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Does Adhesive Thickness Affect Resin-dentin Bond Strength After Thermal/Load Cycling?

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Clinical Relevance

The application of an extra coat of hydrophobic, solvent-free bonding resin, designed to act as an intermediate flexible layer, was not able to minimize the damage caused by thermal/mechanical load cycling in low C-factor cavities.

SUMMARY

This study evaluated the influence of adhesive layer thickness (ADL) on the resin-dentin bond strength of two adhesive systems (AS) after thermal and mechanical loading (TML). A flat superficial dentin surface was exposed with 600-grit SiC paper on 40 molars. After primer application, the adhesive layer of Scotchbond Multipurpose (SBMP) or Clearfil SE Bond (CSEB) was applied in one or two layers to a delimited area (52 mm²) and resin blocks (Filtek Z250) were built incrementally. Half of the sample was stored in distilled water (37°C, 24 hours) and submitted to thermal (1,000; 5°-55°C) and mechanical cycles (500,000; 10kgf) [TML]. The other half was stored in distilled water (72 hours). The teeth were then sectioned to obtain sticks (0.8 mm²) to be tested under tensile mode (1.0 mm/minute). The fracture mode was analyzed at 400x. The BS from all sticks from the same tooth was averaged for statistical purposes. The data was analyzed by three-way ANOVA. The χ^2 test was used ($p<0.05$) to compare the frequency of pre-testing failure specimens. Higher BS values were observed for SBMP regardless of the ADL. The TML reduced the BS values irrespective of the adhesive employed and the ADL. A higher frequency of pre-testing failure specimens was observed for the cycled groups. A thicker adhesive layer, acting as an intermediate flexible layer, did not min-

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imize the damage caused by thermal/mechanical load cycling for a three-step etch-and-rinse and two-step self-etch system.

INTRODUCTION

One of the most important criteria for clinical success with composite restorative materials is the effectiveness and durability of the bonded interface.[1] During their lifetime in the oral environment, composite restorative materials are submitted to different types of challenges, such as thermal and mechanical loading. Initially, the bonded interface must withstand stresses produced by composite polymerization shrinkage. This stress might exceed adhesive bond strength in some regions of the interface and might cause adhesive failures or cohesive fractures within dentin and/or composite.[2] Both failures may lead to the development of marginal gaps and consequential recurrent caries, post-operative sensitivity and marginal discoloration of composite restorations, reducing the clinical performance.

Additionally, interfaces are also subjected to occlusal loading and thermal variations that can potentially aggravate de-bonding and lead to a decrease in bond strength over time.[3] Many efforts have been developed in order to maintain the integrity of the bonded interfaces. This can be achieved by reducing polymerization shrinkage of the composites by controlling the type and amount of matrix monomers, fillers and initiators.[4-5] Other approaches include modification of insertion techniques, such as the oblique incremental technique,[6] association between the different dental materials as composites and glass ionomer[7] or flowable composites,[8-11] which may act as "elastic buffers." The rationale behind this is that they have sufficient flexibility to deform and favorably dissipate stresses produced by polymerization shrinkage, thermal variations, water absorption and occlusal loads across the interface.[9-11]

Filled adhesive resins or thicker, unfilled adhesive resins, along with the hybrid layer, may also create an artificial elastic cavity wall[9-10] and act as "shock absorbers."[12] The benefits of using artificial elastic cavity walls to counteract the effects of resin polymerization shrinkage stresses have been demonstrated in static[9-12] *in vitro* studies. However, the effects of the elastic layer should also be evaluated under thermal and mechanical loading in order to simulate the mechanical stresses to which restorations are prone.[13]

Therefore, this study evaluated the effect of different adhesive layer thicknesses subjected or not to thermal/mechanical loading (TML) on the resin-dentin bond strengths (BS) of two adhesive strategies (etch-and-rinse and self-etch approaches). The null hypothesis tested was that thicker adhesive interfaces provide resin-dentin bond strengths similar to thinner layers, whether or not they are subjected to thermal/mechanical loading.

METHODS AND MATERIALS

Forty extracted, caries-free human third molars were collected after obtaining the patients' informed consent under a protocol approved by the local Institutional Review Board. The teeth were then disinfected in 0.5% chloramine solution and used within six months of extraction. This study was approved by the Ethics Committee of the University of São Paulo. A three-step etch-and-rinse system, Scotchbond Multi-Purpose (3M ESPE, St Paul, MN, USA), and a two-step self-etching primer system, Clearfil SE Bond (Kuraray Medical Inc, Osaka, Japan), were employed in this study (Table 1).

Initially, the samples had their roots cut and a flat superficial dentin surface was exposed after wet grinding the occlusal enamel on 180-grit SiC paper. The teeth were included in PVC rings in order to guarantee the perpendicularity of their bonding surfaces in relation to the tooth long axis. These dentin surfaces were polished on 220, 320, 400 and 600-grit SiC paper for 60 seconds to create a standardized smear layer. The bonding area was demarcated by placing a piece of masking tape with a punched hole 8.45 mm in diameter on the center of the dentin surface. This yielded a standard area of approximately 52 mm^2 for bonding.[14] The samples were divided into eight experimental groups, assigning five teeth for each tested condition, as shown in Table 1.

Table 1: *Characteristics of the Adhesive Systems*

Adhesive System/Batch #	Composition		
	Conditioner	**Primer**	**Adhesive**
Scotchbond Multi-Purpose (3M ESPE, St Paul, MN, USA) 2YF (acid); 3AH (primer); 3NL (bond)	35% phosphoric acid	HEMA, polyalkenoic acid copolymer, water	Bis-GMA, HEMA, initiators
Clearfil SE Bond (Kuraray Medical Inc, Osaka, Japan) 00410A (primer); 00555A (bond)	MDP, HEMA, hydrophilic dimethacrylate, initiators, water		MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, initiators, silanated colloidal silica

Bonding Procedures

All adhesive procedures are described in Table 2. For the SBMP groups, after acid etching with their respective etchants (Table 2), the surfaces were rinsed with distilled water for 15 seconds, followed by air-drying for 30 seconds with oil-free compressed air. The surfaces were then rewetted prior to adhesive application. One coat of primer (equivalent to eight µl—Microbrush International, Grafton, WI, USA), which corresponds to approximately the volume of the primer applied with a regular microbrush (previously measured) was stirred over the dentin surface with a microbrush for 30 seconds. A stream of dry air was applied at a distance of 20 cm until no liquid movement was visible in the dentin surface. Then, in the groups SBMP1, one coat of adhesive (equivalent to 8 µl of adhesive) was applied with a plastic tip (obtained by removal of the fluffs of the microbrush) to avoid extreme variation in the volume of the primer applied, gently air dried to spread film in a uniform layer and light activated for 10 seconds using an Optilux 500 Curing Light unit with a light intensity of 700 mW/cm^2 (Demetron-Kerr Corp, Danbury, CT, USA). In the SBMP2 groups, the adhesive application was repeated after polymerization of the first coat.

For the CSEB groups, one coat of primer (equivalent to 8 µl of the self-etching primer) was applied onto a 10-second air-dried dentin surface using a microbrush. The primer was left undisturbed for 20 seconds, then air dried at a distance of 20 cm until a glossy surface could be seen without perceptible liquid movement with the naked eye. The adhesive application was applied in one or two coats as described in the SBMP groups.

Resin composite build-up "crowns" (Filtek Z250, 3M ESPE) were constructed in three increments of approximately 1.5 mm and light activated for 30 seconds each. A single operator performed all the bonding procedures at a controlled temperature and relative humidity (23°C and 50%, respectively).

Half of the samples from each group (SBMP1y, SBMP2y, CSEB1y and CSEB2y) were submitted to thermal/mechanical cycling (TML) (thermal: 1,000; 5°C-55°C, one minute dwell time, mechanical loading: 500,000; 10 kgf, 4 Hz), while the remaining samples (SBMP1n, SBMP2n, CSEB1n and CSEB2n) were stored in distilled water for the same time period required for TML—72 hours. The mechanical loading was applied as compression and performed under immersion in distilled water.

Microtensile Bond Strength

The specimens were longitudinally sectioned in perpendicular directions across the bonded interface with a diamond saw in a Labcut 1010 machine (Extec, Enfield, CT, USA) to obtain approximately 30 bonded sticks per tooth, each with a cross-sectional area of approximately 0.8 mm^2.

The number of prematurely debonded sticks (D) per tooth during specimen preparation was recorded. The cross-sectional area of each stick was measured with a digital caliper to the nearest 0.01 mm (Absolute Digimatic, Mitutoyo, Tokyo, Japan) and recorded for calculation of the bond strength. The dentin and resin ends of the sticks were individually attached to a Geraldeli microtensile device (Perdigão & others[15]) with a cyanocrylate glue (Loctite Super Bonder Gel, Henkel Ltda, Itapevi, SP, Brazil) that was instant-set with a thin coating of dimethyl-p-toluidine.

The tensile testing was performed in a universal testing machine (Kratos Dinamômetros, São Paulo, SP, Brazil) at a crosshead speed of 1.0 mm/minute. The failure modes were evaluated at 400x magnification (HMV-2, Shimadzu, Tokyo, Japan) and classified as "cohesive" (C) (failure exclusively within dentin or resin composite) and "adhesive or mixed" (A/M) (failure at the resin/dentin interface or mixed with cohesive failure of the adjacent substrates).[14]

Data Treatment

All bond strength values from sticks from the same teeth were averaged for statistical purposes. The speci-

Table 2: *Experimental Groups and the Respective Application Mode*

Adhesive System	# of Coats	Thermal/Mechanical Load Cycling	Group Code	Application Mode*
Scotchbond Multi-Purpose [SBMP]	1	Yes [y]	SBMP1y	a, b, c, d, e1, f, g, h, i
		No [n]	SBMP1n	
	2	Yes [y]	SBMP2y	a, b, c, d, e1, f, g, h, i, g, h, i
		No [n]	SBMP2n	
Clearfil SE Bond [CSEB]	1	Yes [y]	CSEB1y	e_2, f, g, h, i
		No [n]	CSEB1n	
	2	Yes [y]	CSEB2y	e_2, f, g, h, i, g, h, i
		No [n]	CSEB2n	

**a–acid etching (15 seconds); b–rinse (15 seconds); c–air-dry (30 seconds); d–dentin rewetted with 1.5 µL of water; e_1 application of 8 µL of primer brushed for 30 seconds; e_2) application of 8 µL of primer, kept for 20 seconds; f) 20 cm air dry until no liquid movement was visible seen; g) application of 8 µL of the adhesive; h) gentle air dry; i) light cure (10 seconds, 700mW/cm^2).*

Table 3: *Number of Specimens According to the Fracture Pattern* or Premature Debonding (D) for Each Experimental Condition*

Applied Adhesive Volume/TML	Adhesive System					
	SBMP			CSEB		
	A/M	C	D	A/M	C	D
1n	109	5	31	114	4	23
1y	106	2	38	94	0	44
2n	121	9	15	105	0	39
2y	90	1	56	89	2	47

**A/M = adhesive or mixed failure; C = cohesive failure in dentin (no resin cohesive pattern was observed)*

mens were prematurely included in the tooth mean by assigning an arbitrary value that corresponds to approximately half of the minimum bond strength value that could be measured in this study (ca 9.49 MPa).

A three-way ANOVA (Adhesive system vs Adhesive thickness vs Thermal/mechanical load cycling) and Tukey's multiple comparisons test were used to analyze the data at α=0.05. The Chi-square test was used (p<0.05) to test for significant differences in frequency of the prematurely debonded specimens for each main factor.

SEM Characterization

In order to measure the adhesive layer thickness of the experimental groups, 24 extracted, caries-free human third molars were divided into four experimental groups (SBMP1, SBMP2, CSEB1 and CSEB2). After accomplishing all of the bonding and storage procedures as described, the specimens were longitudinally sectioned across the bonded interface in order to obtain three 1.5 mm-thick slabs for each tooth. A total of 72 slabs were obtained. The slabs were prepared as described by Perdigão and others.[15] Initially, the specimens were immersed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 at 4°C for 12 hours, followed by rinsing with 0.2 M sodium cacodylate buffer at pH 7.4 for one hour (three changes) and distilled water for five minutes. The specimens were dehydrated in rising gradations of ethanol (25% for 20 minutes, 50% for 20 minutes, 75% for 20 minutes, 95% for 30 minutes and 100% for 60 minutes). Finally, the specimens were immersed in a solution prepared with equal parts of 100% ethanol and hexamethyldisilazane (HMDS) for 10 minutes, followed by immersion in HMDS for an additional 10 minutes. They were then placed on filter paper inside a recipient and air-dried at room temperature for at least 12 hours. After being mounted on stubs (Extec, Enfield, CT, USA) with the aid of an epoxy resin (Extec), the specimens were gold-coated at 10 mA for one minute and observed under SEM. Digital images were taken from five distinct regions of the bonded slabs under 330x magnification. The thickness of the adhesive layer was measured using the image analysis software Corel Photo-Paint 8 (Corel Corporation Limited, Dublin, Ireland) into three different regions of each picture, which were averaged for statistical purposes. A two-way ANOVA and Tukey's test was employed to compare the adhesive thickness under the different conditions of the current investigation.

RESULTS

The mean cross-sectional area of the sticks ranged from 0.77 to 0.82 mm^2 and no differences among groups were detected (p>0.05). The number of sticks that failed prematurely during specimen preparation and the frequency of each fracture pattern in each group are shown in Table 3.

A Chi-square test was applied to compare the prematurely debonded sticks frequency (α=0.05). With respect to the criteria "Adhesive system," no difference was detected (p=0.3314). Also, the frequency of prematurely debonded sticks on the main factor "Adhesive thickness" was similar for both systems (p=0.8937 and p=0.1924, respectively, for SBMP and CSEB). A higher frequency of prematurely debonded sticks occurred for groups submitted to TML (p=0.0003 and p=0.0038, respectively, for SBMP and CSEB).

The overall means and standard deviations of the bond strength means are presented in Table 4. Only the main factors "Adhesive system" (p=0.0107) and "Thermal/Mechanical cycling" (p=0.0277) were significant. Cross-product interactions were not significant, allowing comparison of mean values for each factor. Table 5 presents means and standard deviations of

Table 4: *Overall Means and Standard Deviations of Bond Strength Means (MPa) for the Experimental Groups*

Adhesive Thickness/TML	Adhesive System	
	SBMP	CSEB
1n	39.55 (1.7)	33.85 (4.7)
1y	39.36 (14.7)	26.89 (8.0)
2n	46.00 (3.6)	31.54 (7.8)
2y	28.34 (10.5)	30.11 (12.5)

bond strength indexes under a reduced ANOVA model that includes "Adhesive system" and "Thermal/ Mechanical cycling" as a single between-subjects factor. High bond strength values were observed for SBMP and non-cycled groups.

The mean thickness of the adhesive layer for the SBMP1 group was 109.51 (± 30.18) µm and 104.16 (± 15.06) µm for the CSEB1 group. For the SBMP2 and CSEB2 groups, the mean thickness was 172.84 (± 47.22) µm and 267.02 (± 69.87) µm, respectively (Figure 1). No significant difference was observed between materials when one coat was applied ($p>0.05$); however, a significantly thicker adhesive layer was observed for CSEB2 compared with SBMP2 ($p<0.05$).

DISCUSSION

Laboratory methods must resemble the varying nature of stresses to which restorations are highly prone in the oral environment. Thermal and mechanical loadings are often employed to simulate *in vivo* challenges.[17] These aging approaches accelerate the deterioration of bonded interfaces, since they can induce crack propagation along this region.[18-19] Additionally, the combination of thermal and mechanical loading has been reported to be the most efficient approach to stressing the resin-dentin bonding interfaces.[3]

This was, in fact, confirmed in the current investigation. Low resin-dentin bond strengths and a higher frequency of prematurely debonded specimens were observed for the TML groups, irrespective of the adhesive system employed. It can be concluded that TML acted as an accelerated *in vitro* model for aging when primarily performed before bond strength testing, as observed in previous investigations.[3] Deformation of the restoration can occur due to mechanical and thermal cycling, which may cause the creation of micro-separations between the cavity floor and the adhesive or plastic deformation of the adhesive interface, regardless the thickness of the adhesive layer.

In this study, SBMP had a better performance than CSEB. One could suppose that the thinner hybrid layer produced by the CSEB could have played a role in the inferior performance of this system. However, it is worth emphasizing that, to date, no study has succeeded in establishing a positive correlation between the thickness of the resin-infiltrated layer and bond strengths, suggesting that the quality, rather than the thickness of the resin-infiltrated layer, is more important for bond strength measurements.[20]

Some studies have shown an enhanced performance of composite restorations when an additional intermediate elastic layer was placed between the resin composite and dentin substrate. A better dissipation of shrinkage stresses,[8-9,21] lower microleakage[8] and improved marginal adaptation[9-10] have already been reported. However, regarding bond strength values, there is no consensus regarding the efficacy of this intermediate elastic layer. Montes and others[12] did not detect any beneficial effect in tensile bond strength when applying low-viscosity intermediate resins or a filled adhesive in one or two coats in low C-factor cavities.

However, marked differences in the failure modes of fractured specimens were observed, with an increase in the number of specimens with partial cohesive failure in dentin.[12] In the study by Zheng and others,[22] high resin-dentin bond strengths were observed when the thickness of the bonding layer was increased for a two-step, self-etch adhesive, while low resin-dentin bond strengths were noticed for the

Table 5: *Means and Standard Deviations of Bond Strength Values (MPa) of the Reduced ANOVA Model*

Adhesive System		TML	
SBMP	CSEB	n	y
38.31 (10.71) A	30.60 (8.41) B	37.74 (7.34) a	31.17 (11.84) b

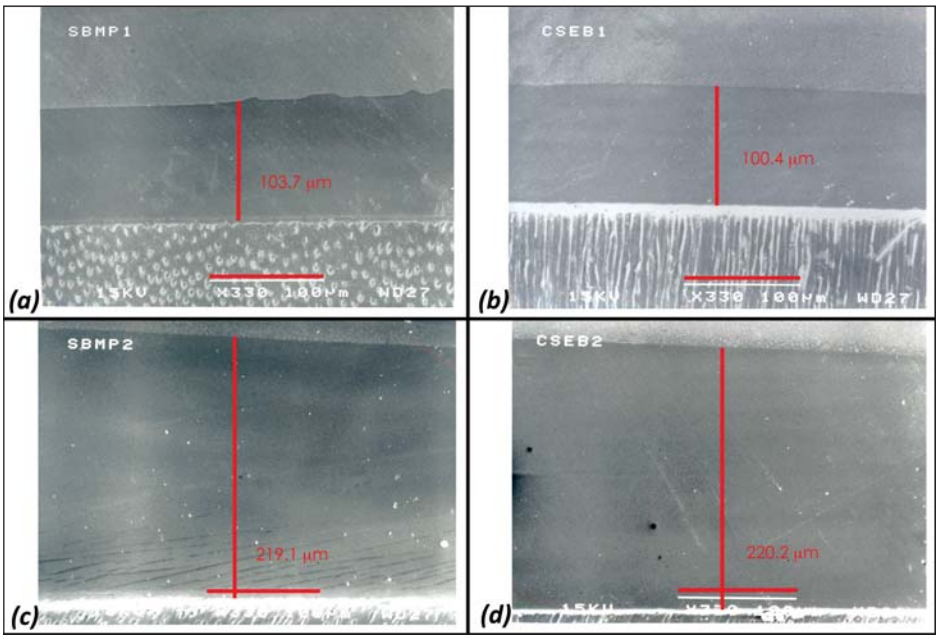

Figure 1. *SEM images and regional thickness of the adhesive interfaces for the two bonding systems applied as recommended and after applying two coats of the adhesive coating. (a) and (b) are representative images of SBMP1 and CSEB1; (c) and (d) are representative images of SBMP2 and CSEB2.*

two-step etch-and-rinse system. It is likely that the differences observed by the aforementioned authors are due to the fact that the adhesive layer employed in the self-etch approach is solvent-free and hydrophobic, while the two-step etch-and-rinse adhesive employed a solvent-rich and hydrophilic layer. This was confirmed by a study by Silva and others.[23] According to the authors, when a hydrophobic, solvent-free bonding layer is applied, the thickness of the adhesive layer does not affect the microtensile bond strength results, which might be the reason why the main factor Adhesive Thickness was not significant in the current investigation.

The current study demonstrated that the placement of an intermediate flexible layer did not have a significant effect in counteracting the stresses generated by the TML. This is in agreement with the findings of De Munck and others,[24] who revealed that placement of an additional elastic layer had no effect on static resin-dentin bond strength when measuring under microtensile bond strength values, but it was contrary to the findings of Pongprueksa and others,[25] who reported that the application of an adhesive or low-viscosity resin had an influence on the microtensile bond strength to dentin of Class V restorations. This controversy encourages the conduction of further studies on this matter.

Although an extra coat of the hydrophobic, solvent-free adhesive layer was applied, one may hypothesize that the adhesive layer was not enlarged sufficiently to guarantee the energy absorption phenomenon.[21] This, however, seems unlikely, due to the relatively thick layers that measured much more than the average of 13 µm[26] reported by other authors.

The thickness of the light-cured primer can vary significantly according to the surface geometry. On smooth, convex surfaces, the adhesive layer can be 60-80 µm, while in concave regions, such as marginal chamfer, the thickness of the adhesive layer might be higher than 200-300 µm, thicker than that obtained in the current investigation.[22-23,27-29]

Another possible explanation for the lack of effectiveness of the increase in the adhesive layer thickness is that, despite the better performance of thicker layers observed in some studies in relation to microleakage[8] and marginal adaptation,[10] the same would not be reflected in bond strength, particularly in the current study, because of the low configuration factor of the specimens employed in this investigation.

It is known that the stress generated in bonding interfaces during polymerization shrinkage is directly proportional to the bonded to unbonded surface ratio (configuration factor or C factor).[30] The bonded area in the current study (approximately 52 mm^2) represents only 32% of the free area (162.27 mm^2). This could have resulted in low stress generation in the bonding interfaces during resin polymerization. Nikaido and others[31] have demonstrated that, in low C factor restorations, such as the one employed in the current study, similar bond strength values were obtained before and after TML. This means that, under favorable conditions, there is no need for an elastic layer between the composite and dentin substrate. The evaluation of the effect of flexible layers in restorations placed under high configuration factors should be encouraged.

Clinically, use of the elastic bonding concept has been primarily advocated for the restoration of Class II and V cavities. An intermediate layer capable of dissipating stresses, such as composites with low elastic modulus or glass-ionomer cements, has been recommended, since these materials are supposed to flex with the tooth structure rather than debond during cervical flexure.[32-33] A recent one- and two-year clinical investigation failed to demonstrate a superior performance on retention rates of microhybrid composite restorations lined with an intermediate layer of flowable composite,[32-33] which is in agreement with the findings of the current investigation. This suggests that the elastic bonding concept may not be as effective, as reported by previous investigations under *in vivo* conditions.

CONCLUSIONS

The application of two coats of an adhesive layer for three-step etch-and-rinse and two-step self-etch systems to act as an intermediate flexible layer did not minimize the damage caused by thermal and mechanical load cycling in a cavity with low-bonding constraint. Within the limitations of the current study, one may conclude that more studies must be carried out in order to elucidate the actual importance of the elastic wall concept.

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