博 士 論 文

Cause-effect relationship of varying bonding thicknesses in dentine adhesion of universal adhesives

ユニバーサル接着材の象牙質接着における接着厚さの変化が及ぼす因果関係

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by

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ACRONYMS

μTBS	Microtensile bond strength
10-MDP	10-methacryloyloxydecyl dihydrogen phosphate
3D	Three-dimensional
4-META	4-methacryloxyethyl trimellitic anhydride
ANOVA	Analysis of variance
Bis-GMA	Bisphenol-A-glycidyl methacrylate
CQ	Camphorquinone
FE-SEM	Field emission - Scanning electron microscope
GP	G-Premio Bond
HEMA	2-hydroxyethyl methacrylate
LED	Light-emitting diode
MB	Clearfil Megabond 2
MDTP	10-methacryloyloxydecyl dihydrogen thiophosphate
Pt-Pd	Platinum-Palladium
RH	Relative humidity
SB	Scotchbond Universal Adhesive
SEM	Scanning electron microscope
SiC	Silicon carbide

ABSTRACT

Today's Dentistry is less invasive and less painful for the patients because of Dr. Buonocore (USA), Dr. Fusayama (Japan), and Dr. Nakabayashi (Japan). Their research works were based on dental adhesives. Dental adhesive bonds restoration directly to tooth tissue. Adhesives marketed now are very sophisticated, however failures happen resulting in bacterial invasion and painful complications, especially in the case of universal adhesives. The performance of the universal adhesives can be greatly affected by their application procedures and inherent formulations.

Therefore, the aim of this study was to evaluate if varying thicknesses of universal adhesives utilising the additional coating strategy would affect their bond strength to dentine and their mechanical properties.

Ninety-nine human maxillary premolars were cut to expose the coronal dentine. The exposed dentine were ground with regular-grit (63 μm) diamond burs to produce clinically relevant smear layers. The ground teeth were randomly distributed to nine groups based on two variables - (1) adhesive: Scotchbond Universal Adhesive (SB; universal), G Premio Bond (GP; universal) and Clearfil Megabond 2 (MB; two-step self-etch; control); and (2) application strategy (one, two or three coats; each coat light-cured).

After adhesive application, the teeth were incrementally restored with light-cure resin composite. The bonded teeth were then stored in distilled water at 37 ° C for 24 h. Resin-dentine sticks from eight premolars per group (each premolar rendering 3 sticks; n = 24 sticks altogether) were prepared for the microtensile bond strength (μTBS) test using a universal testing apparatus, followed by measuring the adhesive thicknesses at their fractured ends using scanning electron microscope. The hardness and elastic modulus of the adhesive layers produced by different coats were evaluated on separate resin-dentine slices (n = 3 teeth per group) with an ultra-microhardness tester. The effects of different coats on the μTBS of the adhesives were tested with Two-way ANOVA followed by Tukey's post hoc test. The adhesive thickness, hardness and elastic modulus data were analysed with Kruskal-Wallis test with Dunn's Bonferroni adjustment. Spearman correlation test was done to check the association between the μTBS and adhesive thickness.

Two coats significantly increased the μTBS ($p < 0.001$) of all the adhesives. Regarding the application of one, two and three coats, the correlation between adhesive thickness and bond strength was positive for GP, but negative for SB. In the case of the adhesive hardness and, elastic modulus,

additional coating significantly increased the values of GP ($p < 0.05$) but did not affect SB and MB ($p < 0.05$).

An additional adhesive coating over the manufacturers' recommendations improved the bond strength of all the adhesives tested. However, as the hardness and, the elastic modulus were found to be not influenced, the beneficial effect of additional curing was material dependent.

Keywords: Dentine; Universal Adhesives; Additional coating; Adhesive thickness; Microtensile bond strength; Hardness; Elastic modulus

CHAPTER 1

INTRODUCTION

The advances in dental adhesive system as well as the composite resin restoration were to a larger extent promoted by the progressive demand of both minimal invasive and esthetic dentistry.[1,2] The expectation from a dental adhesive system begins with the formation and later maintenance of a tight adhesive-tooth interface, with an aim to provide good retention, marginal seal, and clinical durability.[3] To implement such goals, clinicians need to choose from any of the three currently available options: etch-and-rinse, self-etch, or universal adhesive systems,[4] each having its pros and cons.[5,6] Several commercial products that belong to etch-and-rinse and self-etch system satisfactorily passed their criteria as a dental adhesive in laboratory and clinical researches.[5-7]

From 2011, manufacturers have been marketing the multi-mode adhesive systems, known as universal adhesives.[8] These universal adhesives can be used in either of the two adhesion modes: etch-and-rinse or self-etch modes.[9] They are designed similarly to the already existing 'all-in-one' concept utilised in the 'one-step self-etch' adhesives, continuing the shortened application steps than other systems,[10] but modified for more versatile indications.[9,11] Nonetheless, the one-step self-etching approach

complies with the dentine only since it was found to be insufficient for enamel adhesion unless the enamel is pre-etched with phosphoric acid, known as the selective enamel etching. [12,13]

Water is an integral component of the universal adhesives, making them more hydrophilic than the two-bottle systems.[11] Their higher hydrophilicity results in lower dentine bond strengths than the two-step systems, an outcome similar to their predecessor - the one-step adhesives.[14,15] The accusations were less immediate bond strength, higher nanoleakage, increased water sorption or phase separation.[10] Despite such limitations, the multi-functionality, reduced application time, and user-friendliness of the universal adhesives have preserved their demand and constantly increasing use among clinicians.

Including the 10-methacryloyloxydecyl dihydrogen phosphate or 10-MDP in the self-etch systems dramatically improved their bonding performances on dentine.[16,17] Consequently, most of the universal adhesives now include this functional monomer in their compositions.[18] However, even a 10-MDP containing universal adhesive also require a higher concentration of co-monomers or solvents in their composition for proper adhesive-dentine reactivity.[19] Such an increased volume of solvents have been found to greatly modify the physical and mechanical properties of the universal adhesives

and adversely affect the quality of hybrid layer as well as their bonding efficacy.[20]

Several studies have intended to improve the bonding outcome of universal adhesives to dentine by employing different clinically relevant approaches such as covering the adhesive layer with an extra hydrophobic resin layer or enhancing the adhesive application.[21,22] The adhesive application can be enhanced either by applying additional layers and curing after each application (additional coating) or by additional applications but curing only at the end of the application procedure (increased application time).[21,23] A thicker adhesive layer (additional coating), which in turn exerts a cushioning effect against stress, also reduces the oxygen inhibition effects, which leads to better polymerisation.[24,25] In contrast, increased application time can improve the bond strength by better resin infiltration and decreasing residual water; however, the latter effect is material dependent.[24,26]

Adhesive application for an increased time may aggravate adhesive pooling, resulting in a non-homogeneous and poorly polymerised adhesive layer.[27] In contrast, the additional coating strategy can produce a more uniform adhesive layer.[28] Moreover, additional curing with light may also improve the polymerisation via increased monomer conversion.[29] Double coats or an

extra hydrophobic resin layer have been found to improve the bonding efficacy of the universal adhesives.[21,22] According to the findings of a recent study, a new two-step universal adhesive system – G2 BOND Universal (a successor of G-Premio Bond) showed an adhesive thickness as high as 38 µm, with a bonding performance comparable to that of the gold standard two-step self-etch adhesive Clearfil Megabond 2 (22 µm).[30]

Nonetheless, if an adhesive layer is excessively thick, it would rather fail cohesively while bearing the functional load of a restoration due to the concentration of stress inside it. [27] Moreover, a thick adhesive layer could be aesthetically unacceptable at the restoration margins or appear as a caries-like radiolucent area in radiographs[31], unless the adhesive itself is radiopaque. [32] Furthermore, to what extent the increased thickness of a universal adhesive would improve its dentine bond strength or other mechanical properties have not been established by a direct cause-effect relationship utilising the same specimens to evaluate both variables. Therefore, investigating the effects of additional coating of current universal adhesives on their thickness, mechanical properties, and bond strength is crucial.

The purpose of this study was to determine the correlation between the dentine bond strength of universal adhesives with their corresponding

adhesive thickness when applied in an additional coating strategy. The effects of the additional curing on the hardness and, elastic modulus of the adhesive layer were also determined. The null hypothesis tested was that the additional coating strategy would not improve the bond strength of universal adhesives by enhancing their thicknesses and hardness as well as the elastic modulus.

CHAPTER 2

MATERIALS AND METHODS

2.1 Tooth Selection and Specimen Preparation

A total of 99 sound human maxillary premolars were used in this study.[33] The teeth were obtained with the patients' informed consent approved by the local Ethics Committee (protocol # 2018-9). All the teeth were cleaned and stored in a 0.5% aqueous chloramine-T solution at 4° C and used within six months post-extraction.

The methodology of the study is illustrated in Fig 1.

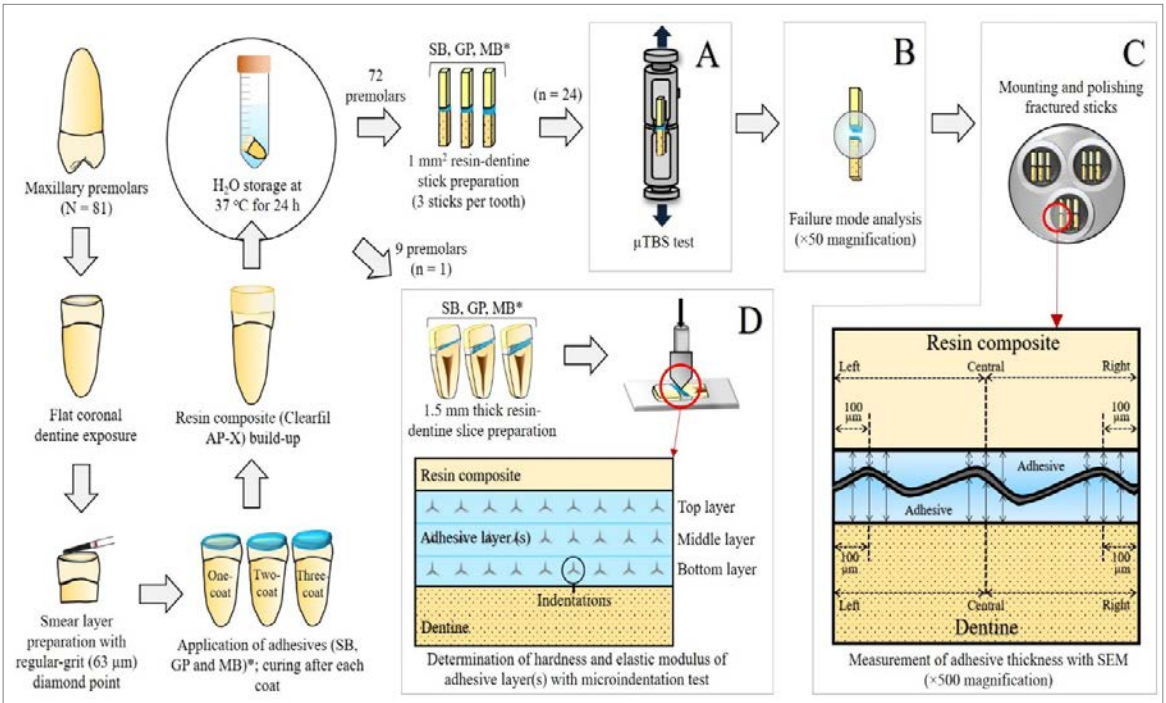

Figure 1. A diagrammatic overview of the methodology. The study employed one (manufacturers' instructions), two or three coats of two universal adhesives (SB and GP)* and one control two-step self-etch adhesive (MB; no priming for two and three coats, only the bond resin was applied)* on bur-cut dentine of maxillary premolars: (A) Microtensile bond strength (μTBS) tests of the resin-dentine sticks, (B) Failure mode analyses, (C) Measurement of adhesive thickness and, (D) Microindentation test
* SB: Scotchbond Universal Adhesive, GP: G-Premio Bond and MB: Clearfil Megabond 2.

The teeth enamel was removed to expose the coronal dentine surfaces that were prepared with five unidirectional gentle strokes of tapered regular-grit (63 μm) diamond burs (diamond point FG, #102R, Shofu, Kyoto, Japan) in a high-speed handpiece with adequate water cooling to simulate clinically relevant smear layers.[33] Each bur was discarded after the preparation of five teeth.[34] The teeth were randomly assigned to nine experimental groups to produce samples based on - (1) adhesives (Table 1): Scotchbond Universal Adhesive [SB; 3M ESPE, St. Paul, MN, USA; Universal], G-Premio Bond [GP; GC, Tokyo, Japan; Universal], Clearfil Megabond 2 [MB; Kuraray Noritake, Osaka, Japan; two-step; control] and (2) their application strategies (Table 2): one-coat (according to the manufacturers' instructions), two-coat, three-coat.

Table 1 Adhesives and their composition

Adhesives (codes/ batch no.)	Composition
Scotchbond Universal Adhesive (SB/ 666963)	10-MDP, Vitrebond copolymer, HEMA, dimethacrylate resins, filler, silane, initiators, ethanol, water
G-Premio Bond (GP/ 1807031)	10-MDP, 4-META, MDTP, methacrylate acid ester, distilled water, acetone, photoinitiators, fine powdered silica
Clearfil Megabond 2 (MB/ Japan/ 000047)	*Primer:* 10-MDP, HEMA, hydrophilic aliphatic dimethacrylate, dl-CQ, water. *Bond:* 10-MDP, bis-GMA, HEMA, dl-CQ, hydrophobic aliphatic dimethacrylate, initiators, accelerators, silanated colloidal silica.

10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; 4-META: 4-methacryloxyethyl trimellitic anhydride; MDTP: 10-methacryloyloxydecyl dihydrogen thiophosphate; CQ: camphorquinone; bis-GMA: bisphenol-A-glycidyl methacrylate

Table 2 Application strategies of the adhesives

	⁋Application strategies		
Adhesives (codes)	*One-coat	Two-coat	Three-coat
Scotchbond Universal Adhesive	1. Apply adhesive and rub for 20 s. 2. Gently dry for about 5 s until it no longer moves, and the solvent evaporates. 3. Light cure for 10 s.	1. Apply the first layer of adhesive following steps 1-3 of the one-coat strategy. 2. Apply the second layer for 20 s without rubbing. 3. Repeat steps 2-3 of the one-coat strategy.	1. Apply two layers of adhesive, following steps 1-3 of the two-coat strategy. 2. Apply the third layer for 20 s without rubbing. 3. Repeat steps 2-3 of the one-coat strategy.
G-Premio Bond	1. Apply adhesive and leave undisturbed for 10 s. 2. Dry thoroughly with maximum air pressure. 3. Light cure for 10 s.	1. Apply the first layer of adhesive following steps 1-3 of the one-coat strategy. 2. Apply the second layer for 20 s without rubbing. 3. Repeat steps 2-3 of the one-coat strategy.	1. Apply two layers of adhesive, following steps 1-3 of the two-coat strategy. 2. Apply the third layer for 20 s without rubbing. 3. Repeat steps 2-3 of the one-coat strategy.
Clearfil Megabond 2	1. Apply primer and leave for 20 s. 2. Gently air blow for > 5 s. 3. Apply bond and gently air blow to make a uniform film. 4. Light cure for 10 s.	1. Apply the first layer of adhesive following steps 1-4 of the one-coat strategy. 2. Apply the second layer following steps 3-4 of the one-coat strategy.	1. Apply the first layer of adhesive, following steps 1-4 of the one-coat strategy. 2. Apply the second and third layers by following steps 3-4 of the one-coat strategy twice.

⁋Self-etch mode

*According to manufacturer's instruction

The adhesive application (Table 2) was followed by incremental build-up with three increments of resin composite (Clearfil AP-X, Kuraray Noritake, Niigata, Japan), with each increment not exceeding 1.5 mm. Each adhesive coat was light-cured for 10 s, and each resin composite increment was cured for 20 s, with an LED curing unit (Pencure 2000, J. Morita Corp., Tokyo,

Japan) having a power output (irradiance) of 1,000 mW/cm2. The irradiance of the curing unit was checked periodically (Radiometer 100, Demetron Kerr, Orange, CA, USA). The bonded teeth were then stored in distilled water at 37 ° C for 24 h.

2.2 Microtensile Bond Strength (μTBS) Test

Resin-dentine sticks (≅1 mm^2 cross-sectional area) were prepared from 72 bonded teeth (8 teeth per group) with a low-speed diamond saw (IsoMet 1000, Buehler, Lake Bluff, IL, USA) according to the non-trimming technique.[33] Three sticks per tooth (n = 24 sticks per group) were selected for testing. Each stick was attached to Ciucchi's jig with cyanoacrylate glue (Model Repair II Blue, Dentsply-Sankin, Tokyo, Japan) and stressed under tension using a 500-N load cell at 1 mm/min crosshead speed in a desktop testing apparatus (EZ-S, Shimadzu, Kyoto, Japan) until failure occurred (Fig 1-A). The strength value at the maximum load of failure was calculated. The data retrieved (in N) were divided by the cross-sectional area (mm^2) and expressed in MPa.

2.3 Failure Mode Analysis

The failure modes were determined immediately after the μTBS test from the sticks' fractured ends (Fig 1-B) using a stereomicroscope at 50× magnification (SMZ-171-TLED, Shimadzu, Kyoto, Japan), paying attention to

the fact that the specimens were not dehydrated.[33] The failure modes were classified into adhesive failure, cohesive failure in dentine, cohesive failure in resin composite, and mixed failure.[35] For simplifying the explanation, these four modes were reclassified into adhesive failure and non-adhesive failure.[36] The failures occurring individually or simultaneously at the resin composite-adhesive interface, adhesive-dentine interface, and cohesively within the adhesive were considered as adhesive failures and the non-adhesive failures included cohesive failure in dentine, cohesive failure in resin composite, and mixed failure.[36]

2.4 Measurement of Adhesive Thickness from Fractured Resin-Dentine Sticks

The adhesive layer thickness was measured using a scanning electron microscope (SEM, FE-SEM, S-4800, Hitachi, Tokyo, Japan). The measurement was done from both ends of the fractured resin-dentine sticks immediately after the failure mode analysis.

Flat plastic rings (diameter = 6 mm; height = 1 mm) were fixed over aluminium stubs (Fig 1-C). The same surfaces of each pair of fractured fragments were demarcated to align the fractured ends of the resin-dentine sticks properly inside the rings. After epoxy embedment, the specimens were sequentially wet-polished with SiC papers (#600, #800, and #1000-grit; Sankyo-Rikagaku Co., Saitama, Japan) and diamond pastes (6, 3, and 1 μm; DP-Paste, Struers,

Denmark). Ultrasonic cleaning was done for 2 min after each polishing step. The specimens were then dried at room temperature (23 ± 2° C; 50 ± 10% RH) for 24 h, sputter coated with Pt-Pd (E-1030, Hitachi, Tokyo, Japan), and observed under the FE-SEM at 500× magnification at an accelerating voltage of 10 kV to measure the adhesive thickness.

The measurement procedure was accomplished with the built-in scaling tool of the SEM image processing software. As shown in Fig 1-C, the adhesive thickness (μm) was calculated from the remaining adhesive thicknesses at three different locations from each fractured end. The locations were at the left lateral, right lateral, and central areas of each end. Each lateral location was approximately 100 μm medial to the respective edge of a fractured end, and the central spot was located approximately halfway between the edges. The value at each location corresponded to the mean of triplicate measurements. The average thickness value of all three spots of one fractured end was considered as its adhesive thickness. Finally, the sum total value of each stick's fractured-end adhesive thickness was considered the approximate adhesive thickness of that stick.

2.5 Evaluation of Hardness and Elastic Modulus of the Adhesive

After 24 h water storage (37 ° C), twenty-seven bonded teeth (n = 3/group) were longitudinally sectioned with an IsoMet diamond saw to form 1.5 mm

thick resin-dentine slices (Fig 1-D). One central slice per tooth was employed for hardness and elastic modulus evaluation. Each slice was sequentially polished with wet SiC of decreasing abrasiveness (1000-, 1200- and 2000-grit) followed by diamond paste polishing up to 1μm grain. Each polishing step followed ultrasonic cleaning with distilled water. The hardness and elastic modulus of the adhesive layer was measured with a dynamic ultra-microhardness tester (DUH-211, Shimadzu, Kyoto, Japan) having a 0.1 μm Berkovich diamond indenter with 115° angulated tip (room condition: 23 ± 2° C and 50 ± 5% RH). The procedure was conducted by indenting at five different spots/ adhesive coat/slice at an interval of approximately 200 μm. Indentations at the excessively thinned adhesive regions were avoided. The indentations were performed at a constant speed of 0.2926 mN/s, and the maximum load employed was 5.04 mN with a 10 s holding-time setting the Poisson's ratio at 0.30.[37] A clear demarcation between two adhesive layers of all three adhesives could be visualized with the built-in microscope of the testing device, permitting the indentation placement approximately in the mid-thickness of each layer. As shown in Fig 1-D, the two-coat groups received indentations at the bottom of the adhesive layer (produced by the first coat) as well as the top adhesive layer (produced by the last coat). Likewise, the three-coat groups received

indentations at the bottom, middle, and top of adhesive layers. Finally, the mean hardness (MPa) and elastic modulus (GPa) values of each adhesive layer were obtained. In the case of two- or three-coat, mean values of the multiple coats were also calculated.

2.6 Statistical Analyses

Data were analysed using SPSS 24.0 (Chicago, IL, USA) at α = 0.05 level of significance. The μTBS data were normal and homogeneous. A two-way ANOVA was done to check the effects of the adhesives and their number of coats on μTBS. The statistical differences among nine study groups were further checked with one-way ANOVA with Tukey's post-hoc test. Adhesive thickness, hardness and elastic modulus data were non-normal and non-homogeneous. Therefore, a Kruskal-Wallis H test, followed by Dunn's post-hoc test adjusted with Bonferroni correction, were performed to demonstrate the effects of different coats of adhesives on their thicknesses and mechanical properties. The resin-dentine stick was considered the statistical unit, and Spearman's rank-order correlation test was done to correlate the μTBS with the same stick's adhesive thickness. For analysing the hardness and elastic modulus of the adhesive layers in multiple-coat groups, Mann Whitney U Test (two-coat) and Kruskal-Wallis H test with pairwise comparisons (three-coat) were performed.

CHAPTER 3

RESULTS

3.1 μTBS

There were no pretest failures. Two-way ANOVA revealed significant effects of adhesives ($F = 476.263$, $p < 0.001$), and number of coats ($F = 51.625$, $p < 0.001$) on the μTBS. The interaction among these variables were also significant ($F = 12.498$, $p < 0.001$). The mean bond strength values are summarised in Table 3.

Table 3 Mean bond strength of the adhesives applied in different coats

Adhesives	μTBS (in MPa) ± standard deviations		
	One-coat	Two-coat	Three-coat
Scotchbond Universal Adhesive	55.9 ± 6.9$^{c-d,e}$	66.7 ± 6.8^{e-h}	50.3 ± 5.5^{e-d}
G-Premio Bond	29.1 ± 7.8^{a}	37.2 ± 5.1^{b}	38.9 ± 3.2^{b,c}
Clearfil Megabond 2	58.5 ± 6.4$^{d-e,f,g}$	68.7 ± 5.4$^{e-h,i}$	57.5 ± 8.0$^{d-e,f}$

n = 24 resin-dentine sticks; values with different superscript lowercase letters indicate statistically significant differences (Tukey's test; $p < 0.05$)

Regardless of the adhesives, the application of two coats resulted in significantly higher μTBS values compared to their one-coat counterparts ($p < 0.05$). However, in the three-coat groups, the μTBS values of SB and MB decreased significantly compared to their corresponding two-coat groups ($p < 0.05$) but were similar to their one-coat groups ($p > 0.05$). In contrast, the μTBS of the three-coat group for GP was not significantly different from its two-coat counterpart ($p > 0.05$).

3.2 Failure Modes

Fig 2 shows the percentage of failure modes. Irrespective of the number of coats, non-adhesive failures were predominant in SB and MB, except for the three-coat group of SB, which mainly showed adhesive failures. All the GP specimens demonstrated adhesive failures only. No cohesive failure in resin composite was observed in the study.

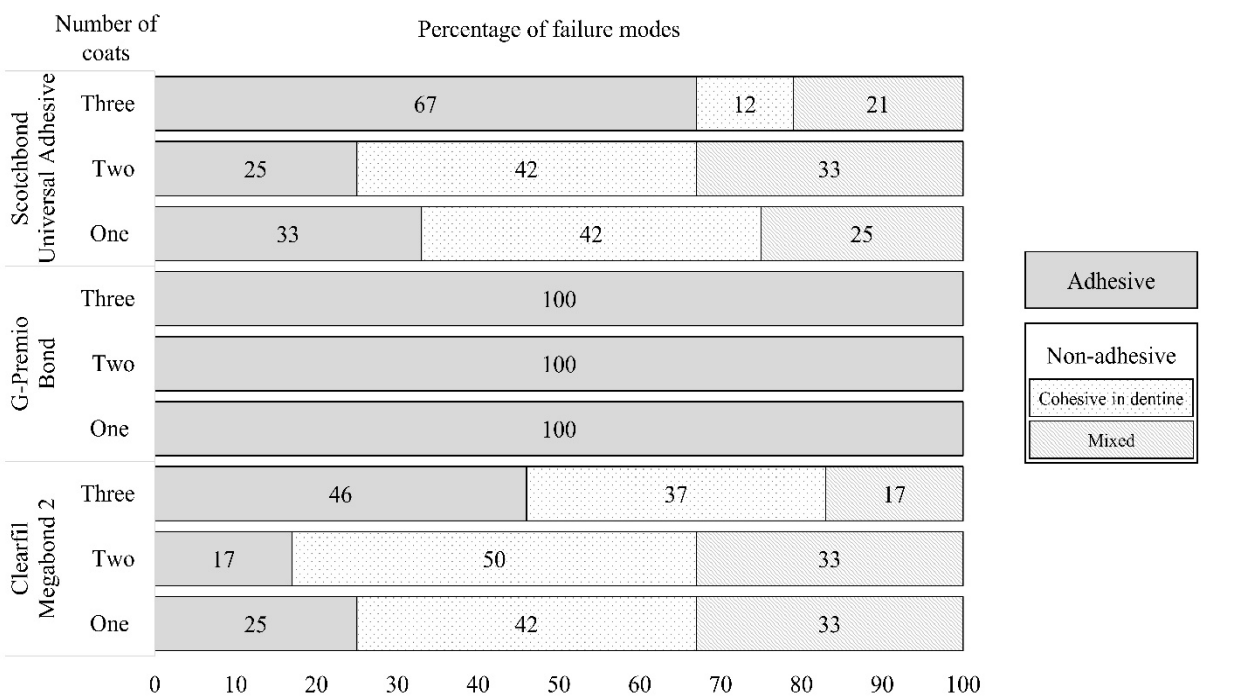

Figure 2. Fracture modes of the tested adhesives applied in one, two or three coats. The non-adhesive failures include cohesive failures in dentine or mixed type of failures. Cohesive failure in resin composite was not observed.

3.3 Adhesive Thickness

The increased number of coats resulted in a significant increase in adhesive thicknesses for all the adhesives ($p < 0.001$). Table 4 shows the mean adhesive thickness values.

Table 4 Mean thickness of the adhesives applied in different coats

Adhesives	Adhesive layer thickness (in μm) ± standard deviations		
	One-coat	Two-coat	Three-coat
Scotchbond Universal Adhesive	$10.4 \pm 3.4^{a,b}$	24.8 ± 7.3^{e}	38.5 ± 5.5^{f}
G-Premio Bond	7.8 ± 2.6^{a}	$14.6 \pm 3.2^{b,c}$	$20.8 \pm 4.4^{d,e}$
Clearfil Megabond 2	$18.9 \pm 5.3^{c,d}$	77.0 ± 11.7^{g}	151.3 ± 19.4^{h}

n = 24 resin-dentine sticks; values with different superscript lowercase letters indicate statistically significant differences (Dunn´s post-hoc test with Bonferroni adjustment; $p < 0.05$)

In the case of one-coat, the thickest adhesive layer was obtained by MB (18.9 ± 5.3 μm) and the thinnest by GP (7.8 ± 2.6 μm). A similar trend continued among the adhesives, after increasing the number of coats. Fig 3 demonstrates representative SEM images of the adhesive thickness measured from the fractured interfaces. MB three-coat group had the thickest adhesive layer (Fig 3-i), SB two-coat group obtained relatively thicker (Fig 3-ii) and, GP one-coat group presented the thinnest adhesive layer (Fig 3-iii).

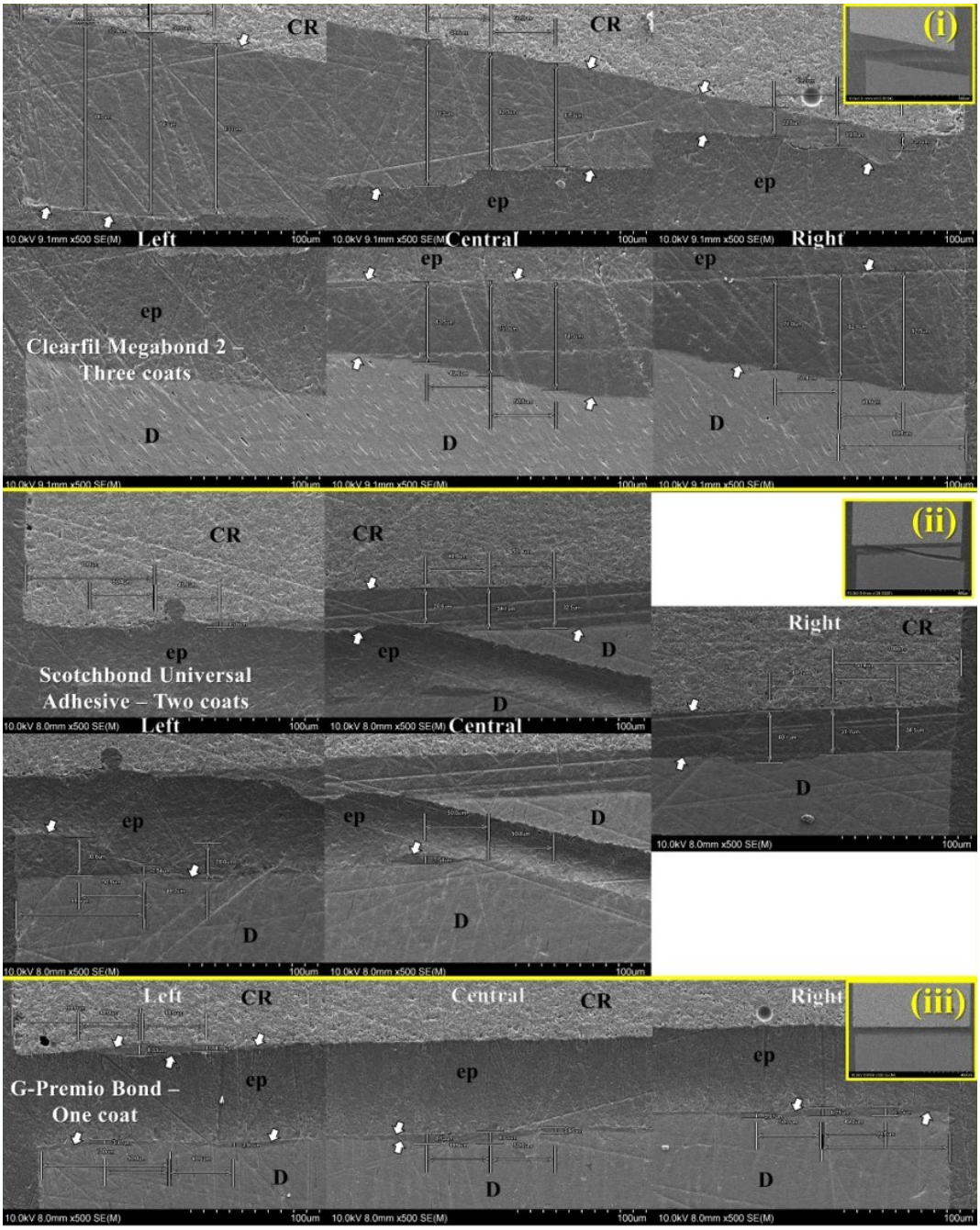

Figure 3. Representative SEM images showing the measuring locations of the adhesive layers of - (i) Clearfil Megabond 2 applied in three coats, (ii) Scotchbond Universal Adhesive applied in two coats, and (iii) G-Premio Bond applied in one coat. Three adhesive groups (i,ii,iii) are separated by yellow lines. White arrows indicate the adhesive layers; ep - epoxy resin; den - dentine; CR - resin composite. The inset-images with yellow boundaries are the fractured ends of both the pairs having the adhesive layers in lower magnification.

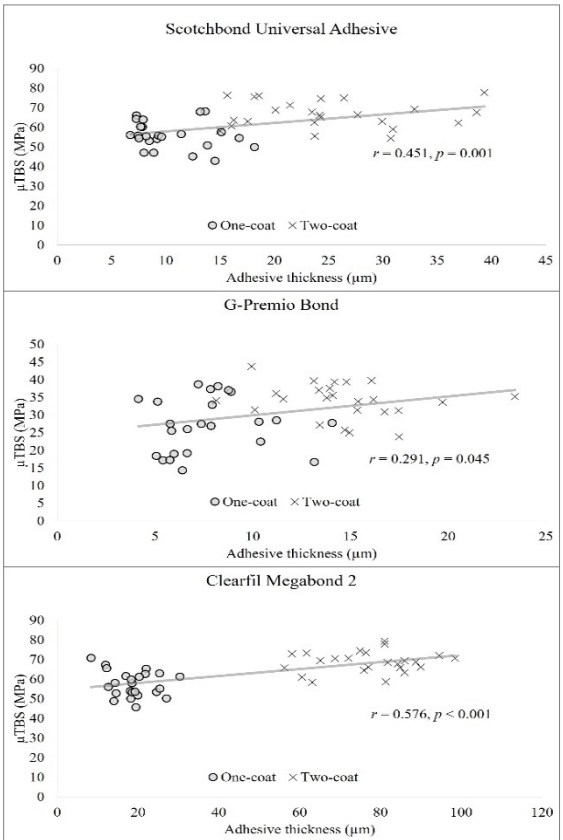

Figure 4. Correlation between adhesive thickness and corresponding microtensile bond strength (μTBS) of the tested adhesives applied in one and, two coats.

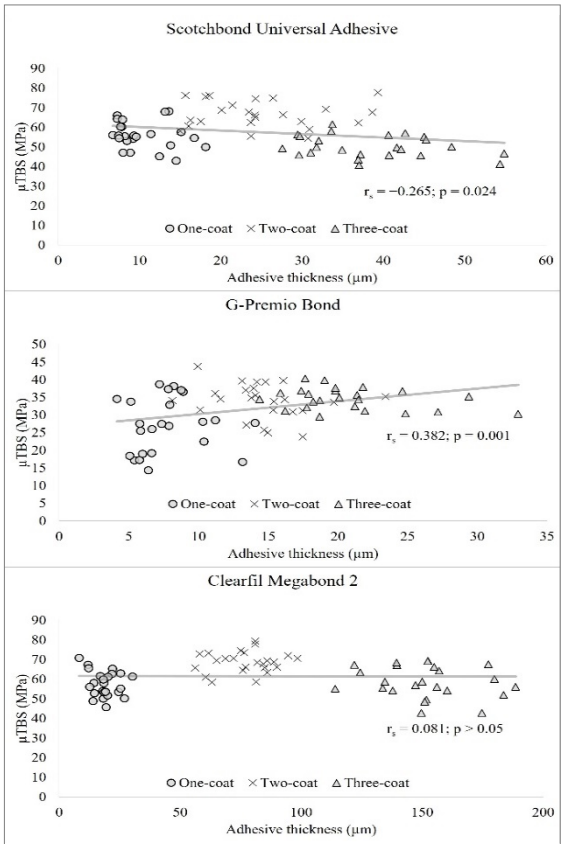

Figure 5. Correlation between adhesive thickness and corresponding microtensile bond strength (μTBS) of the tested adhesives applied in one, two and, three coats.

Fig 4 shows the correlation between the bond strength and the corresponding thickness of the adhesives when applied in two coats. The correlation was significant and positive for all three adhesives: SB ($r = 0.451$, $p = 0.001$), GP ($r = 0.291$, $p = 0.045$) and MB ($r = 0.576$, $p < 0.001$). However, adding the three-coat data (Fig 5) demonstrated significant but weak and negative correlation for SB ($rs = -0.265$, $p < 0.05$), moderately positive correlation for GP ($rs = 0.382$, $p < 0.05$) and, no correlation for MB ($rs = 0.089$, $p >$

0.456). However, the correlations for all three adhesives were weak to moderately positive and significant till their application in two coats (Fig 5): SB (rs = 0.451, p = 0.001), GP (rs = 0.291, p = 0.045) and MB (rs = 0.576, p < 0.001).

3.4 Hardness and Elastic Modulus

The mean hardness and elastic modulus values of the adhesive layer(s) obtained by different application strategies are shown as bar graphs in Fig 5.

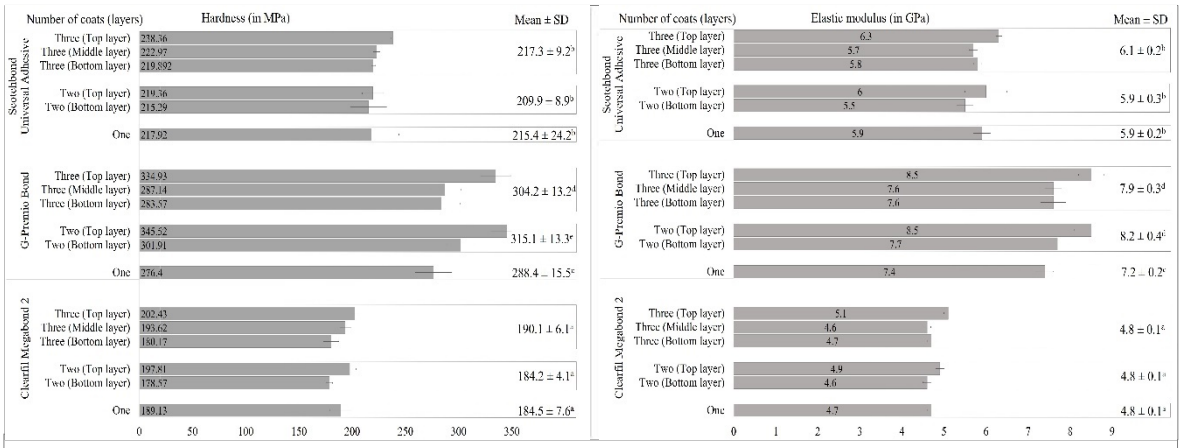

Figure 6. Hardness and elastic modulus of the adhesive layer(s) when applied as one, two or three coats. For two- and three-coat groups, the bottom layer denotes the first coat applied over the dentine, and the top layer indicates the terminal coat just beneath the lining resin composite. The middle layer (only in three-coat) refers to the intermediary layer between the top and bottom. Different lowercase superscript letters beside values indicate statistically significant differences.

The mechanical properties of SB and MB was not affected by the application of multiple coats (p > 0.05). For GP, additional coatings significantly increased both the hardness and elastic modulus values compared to the one-coat counterpart (p < 0.05). GP's two-coat group showed the highest

hardness (315.1 ± 13.3 MPa) and elastic modulus (8.2 ± 0.4 GPa) values among all its groups. However, in general, regardless of the adhesive, in the cases of two and three coats, the top adhesive layers always showed higher values compared to their bottom layers.

CHAPTER 4

DISCUSSION

Several studies have been performed that established a connection between bond strength and adhesive layer thickness subjecting the specimens with predetermined adhesive thicknesses to stress.[27,29,38,39] One μTBS study demonstrated adhesive thickness measured from intact, non-fractured beams.[40] The present study also employed the measurement of adhesive thickness from sectioned beams but from the fractured ones after submitting them to microtensile stress with an aim to establish a more factual correlation between μTBS and adhesive thickness.

Clinically, multiple coats of an adhesive can be applied either by finally polymerizing once after applying all the coats or by separately polymerizing each coat.[21,41] The current study emphasized on adhesive layer thickness and hence followed the latter strategy as separate polymerization is known to distinctly increase the adhesive thickness compared to final polymerization.[40-42] Correspondingly, substantial variation in thicknesses among different coats were also observed in this study (Table 2; Fig 5). An additional coating strategy could increase the adhesive layer's thickness, which led to enhancements of the dentine bond strength.[40,43] Ausiello et al. evaluated the effects of increased adhesive thickness by

3D finite element analysis.[24] They concluded that an optimal adhesive layer thickness could lead to maximum stress relief, which can improve the dentine bond strength. The present study results, which focused on obtaining the bond strength values and adhesive layer thicknesses of the same resin-dentine sticks, are also in agreement with these reports. In our case, after the 24 h-bond strength test, the fractured ends of the sticks were immediately subjected to failure mode analysis, and the adhesive layer thickness was measured using SEM. Thus, the evaluation method employed in this investigation made it possible to determine the direct and representative cause (adhesive thickness) and effect (bond strength) relationship by using the resin-dentine sticks for μTBS test, followed by measurement of the adhesive thickness from the fractured ends of the same sticks with SEM.

In this study, the overall thickness of MB was the highest, followed by SB and the lowest in GP (Table 2). MB, as a 2-step self-etch adhesive, comprises of a separate non-solvented relatively more hydrophobic bonding resin over the primed surface, which might have been accountable for its pronounced thicker dimension compared to the other two single-component 1-step self-etch adhesives SB and GP.[22,30] Regarding the film adhesive thickness comparison between two universal adhesives, SB1 was non-significantly

higher than GP1, Adhesive thicknesses of one coat of SB (around 10 μm) and GP (around 8 μm) were not significantly different. However, in both the cases of two and three coats, SB was significantly higher than GP ($p < 0.001$). SB contains ethanol and water as solvents, requiring gentle air blow after an active application, whereas, the HEMA-free GP requires desiccation to remove the excess water, as the highly volatile acetone solvent in it, cannot form an effective azeotrope, like ethanol [45], which can be reasoned to GP's thinner film. Again, filled adhesives are known to produce more viscous and thicker adhesive layer with a single application compared to the unfilled ones forming a less susceptible to oxygen-inhibition layer.[44-46] TEM observation in a previous study demonstrated the presence of nano-sized filler particles among all the three adhesives used in this study, with SB having the highest and GP having the lowest concentration of fillers [47], which can be considered as another reason of thicker SB layer than GP.

Microtensile method of testing the bond strength was employed in this study as it has already been proved versatile and has been found to be frequently conducted in thousands of studies,[48] including many adhesive thickness related works.[27,40,49] The bond strength can originate from hybridization, resin tag formation, and chemical interaction.[50-52]

The results of this study revealed that, regardless of adhesive, a second coat on top of one significantly increased ($p < 0.05$) the adhesive thickness and also the bond strength (Fig 4). On the contrary, a third coat, despite forming the thickest adhesive layer, did not improve the bond strength any further, but rather decreased in SB. This finding was complemented by the weak but significant and inverse correlation for SB ($rs = -0.265$) and no correlation for MB ($rs = 0.089$, $p = 0.456$) (Fig 5). Despite showing a moderately positive correlation ($rs = 0.382$, $p < 0.05$), the mean bond strength of the three-coat group of GP (38.9 ± 3.2 MPa) was indifferent ($p > 0.05$) to its two-coat counterpart (37.2 ± 5.1 MPa) (Table 2), indicating achievement of a plateau at two-coats.

The significantly improved bonding performances of SB and MB two-coat groups were substantiated by the increasing non-adhesive failure percentages (Fig 2). According to the previous reports, the predominant failure in HEMA-free GP is the adhesive-dentine interfacial failures which occurs as a consequence of phase separation.[53,54] Also, in this study, the failure pattern of GP was always adhesive regardless of the adhesive thickness and bond strength. Nevertheless, the multiple light exposure radiating through the additional thin films in the cases of GP's two or three-coat groups might have benefitted the bottom layer [55] promoting the bonding ability.

Therefore, it seems that in all the tested adhesives, two-coat applications led to an optimum thickness at which the bond strength reached its peak (Table 2). This observation is in agreement with previous reports.[21,25,28,43]

All the adhesives used in this study contain a functional monomer 10-MDP in their formulations (Table 1). 10-MDP has already been superficially ranked because of their ability to establish a very intensive and stable chemical interaction with the hydroxyapatite.[56,57] However, dimers and impurities can hinder the adhesive capability of 10-MDP.[58] Furthermore, different comonomers, solvents and catalysts can lead to variations in their film properties, reactivities and bonding capabilities to dentine.[10] Therefore, in the present study, the rise and fall in μTBS values associated with the progressive thicknesses (Fig 4, 5) might be owing to more mechanical rather than chemical factors as after curing the first coat, further resin penetration and chemical interaction, as well as hybridization, did no longer follow.[59,60]

As observed during the SEM observation thickness measurement of the fractured interfaces in SEM, most of the adhesive failures of SB's three coat group occurred cohesively inside adhesive (Appendix A). Despite having substantially thicker dimension, MB's two-coat group (approximately 77 μm) did not demonstrate such predominance in adhesive failure, which might be

the possible result of stress relief. It is possible that, depending upon the type of material, such cohesive failures even with a reasonable bond strength value, would be likely beyond a certain thickness, when Young's modulus can no longer contribute in resisting the elastic deformation under stress. This might be the reason one of the reasons of lower descending bond strength of SB's three coat group compared to its other counterparts, albeit having the lowest Young's modulus value.

The hardness and elastic modulus of an adhesive, can modify the bond strength by influencing the fracture resistance of the adhesive.[61] For instance, increased stiffness and lower flexibility of a material can increase the chances of adhesive failure. We hypothesised that the additional curing steps would contribute to an enhancement of the mechanical properties of the adhesive layer(s), leading to improved μTBS. Therefore, we evaluated the hardness and elastic modulus values of the different adhesive layers (bottom, middle, and top) as an indirect indicator of the degree of conversion.[62] However, the mean hardness and elastic modulus values of the tested groups (one-coat, two-coat, and three-coat) showed material dependency. Only GP demonstrated a significant increase in both properties with additional curing. In contrast, in SB, only hardness was

increased in the three-coat group ($p < 0.05$), influencing the bond strength. MB did not show any change ($p > 0.05$).

Filled or thicker adhesive layer along with the hybrid layer can form an elastic cavity wall, which can counterbalance the polymerization shrinkage stress. [63-65] A previous study demonstrated inverse correlations between elastic moduli of both the three or two-step etch-and-rinse and two or one-step self-etch adhesives and their bond strength observed an inverse correlation between elastic modulus of the adhesive layer and bond strength, implying that low elastic modulus leads to higher bond strength.[66] The microindentation test results of the present study (Figure 6) also showed that fluctuation of elastic modulus values of the adhesive layers occurred with the variation in products and their thicknesses and exhibited an opposite trend compared to their μTBS values (Table 3), except for SB's three coat group, which showed the lowest elastic modulus values when SB was applied in three coats (Figure 5).

Nevertheless, Taschner et al. reported sufficient curing capability with a high degree of conversion of SB and concluded that additional coating would not improve it any further.[23] Similarly, the new, improved MB contains an additional photoinitiator and a new accelerator, both of which have been claimed to be responsible for a high degree of conversion.[67]

Interestingly, regardless of the adhesive, with an additional coating, the top adhesive layers demonstrated significantly higher hardness and elastic modulus values ($p < 0.05$) compared to the bottom layers (Fig 6). This phenomenon may be a combined result of the following: firstly, the resin composite may have 'dislodged or absorbed' some residual monomers from the underlying top adhesive layer, resulting in a better degree of conversion, and secondly, the heat generated during polymerisation of the resin composite may also have resulted in improved monomer conversion at the adjacent adhesive layer.[25]

The present study results revealed that applying an additional coat (two-coat) was beneficial for all the adhesives tested. In GP, the application of two coats improved both adhesive thickness, hardness and, elastic modulus leading to increased μTBS. On the contrary, the bond strength improvement of SB and MB resulted from increased adhesive thickness only. Therefore, the null hypothesis that increasing the coats would not improve the bond strength of universal adhesives by the increased thickness and enhanced hardness and, elastic modulus had to be partially rejected.

From a clinical perspective, a thicker adhesive layer created with additional coating beneath a resin composite restoration may trigger aesthetic or diagnostic concerns. A crack or craze in an enamel surface is

not always visible unless illuminated or possesses the size of a hairline.[68] The diameter of a single hairline usually ranges from 60 to 100 µm.[69] In addition, as claimed by the BBC Science Focus, a pair of well-functioning human eyes can detect objects as small as 40 µm.[70] The polymeric structure of fillers and the surface roughness of an adhesive can result in the accumulation of stains inside the oral cavity from various sources over time, leading to marginal discolouration.[71] Such a discoloration might be esthetically unacceptable if the area goes beyond the size of a hairline or 40 µm, especially in case of anterior restorations. Furthermore, referencing Jorgensen, Fusayama reported that the minimal visible space between preparation and an inlay was 50 µm.[72] Therefore, adhesive thicknesses below this limit may be aesthetically tolerable. However, according to Opdam et al., an adhesive layer thicker than 40 µm would be detectable as a radiolucent area underneath the restoration.[73] The addition of some degree of radiopacity to the current adhesives could avoid misdiagnosing them as caries. Thanks to the new Scotchbond Universal Plus Adhesive, a successor of the SB, claimed to contain a novel type resin providing the adhesive layer dentine-like radiopacity.[32]

While one additional adhesive coat being promising with improved immediate performance, long-term bonding outcome should confirm the eligibility of

such application. Because an additional adhesive layer might contain non-neutralized acidic monomers and solvents, provoking internal plasticization of the layer itself, lowering the cohesive forces between the polymer molecules.[74] Future studies involving such application strategies should consider thermo-cycling or prolonged water storage of the specimens.

CHAPTER 5

CONCLUSION

Within limitations, the findings of the current study lead to the following conclusions:

1. An additional adhesive coating over the manufacturer's recommended adhesive layer improved the bond strength of all the adhesives tested.

2. While increased adhesive thickness resulting from additional coating favoured all the adhesives' bond strengths, the beneficial effect of additional curing was material dependent.

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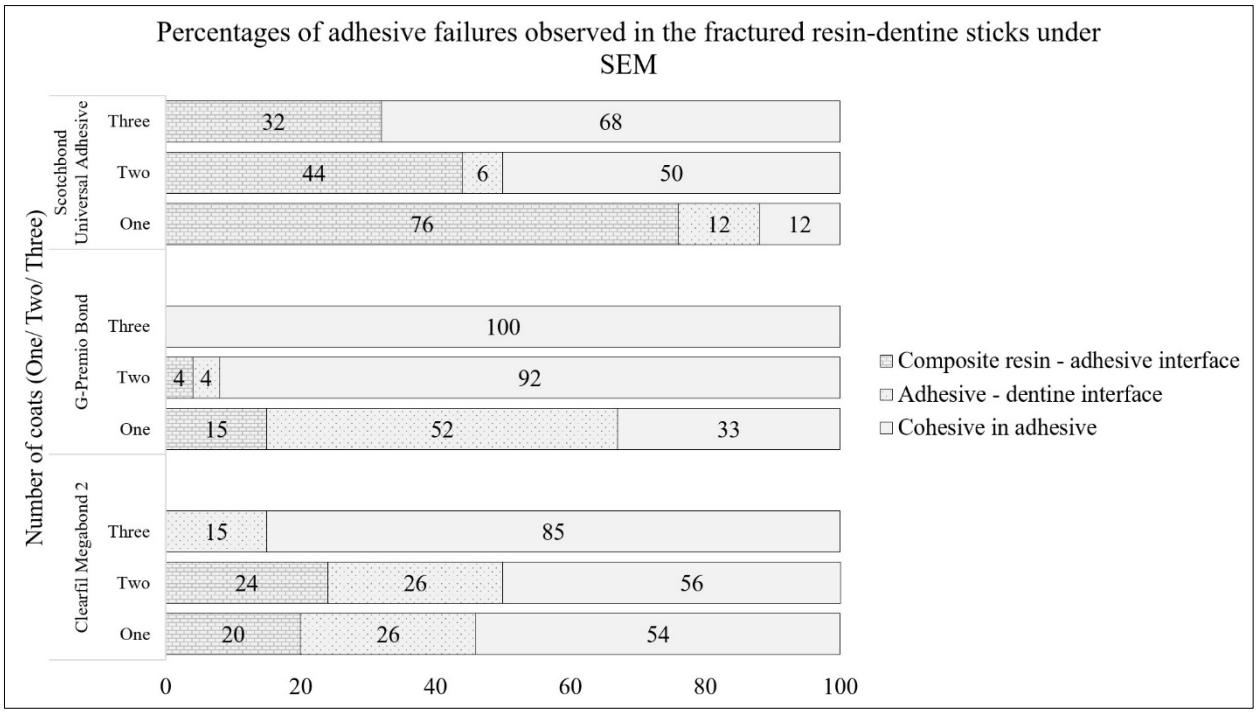
APPENDIX - A


Percentages of adhesive failures observed in the fractured resin-dentine sticks under SEM
Number of coats (One/ Two/ Three)
Scotchbond Universal Adhesive
G-Premio Bond
Clearfil Megabond 2
Three
Two
One
32
68
44
6
50
76
12
12
100
4
4
92
15
52
33
15
85
24
26
56
20
26
54
0
20
40
60
80
100
Composite resin - adhesive interface
Adhesive - dentine interface
Cohesive in adhesive