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How Do the Etching Mode and Thermomechanical Loading Influence the Marginal Integrity of Universal Adhesives?

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Clinical Relevance

Adhese Universal exhibited better marginal integrity than Peak Universal, and for both adhesives, thermomechanical loading degraded the bonding durability, particularly for nonetched enamel and pre-etched dentin.

SUMMARY

Objective: This study evaluated the effect of etching mode and thermomechanical loading on universal adhesives.

Methods and Materials: Two universal adhesives, Peak Universal and Adhese Universal, were used in two etching modes as the experimental groups: Peak Universal etch-and-rinse (PER), Peak Universal self-etch (PSE), Adhese Universal etch-and-rinse (AER), and Adhese Universal self-etch (ASE). Two adhesives considered gold standards were used as control groups: OptiBond FL (OER) was used as a control group for the etch-and-rinse (ER) mode, and Clearfil SE Bond (CSE) was used as a control group for the self-etch (SE) mode. Standardized class V cavities were created on the buccal and lingual surface in 30 extracted caries-free human third molars. Each adhesive and resin composite was applied according to the manufacturer's instructions. The specimens were subjected to thermomechanical loading (TML) immediately after the fillings were placed. Before and after TML, replicas and photographs of the fillings were performed and evaluated quantitatively and qualitatively. The Mann-Whitney U-test or Kruskal-Wallis test was used for quantitative analyses, and Fisher exact test was used for qualitative analysis.

Results: Adhese Universal achieved a significantly higher percentage of continuous margin in the enamel than Peak Universal for the two types of etching both before and after TML

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(except for the SE group after TML). In dentin, the greatest percentage of continuous margin was achieved for Adhese Universal in the ER group (100%) before TML and for both universal adhesives in the SE groups (61%) after TML. For both etching modes and both time points, Adhese Universal had a greater percentage of continuous margin than Peak Universal for the whole margin. For the ER approach, significant differences were observed both before and after TML, and for the SE approach, significant differences were observed before TML. TML did not cause a significant decrease in the percentage of continuous margin in the enamel, but the results were the opposite in dentin. A qualitative assessment using World Dental Federation criteria did not show statistically significant differences between the groups.

Conclusions: Scanning electron microscope assessment of marginal integrity showed that the evaluated factors such as etching mode and TML significantly influenced the marginal integrity of the universal adhesives. The replica method shows that laboratory and clinical assessment methods complement each other and give a broader view of marginal integrity.

INTRODUCTION

The effectiveness of adhesive systems can be evaluated by analyzing different factors (eg, the bond strength and marginal integrity of the resin-tooth interface).[1] Good marginal integrity is achieved when there are no marginal microgaps around the filling, with sufficiently deep penetration of the adhesive into the tissue. The gaps occurring at that interface contribute to the penetration of bacteria and fluids, which may provoke hypersensitivity, pulpitis, marginal staining, and debonding with retention loss.[2,3] Marginal integrity might be greatly influenced by shrinkage stress on the restorative material, the size and type of the cavity, the insertion and polymerization technique, the composition and type of the adhesive system used, and the etching approach.[4-6]

Etching pretreatment of the tooth surface and bonding between the tooth tissue and adhesive can be achieved through an etch-and-rinse (ER) or self-etch (SE) approach.[7] Compared with the SE technique, the ER technique, which commonly employs a 30% to 40% phosphoric acid gel, results in more effective and stable bonding to the enamel.[7] Unfortunately, in dentin, this etching mode can ultimately result in the collapse of collagen fibrils demineralized by acid etching, which leads to weaker bond strengths and a low-quality hybrid layer.[8] Compared with the ER technique, the SE technique is simpler and more favorable for dentin. SE adhesives are designed to infiltrate the regions that they have demineralized and should produce a hybridized complex comprising the residual smear layer and a thin, partially demineralized dentin collagen matrix.[9] To combine the advantages of these two modes, new universal adhesives have been introduced. These adhesives can be used as ER or SE adhesives or can be applied using the selective enamel etching approach. The aim of universal adhesives is to accelerate and simplify the process, with the end result of making the procedure easier (ie, less technique sensitive) and improving the clinical effectiveness of adhesives, including marginal integrity, bond durability, and long-term esthetics.[10] In 2015, Loguercio and others[11] applied field-emission scanning electron microscopy (direct and replica techniques) to assess qualitatively the influence of enamel preparation on the application of five universal adhesives. The authors found that the ER approach resulted in a deeper and more pronounced etching pattern in the enamel than the SE method. For the SE technique, the etching pattern was poorer in passive application than active application of the adhesive to enamel. However, there is still a lack of laboratory studies on quantitative assessments of the marginal integrity of universal adhesives under different conditions.

Laboratory tests, especially those with thermomechanical loading (TML), allow researchers to predict the clinical performance of adhesive systems and filling materials under different use conditions and to evaluate their mechanical and structural characteristics during aging.[12] The clinical assessment of marginal integrity is one of the functional properties of the World Dental Federation (FDI) criteria, which were introduced in 2007; the criteria are more rigorous for identifying differences in composite resin restorations than previous criteria.[13] The greatest opportunity for connecting laboratory and clinical research is the replica technique, in which a model based on an impression taken *in vivo* or *in vitro* is analyzed. Therefore, the purpose of this study was to evaluate the external marginal integrity of universal adhesives for different etching modes before and after TML *in vitro* in noncarious cervical lesions (NCCLs) using the replica technique and FDI criteria and to compare the two assessment methods. The first null hypothesis was that there are

no differences in the extent of marginal integrity when using universal adhesives with the ER or SE strategy. The second null hypothesis was that the universal adhesives were characterized by similar marginal integrity to "gold standard" adhesives. The third null hypothesis was that the results for the two methods of assessment of marginal integrity are comparable.

METHODS AND MATERIALS

Tooth Preparation and Bonding Procedures

The experimental part of this study is presented in Figure 1. Thirty extracted caries-free human third molars were cleaned from concrements and soft-tissue remnants and used in this research. The teeth were stored in aqueous solution of 0.5% chloramine-T and used within six months after extraction. Standardized class V cavities were created on the buccal and lingual surface of each specimen with a round diamond drill (EDENTA-801.314.012), which was replaced after five preparations. The cavities were 4-mm wide, 4-mm tall (2 mm above and below the cement-enamel junction), and 2-mm deep (controlled by a caliper). Fine-grained diamond burs were used for finishing the preparations and placing the enamel bevel (EDENTA-862.204.012). Next, the teeth were randomly allocated into six groups of 10 specimens each, divided according to the adhesive and etching approach. Two universal adhesives, Peak Universal and Adhese Universal, were applied with two etching modes as experimental groups: Peak Universal etch-and-rinse (PER), Peak Universal self-etch (PSE), Adhese Universal etch-and-rinse (AER), and Adhese Universal self-etch (ASE). Two adhesives considered gold standards were used as control groups: OptiBond FL (OER) was used as a control group for the ER group, and Clearfil SE Bond (CSE) was used as a control group for the SE group. The composition, manufacturer, batch number, and application technique of each adhesive system are shown in Table 1. After application of the adhesive systems, the cavities were filled with two increments of composite resin, which were polymerized (Demi Plus, Kerr, Orange, CA, USA) according to the manufacturer's instructions (Table 1). Immediately after the filling procedure, the restorations were finished with a flexible disc (Soflex, 3M ESPE, Maplewood, MN, USA).

Thermomechanical Loading

The specimens were subjected to combined thermal and mechanical loading immediately after the fillings were applied. Thermal cycling consisted of 3000 cycles in water at a temperature of 5°C and 55°C with a dwell time of 20 seconds in each temperature bath and a transfer time of 13 seconds (SD Mechatronik GmbH, Feldkirchen-Westerham, Germany). Mechanical loading was performed over 100,000 cycles with a load of 110 N at a frequency of 2 Hz using a rounded tip as an antagonist (Walter +Bai Dynamic Testing Systems LFV-50kN, Walter +Bai, Löhningen, Switzerland).

Assessment of External Marginal Integrity by Scanning Electron Microscopy

Before and after TML, impressions of each restoration were acquired with an A-polyvinylsiloxane material (Aquasil Ultra XLV, Densply Sirona, York, PA, USA). Next, to obtain positive replicas, the impressions were filled with epoxy resin (EpoFix, Struers, Torrance, CA, USA). The replicas were sputter coated with gold-palladium and subjected to margin analysis using scanning electron microscopy (SEM) at 15 kV and 35× or 200× magnification (SU-70, Hitachi, Tokyo, Japan). The obtained SEM images were analyzed using ImageJ software (National Institutes of Health, Bethesda, MD, USA) to estimate the length and percentage of the continuous margin in relation to the entire assessable margin before and after TML.

Assessment of Marginal Integrity by FDI Criteria

Before and after TML, photographs of the fillings were acquired using a surgical microscope with a camera (Karl Kaps SOM 62 No. 16810). The photographs of each filling were rated according to the following FDI criteria for marginal adaptation, as suggested by Hickel and others[13]:

1. VG: clinically very good, which means harmonious outline, no gaps, no white or discolored lines
2. GO: clinically good, which means a marginal gap <150 μm, white lines or small marginal fractures removable by polishing or slight ditching, slight steps/flashes, minor irregularities
3. SS: clinically sufficient/satisfactory, which means a gap <250 μm that is not removable, several small marginal fractures or major irregularities, ditching or flashes, steps
4. UN: clinically unsatisfactory, which means a gap >250 μm, dentin/base exposed or severe ditching, marginal fractures, large irregularities or steps (repair necessary)

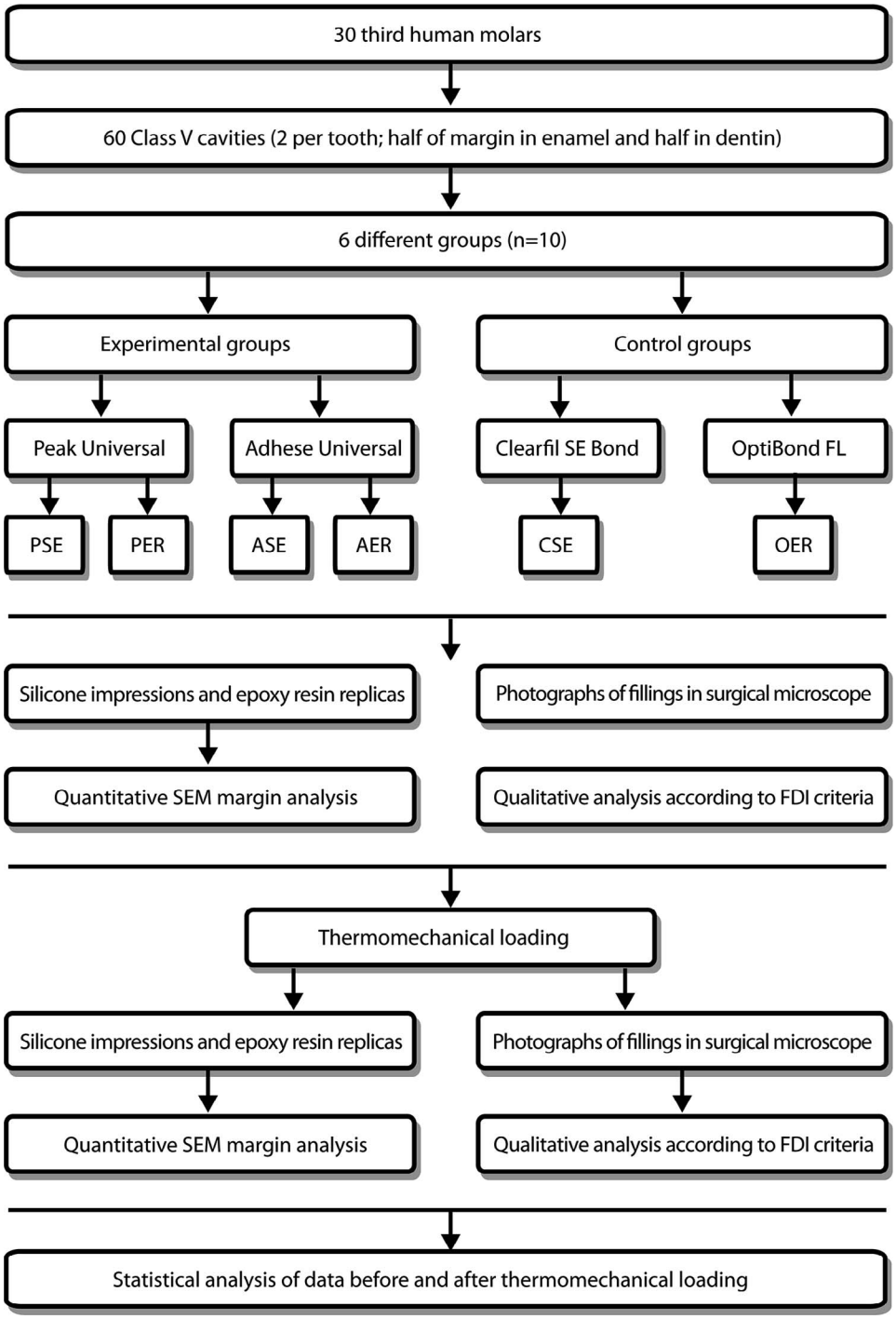

Figure 1. *Diagram of the current study.*

5. PO: clinically poor, which means a loose *in situ* restoration (complete or partial), major gaps or irregularities

Statistical Analysis

The Kolmogorov-Smirnov test revealed a nonnormal distribution of data in some groups; therefore, nonparametric tests (Mann-Whitney U test and Kruskal-Wallis test) were used for quantitative analyses. The Kruskal-Wallis test was used for testing whether samples originate from the same distribution (for testing more than two groups concurrently). The test determines whether the medians of the two or more groups are different, but it does not show the differences that exist between the groups. The Mann-Whitney test was

Table 1: *Adhesive and Composite, Application Mode, Composition, Manufacturer, and Batch Number for the Experimental and Control Groups*

Group	Adhesive (Manufacturer; Lot No.)	Application Mode	Composition	Application Method	Composite (Manufacturer; Lot No.)
Experimental					
PER	Peak Universal Bond (Ultradent, South Jordan, UT, USA; 5135 BBBXD)	Etch-and-Rinse	Ethyl alcohol, 2-hydroxyethyl methacrylate, methacrylic acid, 0.2% chlorhexidine di(acetate)	1. Acid etching for 20 s 2. Rinse for 5 s 3. Remove excess moisture 4. Apply adhesive 5. Air stream for 10 s 6. Light cure for 20 s	Amelogen Plus (Ultradent; 9031 BB5BJ)
PSE		Self-Etch		1. Apply Peak SE Primer for 20 s 2. Dry 3. Apply adhesive 4. Air stream for 10 s 5. Light cure for 20 s	
AER	Adhese Universal (Ivoclar Vivadent, Schaan, Lichtenstein; U23288)	Etch-and-Rinse	MDP, bis-GMA, HEMA, MCAP, D3MA, ethanol, water, initiator, stabilizers, silicon dioxide	1. Apply etchant for 15 s 2. Rinse for 5 s 3. Dry until chalky white 4. Apply adhesive 5. Gently air thin for 5 s 6. Light cure for 10 s at 1200 mW/cm^2	IPS Empress Direct (Ivoclar Vivadent; T39377)
ASE		Self-Etch		1. Scrub (ACTIVE[b]) or leave undisturbed (PASSIVE) one coat of adhesive for 20 s 2. Gently air thin for 5 s 3. Light cure for 10 s at 1200 mW/cm^2	
Control					
OER	OptiBond FL (Kerr, Orange, CA, USA; 5457273)	Etch-and-Rinse	Primer: HEMA, glycerol phosphate dimethacrylate, mono-2-methacryloyloxyethyl phthalate, water, ethanol Bond: Bis-GMA, HEMA, glycerol dimethacrylate, filler particles (fumed SiO_2, barium aluminoborosilicate, Na_2SiF_6)	1. Apply etchant for 15 s, rinse and blot dry 2. Apply primer with scrubbing for 15 s 3. Gentle air stream 4. Apply adhesive with brushing motion for 15 s 5. Light cure for 20 s	Herculite XRV Ultra (Kerr; 5136056)
CSE	Clearfil SE (Kuraray Noritake Dental, Tokyo, Japan; 000147)	Self-Etch	Primer: water, 10-MDP, HEMA, hydrophilic aliphatic dimethacrylate, accelerators, dl-camphorquinone Bond: 10-MDP, bis-GMA, HEMA, initiators, colloidal silica, dl-camphorquinone, accelerator	1. Apply primer with brushing motion for 20 s 2. Air dry for 5 s 3. Using the same applicator, apply adhesive with light brushing motion for 15 s 4. Air thin for 3 s 5. Light cure for 10 s at 1200 mW/cm^2	Clearfil Majesty (Kuraray Noritake Dental; 3J0009)

used to compare the two groups. Fisher's exact test was used to analyze the FDI criteria score. The level of significance was established as $p=0.05$ for all tests.

RESULTS

Enamel Marginal Integrity

Adhese Universal achieved a significantly higher percentage of continuous margin in the enamel than Peak Universal for the two types of etching both before and after TML (except for the SE group after TML). Both experimental Peak Universal groups achieved a significantly lower percentage of continuous margin than the control groups. For Adhese Universal, a significantly lower percentage of continuous margin was observed only for the ASE group after TML compared with Clearfil SE Bond. Both before and after TML, significantly better results were observed in the experimental ER groups than the experimental SE groups (Table 2). A significantly greater percentage of continuous margin in enamel than dentin was observed for the AER ($p=0.0001$), PER ($p=0.0004$), and CSE ($p=0.0233$) groups after TML (Table 2). The largest decrease in the percentage of continuous margin after TML was observed

Table 2: *Percentage (%) of Continuous Margin in Enamel, Dentin, and Enamel and Dentin Together Before and After Thermomechanical Loading (TML) for Different Etching Modes*[a]

Time	Tooth Tissue	Etch-and-Rinse			Self-Etch		
		PER	AER	OER	PSE	ASE	CSE
Before TML	Enamel	94 ± 6^{bde}	99 ± 1^{ad}	100 ± 1^{ce}	86 ± 11^{abc1}	96 ± 4^{ac1}	97 ± 5^{b1}
	Dentin	81 ± 16^{fgh2}	100 ± 1^{g2}	100 ± 0^{h2}	97 ± 5^{f12}	99 ± 3^{12}	100 ± 0^{f12}
	Enamel + dentin	88 ± 8^{kl2}	99 ± 1^{ik2}	100 ± 1^{l2}	90 ± 7^{ij2}	97 ± 2^{ik2}	98 ± 3^{j2}
After TML	Enamel	81 ± 15^{AC1}	99 ± 2^{AD1}	96 ± 7^{BC}	63 ± 19^{A}	72 ± 21^{BD}	92 ± 5^{AB1}
	Dentin	34 ± 20^{EF12}	49 ± 18^{G12}	89 ± 13^{FG2}	61 ± 29^{E2}	61 ± 23^{2}	78 ± 24^{12}
	Enamel + dentin	60 ± 11^{J2}	81 ± 9^{JK2}	93 ± 9^{HJ2}	62 ± 12^{I2}	67 ± 14^{HK2}	86 ± 10^{HI2}

[a] *N=10, mean ± SD. The same lowercase superscript indicates a difference at the 5% significance level between groups before TML in the same tooth tissue. The same uppercase superscript indicates a difference at the 5% significance level between groups after TML in the same tooth tissue. The superscript number "1" for the same group and time indicates a difference at the 5% significance level between tooth tissues. The superscript number "2" for the same group and tooth tissue indicates a difference at the 5% significance level between times.*

for both experimental SE groups; however, neither the experimental nor the control group showed a significant decrease in continuous margin (Figure 2).

Dentin Marginal Integrity

In the ER mode, Adhese Universal had a higher percentage of continuous margin than Peak Universal both before and after TML (before TML, the differences were significant). For the SE approach, Adhese Universal and Peak Universal had comparable percentages of continuous margin both before and after TML. Peak Universal exhibited a significantly lower percentage of continuous margin than the control group both before and after TML. Compared with the control group, the AER group had similar results before TML and significantly worse results after TML. Both universal adhesives in the SE approach before and after TML had a lower percentage of continuous margin than the control groups, and for the PSE group, this difference was significant. A significantly greater percentage of continuous margin in the dentin than the enamel was observed for the ASE ($p=0.0255$), PSE ($p=0.0306$), and CSE ($p=0.0306$) groups before TML. After TML, the experimental and control groups had a lower percentage of continuous margin in the dentin than in the enamel; this difference was significant for both universal adhesive ER groups. TML led to a significant decrease in the percentage of continuous margins for both the experimental and controls groups, and the largest decrease was observed for the two universal adhesives with the ER approach (Figure 2).

Enamel and Dentin Marginal Integrity

For both etching modes and both time points, Adhese Universal had a greater percentage of continuous margin than Peak Universal (Table 2). For the ER approach, the differences were significant both before and after TML, while for the SE approach, the differences were significant only before TML. Peak Universal exhibited a significantly lower percentage of continuous margin than the control groups for both etching approaches and both time points. Adhese Universal exhibited a lower percentage of continuous margin than the control groups for both etching approaches and both time points, but the differences were significant for the SE approach only.

Qualitative SEM Assessment of Marginal Integrity

Representative SEM micrographs of the PSE and PER, ASE and AER, and control groups are presented in Figures 3, 4, and 5, respectively. The influence of TML on the resin-enamel and resin-dentin interfaces of the same region is presented in these figures.

Qualitative Assessment According to FDI Criteria

Each filling received a "very good" or "good" rating based on the five-level scale. The best scores were obtained by the AER and OER groups both before and after TML, with 10 scores of "very good" in each group (Table 3). There were no significant differences for any single group before and after TML or between different groups at a single time point ($p>0.05$).

DISCUSSION

The outcomes of an adhesive procedure can differ widely depending on the adhesive and clinical situation. Therefore, the influence of application mode of universal adhesives on enamel and dentin bond efficacy was investigated. In this study, statis-

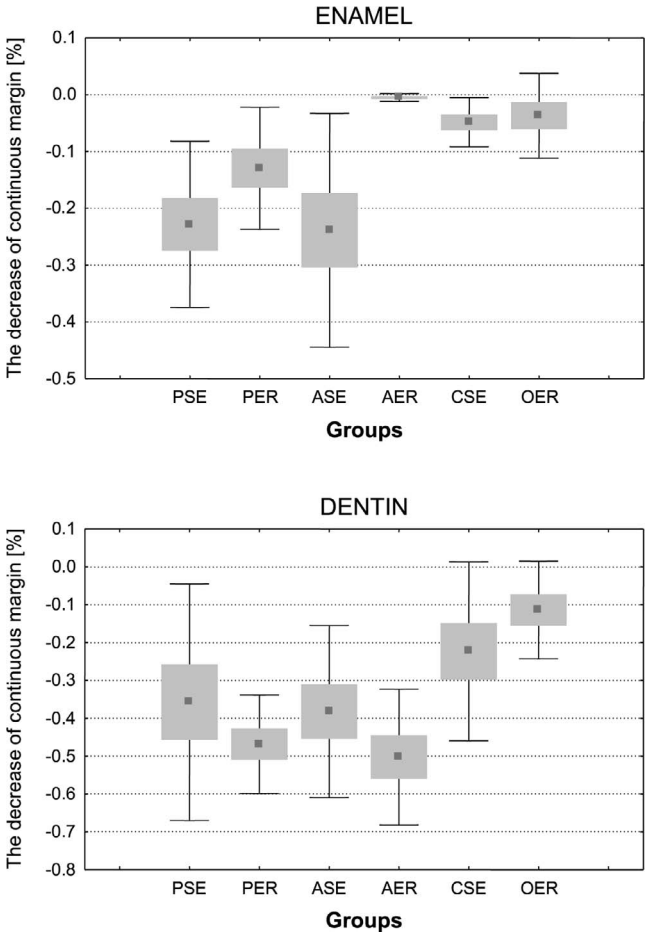

Figure 2. *The decrease in the continuous margin in enamel (upper graph) and dentin (lower graph) after TML as a percentage (%).*
Mean
±standard error
±standard deviation

tically better marginal integrity was observed for both universal adhesives with the ER method for enamel before and after TML and with the SE technique for dentin (except for Adhese Universal before TML); thus, the first null hypothesis was rejected.

This study is the first to evaluate the marginal integration of universal adhesives with the replica technique. This approach is a nondestructive technique for tooth specimens that enables the assessment and comparison marginal defects at different time points, as well as before and after applying different stresses to the tooth specimens, such as TML.[14] SEM is widely used to evaluate marginal integrity and allows marginal gaps to be distinguished from marginal irregularities or tooth fractures; moreover, areas of concern can be evaluated at a higher magnification.[15]

This study was performed using adhesive and resin composite from the same manufacturer, and the same operator performed all restorations to avoid variability.[14,16,17] Before the study, the teeth were stored in 0.5% chloramine-containing water to prevent bacterial growth, according to ISO Technical Specification 11405[18] and previous studies.[19,20] This study was based on class V cavities because they are easy to create with low researcher variability, and most studies evaluating the effectiveness of adhesives have used NCCLs.[12,16,21] NCCLs have no mechanical retention form and are located in enamel and dentin, facilitating a comparative assessment of the resin-dentin interface and the resin-enamel interface at the same time.[22,23] Moreover, cervical restorations are clinically challenging because of difficulties in moisture control, caries access, and proximity to the gingival margin.[24]

TML was performed to assess changes in the resin-tooth tissue interface under oral conditions. Thermocycling is commonly used as an artificial aging method because it creates repetitive expansion and contraction stresses along the resin-tooth tissue interface.[25,26] Hot water may also accelerate the hydrolysis of areas not covered by adhesive collagen and may extract poorly polymerized resin composite.[27] Similarly, mechanical loading imitates chewing on the sample, which causes tooth deformation and generates stress on the restoration margins.[28] These stresses are expected to increase the length of existing gaps or lead to the development of new gaps. Although there is a lack of standardized thermocycling protocols, the parameters used in this research are comparable with those used in other studies.[27] Mechanical loading is an additional factor that causes tension in the resin-tooth interface, leading to deformations such as microcracks or gaps.[29] In the literature, there is no standard protocol for mechanical loading; other studies have applied forces ranging from 50 N[30,31] to 100 N/250 N[21] at frequencies from 0.5 Hz[30] to 1.5 Hz[31] over 10,000[21] to 250,000 cycles.[31] Comparing the results before and after TML revealed that all groups, except the AER group for enamel, showed a significant decrease in the percentage of continuous margin (Table 2). Greater loss occurred in the experimental ER group at the composite-dentin interface than the composite-enamel interface. The opposite trend was observed for the experimental SE group. A decrease in the continuous margin after TML was also observed in other studies testing Clearfil SE Bond,[32] Silorane System Adhesive,[33] Syntac, XP Bond, Single Bond Plus, AdheSE, and Clearfil SE Bond.[30]

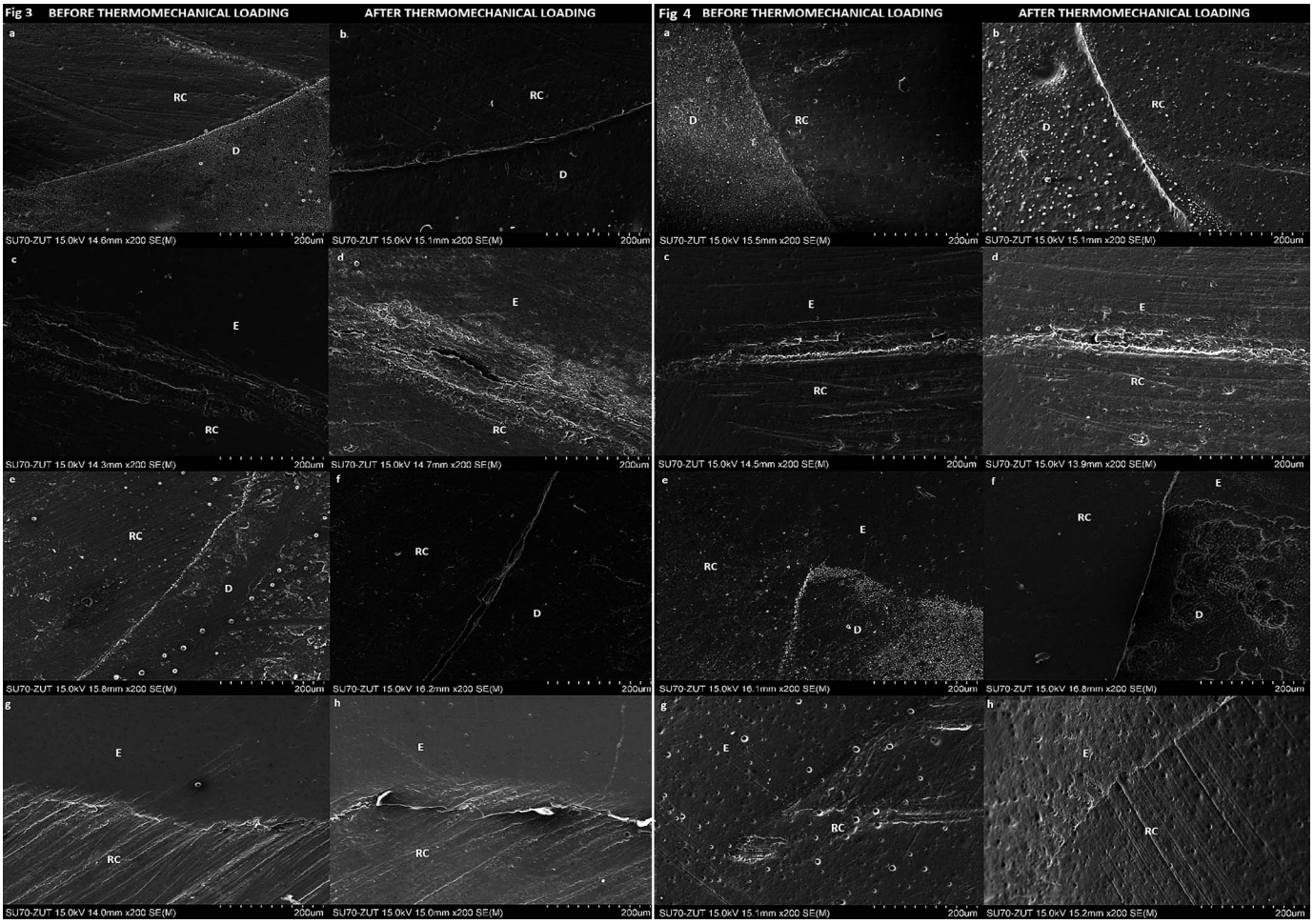

Figure 3. *Representative SEM micrographs from the same region of the PSE (a-d) and PER (e-h) groups before and after TML. The micrographs show the appearance of gaps in the resin-dentin interface (a, b and e, f) and an increase in current gaps at the resin-enamel interface (c, d and g, h) in the PSE and PER groups, respectively, after TML. E, enamel; D, dentin; RC, resin composite.*

Figure 4. *Representative SEM images from the same region of the ASE (a-d) and AER (e-h) groups before and after TML. (a-d): The micrographs show no visible changes in the resin-dentin interface (a, b) and increased irregularity in the resin-enamel interface (c, d) for the ASE group after TML. (e-h): The micrographs show a manifestation of gaps in the resin-dentin interface (e, f) and free gaps in the resin-enamel interface (g, h) for the AER group after TML. E, enamel; D, dentin; RC, resin composite.*

In this study, two universal adhesives were compared for different etching modes. It was found that the ER approach had higher percentages of continuous margins in enamel than in dentin and that the SE approach achieved better results in dentin than in enamel (except for the AER and ASE groups before TML), which was more prominent after TML. Similar conclusions were obtained by Bortolotto and others,[16] who tested 12 adhesives, and Gregor and others,[33] who investigated Silorane System Adhesive. Moreover, Casselli and others[17] demonstrated that the gap width also can vary depending on the etching approach and tooth tissue. Casselli and others[17] and Blunck and Zaslansky[19] demonstrated that the margins in enamel observed after using one-bottle one-step SE adhesives are much poorer than the margins of restorations in which phosphoric acid etching was used, which is consistent with the obtained results. This result occurs because in the ER technique, the adhesive fills the space surrounding the etched region in the enamel and envelops individual hydroxyapatite crystals, creating a stable bond with the enamel.[7] After TML, in dentin, poorer results were observed for the ER group than the SE group (the difference was significant in the Peak Universal group), which may be due to excessive etching in the ER approach. When etching dentin with phosphoric acid, the adhesive may not penetrate to a sufficient depth, and overdrying the cavity may cause the collagen network on the dentin surface to collapse, which reduces bond durability.[34] With regard to the whole

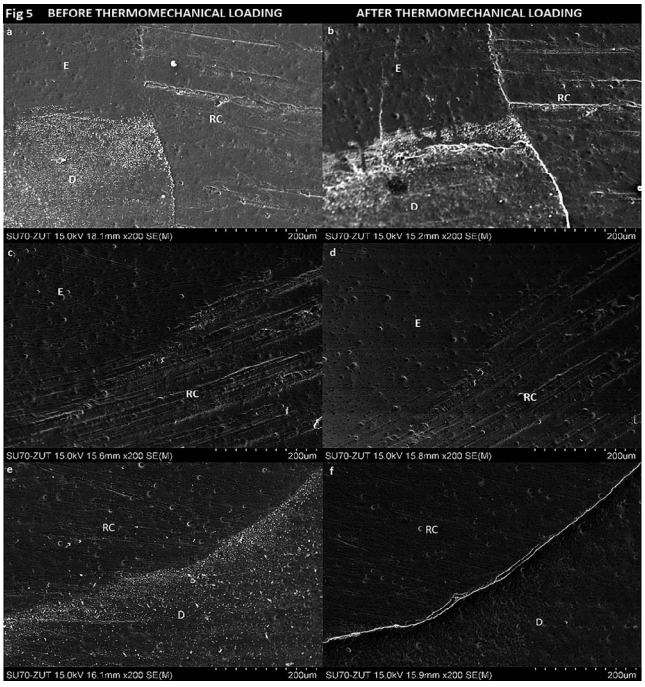

Figure 5. *Representative SEM micrographs from the same region of the CSE (a-b) and OER (c-f) groups before and after TML. (a, b): The micrographs show greater irregularity in the resin-dentin interface than the resin-enamel interface in the CSE group after TML. (c, d): The resin-enamel interface is barely detectable in the OER group both before and after TML. (e, f): The micrographs show the manifestation of gaps in the resin-dentin interface in the OER group after TML. E, enamel; D, dentin; RC, resin composite.*

margin in enamel and dentin, the AER group had a smaller decrease than the PER group, but the decrease was still larger than that observed for Scotchbond Universal applied with the ER technique, as reported by Bortolotto and others,[35] who found that the continuous margin decreased from 98.5% before TML to 97.8% after TML. However, there is still a lack of laboratory studies assessing universal adhesives under different conditions.

This study compared the marginal integrity of universal adhesives with two gold standard adhesives: OptiBond FL and Clearfil SE Bond. Peak Universal Bond had statistically worse marginal integrity than the control groups in both etching techniques and time points (except the PSE group in dentin after TML) in contrast to Adhese Universal. When comparing this system to the control group, a significant difference was observed only in the ER technique in dentin after TML, so the second null hypothesis is partially rejected. OptiBond FL presented a relatively equal marginal adaptation on enamel and dentin, both before and after TML, which is comparable with results from other studies[16,35]; this adhesive and Clearfil SE Bond are considered the gold standard of ER and SE adhesives, respectively.[36-39] Compared with other studies, this study obtained both similar[40] and contrasting[30] results for Clearfil SE Bond, confirming that adhesion to dentin is unpredictable and may be worse if the dentin is etched before the application of SE adhesive. This study also emphasized that preetching of enamel by phosphoric acid remains the most reliable, durable, and fatigue-resistant mode of enamel bonding,[10,40,41] as reported by Körner and others[42] and Bortolotto and others.[43]

The composition of an adhesive also influences its bond stability. Peak Universal had the smallest continuous margin before and after TML (not significant in all cases; Table 2), despite containing chlorhexidine (Table 1), which is widely used as an antimicrobial agent. Chlorhexidine may inhibit matrix metalloproteinases and, consequently, prevent the degradation of collagen fibrils at the resin-dentin interface[44,45] and partially conserve the integrity of the hybrid layer to improve bond durability.[46] However, the small continuous margin observed for Peak Universal in both etching modes may be due to the lack of 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) monomer, which is present in the composition of Adhese Universal and Clearfil SE (Table 1). 10-MDP is primarily used as an etching monomer due to its dihydrogen phosphate group, which dissociates in water to form two

Table 3: *Number of Evaluated Restorations Classified According to the World Dental Federation (FDI) Criteria*[13]

FDI Criteria	Time											
	Before TML						After TML					
	PER	PSE	AER	ASE	OER	CSE	PER	PSE	AER	ASE	OER	CSE
VG	9	7	10	8	10	9	9	6	10	6	10	9
GO	1	3	—	2	—	1	1	4	—	4	—	1
SS	—	—	—	—	—		—	—	—	—	—	—
UN	—	—	—	—	—	—	—	—	—	—	—	—
PO	—	—	—	—	—	—	—	—	—	—	—	—
N	10	10	10	10	10	10	10	10	10	10	10	10

protons. 10-MDP is hydrophobic (its long carbonyl chain favors ethanol and acetone as solvents) and hydrolytically stable.[47,48] 10-MDP monomers can form an ionic bond with the calcium in hydroxyapatite and can hydrolytically nucleate stable 10-MDP-calcium salts.[39] To improve bonding stability, it could be useful to connect MDP-10 monomer and chlorhexidine in a single adhesive to combine their advantages.

This study compared clinical and laboratory methods for assessing marginal integrity. The AER and OER groups received the best scores in FDI assessment both before and after TML; however, there were no significant differences between any of the groups or time points in the FDI assessment. Thus, the third null hypothesis was rejected. Similar conclusions were reported by Lopes and others,[49] who assessed the universal adhesive Xeno Select in clinical studies. However, Loguercio and others,[50] who evaluated Scotchbond Universal Adhesive, noted a significantly worse rating of marginal integrity after a three-year trial, although the application method did not influence this parameter at the baseline or at later time points. Laboratory (SEM) and clinical (FDI criteria) assessment methods are crucial because gaps seen in the laboratory that are imperceptible clinically may cause postoperative sensitivity, marginal staining, or retention loss.[2] Moreover, this short-term study is the first stage of research and did not include water degradation of adhesives; thus, there is a need to perform long-term research using the replica method in a laboratory and clinical study.

CONCLUSION

Within the limitations of this laboratory study, the evaluated factors such as etching mode and TML influenced the marginal integrity of universal adhesives. For both etching modes and both time points, Adhese Universal offered better results in both enamel and dentin than Peak Universal, and its marginal integrity is comparable to the gold standard adhesives. For the ER approach, the differences in the percentage of continuous margin were significant both before and after TML, while for the SE approach, the differences were significant only before TML. Unfortunately, the new universal adhesives do not resolve the problem of dentin adhesion, because in each group, a significant decrease in the percentage of continuous margin was observed for dentin and thus for the whole margin. Moreover, the replica method shows that clinical and laboratory tests complement each other and give a broader view of marginal integrity.

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the Local Ethics Committee of Pomeranian Medical University in Szczecin, Poland. The approval code for this study is KB-0012/82/11/2014.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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