The effect of preliminary acid etching and the addition of an intermediary layer of flowable composite on the microtensile bond strength of two current simplified adhesive systems to dentin

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Abstract: Objectives: This *in vitro* study was done to compare the microtensile bond strengths (µTBS) of two current simplified adhesive systems, two-step (etch-and-rinse) and one-step (all-in-one) adhesives, as well as, to investigate the effect of dentin surface conditioning with phosphoric acid before the application of the all-in-one adhesive on its bond strength to dentin, in addition to, examining the effect of the application of an intermediary layer of flowable composite on the bond strength of both adhesive systems. Materials and methods: 30 extracted human molar teeth were ground flat occlusally to remove enamel and expose dentin. The roots were removed under the cemento-enamel junction and the pulps were accessed and removed from furcation direction. The remaining root trunks were connected to a perfusion system to deliver a simulated pulpal pressure. Teeth were divided randomly into six groups according to the restoration protocol used; which were all done under the effect of a simulated pulpal pressure of 15 cm H_2O; that was maintained for 24 hours of storage in distilled water bath at 37° C before cutting the restored teeth to produce beam specimens of diameter ~ 1mm^2 for microtensile bond strength testing. Data was collected and analyzed by one-way ANOVA and Tukey's post hoc-test for inter-group comparison and t-test for collective comparison of the tested variants, both were at ($P<0.05$). Results: Adper Single bond 2 showed significantly higher µTBS than Adper Easy One, preliminary dentin etching was found to significantly improve µTBS of the later and the addition of intermediary flowable layer was found to increase the µTBS of Adper Single bond 2 and to decrease that of Adper Easy One, yet in both conditions the change was insignificant.
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1. Introduction

The adhesive dentistry is in constant state of evolution and with the introduction of new materials and techniques clinicians are puzzled in selection of both the appropriate materials and the best application techniques in their buildup of the restorative system in order to reach to the utmost successful restorations without endure neither prolonged procedures nor very sensitive techniques.

In three-step, etch-and-rinse, approach the dentin substrate is first conditioned with an acid and rinsed off followed by dentin priming and lastly the application of the resin adhesive over the dentin surface. However, with such approach the lengthy steps and technique sensitivity were the major issues (Stangel et al., 2007). Simplification and the production of user-friendly materials has been a focal objective in the development of dental adhesives, not only to shorten clinical application procedures, but also to limit handling errors (Mak et al., 2002; Ilie and Hickel, 2011).

Simplified two-step etch-and-rinse adhesives combine the primer and adhesive resin into one application (De Munck et al., 2004). For further simplification; the one-step self-etch adhesives were introduced and referred to as all-in-one adhesives. They does not require a separate step of acid etching, but combining the three steps of dentin conditioning, priming and resin impregnation in one step by simultaneous demineralization, dentin priming and resin infiltration of the tooth surface to the same depth; ensuring complete penetration of the adhesive (De Munck et al., 2004; Carvalho et al., 2005). These adhesives have been reported to show less than optimum performance in dentin bonding because of a variety of problems associated with their higher hydrophilic tendency and low viscosity (Van Landuyt et al., 2006). Moreover, all-in-one adhesives result in the presence of a smear layer barrier which is difficult to be removed with those "non-rinse" adhesive systems causing calcium salts, amorphous calcium phosphate, and dissolved proteins to remain on top of dentin and mix with the monomer at the bonding site (Stangel et al., 2007). While the removal of the smear layer and smear plugs from dentinal tubules by acidic conditioners, used in etch-and-rinse adhesives, increases the outflow of fluid through dentin due to the physiological pulpal pressure (Pereira et al., 1999; Murray et al., 2001). The hydration state of dentin surface represents a critical variable during bonding procedures (Sauro et al., 2007; Mazzitelli et al., 2008) which in turn offers a favorable condition when using wet bonding in etch-and-rinse adhesive strategy (Hosaka et al., 2007a), but this is not the case in all-in-one adhesive systems; as their bond strength to dry dentin was found to be significantly greater than to wet dentin, which could be attributed to their adsorption of water from dentinal tubules during bonding leading to water trapping and nanoleakage formation and thus a decline in bond strength (Hashimoto et al., 2008; Hashimoto et al., 2009).

Likewise, contradictory results have been reported regarding the effect of preliminary phosphoric acid etching on the bond strength of self-etch adhesives to dentin substrate (Van Landuyt et al., 2006; Osorio et al., 2010; De Munck et al., 2003). On the other hand, one of the methods suggested for reducing the effect of stresses during polymerization shrinkage is the application of an intermediary low-viscosity, low-modulus flowable resin composite between the bonding agent and the restorative resin composite to act as an elastic buffer or stress breaker (Braga et al., 2003). Flowable composites have excellent handling properties, injectable and possess a good wetting ability which favor they adaptation to the cavity walls (Chuang et al., 2001), yet they have lower filler loading than conventional composites, which affects their overall mechanical properties (Bayne et al., 1998).

So that, this *in vitro* study was conducted to investigate some of the current resin composite application protocols on dentin substrate in order to help in reaching the most appropriate selection of the clinical treatment modality.

2. Material and Methods

Thirty non-carious human molar teeth were used in this study. All teeth were examined using a 7× magnification lens to exclude teeth with cracks or other structural defects. Teeth were placed in water containing 0.5% chloramine at 4°C and were used within 1 month after extraction.

Molar teeth were used in this study because of the larger size of occlusal table than any other human teeth which offered greater surface for application of the adhesives and resin composite, as well as, producing adequate number of beam sticks. Furthermore, the hydraulic conductance of dentin in the molars was found to be significantly higher than other teeth even when having the same dentin thickness (Pashley et al., 1981). The roots of teeth were cut and removed 3–4 mm below the cemento-enamel junction. The pulp chambers were exposed at the furcation level and the contents were carefully removed with an endodontic barbed nerve broach and cotton pliers; avoiding to disturb the pulp chamber walls and altering the predentin surface, thus preserving the odontoblastic layer (Sauro et al., 2007; Hosaka et al., 2007a; Hosakaet al., 2007b).

The occlusal surface of each tooth was ground flat in order to expose the dentin surface by means of a flat-end cylindrical diamond stone, ISO #111/014, (Mani inc. Utsunomiya, Tochigi, Japan) mounted in a high speed handpiece accompanied with copious air-water spray.

A flat occlusal table was utilized for bonding rather than a cavity preparation as this is a common protocol used for microtensile bond testing to minimize the effect of cavity configuration factor (C-factor), thus minimizing the effect of polymerization shrinkage stresses on the resultant bond strength (De Munck et al., 2005; Gernhardt et al., 2008; Vachiramon et al., 2008; Boaro et al., 2014) and allowing for unconfined adhesive flow during polymerization (Silva et al., 2006).

Table 1. Materials used

Material	Composition
Scotchbond Etching Gel	Phosphoric acid gel 35%, silica.
Adper Single Bond 2 (two-step etch-and-rinse adhesive)	Bis GMA, HEMA, dimethacrylates, ethanol, water, photoinitiator, a methacrylate functional copolymer of polyacrylic and polyitaconic acids, silica filler
Adper Easy One (all-in-one adhesive)	2 HEMA, Bis GMA, methacrylated phosphoric esters, 1,6 hexanidol dimethacrylate, methacrylate functionalized polyalkenoic acid, camphorquinone, ethanol, water, silica filler, and stabilizer
Filtek Flow Composite	Bis-GMA, TEGDMA, dimethacrylate polymer, zirconia–silica fillers
Filtek Z250 Hybrid Resin Composite	Bis-GMA, UEDMA, Bis-EMA, zirconia–silica fillers

Materials used which were all selected from one manufacture: (3M ESPE, St. Paul MN, USA).

After removal of enamel, the dentin thickness was measured and repeatedly checked while grinding by means of a pincer Iwanson thickness caliper (Renfert

GmbH, Hilzingen, Germany) and was adjusted to 3 mm measured from the occlusal dentin surface (outer surface) to the central area of the pulp chamber roof (inner surface). The dentin thickness was kept at a constant value of 3 mm because as dentin is made thinner, the tubules become shorter and hyper-conductive relative to thick dentin; so that, the dentin becomes more permeable (Trowbridge, 1981), hence the hydraulic conductance of dentinal tubules is inversely related to their length and directly related to their radius (Richardson et al., 1991). The exposed dentin surface was then polished with 600-grit silicon carbide paper under running water for 30 seconds to create a standardized uniform smear layer (Hashimoto et al., 2004a; Hashimoto et al., 2004b; Hiraishi et al., 2009; Zheng et al., 2000).

Pulpal pressure simulation: A plastic water bath container (Figure 1) was constructed with a lid, to minimize heat loss and evaporation. The lid had an opening to accommodate mounting a digital temperature control unit to ensure constant temperature (37°C ± 0.1) and to keep the storage water moving through an immersed fan. All teeth were mounted to a perfusion system containing distilled water, with a normal physiologic pulpal pressure of 15 cm H_2O (equivalent to 1.5 kPa or 11.1 mm Hg).

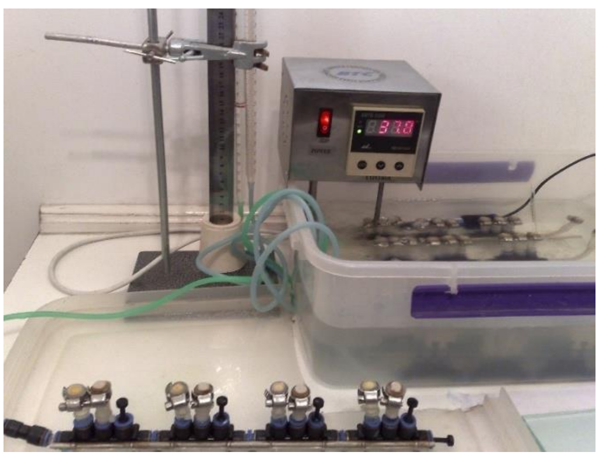

Figure 1. The water bath container connected to the perfusion system and the temperature controlling unit is showing the water temperature. A raw of teeth was removed from the container for adhesive application and composite buildup.

Different solutions have been used by researchers for this purpose, mostly phosphate buffered or physiologic saline were used (Prati and Pashley, 1992; Augustin et al., 1998), although salt precipitation had been reported to occur in dentin stored in sodium phosphate buffered saline resulting in decrease in its permeability over time (Pashley et al., 1984). Protein containing solution as horse serum or bovine serum has been tried (Augustin et al., 1998; Krejci et al., 1993) and more recently human plasma have been used for perfusion of dentin (Gernhardt et al., 2005).

So that, distilled water was selected for dentin perfusion, as it was reported that it causes adhesives to produce higher bond strength values than other fluids as plasma serum and saline (Gernhardt et al., 2005; Gernhardt et al., 2006).

Teeth were perfused 24 hours prior to bonding and resin composite buildup procedures to assure complete hydration of dentinal tissue. During performing the adhesive and restorative procedures, the related pipeline row of teeth was raised from the water bath container and placed on the bench top at the same level of the bottom of the container which allowed bonding and resin buildup procedures to be accomplished in dry condition, while perfused and staying under the effect of pulpal pressure, thus keeping the teeth at a constant relation with the burettes position level and maintaining the pressure gradient.

Teeth were randomly divided into six equal groups of five teeth each according to the restoration protocol employed as shown in table 2.

Table 2. Groups with dentin surface treatment and restorative system buildup

Group	Dentin treatment and restorative buildup
G1	Phosphoric acid etching + Adper Single Bond 2 + Z250 resin composite.
G2	Phosphoric acid etching + Adper Single Bond 2 + Filtek Flow + Z250 resin composite.
G3	Adper Easy One + Z250 resin composite.
G4	Adper Easy One + Filtek Flow + Z250 resin composite.
G5	Phosphoric acid etching + Adper Easy One + Z250 resin composite.
G6	Phosphoric acid etching + Adper Easy One + Filtek Flow + Z250 resin composite.

Etching and bonding procedures were done according to the manufacturer instructions. Excess water was blotted dry with an absorbent pellet of sponge leaving the dentin surface visibly moist (wet bonding) followed by the application of Adper Single Bond 2. Agitation was done during the application of Adper Easy One, when no preliminary phosphoric acid etching was applied. Two consecutive layers of each adhesive were applied and gently air-dried for 5 seconds at a distance of 5 cm and each adhesive layer was light cured for 10 sec. For the groups receiving intermediary layer of flowable composite; a thin layer of flowable composite was applied and cured separately. A hybrid resin composite, Filtek Z250 universal restorative, was used for buildup. Two consecutive increments, of two mm thickness, were applied and light-cured separately for 40 seconds.

Microtensile bond strength measurement was selected for testing, as bond strength tests are the most frequently used tests to screen adhesives. The rationale behind this testing method is that the stronger the adhesion between tooth and tooth structure, the better it will resist stress imposed by resin polymerization and oral function (Sano et al., 1994; Pashley et al., 1995; Pashley et al., 1999).

The teeth were sectioned into a series of slabs under continuous water spray cooling, using a low-speed diamond saw, in axial direction to the crown. Then, by rotating the slabs 90° around the long axis of the tooth and again sectioning it in a direction perpendicular to the first one to obtain beam sticks of ~1 mm2 cross-section area. Peripheral beams where discarded; while four central beams were selected from each tooth for testing to make a total number of 20 beam specimens for each group. Beam thickness was checked using pincer Iwanson thickness caliper. Since specimen's geometry plays a major role in µTBS testing results, so that the non-trimming method was used to obtain beam sticks which was claimed to be easier in preparation with fewer induced flaws; which might affect bond strength results (Ghassemieh, 2008).

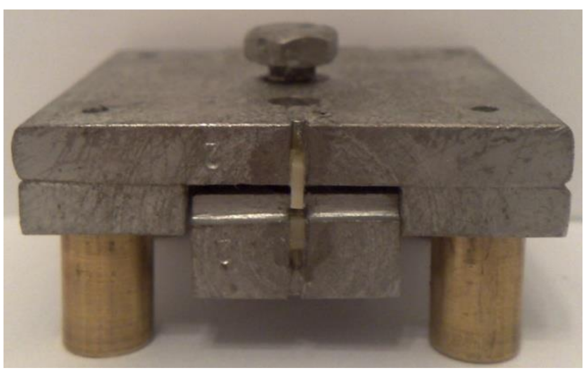

Figure 2. Attachment jig with a glued beam specimen immediately after its tensile straining to failure.

Because the method of fixing the specimen to the jig has a great effect on the type of resulting force (Poitevin et al., 2007). So that, a specially designed jig was used which was a modified design from that described by El Zohairy et al. (2004). Each beam was attached and had its ends glued with cyanoacrylate to the attachment jig (Figure 2) that consisted of two stainless steel articulating members, a fixed part and moving component. A flexible plastic sheet attached the articulating member to the fixed part at one end (on the back side). This attachment allowed hinge movement of the moving component between the free ends of reversed U-shaped fixed component. The force was applied to the moving component via a steel rod that was loosely fitted in an outlet of the fixed component. The modification was done by the addition of a vertical groove on the jig face extending in its two components (fixed and moving) that allowed better orientation and fixation of specimens. Moreover, it offered homogeneous stress distribution and assured the production of more pure tensile force; i.e. reduced the bending action resulting from moving the two components apart while fixing the specimen from only one face.

Universal testing machine (Model LRX-plus; Lloyd Instruments Ltd., Fare ham, UK); was used with a load cell of 5 kN; to produce a tensile load with compression mode of force at a crosshead speed of 0.5 mm/min. The applied tensile force resulted in de-bonding or failure along the substrate-adhesive interface. The exact dimensions data of each beam (width and breadth) were fed to the testing machine computer and the load required for failure or debonding of each beam stick was recorded in megapascals.

The results were collected and statistically analyzed for significance between the groups using SPSS for Windows (version 22, IBM, Corp., Chicago, IL, USA) employing one-way analysis of variance (ANOVA) and Tukey's post-hoc test ($P < 0.05$). The same software was used to compare the collective results of each tested variant by applying t-test ($P < 0.05$).

3. Results

One-way ANOVA and Tukey's post-hoc test for inter-group comparison showed that Adper Single Bond 2 recorded a significantly higher µTBS (27.34 MPs ± 5.57) than Adper Easy One (15.38 MPs ± 5.29), when the later was applied without preliminary dentin surface acid conditioning (table 3).

Table 3. Microtensile bond strength results of groups

Group	Mean µTBS (MPs) ± S.D.
G1	27.34 ± 5.57
G2	32.35 ± 7.15
G3	15.38 ± 5.29
G4	13.62 ± 5.50
G5	25.12 ± 5.78
G6	31.26 ± 6.04

A significant improvement in Adper Easy One µTBS was observed after preliminary acid conditioning of dentin to reach (25.12 MPs ± 5.78), which was statistically insignificant from that of Adper Single Bond 2.

Insignificant increase occurred in µTBS of Adper Single Bond 2 after the application of a thin intermediary layer of flowable composite to record its highest value among all groups (32.35 MPs ± 7.15), while the application of flowable composite over Adper Easy One, without dentin acid conditioning step, resulted in insignificant decrease of its bond strength to yield the lowest results among all groups (13.62 MPs ± 5.50).

But, Adper Easy One recorded its best µTBS value when phosphoric acid conditioning of dentin and flowable composite application were combined to record (31.26 MPs ± 6.04) with insignificant difference from Adper Single Bond 2 (32.35 MPs ± 7.15), when the same protocol was followed.

On the other hand, statistical analysis by applying t-test for the collective result of Adper Single Bond 2 was significantly higher than that of Adper Easy One, whether flowable layer was applied or not (table 4).

Table 4. Microtensile bond strength collective results of both types of adhesives

Adhesive type	Groups	Mean µTBS (MPs) ± S.D.
Etch-and-rinse	G1 and G2	29.85 ± 6.36
All-in-one	G3 and G4	14.50 ± 5.40

Again, the collective results of Adper Easy One increased significantly after dentin preliminary conditioning, regardless intermediary flowable composite was used or not (table 5).

Table 5. Effect of dentin preliminary conditioning on µTBS of all-in-one adhesive

Dentin acid conditioning	Groups	Mean µTBS (MPs) ± S.D.
No dentin conditioning	G3 and G4	14.50 ± 5.40
Dentin pre-conditioning	G5 and G6	28.19 ± 5.91

The effect of adding an intermediary layer of flowable composite showed insignificant difference, when results of the three groups in which flowable composite was employed were pooled together (25.75 ± 10.63) and compared to the other groups when no flowable composite was applied (22.61 ± 7.56) (table 6).

Table 6. Effect of adding an intermediary layer of flowable composite on µTBS

Intermediary flowable composite	Groups	Mean µTBS (MPs) ± S.D.
No flowable	G2, G4 and G6	22.61 ± 7.56
With flowable	G1, G3, and G5	25.75 ± 10.63

4. Discussions

Since no-pulpal pressure is not representing any vital tooth restoration clinical condition, simulated pulpal pressure of a value of 15 cm H_2O delivered through a perfusion system was applied during adhesive and resin composite application to mimic that of the normal physiological pulpal pressure (Gerzina et al., 1995; Zheng et al., 2000; Özok et al., 2004; Hosaka et al., 2007b; Mazzitelli et al., 2008; Hashimoto et al., 2009). The perfusion system used in the current study allowed the presence of pulpal pressure during the procedures of acid etching, bonding and resin composite build up, while working in a dry condition outside the water storage container.

Feitosa et al. (2014) described a simple technique to simulate the effect of pulpal pressure, simply by fixing the examined teeth at the bottom of a container under the desired water bath height, while leaving the pulp chambers open through the roots; instead of using a sophisticated perfusion system. The technique could be useful only during storage for a long period of time, but not applicable during the bonding procedures and composite buildup throughout the pre-gel phase; in which pulpal pressure is expected to be more critical and influential. Likewise, the technique described by Gupta and Tewari (2006) that offered pulpal pressure to be delivered through injection syringes filled with distilled water and fixed at a higher level than that of teeth. Each syringe, with their pistons in place, was connected to a single tooth root via a tube, but the technique did not ensure exerting positive pulpal pressure. Instead, it is lacking the presence of any opening to allow air replacement and to permit free running of the perfusion liquid without creating any negative pressure. In the device used in the current study, burettes were filled with distilled water to the level of 15 cm, while their tops were kept open to permit air entrance that prevented the occurrence of reversal vacuum.

The results of the present study showed that etch-and-rinse adhesive recorded a significantly higher bond strength values than all-in-one. The explanation of this finding is that one-bottle self-etching adhesives might adsorb the water from dentinal tubules during bonding, leading to nanoleakage formation and thus a decline in their bond strength (Hashimoto et al., 2008; Hashimoto et al., 2009). This finding was in agreement with Knobloch et al. (2007) who compared the µTBS of one-step and two-step self-etching adhesives to that of two-step etch-and-rinse adhesive and found the later showed significantly higher µTBS than any of the one-component all-in-one adhesives examined. In agreement too; Alves et al. (2013) tested µTBS of the same adhesives used in the current study to primary dentin and found Adper Single Bond 2 to give a significantly higher bond strength than Adper Easy

One. Also, Hegde and Manjunath (2011) tested the same adhesives and their findings agreed with the results of the current study on wet dentin, while the difference was insignificant on dry dentin. In agreement too; De Munck et al. (2004), in their critical review article, spotted that all-in-one adhesives recorded lower bond strength values, in the studied literatures, than the two-step self-etch adhesives that showed a comparable results to that of two-step total-etch adhesives.

The results of the current study, also showed that dentin surface conditioning with phosphoric acid prior to the application of the all-in-one adhesive, Adper Easy One, to significantly improve its bond strength values. In agreement with this finding; Osorio, et al. (2010) found that preliminary acid etching recorded higher µTBS values, while following the manufacturer instructions, by not to apply preliminary dentin etching, to yield lower values. On the other hand, De Munck et al. (2003) found acid etching prior to the application of self-etch luting resin cement to decrease their µTBS to dentin and they attributed that to the effect of acid etching that although totally removed the smear layer, but it resulted in a demineralized and poorly infiltrated non-resin un-reinforced collagen mesh layer that remained attached to the resin cement after µTBS testing. In disagreement to the present study results, Van Landuyt et al. (2006) found phosphoric-acid etching prior to the application of Clearfil SE Bond, a two-step self-etch adhesive, to significantly decrease its µTBS to dentin. This disagreement could be attributed to the difference in chemical composition of the Clearfil SE Bond that contains MDP monomer and the difference in material clinical steps of application, as Clearfil SE Bond is a two-step self-etch adhesive converted, in their study, into a three-step which they shown to result in a significantly lower µTBS.

The explanation of variations in some results reported by different researchers could be attributed to the drop of bond strength of total-etch adhesives to dentin due to over-drying of dentin by air leading to collagen fibers' collapse and yielding lower bond strengths (Pashley et al., 1998). Ikeda et al. (2008) evaluated the effect of air-drying during dry bonding procedures on µTBS of HEMA-rich and HEMA-free one-step adhesives on the evaporation degree of residual monomers and they found that long air-drying time resulted in higher µTBS in Hema-rich adhesives compared to the HEMA-free.

The results showed insignificant increase in µTBS of Adper Single Bond 2 when intermediary flowable composite was applied. Whereas in case of Adper Easy One the addition of the flowable composite, without dentin surface acid pre-conditioning resulted in insignificant reduction of its bond strength. The collective results showed that the application of a thin intermediate layer of flowable composite to insignificantly increase µTBS. In agreement with this finding Abdalla (2010) reported that the addition of flowable composite resulted in insignificant increase in the µTBS for all tested adhesive systems, a total-etch, and two two-step self-etch adhesives, Admira Bond, Futurabond DC and Clearfil SE Bond. The explanation of the part of disagreement regarding the increase of µTBS of the self-etch adhesives is that the difference in µTBS was insignificant and that there was a significant difference in the bond strengths to dentin between the all-in-one (one-step) and two-step self-etching adhesives (De Munck et al., 2004), as well as, the different physical and chemical compositions of the two adhesives used than Adper Easy One; as the first one was Futurabond DC, a dual-curing self-etch-bond with nano-fillers, and the other one was Clearfil SE Bond containing MDP monomer (10-methacryloyloxydecyl dihydrogen phosphate) that is said to be able to chemically interact with hydroxyapatite (Yoshida et al., 2004; Fukegawa et al., 2006). Similarly, De Goes et al. (2008) found that placement of a low-viscosity flowable resin after adhesive application increased the microtensile bond strength for all the four tested adhesive systems (two etch-and-rinse, one two-step self-etch and one all-in-one). They reported that such increase was insignificant except for in Clearfil SE Bond the two-step self-etch adhesive. They attributed that to be material-dependent. In agreement too, Cavalcant et al. (2007) who evaluated the effect of adhesive systems and flowable composite lining on bond strength to gingival margins of class II restorations in bovine teeth after thermal/mechanical stresses. They used three two-step etch-and-rinse adhesives and found no significant difference to occur between bond strength values whether or not flowable layer was applied. In agreement too, Knobloch et al. (2007) reported that the use of an intermediary layer of flowable elastic resin did not show an effect on the µTBS of the self-etching adhesives tested. Yet, Miguez et al. (2004) reported that the use of flowable resin increased the bond strength of one adhesive but not the other one, although both were etch-and-rinse two-step systems.

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