Dentin Bonding on Different Walls of a Class II Preparation

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Purpose: To evaluate the bond strength on different cavity walls of Class II preparations. Different bonding systems and the effect of thermomechanical cycling were investigated.

Materials and Methods: Human third molars received MOD preparations with dentin margins. Teeth were randomly assigned to 18 groups (n = 5) according to the combination of cavity wall (axial, occlusal, and gingival), bonding system (Single Bond Plus, Clearfil SE Bond, and Adper Prompt) and the occurrence of thermomechanical cycling. Restorations were concluded with Filtek Z250 composite. Specimens were sectioned according to the respective cavity wall (4 slabs/restoration), and the adhesive interface was trimmed to an hourglass shape (1 mm^2). Slabs were tested under tension, and failure mode was observed. Bond strength data were analyzed with three-way ANOVA/Tukey's test.

Results: Single Bond Plus and Clearfil SE Bond performed similarly under most experimental conditions. Single Bond Plus presented similar bond strength on the three cavity walls, regardless of the aging condition. Clearfil SE Bond exhibited significant differences among cavity walls: the occlusal wall showed higher means in both aging conditions. Non-aged gingival walls and aged axial and gingival walls yielded lower means. Non-aged Adper Prompt produced similar bond strengths on the three cavity walls. After thermomechanical cycling, the gingival wall showed lower means.

Conclusion: The effect of cavity walls was dependent on the bonding system and thermomechanical cycling. Adper Prompt demonstrated bond strengths lower than Single Bond Plus or Clearfil SE Bond under most experimental conditions.

Keywords: dental adhesion, class II restoration, fatigue, thermal cycling, bond strength testing.

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Early failures of adhesive restorations are common events, particularly when composite restorations are placed in posterior teeth and margins of cavity preparations are located in dentin/cementum.[9,18] Dentin bonding might be affected by factors such as localization and depth of cavity walls, and orientation and density of dentinal tubules.[4,6,11,12,14,16,18,21,30] Complex three-dimensional cavities present different dentinal sites for adhesion.[11,12] Thus, an effective adhesive system must present an adequate and uniform performance at the different walls of a cavity preparation.

The basic mechanism of bonding to enamel and dentin involves replacement of minerals removed from the hard dental tissue by resin monomers which, upon setting, become micromechanically interlocked in the porosities created.[15,28] Contemporary adhesive systems present distinct approaches for dentin bonding.[25] Etch-and-rinse systems use previous acid etching of the dentin to remove the smear layer, demineralize dentin, and expose a scaffold of collagen fibrils that is nearly totally depleted of hydroxyapatite.[27,28] The exposed collagen fibrils function as a microretentive network for micromechanical interlocking of resin polymers.[27] The acid-etching step is followed by a priming step and adhesive monomer application. Simplified etch-and-rinse sys-

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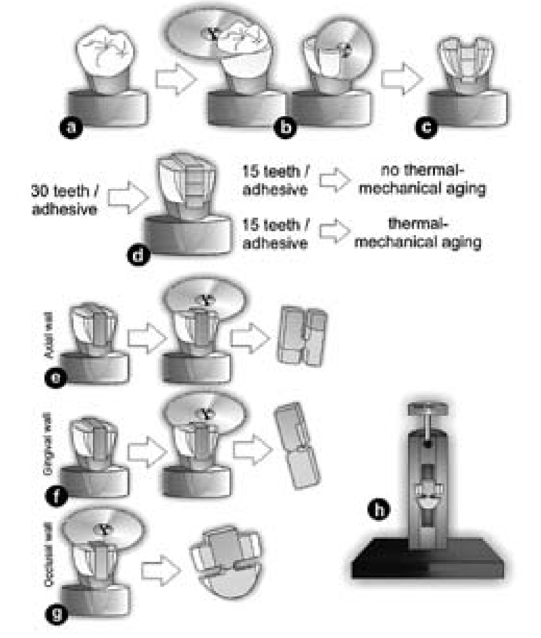

Fig 1 Schematic study design. (a) Human third molar embedded in polystyrene resin; (b) section of enamel occlusal and proximal surfaces; (c) MOD cavity preparation; (d) restorative procedure; (e) slabs from axial wall; (f) slabs from gingival wall; (g) slabs from occlusal wall, and (h) microtensile testing.

tems include the primer and the adhesive monomers in one solution.[15]

Self-etching systems present a bonding mechanism different from that of etch and rinse systems, characterized by simultaneous conditioning, demineralizing, and infiltrating tooth substrates.[27,28,31] Self-etching systems are classified as "two-step" or "one-step". One-step self-etching systems combine the etching and the bonding procedures and are often referred as "all-in-one" adhesives. Self-etching systems have become popular, since they eliminate the clinical assessment of residual dentin moisture. The elimination of the rinsing step reduces both the application time and technique sensitivity.[15,27,31] These systems might also be less sensitive to the regional variability of dentin.[6,14]

Long-term clinical trials of adhesive restorative materials are the most efficient methods of evaluating adhesion durability.[10] However, the drawbacks of such studies are high cost and length of time. These characteristics make them unable to follow the fast evolution of adhesive materials.[2] In order to overcome these limitations, methods were developed to simulate some conditions of the oral environment in vitro, such as thermal and mechanical cycling.[3,5,8] Therefore, restorative materials can be tested under clinically simulated conditions.

The purpose of the present study was to evaluate the bond strength of three adhesive systems (an etch and rinse system, a two-step self-etching system, and a one-step self-etching system) on different walls of Class II preparations, as well as the effect of thermomechanical cycling on dentin bonding. The null hypotheses tested were:

1. bond strength of adhesive systems is not affected by the cavity wall location (axial, occlusal, or gingival);
2. thermal and mechanical stresses do not decrease bond strengths.

MATERIALS AND METHODS

Specimen Preparation

Figure 1 presents the methods of the present study.

As approved by the Ethics Commission of Piracicaba Dental School (# 039/2005), 90 recently extracted noncarious human third molars were stored in 0.1% thymol solution and used within 6 months. Roots were embedded in cold-curing polystyrene resin cylinders (Cromex; Piracicaba, SP, Brazil) (Fig 1a). Occlusal surfaces were transversally sectioned to expose the dentinal surface 4 mm above the cementoenamel junction (CEJ) using a low-speed diamond saw under water cooling (Isomet 1000, Buehler; Lake Bluff, IL, USA). Proximal surfaces (mesial and distal) were also sectioned in the buccal-lingual direction to remove the enamel, so that the proximal margins of Class II preparations were just surrounded by dentin (Fig 1b).

To produce standardized Class II cavity preparations (mesio-occlusal-distal/MOD), a new coarse diamond bur (3135, KG Sorensen; Barueri, SP, Brazil) was used every five preparations, operated in a high-speed handpiece under profuse water cooling (Fig 1c). Preparations were finished with superfine diamond burs (3135FF, KG Sorensen). Inner angles of the cavities were rounded and the margins were not beveled. Cavity dimensions were standardized to the following mean (standard deviation/SD):[3] occlusal box – width 4.0 (0.3) mm, length 5.6 (1.1) mm, height 1.9 (0.2) mm; axial wall – width 4.0 (0.3) mm, height 3.0 (0.3) mm; gingival wall – width 4.0 (0.3) mm, depth 1.4 (0.4) mm. Both proximal boxes ended 1.0 mm below the CEJ, and all margins of the preparation were located in dentin tissue.

Preparations were randomly assigned to three groups (n = 30) according to the adhesive system used: etch and rinse system (Single Bond Plus); two-step self-etching system (Clearfil SE Bond); one-step self-etching system (Adper Prompt). Adhesive systems were applied following manufacturers' instructions (Table 1).

After the bonding procedure, Filtek Z250 composite (3M ESPE; St. Paul, MN, USA) was applied in 2.0 mm horizontal increments and light cured for 20 s through the occlusal surface (Fig 1d). Proximal boxes were filled with three increments. Mesial and distal increments were inserted simultaneously, so both increments received the same amount of light energy. Subsequently, the occlusal box was filled with the composite. A 1-mm overfill was left on the occlusal surface to enable mechanical loading over the restorative material only.[1] During all restorative procedures, the light output of the light-curing unit (Optilux 501, Sybron Kerr; Danbury, CT, USA) was measured and found to be greater than 660 mW/cm^2. After restorative procedures, specimens were

Table 1 Adhesive systems, composition, and mode of application

Product name, Lot #	Composition	Mode of application
Single Bond Plus 3M ESPE; St Paul, MN, USA Lot: 4BU	Etchant: 35% Phosphoric acid bis-GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, methacrylate functional copolymer of polyacrylic and polyitaconic acids, 5 nm colloidal filler (10% weight)	Apply etchant to dentin. Wait 15 s, rinse for 10 s, blot excess water using a cotton pellet. Apply 2 consecutive coats of adhesive for 15 s with gentle agitation using a fully saturated applicator. Gently air thin for 5s to evaporate solvent. Light cure for 10 s.
Clearfil SE Bond Kuraray; Okayama, Japan SE Primer Lot: 537A SE Bond Lot: 756A	Primer: 10-MDP, HEMA, dimethacrylate, photoinitiator, water Bond: 10-MDP, HEMA, bis-GMA, dimethacrylate, microfiller, photoinitiator	Apply SE Primer and leave undisturbed for 20 s. Dry thoroughly with mild air flow to evaporate solvent. Apply SE Bond, air blow gently. Light cure for 10 s.
Adper Prompt 3M ESPE Lot: 170127	Solution-A: bis-GMA, methacrylated phosphoric esters, initiators based on camphorquinone, stabilizers Solution-B: water, HEMA, polyalkenoic acid, stabilizers	Dispense one drop from Solution-A and one drop from Solution-B into the disposable mixing well. Mix the solution aggressively for 5 s. Apply adhesive to the entire surface of the cavity, rubbing in the solution with moderate finger pressure for 15 s. Use a gentle stream of air to thoroughly dry the adhesive to a thin film. Light cure for 10 s.

Bis-GMA = bisphenol A diglycidyl ether dimethacrylate; HEMA = 2-hydroxyethyl methacrylate; 10-MDP = 10-methacryloxydecyl dihydrogen phosphate.

stored in distilled water at 37°C for 24 h. After this period, they were finished and polished with Al_2O_3 abrasive disks (Sof-Lex Pop-On, 3M ESPE).

Thermal and Mechanical Cycles

After restorative procedures, 15 specimens from each experimental group were submitted to thermal and mechanical cycling. Thermal stresses were induced in a thermocycling machine (MCT2-AMM2; São Paulo, SP, Brazil). Teeth were submitted to 2000 thermocycles in water between 5°C (± 2) and 55°C (± 2) with a 1 min dwell time at each temperature and transfer time of 5 s.

After thermal cycling, teeth were placed in the mechanical loading machine (ER-FOP 10, Erios Internacional; São Paulo, SP, Brazil). The loading device had a block of bovine enamel (8 x 5 x 2 mm) on its extremity, and this block was placed in contact with the entire occlusal surface of the restoration. The loading device delivered an intermittent axial force of 50 N with 2 Hz (cycles/s), totaling 100,000 cycles in water at 37°C.

Microtensile Bonding Test (μTBS)

For microtensile evaluation, specimens were sectioned with a low-speed diamond saw under constant water coolant. In each subgroup (n = 5), a specific sectioning was performed according to the cavity wall to be evaluated.

- Axial and gingival wall specimens (Figs 1e and 1f): restorations were sectioned in the mesiodistal direction into 1-mm-thick slabs (n = 2 per proximal box). Then, a perpendicular section divided the restoration in two parts, resulting in four slabs per restoration.
- Occlusal wall specimens (Fig 1g): each restoration was sectioned in the labial-lingual direction into 1-mm-thick slabs (n = 4 per tooth).

Each slab was trimmed and shaped to form a gentle curve with a narrowest portion at the specific adhesive interface, using a super-fine diamond bur (1090FF, KG Sorensen; Barueri, SP, Brazil) in a high-speed handpiece under constant water cooling.[20] These procedures yielded bonded surface areas of approximately 1.0 mm^2. The number of slabs prematurely debonded during specimen preparation was recorded; pre-test failures were not included in the statistical analysis. The distance between the adhesive interface and the nearest portion of pulp chamber was designated as the remaining dentin thickness (RDT).

Specimens were attached to the flat grips of a μTBS testing device with cyanoacrylate glue (Super Bonder, Henckel Loctite; Itapevi, SP, Brazil) and tested under tension in a Universal Testing Machine (EMIC DL 500; São José dos Pinhais, SC, Brazil) at a crosshead speed of 0.5 mm/min until failure (Fig 1h). Means and standard deviations were calculated and expressed in MPa. The bond strength of each tooth was obtained from the arithmetical mean of its four slabs. Bond strength data were analyzed with three-way ANOVA (adhesive system x cavity wall x thermomechanical cycling). All

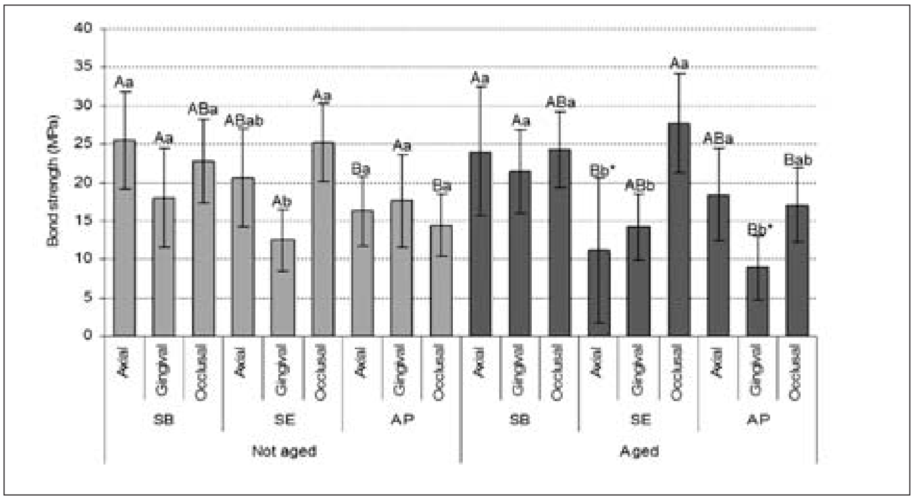

Fig 2 Mean values (standard deviations) of microtensile bond strength (MPa).
Same letters are not statistically different (3-way ANOVA/Tukey's test, $\alpha = 0.05$). Upper case letters compare adhesive systems within cavity wall/aging condition. Lower case letters compare cavity walls within adhesive system/aging condition. * represents differences between groups aged and not aged. Coefficient of variation = 29.86%.

Table 2 Distribution of failure modes

Aging condition	Adhesive system	Cavity wall		
		Axial wall (%)	Gingival wall (%)	Occlusal wall (%)
No thermomechanical cycling	Single Bond Plus	I (30); A (10); M (60) npf = 1	I (55); M (45) npf = 8	I (10); M (90) npf = 3
	Clearfil SE Bond	I (25); M (75) npf = 0	I (37); M (63) npf = 6	I (56); M (44) npf = 1
	Adper Prompt	M (100) npf = 6	I (100) npf = 15	M (100) npf = 11
Thermomechanical cycling	Single Bond Plus	I (17); A (17); M (66) npf = 3	I (18); M (55); C (27) npf = 3	M (100) npf = 2
	Clearfil SE Bond	I (67); M (33) npf = 3	I (29); M (71) npf = 9	I (50); M (50) npf = 2
	Adper Prompt	I (12); M (88) npf = 5	M (100) npf = 13	I (10); M (90) npf = 9

npf = number of premature failures (total number of slabs per group = 24). I = interfacial failures; A = cohesive failure within adhesive resin; C = cohesive failure within composite; D = cohesive failure within dentin; M = mixed failure.

possible interactions were included in the model. Multiple pairwise comparisons were performed with Tukey's test. Statistical analysis was carried out in SAS 8.0 (SAS Institute; Cary, NC, USA) at a significance level of 5%.

Failure Mode

After μTBS testing, both sides of fractured specimens were dried, mounted on aluminum stubs, gold sputter coated (Denton Vacuum Desk II, Denton Vacuum LLC; Moorestown, NJ, USA), and observed with a scanning electron microscope (JSM 5600 LV, JEOL; Tokyo, Japan) to evaluate the fracture mode. Failure patterns were classified into one of five categories: I – interfacial failure (between the top and bottom of the hybrid layer); A – cohesive failure within adhesive resin; C – cohesive failure within composite; D – cohesive failure within dentin; and M – mixed failure (association of two or more failures).

RESULTS

Microtensile Bond Strength

Figure 2 shows bond strength values and standard deviations. Three-way ANOVA/Tukey's test showed a significant interaction ($p = 0.03$) among main factors: adhesive system, cavity wall, and thermomechanical cycling.

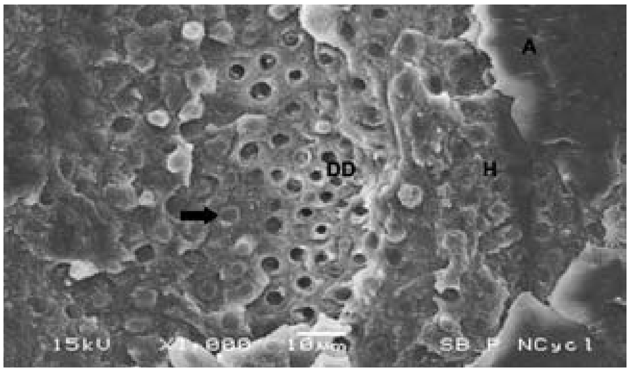

Fig 3 Scanning electron micrograph of fractured surface classified as a mixture of interfacial failure and cohesive failure within adhesive resin (I/A). Dentin side of a specimen from occlusal wall bonded with Single Bond Plus and not aged. Adhesive resin (A), hybrid layer (H), demineralized dentin (DD). Tubules filled with the adhesive resin can be observed (arrow). Magnification: 1000X.

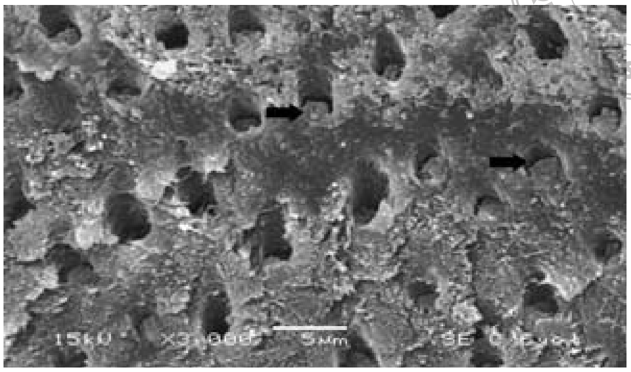

Fig 4 Scanning electron micrograph of fractured surface classified as interfacial failure (I). Dentin side of a specimen from gingival wall bonded with Clearfil SE Bond and aged. Tubules partially filled can be observed (arrow). Magnification: 1000X.

The etch-and-rinse system Single Bond Plus performed similarly on the three cavity walls, regardless of the aging condition. The two-step self-etching system Clearfil SE Bond exhibited significant differences among cavity walls. When Clearfil SE Bond was not aged, the highest bond strength was observed on the occlusal wall and the lowest on the gingival wall. After thermomechanical cycling, Clearfil SE Bond presented higher bond strengths on the occlusal wall than on the axial and gingival walls. The one-step self-etching system Adper Prompt presented bond strength means significantly lower than Single Bond Plus on the non-aged axial wall and the aged gingival wall. The bond strength of Adper Prompt was significantly lower than that of Clearfil SE Bond on the occlusal wall, under both aging conditions. Non-aged Adper Prompt presented similar bond strengths on the three cavity walls. However, after thermomechanical cycling, the gingival wall showed lower means.

The effect of thermomechanical cycling was statistically significant for the two-step self-etching system on the axial wall and for the one-step self-etching system on the gingival wall. Both systems/cavity walls presented lower means after thermomechanical cycling.

The number of premature failures is summarized in Table 2. Those failures occurred during the preparation of specimens, mostly during hourglass shaping. The remaining dentin thickness of the cavity walls indicated that they were all located in deep dentin. The RDT values found were (mean/SD): axial wall (1.60 mm/0.6), gingival wall (1.36 mm/0.34) and occlusal wall (2.21 mm/0.39).

Failure Mode

The distribution of failure modes is summarized in Table 2. Fracture modes were mainly classified as a mixture of more than one type of failure (M), irrespective of the experimental condition (Fig 3). Failures restricted to the bonded interface (I) were also frequently observed (Fig 4). These failures occurred at the bottom of the hybrid layer leaving the dentinal tubules partially occluded. No cohesive failures in dentin were found.

DISCUSSION

In the present study, bonding systems of different composition were investigated under a clinically simulated condition. Class II preparations were performed in human molars and the dentin bonding efficacy was tested at various walls of MOD preparations (occlusal, axial, and gingival). Restorations were also challenged under thermal and mechanical stresses.

An ideal bonding system should be effective regardless of the regional characteristics of the tooth structure.[12] However, it appears that the variability of dentin structure can significantly affect bonding system performance,[11,12,16,18] and may be one of the reasons bond strengths are not uniform inside a cavity.[11,12,21] Previous studies investigated the effect of dentin variability on the bond strength obtained on flat dentin surfaces, thus eliminating the effect of the cavity configuration factor created by cavity walls.[6,11,21] Results observed in those studies are very useful to demonstrate the bonding performance in different dentin substrates. However, the bond strength obtained on flat surfaces might overestimate the values reached in a clinical situation.[3,12] In contrast, laboratory research using cavity configurations can reproduce the shape of typical in vivo preparations, and the way and amount of light energy received by the restoration.[17]

The orientation of a dentinal tubule on a cavity wall depends on its location.[4,7] In Class II preparations, tubule orientation can be perpendicular to the surface (occlusal and axial walls) or parallel/oblique to the surface (gingival wall).[7] The three cavity walls investigated in the present study were located in deep dentin,[14] which is a substrate generally associated with lower bond strengths.[29,30] The adhesion to deep dentin might be negatively affected by the difference in the amount of solid dentin available for bonding.[29] In deeper cavities, several factors – eg, configuration factor, polymerization shrinkage, increased permeability of dentin, failure of primer solvent to evaporate completely, and inability of air drying to remove excess water – may combine to result in lower bond strengths.[18,30] Furthermore, in a deep cavity, light intensity is attenuated, decreasing the depth of cure and impairing adequate monomer conversion on the bottom surface of a restoration.[26]

Bonding systems evaluated in the present study present different composition and use different approaches for dentin bonding. Single Bond Plus is a nanofilled etch-and-rinse system, while Clearfil SE Bond and Adper Prompt are self-etching systems classified as two-step and one-step, respectively. In the present study, Single Bond Plus and Clearfil SE Bond demonstrated similar bond strengths, except on the axial wall under aging conditions, where the two-step self-etching system presented lower bond strengths. Previous studies reported similar dentin bond strengths between Single Bond Plus and Clearfil SE Bond,[13] and also between Clearfil SE Bond and nonfilled Single Bond.[19] On the other hand, the one-step self-etching system Adper Prompt presented lower bond strength in most conditions tested. Relatively low bond strengths obtained with all-in-one systems is a frequently observed finding.[22,25,28] Reasons for this lower bonding performance to dentin as compared to more conventional etch-and-rinse and two-step self-etching systems include: inhibition of polymerization of the restorative composite on top due to the higher acidity of one-step self-etching systems, incomplete wetting, and insufficiently thick adhesive layer and phase separation between hydrophilic and hydrophobic ingredients.[22] In addition, due to their higher hydrophilicity, cured one-step self-etching adhesives may act as permeable membranes, permitting water movement across the adhesive layer.[24]

The etch-and-rinse system presented uniform bonding on the various cavity walls evaluated, regardless of the aging condition. The effect of cavity walls was not significant for the non-aged one-step self-etching system as well. On the other hand, differences among cavity walls were observed for non-aged (gingival wall with lower mean) and aged (axial and gingival walls with lower means) Clearfil SE Bond, and for Adper Prompt after thermomechanical cycling (gingival wall with lower mean). These results are not in accordance with those of previous studies,[6,14,16] which stated that self-etching systems are less affected by the regional variation of dentin because they leave partially dissolved smear plugs occluding the tubules. However, the findings of the present study are in agreement with research which reported lower bond strength values for a two-step self-etching system on the gingival wall of a MOD cavity configuration.[8] Two-step self-etching systems consist of a hydrophilic aqueous primer solution and a separate hydrophobic adhesive resin; one-step self-etching systems are complex mixtures of both hydrophilic and hydrophobic components with a high concentration of water.[28] Incomplete water removal can have a negative effect on the polymerization of adhesive layers.[23] Areas of incomplete polymerization may permit greater diffusion of water through the hybrid layers, which could accelerate water sorption and extraction of unpolymerized or degraded monomers.[23] In addition, higher concentrations of solvents added to self-etching systems may cause incomplete resin polymerization if evaporation is incomplete.[27,28] Accomplishing complete water removal and solvent evaporation could be more difficult in a cavity configuration. In addition, the greater distance between the tip of the light-curing unit and the cavity walls might have an additional effect on the incomplete polymerization of the self-etching systems on the gingival and axial walls. These questions must be addressed in further studies.

Specimens bonded with Clearfil SE Bond on the axial wall and those bonded with Adper Prompt on the gingival wall showed significantly lower bond strength after thermomechanical cycling. The decrease in bond strength on those cavity walls was probably caused by the deformation of the restoration due to loading and thermocycling, which might create microseparations between the cavity wall and the adhesive resin, or plastic deformation of the adhesive interface.[10] In the present study, restorations were aged through 2000 thermal and 100,000 mechanical cycles. It was observed in a previous study[8] that the combination of thermal and mechanical cycling could significantly decrease bond strength values obtained on the gingival wall of Class II composite restorations. Those authors also found that specimens restored with the self-etching system Clearfil SE Bond did not resist for the 200,000 and 500,000 mechanical cycles associated with thermocycling.[8] Therefore, the number of mechanical cycles of the present study was chosen to provide significant aging but also to preclude a higher number of premature failures. Nevertheless, a high incidence of premature failures was found, even in specimens not submitted to thermomechanical cycling.

Fracture mode analysis showed that, regardless of the experimental condition (ie, adhesive system, cavity wall, and thermomechanical cycling), most failures were interfacial and cohesive within adhesive resin, whether or not they were together. Failures located at the adhesive interface might represent low bond strength values.[14] In the present study, low bond strength values appeared to be directly related to the cavity configuration and the dentin depth used.

CONCLUSION

The null hypotheses tested in the present study had to be rejected, since adhesive systems presented significant differences on the three cavity walls. Moreover, the axial wall bonded with the two-step self-etching system and the gingival wall bonded with the one-step self-etching system exhibited decreased bond strengths after aging by thermal and mechanical stresses. The effect of cavity walls was adhesive-system dependent. The nanofilled etch-and-rinse system and the two-step self-etching system performed similarly, except on the axial wall after aging. Although non-aged Adper Prompt presented similar bond strengths on the three cavity walls; the one-step self-etching system demonstrated bond strengths lower than Single Bond Plus or Clearfil SE Bond under most experimental conditions.

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Clinical relevance: The one-step system demonstrates weak dentin bonding on most of the Class II preparations walls, when compared with the nanofilled etch-and-rinse system or the two-step self-etching system.