mm in height, were constructed incrementally (2 mm) with a microhybrid

Table 1. Compositions of the solutions employed for the pH cycles.

Solution (pH)	Composition
Demineralizing (4.5)	2.2 mM calcium chloride phosphate ($CaCl_2$) 2.2 mM monosodium phosphate (NaH_2PO_4) 0.05 M sodium acetate 0.05 M acetic acid 1 ppm fluoride (NaF)
Remineralizing (7.0)	1.5 mM calcium chloride phosphate ($CaCl_2$) 0.9 mM monosodium phosphate (NaH_2PO_4) 0.15 M potassium chloride (KCl) 0.1 M Tris buffer
Hyper-remineralizing (7.0)	1.5 mM calcium chloride phosphate ($CaCl_2$) 0.9 mM monosodium phosphate (NaH_2PO_4) 0.15 M potassium chloride(KCl) 0.1 M Tris buffer 10 ppm fluoride (NaF)

Table 2. Bonding agents, compositions, and modes of application used in the experimental groups.

Bonding agent (manufacturer) and batch no.	Composition	Mode of application
Single Bond (3M ESPE) 1FH	HEMA, bysphenyl glycidyl methacrylate, dimethacrylates, amines, water, methacrylate-functional, copolymer of polyacrylic and polyitaconic acids, ethanol.	Etch for 15 seconds. Rinse with water spray for 10 seconds, leaving tooth moist. Apply 2 consecutive coats of the adhesive with a fully saturated brush tip. Dry gently for 5 seconds. Light-cure for 10 seconds.
Clearfil SE Bond (Kuraray America, Inc.) 352	Primer: 10-MDP, HEMA, hydrophilic dimethacrylate, di-camphorquinone, water, N,N-diethanol-p-toludine. Bond: 10-MDP, HEMA, hydrophobic dimethacrylate, N,N-diethanol-p-toluidine, di-camphorquinone, bis-phenol A diglycidylmethacrylate, silanated colloidal silica.	Apply primer for 20 seconds. Mild air stream. Apply bond. Dry with gentle air stream. Light-cure for 10 seconds.

Abbreviations: HEMA, 2-hydroxyethyl methacrylate; 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate.

resin composite, Filtek Z250 (3M ESPE). Each layer of the composite was light-cured for 40 seconds with a halogen light-curing unit (XL 2500, 3M ESPE). Bonded specimens were stored in distilled water for 24 hours at 37°C.

After storage, resin-dentin bonded specimens were sectioned into slabs and trimmed to an hourglass shape with a fine diamond bur, giving a cross-sectional area of 1 mm^2. Specimens (4 slabs per tooth) were attached to a prefabricated testing apparatus (Mortise & Tenon MTJIG) with a cyanoacrylate adhesive, and μTBS stressed (EMIC Equipamentos e Sistemas de Ensaio LTDA) at a crosshead speed of 0.5 mm/min. The cross-sectional area at the site of failure of the fractured specimens was measured to the nearest 0.01 mm^2 with a digital caliper (Mahr GmbH). Fractured specimens were examined in a stereomicroscope (Meiji Techno America) at 40X magnification to determine the mode of failure. Failure modes were classified as predominantly adhesive, mixed, or predominantly cohesive.

The quality of the artificially induced CAD and CID pH-cycling was confirmed by the means of subsurface dentin Knoop microhardness number (KHN) evaluations.[13] Following bond strength testing and fracture mode evaluation, the lateral aspects—which were the nonbonded surfaces—of the resin-dentin interface of 2 debonded specimens per tooth were polished with 1000 grit SiC abrasive papers and diamond pastes of 6, 3, and 1 μm (Arotec SA). Specimens were sonicated for 10 minutes to remove the debris in an Ultrasonic Cleaner (T.1440D, Odontobras). KHN indentations were performed 50 μm below the adhesive/dentin interface using a hardness tester (FM-1e, Future-Tech), under a load of 5 g for 15 seconds.[14] Each specimen received 3 indentations at 150 μm intervals. The average of the 3 indentations was used as the value for each specimen.

Additional specimens for each group were submitted to the pH-cycling regimen (as previously described) and prepared to be evaluated by scanning electron microscopy (SEM). Bonded resin-dentin interfaces were perpendicularly sectioned, gently decalcified (37% phosphoric acid for 10 seconds) and subsequently deproteinized (2% NaOCl solution for 1 minute). Samples were maintained in a desiccator for 48 hours, mounted in aluminum stubs, sputter-coated with gold, and observed under SEM (JSM 5600LV, JEOL Ltd.).

The μTBS data obtained were subjected to 2-way ANOVA ($P = 0.05$) and Tukey's post hoc test at a significance level of 5%. In a secondary, supportive analysis, KHN data of each adhesive system were separately subjected to Student's t-test in order to compare SD and CAD KHN values. The analyses were performed with SAS System 6.11 software (SAS Institute, Inc.).

Results

The μTBS values are summarized in Table 3. The adhesive system used was not a significant factor ($P = 0.227$), while the dentin type had a significant influence on bond strength ($P < 0.001$). The interaction between the independent variables was not significant ($P = 0.125$).

Table 3. Mean µTBS values (standard deviations) of tested adhesives bonded to sound dentin (SD), artificially-created caries-affected dentin (CAD), and caries-infected dentin (CID). Failure mode distribution is listed as percentages. For each adhesive/dentin combination, n = 35.

	SD					CAD					CID				
Dentin adhesive	MPa	A	M	C	KHN	MPa	A	M	C	KHN	MPa	A	M	C	KHN
Single Bond	31.0 (4.3)[a]	33	67	0	65.4 (5.1)[1]	24.0 (5.9)[b]	42	53	5	37.3 (8.4)[2]	13.6 (5.4)[c]	57	37	6	12.8 (3.6)[3]
Clearfil SE Bond	28.0 (6.0)[a]	40	59	1	63.8 (5.6)[1]	19.3 (6.7)[b]	45	50	5	28.9 (6.6)[2]	15.8 (4.6)[c]	51	42	7	11.6 (3.8)[3]

Abbreviations: A, predominantly adhesive; M, mixed; C, cohesive in dentin; KHN, Knoop hardness number. Standard deviations (SD) were measured for MPa and KHN values. Statistically significant differences are expressed by different lowercase letters ($P < 0.05$). Different numbers indicate statistically significant differences for KHN values between dentin substrates ($P < 0.05$).

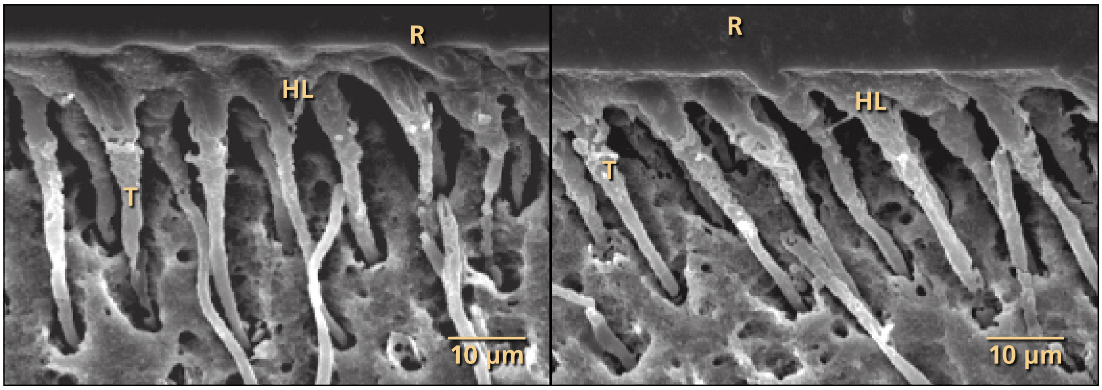

Fig. 1. Scanning electron microscopy (SEM) images of resin in sound dentin polished cross sections *Left.* Bonded with Single Bond etch-and-rinse (SB) system. *Right.* Bonded with Clearfil SE Bond self-etch (SEB) system. The SB system tends to model a thicker hybrid layer than the SEB system. For both adhesives, a regular formation of hybrid layer and resin tags is observed. (2000X). Abbreviations: R, resin composite; T, resin tags; HL, hybrid layer.

Post hoc comparisons revealed that the bonding of both adhesives to CAD generated significantly lower mean µTBS values than those obtained with SD. CID presented the lowest µTBS values. No significant differences between the SB and the SEB systems were detected in the dentin substrates.

An evaluation of KHN followed the same trend as the µTBS results: SD produced significantly higher KHN values than CAD which, in turn, were significantly higher than those obtained in CID. The percentage of adhesive failures was higher when bond strength decreased. Dentin cohesive failures were frequently observed in CAD and CID interfaces.

SEM examinations of the resin-dentin interfaces are shown in Figures 1-3. When bonded to SD, the SB and SEB systems were able to penetrate into dentin forming an extensive resin-dentin interdiffusion zone. When the SB was used, 4-5 µm-thick hybrid layers were created, while approximately 1 µm-thick hybrid layers were produced by the SEB system. Relatively thick and irregular hybrid layers were produced in CAD and CID for both adhesive systems. Mineral casts in CAD were only partially removed from the tubules, allowing the creation of spongy resin tags. In CID, the intertubular dentin matrix appeared to be overetched by the conditioning agents, as poor adhesive resin penetration into the demineralized dentin zone was observed. Compared to SD or CAD, the hybrid layer was more porous, with collapsed resin tags comprising residual dentin chips and denatured collagen.

Discussion

Laboratory investigations that evaluate dentin bond strength are normally conducted on noncarious dentin substrates, which do not necessarily represent the most common dentin type encountered during restorative procedures in clinics. Therefore, this study aimed to evaluate the µTBS of etch-and-rinse and self-etching adhesive systems to artificially created CAD and CID surfaces. Bonding to SD with tested adhesive systems in this study produced significantly higher µTBS values than those with artificially created CAD and CID.

The type of dentin retained following caries excavation can affect the results of bond strength tests.[5,15] Thus, there are several problems that may affect bonding efficacy when etch-and-rinse and self-etch adhesive systems are used on CAD and

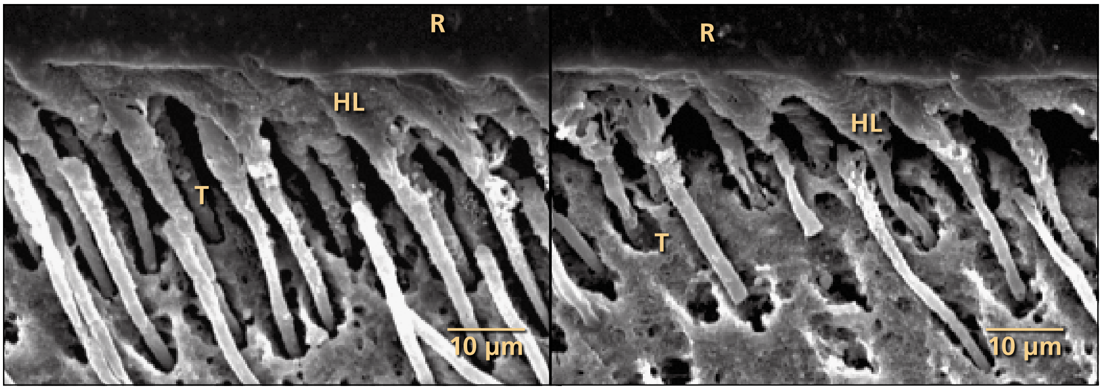

Fig. 2. SEM images of bonded resin-dentin interfaces in artificially induced caries-affected dentin. *Left.* Bonded with SB system. *Right.* Bonded with SEB system. Bonded interfaces presented irregularly shaped resin tags and thicker hybrid layers than those observed for normal dentin (2000X). Abbreviations: R, resin composite; T, resin tags; HL, hybrid layer.

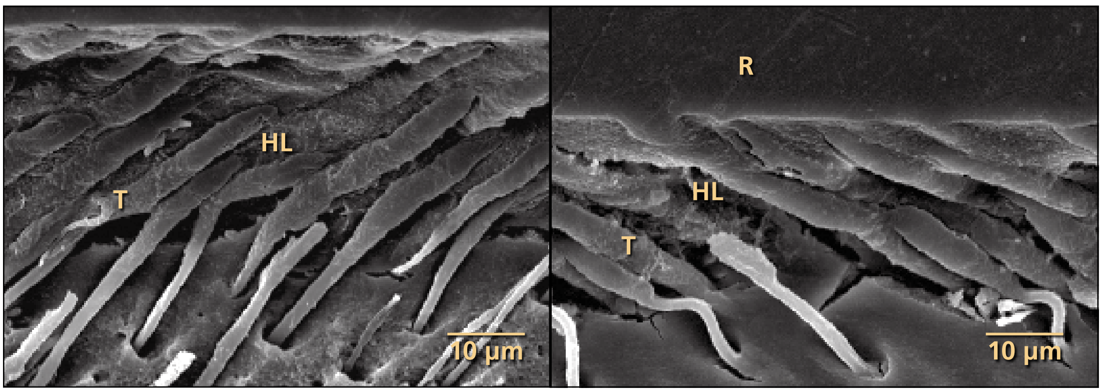

Fig. 3. SEM images of artificially induced caries-infected dentin bonded interfaces. *Left.* Bonded with SB system. *Right.* Bonded with SEB system. Dentin matrix was completely dissolved from tubules close to the surface, with the resultant spaces poorly filled with the adhesive resins. (2000X). Abbreviations: R, resin composite; T, resin tags; HL, hybrid layer.

CID.[8,16,17] There were no significant differences in this study between the SB and SEB systems in µTBS values to the respective dentin substrates. The lower bond strengths attained when bonding to CAD compared to SD were probably related to the lack of resin tag formation due to the presence of acid-resistant whitlockite mineral casts within the tubules, and decreases in the modulus of elasticity and in dentin cohesive strength.[8,17-19] Regarding CID, a complete loss of its mineral phase and denaturation of the collagen matrix may have led to a decrease in the chemical bonding between carboxylic or phosphate derivatives of the resin monomers.[16-18] As a consequence, µTBS values attained in CAD and CID in this study were not unexpected, and corroborate previously published investigations that used natural carious dentin lesions.[13,14,17] Therefore, the proposed pH cycling method applied in this study was effective in producing different dentin substrates.

Since the accomplishment of CAD and CID surfaces has been performed in vitro by means of a pH-cycling regimen, great care was taken to characterize the bonded substrate.[11,20,21] Bovine teeth were used instead of human teeth, as it is easier to control these teeth in terms of age, sclerosis, and the amount of wear of the substrate.[21-23] According to Schilke et al, bovine coronal dentin is considered a suitable substitute for human molar dentin in adhesion studies.[24] The mineral phase of experimental dentin samples was remodeled by dynamic sequences of demineralization/hyper-remineralization for CAD or by sequences of demineralization/remineralization for CID, aiming to simulate the 2 layers of carious dentin in vivo.[13,25] In fact, artificially created CAD specimens had lost half of their hardness, but their tubules were so full of mineral material that resin tag formation was hindered when compared to SD. The same trend was observed for CID. It is likely that the loosely arranged mineral casts in the dentin surface permitted a deeper etching of intertubular dentin.[16] The presence of a denatured collagen matrix may have prevented proper resin infiltration during bonding, lowering final bond strengths.[8,17]

These changes in the permeability and acid-resistance of artificially created dentin samples after the different pH-cycling regimens were morphologically evident in the SEM images. Analysis of CAD interfaces showed that both adhesive systems were not able to perfectly permeate dentin, forming thicker and more irregular hybrid layers than SD. An easier diffusion of acidic conditioners in CAD was a result of an increased porosity in the intertubular dentin. This increased porosity did not imply a correct infiltration of the adhesive monomers: resin penetration was hampered by the presence of acid-resistant mineral casts in the dentinal tubules.[13,14,17,19] A more pronounced variation was found in CID interfaces. Adhesive resins penetrated through the loose, degraded dentin matrix to a higher depth than CAD, forming resin tags that were fused together with residual mineral deposits and denatured collagen. Tubule fusion due to loss of peritubular dentin and enlargement of lateral branches has been previously described.[8,26]

Significant differences were observed between all dentin types in terms of microhardness evaluations. The lowest KHN values were obtained for artificially induced CAD and CID, which are similar to those reported by other authors.[14,27,28] The lower KHN values in CID and CAD are related to a smaller number of larger apatite crystals that no longer fit properly into inter- and intrafibrillar spaces in a normal collagen matrix, as well as to a lower cohesive strength of the disorganized collagen matrix.[8,10,18] Therefore, the attained differences in microtensile and microhardness evaluations for these dentin conditions showed the consistency of the proposed dynamic pH-cycling as a method to artificially obtain CAD and CID.

The findings of this study cannot be compared with previous in vitro studies that performed bonding to artificial CAD and CID lesions.[9,11,20,28,29] The relative effectiveness of the hyper-remineralizing solution (used for CAD simulation in the present study) is partially a result of its fluoride content. Fluoride at 10 ppm may have produced a consistent growth of very small apatite crystallites in the gap region of collagen fibrils to form intra- and interfibrillar mineralization.[20,30,31] These precipitations arose from the reaction of fluoride, calcium, and phosphate ions contained in the demineralized dentin layer.[31] To obtain artificial CAD, surfaces were demineralized 8 times for 3 hours but remineralized for 8 cycles of 45 hours each. This means that a much longer remineralizing time was used. The main difference of the proposed pH-cycling method is that in this study, a dynamic process of de-hyperemineralization for CAD or de-remineralization for CID was performed repeatedly, in order to reproduce more closely the real situation that takes place in the oral environment. This technique provided a much better simulation of carious dentin than that of previously published literature.

Many factors present in natural CAD and CID lesions may play an important role in the final bonding performance of resin-based materials. During the caries process, the organic matrix is exposed to breakdown by bacterial- and host-derived enzymes, such as the matrix metalloproteinases present within the dentin and derived from saliva.[32,33] Moreover, the Maillard reaction between sugar and proteins that occurs during the caries process induces the addition of metabolites and glycoxidation products to the carious dentin matrix collagen, modifying the dentin organic matrix.[34] Clinically, natural CID contains a necrotic collagen matrix and collapsed dentin tubules that are highly infected by bacteria that induce cytokine reactions, which may elicit a chronic pulpal inflammation.[27] Although the use of natural CAD and CID is desirable, the in vitro method attained results with the proposed pH-cycling model, providing data that suggests that manipulation of parameters involved in de- and re-mineralization events has a significant effect on the behavior of the dentin surface, particularly in the bonding phenomenon.

Conclusion

Based on the low bond strengths observed in CAD and CID, it could be suggested that there is a need for development of further bonding alternatives in order to improve the adhesion of resin composites to these different substrates. However, it must be emphasized that if the cavity to be restored presents enamel or even SD margins, such a problem might not be so severe.[35,36] The clinical decision of leaving remaining CID underneath bonded restorations is still a subject of substantial debate and deserves further investigation. The findings of the present study highlight the possibility of this pH-cycling method to be used as a standardized in vitro model for the simulation of pathologically altered dentin surfaces for bonding evaluations. Bond strengths of etch-and-rinse and self-etch adhesive systems to artificially-induced CAD and CID were significantly lower than those attained in SD. Regardless of the substrate, both adhesive systems presented similar bond strengths.

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Manufacturers

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