



Effect of 9.3 μm CO₂ and 2.94 μm Er:YAG Laser vs. Bur Preparations on Marginal Adaptation in Enamel and Dentin of Mixed Class V Cavities Restored With Different Restorative Systems

Clara Isabel Anton y Otero^{1*}, Enrico Di Bella², Ivo Krejci^{1*} and Tissiana Bortolotto¹

¹ Division of Cariology and Endodontology, Faculty of Medicine, CUMD, University Clinics of Dental Medicine, University of Geneva, Geneva, Switzerland, ² Department of Economics and Quantitative Methods, University of Genoa, Genoa, Italy

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*Correspondence:

Clara Isabel Anton y Otero
clara.antonyotero@unige.ch
Ivo Krejci
Ivo.Krejci@unige.ch

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This study aimed to compare marginal adaptation in enamel and dentin before and after aging of laser vs. bur-prepared mixed class V cavities restored by different restorative systems. Seventy two caries-free human molars were distributed to nine experimental groups; cavities were prepared using two different lasers: a handpiece -integrated 2.94 μm Er:YAG laser at 4.5 W, 300 mJ, and 0.75 W, 50 mJ with 15 Hz (LiteTouch, Light Instruments, Israel) and a novel CO₂ laser at 12.95 W, 19.3 mJ, and 4.1 W, 6.11 mJ with 671 Hz (Solea 9.3 μm , Convergent Dental, USA). Cavities prepared with conventional diamond burs (Intensiv, Switzerland) in a red contra angle at high speed under maximal water cooling served as control. Cavities were prepared under simulation of dentinal fluid and restored using three different self-etching universal adhesives in combination with three nanohybrid composites, applied in two layers: Scotchbond Universal with Filtek Supreme XTE (3M, USA), G-Premio BOND with Essentia Universal (GC, Japan), and OptiBond Universal with Harmonize Universal (Kerr, USA). After restorations' polishing and simultaneous thermal (5–50°C, 2 min each) and mechanical loading (max. 49 N; 200,000 cycles), replicas of restoration margins were examined under SEM at $\times 200$ magnification. Percentages of continuous margins (CMs) were quantified before and after the fatigue test and statistically compared (two-way ANOVA with Fisher's least significant difference [LSD] *post hoc* test). Significant differences were found in almost all groups between the results before and after the fatigue test, as well as between the different preparation tools and restorative materials ($p < 0.05$). Traditional bur preparations are confirmed as gold standard in enamel and dentin, as all three tested restorative systems provide results of marginal adaptation of more than 80% CM after loading. Er:YAG laser preparations can be equally effective in combination with SBU/Filtek Supreme XTE. CO₂ laser ablation could not provide convincing results with the tested self-etching restorative systems. Marginal adaptation has been highly dependent on the substrate and showed impaired adhesion, especially in enamel.

Scotchbond Universal/Filtek Supreme XTE showed the highest and most stable values of CM. The other two restorative systems were highly dependent on the preparation device of the substrate.

Keywords: dental hard tissue preparation, marginal adaptation, adhesion, Er:YAG laser, CO₂ laser, laser cavity preparation, universal adhesive system

INTRODUCTION

Minimally invasive dentistry strongly relies on both adhesion due to micromechanical retention owing to mineral replacement by resin monomers and a potential chemical bonding of reactive monomers to hydroxyapatite (1). Likewise, maximum preservation of sound tooth substance is possible thanks to selective ablation methods for caries removal, contributing to patients' quality of life by preserving their own dental tissue up to an advanced age.

Since the introduction of adhesion by Buonocore (2) seven generations of adhesive systems have been developed. Currently available bonding systems can be hierarchically classified into two major categories: etch-and-rinse and self-etch (3, 4). High diversity on the market and differences in the composition of materials and the manner in which they are applied resulted in an increased demand for simpler, more user-friendly, and less technique-sensitive adhesives (3). This is why the seventh generation of one-step universal adhesives has been developed by diverse manufacturers by applying their own concepts and their proprietary ingredients, resulting in products with different characteristics that lead to diverse *in vivo* and *in vitro* results (5, 6).

Preparation of dental hard tissue is an important factor with regard to tissues' surface morphology and thus to the general interaction of adhesive materials to enamel and dentin, resulting in differences in bond strength, in microleakage, in quality of marginal adaptation, and finally in restorations' clinical success (7–9). As shown in previous studies, (7–11) lasers with emission wavelengths which are strongly absorbed in water and hydroxyapatite offer new possibilities in selective and minimally invasive ablation of caries-infected dental tissues. Herein, 2.94 μm Er:YAG lasers and the recently introduced 9.3 μm CO₂ lasers can be used. The Er:YAG laser, in particular, can promote equal adhesion results to conventional bur treatments when using a self-etching universal adhesive, when enamel and dentin cavities are prepared with optimized laser settings with regard to power, frequency, water amount, and air pressure (7, 8). In this context, it has been shown that finishing or rather smoothening of the cavity surface with less powerful settings seems to be essential for optimal marginal adaptation and adhesion (8).

With respect to enamel adhesion, 9.3 μm CO₂ laser preparations seem to adversely affect adhesion of adhesives (10). This might be due to chemical changes in the substrate caused by the occurrence of high temperatures ($\sim 1,000^\circ\text{C}$) next to melting of the surface, which may explain the smooth and glazed surface micromorphology after laser use. Contrarily, Er:YAG laser preparations do not exceed temperatures of 250–300 $^\circ\text{C}$ and produce honeycomb patterns,

which can be—due to its micro retentive properties—considered as favorable to bonding procedures in enamel without the necessity of previous acid etching, under the condition that subsurface damage is not induced by the procedure.

Regarding dentin, both laser types delivered good results in terms of micromechanical adhesion in dentin without previous phosphoric acid etching (11–13).

Conflicting outcomes have been reported in the literature with regard to different one-component universal adhesives, as well as new laser technologies. Success of laser preparation can be directly related to its wavelength as well as to the applied power, water spray settings, and its morphological effects on dental hard tissues (8, 10, 14, 15).

The performances of adhesive systems are strongly dependent on differences in the composition of hydrophobic and hydrophilic monomers, photoinitiators and co-initiators, organic solvents as well as additives such as nanofillers and bioactive components, and also the manufacturers' instructions for application and not only by the bonding approach (additional step for etching or self-etching) (16–20).

There is a need for further investigations and analysis of the interaction between simplified universal adhesives, preparation methods, and their interaction with enamel and dentin substrates (21). Therefore, this study aimed to assess the quality of marginal adaptation delivered by three different commercially available restorative systems: one-component universal adhesive systems and resin composites on restorations with enamel and dentin margins prepared by two lasers (Er:YAG and a recently developed 9.3 μm CO₂ laser) where conventional bur preparation served as the positive control.

The null hypothesis tested was that there were no differences in terms of marginal adaptation in enamel and dentin, between the three ways of cavity preparation, or between the three restorative systems tested.

MATERIALS AND METHODS

Seventy-two caries-free human molars were randomly assigned to nine experimental groups of equal size (Tables 1, 2). They were stored immediately after extraction in 0.1% thymol solution and ultrasonically cleaned and brushed with a rotative brush embedded in toothpaste (Signal RDA 50, Colgate-Palmolive, New York, NY, USA). The teeth were prepared for the simulation of dentinal fluid as earlier detailed by Krejci and co-workers (22). To this purpose, the apices were sealed with an adhesive system (OptiBond FL, KaVo Kerr, Orange, CA, USA), and the roots were fixed in the center of custom-made specimen holders using a cold polymerizing resin (Technovit 4071 resin

TABLE 1 | Parameters used for the cavity preparation by the two lasers (Grades 4 to 9) and for the bur drilling (Grades 1 to 3).

	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group 7	Group 8	Group 9
Preparation tool	Red hand peace			CO ₂ laser (Solea, Convergent Dental, Inc., Natick, MA)		Er:YAG laser (LiteTouch Orcos REF LI-FG0012A)			
Wavelength	–			9.3 μm		2.94 μm			
Tip/spot characterization (name, size)	Intensiv Suisse Football (white ring, 25 μm; FG3255/6)			1.25 mm		AS7066(X) 1.3 × 14 mm			
Working distance	In contact			4–15 mm (~10 mm)		1–2 mm			
Spray amount	Maximum			A+B: 100%		A: 5 arb. unit, B: 3 arb. unit			
Pulse energy	–			A: 19.3 mJ, B: 6.11 mJ		A: 300 mJ, B: 50 mJ			
Pulse frequency	–			671 Hz		15 Hz			
Power	–			A: 50% → 12.95 W, B: 20% → 4.1 W		A: 4.5 W, B: 0.75 W			
Restoration	One-bottle self-etching universal adhesive system with two layers of corresponding composite resin								
Light curing	Each layer (adhesive system and composite resin) 20 s (light curing unit: VALO 1,000 mW/cm ²)								

(A) Parameters for preparation and (B) parameters for finishing and surface smoothing.

TABLE 2 | Description of the materials used in the study.

Group	Adhesive system	LOT#	Composition	pH	Application mode according to the manufacturers	Composite resin
1, 4, 7	Scotchbond Universal (3M-ESPE, St. Paul, MN, USA)	LOT: 90624A	10-MDP, Bis-GMA, 2-HEMA, D3MA, MPTMS decamethylene dimethacrylate, ethyl methacrylate, propanoic acid, methyl reaction products with decanediol and phosphorous oxide, copolymer of acrylic and itaconic acid, dimethylaminobenzoate, methylethylketone, ethanol, water, silane-treated silica, initiator	2.7	<ol style="list-style-type: none"> 1. Apply the adhesive to the prepared tooth and rub it in for 20 s. 2. Gently air-dry the adhesive for approximately 5 s to evaporate the solvent. 3. Light-cure for 10 s. 	Filtek Supreme XTE , Shade A2; LOT NA39611
2, 5, 8	G-Premio BOND (GC, Tokyo, Japan)	LOT: 1902182	10-MDP, 4-MET, MEPS, TEGDMA, methacrylate monomer, acetone, water, initiator [diphenyl(2,4,6-trimethylbenzoyl)]phosphine oxide, silica	1.5	<ol style="list-style-type: none"> 1. Immediately apply the adhesive to the prepared enamel and dentin surfaces using the disposable applicator. 2. Leave undisturbed for 10 s after the end of application. 3. Dry thoroughly for 5 s with oil-free air under maximum air pressure. 4. Light-cure for 10 s 	Essentia Universal ; LOT: 1803261
3, 6, 9	OptiBond Universal (Kerr, Orange, CA, USA)	LOT: 6920139	Acetone, HEMA, glycerol dimethacrylate, glycerol phosphate dimethacrylate acetone, water, ethanol	2.1	<ol style="list-style-type: none"> 1. Apply a generous amount of the adhesive to the enamel/dentin surface. Scrub the surface with a brushing motion for 20 s. 2. Dry with gentle air first and then medium air for at least 5 s with oil-free air. The surface should have a glossy uniform appearance. If not, repeat steps 1 and 2. 3. Light-cure for 10 s. 	Harmonize Universal Dentin A2D; LOT: 7259723

Bis-GMA, 2,2-bis(p-(2-hydroxy-3-methacryloxy propoxy)phenyl)propan; HEMA, 2-hydroxyethyl methacrylate; MDP, 10-methacryloyloxydecyl dihydrogen phosphate; MEPS, methacryloyloxyalkylthiophosphate methyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; D3MA, decanediol dimethacrylate.

cold curing, Kulzer, Hanau, Germany). Throughout the study, during cavity preparation, adhesive procedures related to cavity filling, and thermomechanical loading, teeth were flooded for simulation of dentin fluid with 1:3 diluted horse serum (donor horse serum, Bioswisstec, Schaffhausen, Switzerland) and phosphate-buffered saline (PBS, Bioswisstec, Schaffhausen, Switzerland), using a dentinal fluid simulation device (PAA Laboratories, Linz, Austria). Therefore, a cylindrical hole was drilled at the side surface of the tooth at the cemento-enamel junction, and a metal tube (needle TERUMO with a diameter of 1.2 mm, Somerset, NJ, USA) was inserted, luted with an adhesive system (OptiBond FL), and then connected to a flexible silicone hose. On each tooth, one saucer-shaped class V cavity was prepared at the dentino-enamel junction under $\times 20$ stereomicroscope magnification with a $2.94\ \mu\text{m}$ Er:YAG laser (LiteTouch III, Light Instruments, Israel) in focus with a tip-tissue distance of 1–2 mm, a $9.3\ \mu\text{m}$ CO₂ laser (Solea $9.3\ \mu\text{m}$, Convergent Dental, USA) with a focus distance of about 10 mm—both measured once per sample with a periodontal probe and a $25\ \mu\text{m}$ diamond bur in the shape of a football (see **Table 1**). The groups prepared with burs served as the positive control.

Cavities were prepared at the dentino-enamel junction with standardized dimensions of 3.0–3.5 mm length by 2.5–3.0 mm height and a depth of 1.5 mm, which have been verified with a periodontal probe. The enamel half of the margin was beveled to a crescent shape with a maximal width of 1.2 mm (23).

Prepared teeth were air-dried for 5 s before bonding to three different adhesive systems following the manufacturer's instructions for the corresponding self-etch protocol and light-cured for 20 s with a distance of 1 mm (VALO Cordless standard power $1,000\ \text{mW}/\text{cm}^2$, Ultradent, Salt Lake City, UT, USA) (**Table 2**). After bonding, cavities were filled with a corresponding universal composite in two oblique layers, and each was light-cured for 20 s. The first layer was placed in the cervical half of the cavity and the second one occlusally, completing obturation. Finishing and polishing were then performed with flexible disks (Sof-Lex, 3M, St. Paul, MN, USA), and for concave areas, rubber polishing tips (Brownie and Greenie, Shofu, Kyoto, Japan) were used under $\times 20$ stereomicroscope magnification.

To obtain resin replicas of each restoration, impressions with a polyvinylsiloxane impression material (PRESIDENT light body, Coltène Whaledent, Altstätten, Switzerland) were taken after brush-cleaning the surface with toothpaste (Signal).

The restored teeth were then subjected to repeated thermal and mechanical stresses in a chewing machine (MW-Basis CH [230 V/50 Hz], JULABO Labortechnik GmbH, Seelbach, Germany), under constant simulation of dentin fluid flow (mechanical stress $200,000 \times$ with max. 49 N and thermal stress between 5°C and 50°C) (7, 8).

After loading, replicas were taken again following the above-described procedures.

For the evaluation of marginal adaptation, replicas before and after aging were poured out with an epoxy resin (EpoFix, Struers, Willich, Germany), gold sputtered, and subjected to a quantitative marginal analysis in a scanning electron microscope under $\times 200$ magnification (Zeiss Gemini, Sigma 300 VP, Karl

Zeiss Microscopy, Cambridge, UK) and a custom-made module programmed within an image processing software (Scion Image, Scion Corp, Frederik, MA, USA) (7, 8, 24).

For statistical analysis of the normally distributed data, Kolmogorov–Smirnov and Shapiro–Wilk tests were run to assess normality assumption, and two repeated-measures analysis of variance (ANOVA) on the differences between data before and after loading were performed, the first one on enamel and dentin measurements of continuous margins (CMs) and the second on measurements of the entire margin length. Pairwise differences in group means were evaluated using Fisher's *post hoc* test. The level of confidence was set to 95%.

RESULTS

Percentages of CMs (%CM) before and after aging (thermomechanical loading) on enamel, on dentin, and at the entire margin (total margin length [TML]) are presented in **Table 3**. **Figures 1A–C** demonstrate an example with a restoration after CO₂ preparation with non-continuous (**Figure 1B**) and continuous (**Figure 1C**) margins. Significant differences before and after aging were observed between several experimental groups (*p*-values 1, 2, 3 in **Table 3**, statistically significant differences at $p < 0.05$).

At the TML, marginal adaptation in the three bur-prepared groups (Groups 1, 2, and 3) and in one Er:YAG-prepared group (Group 7) was still above 80% after loading (**Table 3**).

Aging due to thermomechanical loading led to significant marginal degradation in all groups (**Table 3** in Groups 5, 6, 8, and 9 *p*-values₁ < 0.001 ; Group 1 $p = 0.048$, Group 3 $p = 0.025$, and Group 4 $p = 0.004$) except Group 2 (G-Premio BOND [GPB] in bur-prepared cavities) and Group 7 (Scotchbond Universal [SBU] in Er:YAG laser-prepared cavities); in these two groups, no significant differences could be detected between the results before and after aging.

With respect to the performance of the adhesive systems, SBU provided the most stable results with all three preparation methods (**Figure 2A**). Said differently, SBU was the only adhesive to perform well on bur-, CO₂-, and Er:YAG-prepared cavities.

On enamel, both material and ablation devices as well as their interaction significantly influenced the %CMs ($p < 0.001$). Fatigue loading led to a significant decrease of marginal adaptation for all groups prepared with both lasers (**Table 3** *p*-values₂: Group 4 $p = 0.009$ and Groups 5, 6, 8, and 9 $p < 0.001$) with the exception of one group prepared by Er:YAG laser (Group 7) and bur-prepared ones. In these four groups, %CMs were still above 80% after aging. On enamel, SBU provided the most stable results for all three preparation devices (**Figure 2B**).

On dentin, aging and interaction of materials and ablation devices significantly influenced the performance of restorations in contrast to the preparation device alone ($p < 0.0059$). Fatigue loading in all three devices led to a significant reduction of the values in combination with specific materials. GPB in combination with bur and CO₂ laser ablation as well as OptiBond Universal (OBU) with Er:YAG laser did not degrade significantly

TABLE 3 | Mean values (%CM) baseline and aged.

	Total margin						Enamel margin						Dentin margin									
	Baseline		Aged		SD ₁	SD ₂	Baseline		Aged		SD ₁	SD ₂	Baseline		Aged		SD ₁	SD ₂	p-values ₃		p-values ₄	
Group 1 SBU+BUR	94.7	3.4	89.0	3.5	0.048	97.0	2.2	94.3	2.3	0.434	91.7	7.1	81.8	8.1	0.010	0.023						
Group 2 GPB+BUR	97.6	1.8	95.6	2.6	0.476	97.4	2.1	94.4	4.7	0.386	98.1	3.5	97.5	4.0	0.872	0.570						
Group 3 OBU+BUR	95.5	2.2	89.0	5.5	0.025	97.0	2.6	94.3	3.1	0.447	93.6	3.8	83.8	9.9	0.011	0.057						
Group 4 SBU+CO ₂	80.0	7.6	71.5	8.5	0.004	78.7	8.5	69.3	11.4	0.009	80.6	16.0	72.2	18.1	0.028	0.604						
Group 5 GPB+CO ₂	88.5	8.0	60.3	12.3	0.000	80.8	13.8	28.0	21.9	0.000	99.8	0.3	99.3	0.8	0.904	0.000						
Group 6 OBU+CO ₂	76.5	10.2	44.6	8.2	0.000	63.7	18.1	16.2	16.8	0.000	95.7	2.9	85.3	12.0	0.007	0.000						
Group 7 SBU+Er:YAG	90.9	3.9	86.2	5.8	0.107	93.7	3.9	92.2	4.5	0.678	87.0	8.4	77.5	15.2	0.014	0.008						
Group 8 GPB+Er:YAG	95.5	3.9	79.1	17.4	0.000	96.3	2.5	83.7	11.7	0.001	94.0	9.7	71.9	29.7	0.000	0.032						
Group 9 OBU+Er:YAG	93.9	2.4	73.1	9.6	0.000	92.3	5.1	59.7	16.9	0.000	96.00	4.6	91.6	3.6	0.239	0.000						

p-values₁, differences between baseline and aged values of %CMs in the TML; *p-values₂*, differences between baseline and aged values of %CMs in the enamel margins; *p-values₃*, differences between baseline and aged values of %CMs in the dentin margins; *p-values₄*, differences between aged enamel and dentinal values of %CM; values printed in bold type are considered to be statistically significant ($p < 0.05$). SBU, Scotchbond Universal; GPB, G-Premio BOND; OBU, OptiBond Universal.

due to fatigue. However, median values of all bur and laser groups (except G4 SBU with CO₂ laser) ranged between 80% and 100% of CM. Significant differences between materials could be seen with all preparation devices (see **Figure 2B**). The highest values of aged CMs have been attained with GPB and bur or CO₂ laser ablation without significant reduction of CM values due to loading.

In **Table 3**, *p-values₄* show differences between aged enamel and dentin %CMs. Only Group 2 (GPB with bur) and Group 4 (SBU with CO₂ laser) attained similar %CM in enamel and dentin. Groups 5, 6, and 9 with p -values < 0.001 , Group 7 with $p = 0.08$, Group 8 with $p = 0.032$, Group 1 with $p = 0.023$, and Group 3 with $p = 0.057$ are also close to statistically significant. Only bur groups (Groups 1–3) presented %CM above 80%, on both enamel and dentin margins.

DISCUSSION

To attain well-tolerated restorations and ensure their long-term clinical success, challenges such as biocompatibility of materials and technique sensitivity must be faced, in addition to the presence of moisture in the working area. This is further influenced by chemical and micromorphology changes in dental surfaces induced by distinct ablation mechanisms of traditional bur, Er:YAG laser, and CO₂ laser, as well as diversity in compositions and application recommendations of adhesive systems (5). Since one-component self-etch universal adhesive systems and lasers offer new possibilities in minimally invasive adhesive dentistry, this study aimed to analyze the influence of cavity preparation with an Er:YAG laser and a novel 9.3 μm CO₂ laser combined with three one-component self-etch universal adhesives and to compare the quality of marginal adaptation reached with these lasers to conventional bur preparation. In view of the results of the present study, adhesive systems performed significantly differently, depending on the device used for tissue ablation. While all three one-component self-etching universal adhesives delivered high results of marginal adaptation on bur-prepared cavities, the Er:YAG laser performed distinctly depending on the adhesive system, and the 9.3 μm CO₂ laser showed impaired adhesion for all tested materials especially on enamel. In this sense, the null hypotheses stating that all three ablation devices and adhesive systems would perform equally on both enamel and dentin before and after fatigue loading had to be rejected.

The testing protocol for this study was designed based on a previous publication, (7, 8) as well as on the parameters for Er:YAG and CO₂ lasers provided by the manufacturers. The selection of the three one-component self-etch universal adhesives was based on differences in their principal attributes such as pH, functional monomers, solvents, initiator systems, and application methods. These characteristics may affect the bonding effectiveness due to their influence on hydrophilicity, wettability, viscosity, and conversion rate of resin monomers, which may consequently modify the stability and thickness of the hybrid layer (25–27).

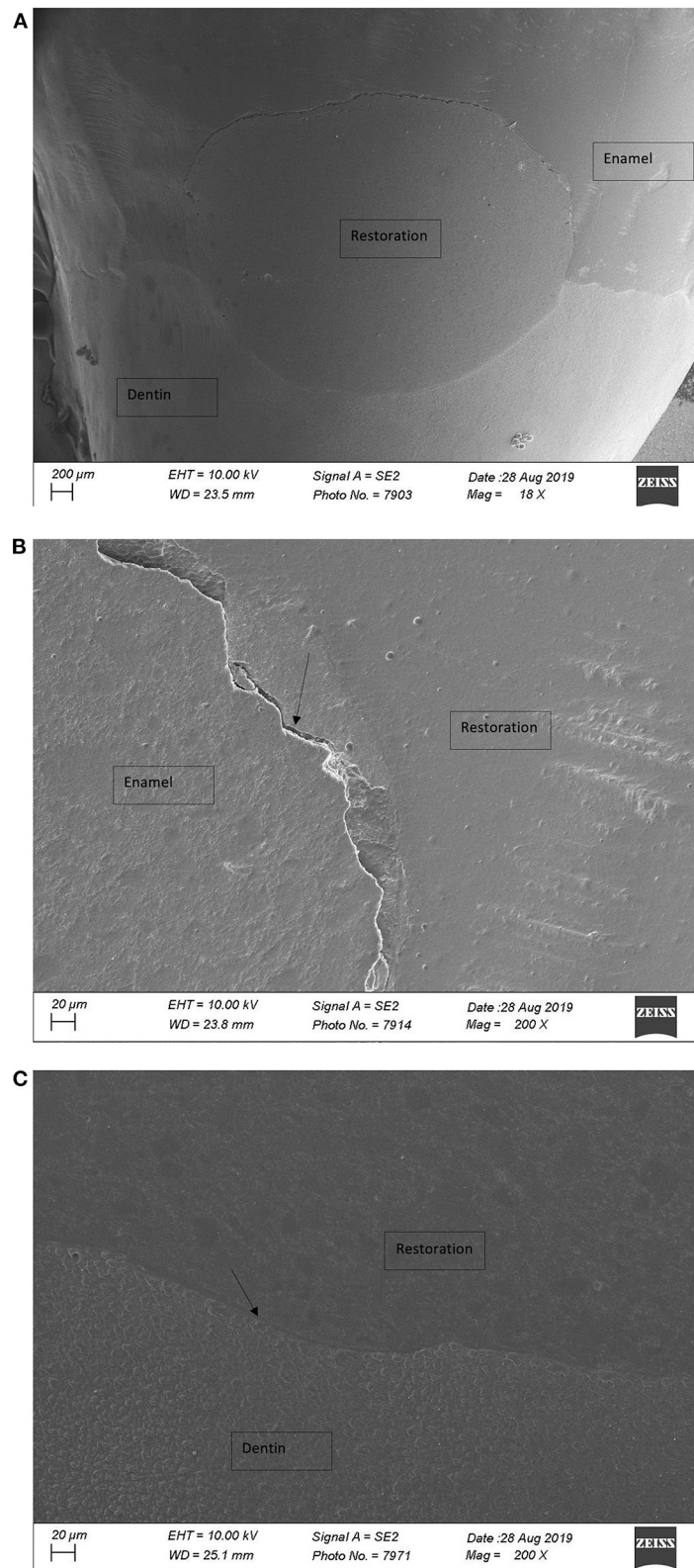


FIGURE 1 | (A) Overview over restoration after CO₂ laser preparation ($\times 18$; FE-SEM); non-CM in enamel and CM in dentin. **(B)** Restoration margin after CO₂ laser preparation ($\times 200$; FE-SEM), non-CM. **(C)** Restoration margin after CO₂ laser preparation ($\times 200$; FE-SEM), CM.

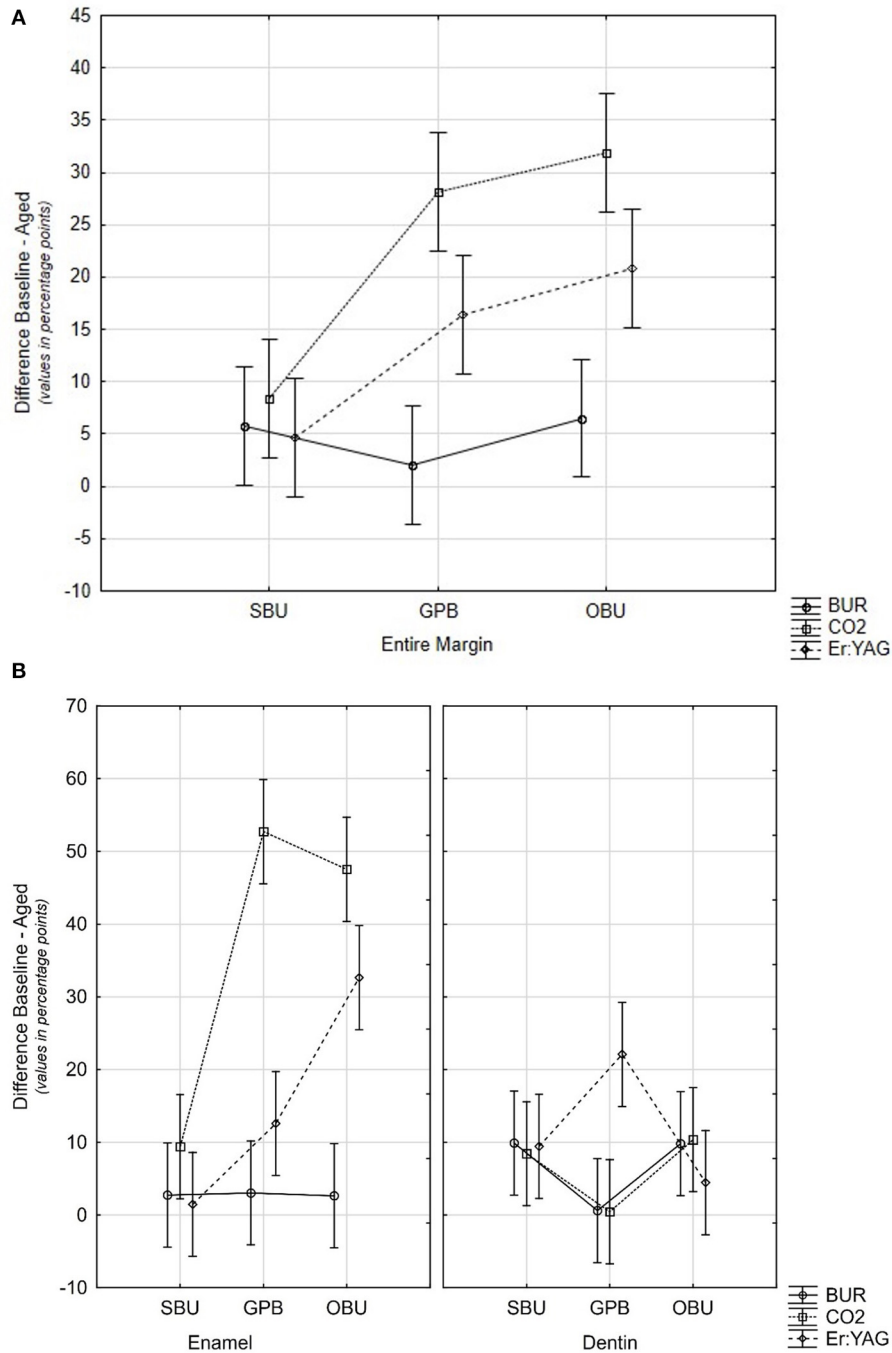


FIGURE 2 | (A) Graph showing differences between baseline-loaded %CM at the entire margin (TML) for the different materials and for the different methods of cavity preparation. Confidence intervals including zero values of the y-axis are not statistically different. **(B)** Plot showing differences of baseline-loaded values in percentage points for materials and devices on enamel and dentin. Confidence intervals including zero values of the y-axis are not statistically different. SBU, Scotchbond Universal; GPB, G-Premio BOND; OBU, OptiBond Universal.

Tooth sample preparation with dentinal fluid simulation was performed according to an established protocol (28) to ensure proximity to *in vivo* conditions and to avoid impaired interaction of laser and adhesive systems due to excessive tooth

dehydration. With respect to fatigue test, mild forces of 49 N were applied to simulate chewing forces occurring in oral conditions for approximately 1 year (22, 29). The analysis of quality of marginal adaptation under SEM based on percentages

of continuous restoration margins before and after thermal and mechanical loading/fatigue may indicate results for clinical long-term performances of the adhesive systems and may therefore be considered as closer and relevant to clinical reality (8, 30).

The discussion of the underlying results focuses on the interaction of different universal adhesive systems to dentin and enamel without specifically considering potential influences in terms of shrinkage stress due to differences between the resin composites between the experimental groups. Universal composites are in all groups accompanied by a resin composite from the same manufacturer's product line to avoid potential adverse interferences in combining different product lines. However, it should be pointed out that differences in the composite resin's composition might also influence results, especially in view of shrinking stress (31, 32).

In restorations where cavities were prepared with conventional burs, all three adhesive systems delivered %CMs higher than 80%. However, even in the bur-prepared cavities, especially in dentin-surrounded parts of the margins, statistically significant differences in %CMs were observed between the tested adhesives (see **Figure 2B**): GPB presented highly load-stable results, in contrast to SBU and OBU, which showed significantly lower but still high %CM values of over 80% after loading. These results might be explained by differences in composition and chemical behavior of the adhesive systems, as well as by their way of application as described below. Significantly different results in bur-prepared cavities after loading in dentin may be explained by the fact that dentin has a complex micromorphology, composition and heterogeneity of water, and organic and inorganic components, resulting in a more challenging substrate for adhesion than enamel, where results did not differ significantly.

Restorations where cavities were prepared by the 9.3 μm CO₂ laser presented a poor marginal adaptation on enamel (**Table 3** and **Figures 1A–C**). This might be explained by the laser ablation process itself, which may induce chemical and morphological alterations of the enamel. The wavelength of 9.3 μm has a strong absorption in hydroxyapatite, leading to important heating and water vaporization, decompensation of proteins, reduction of organic components, and melting as well as rapid recrystallization of the hard tissue with a loss of the carbonated phase and a change in the calcium/phosphate ratio, which makes enamel more acid resistant (33, 34). Enamel surfaces appeared glazed, probably due to high temperatures and melting of dental substrates. In a previous study, we examined the micromorphological changes of dental tissues under SEM (7). Enamel surfaces appeared homogenous and totally even without microroughness. We further found that the acidic monomers in the applied universal self-etching adhesive system (One-Coat 7 Universal from Coltène Whaledent) has not been able, in contrast to 35% phosphoric acid, to ablate this superficial laser-altered enamel layer (7). The underlying study with different universal adhesive systems that have diverse pH values confirms these findings. %CMs dropped significantly after the fatigue test, which may indicate an insufficient etching pattern by one-component self-etching universal adhesives (GPB and OBU) on the chemically modified and more acid-resistant surface

(**Figure 1B**). These findings support the need of enamel etching with phosphoric acid before the application of a self-etching adhesive, at least after the preparation with the 9.3 μm CO₂ laser, which is coherent with the available literature (7, 10). On CO₂ laser-ablated enamel surfaces, SBU values decreased significantly due to loading but did not drop as much as in the GPB and the OBU groups (**Table 3**, Groups 5 and 6). The explanation for these findings might be related to a more stable chemical interaction of enamel with SBU than that with GPB and OBU. SBU, in contrast to the other two adhesives tested, contains polycarboxylic acids. Since the 1970s, polycarboxylate-based adhesive materials, such as glass ionomer cements (GICs), have been shown to bond chemically to dentin (35). Studies have found, for glass ionomer restoration of non-cariou cervical lesions, higher retention rates compared to composite resins/adhesive combinations (16). The liquid of conventional GIC contains a copolymer of polyacrylic acid and other polyalkenoic acids, as is the case with SBU. The phosphate ions in the hydroxyapatite of enamel and dentin may thus be replaced by carboxyl groups of the polyalkenoic acids, which may lead to an ionic bond with the contained calcium. Polyalkenoic acids interact in the same way regardless of their concentration and pH (36, 37). This might explain the results obtained after loading with this adhesive. CO₂ laser-prepared dentin presents a smear-layer-free surface with mainly open tubules, only partly occluded by drops of melted material. The surface does not appear glazed and completely even. As seen in a previous study, the micromorphological differences between bur-, Er:YAG laser-, and CO₂ laser-ablated dentin is clearly less obvious than those in enamel (7). This might be an explanation for the findings of this study that values for CM in dentin showed comparable results to bur and Er:YAG laser preparations (**Table 3** and **Figure 1C**). Within the initial dentin values, SBU showed significantly lower values with both lasers than with bur preparations. This leads to the assumption that the establishment of mechanical interlock with resin tags might have been impaired in these groups (Groups 4 and 7). The differences between laser and bur preparations in dentin may be explained by the laser-promoted dentin fluid exudation due to opening of dentinal tubules caused by smear layer removal and the chemical interaction of the consequently higher amount of liquid to the adhesive systems. Bur preparation produces a smear layer that blocks dentinal tubules and might therefore reduce dentinal fluid exudation (7).

Marginal adaptation of Er:YAG laser-prepared restorations attained high %CMs. High initial values of CMs in enamel and dentin obtained with all three adhesive systems might have been due to the advantageous rough micromorphology of laser-ablated surfaces (7). Values after fatigue did not drop significantly in enamel with SBU, in contrast to GPB and OBU, which may be explained by impaired interaction of adhesive systems to chemically changed substrates due to laser ablation as described above. Results were stable in dentin for OBU but showed a significant decrease after loading for SBU and GPB. Initial dentin values of the SBU group showed already lower %CMs in comparison to the other two products, as already seen within the CO₂ laser groups. Reduction of dentin values due to loading may also be explained with the laser-specific dentin

micromorphology with open dentinal tubules and therefore pronounced dentin fluid exudation that may interact with the adhesive system. According to previous investigations, this effect might be more important for Er:YAG than for CO₂ laser, as Er:YAG does not melt the substrate (7). The CO₂ laser leads to sealing and formation of recrystallized droplets which partly occlude dentinal tubules. Thus, the liquid exudation might be even more important in the Er:YAG laser groups than in the CO₂ groups. However, this does not explain the significantly different behavior of GBP and OBU between both laser groups (7). Nevertheless, one possible explanation for the lower values after loading of SBU and especially GPB might be, next to the laser-specific dentin properties, the adhesive system's water content. Literature reports the double amount of water in GPB in comparison to SBU, which might be more difficult to evaporate in the microporosities of Er:YAG laser dentin (19). It may be even further speculated that the more acidic GPB compared to SBU and OBU demineralizes deeper on the already laser-etched surface with its microporosities where the adhesive system itself does not penetrate entirely, thus producing an incomplete hybrid layer, potentially explaining its lower resistance to loading (38).

The pH value of the adhesives may be considered as one major factor influencing adhesion, due to its influence on the extent of demineralization and dissolution of the smear layer. Nevertheless, in view of the different performance of the three adhesive systems, our results did not seem to depend on the pH (39, 40). There is no supporting evidence that a lower pH of the adhesive systems will improve the material's adhesion to enamel (and to dentin), as both mild adhesive systems (41), OBU (pH 2.1) and SBU (pH 2.7), performed equally well in bur groups before and after thermomechanical loading in enamel and performed better than the intermediate one, GPB (pH 1.5). The more load-stable performance of SBU might further be explained by its solvents ethanol and butanone, compared to OBU and GPB whose solvents are based on water and acetone (see **Table 2**). Solvents such as acetone or ethanol are added to enhance monomer miscibility into one solution and to accelerate water elimination from the adhesive surface. Evaporation of these solvents may lead to phase separation of the water (adding to the dissociation of the acidic monomers and thus for etching) from the other adhesive ingredients due to changes in the solvent–monomer balance, leading to water droplet formation at the bottom of the adhesive layer, adjacent to the hybrid layer, and potentially enhancing low fatigue resistance (42, 43). Literature reports that due to the low vapor pressure, water droplets moved upwards and evaporated completely after 4 to 10 min of application time. Since the vapor pressure (at 25°C) for acetone is four times higher than that for ethanol, acetone is more volatile, potentially leading to more water-remnant droplets and thus instabilities and interferences with the polymerization reaction, causing blister formation or intensifying permeability of the adhesive layer, triggering hydrolytic degradation of polymers and collagen, which is potentiated by the acidic pH of the monomer (44–49). It seems therefore to be crucial, next to a coordinated timing of solvent evaporation, to accelerate and

improve water droplet removal and evaporation with strong air-drying of the adhesive system prior to polymerization, as acetone is evaporated much faster than water (50). These facts may support the hypothesis that differences in recommendations for air-drying may be at least partly responsible for the distinct performance of OBU and GPB in dentin. Literature reports a significant improvement of self-etching universal adhesives by increasing the time for air-drying from 5 to 15 s but also by varying the air pressure (51, 52). This may lead to the suspicion that OBU performs better with prolonged air-drying, as already recommended for GPB by its manufacturer. Furthermore, excessive air-drying (as recommended for GPB) helps to thin the adhesive layer, which is reported to have a positive impact on water and solvent evaporation and polymerization shrinkage as well as on stress concentration, potentially improving bond strength (53).

Besides the differences in solvent agents and application instructions, monomer type may influence the interaction between the adhesive and the substrate. SBU contains Bis-GMA, in contrast to the other two adhesive systems tested. Ethanol and Bis-GMA tend to make the material more viscous than acetone and TEGDMA. The lower volatility and diffusivity of Bis-GMA may hinder infiltration into dentin, but on the other side, it may improve the photopolymerization and conversion rate due to autoacceleration. Moreover, Bis-GMA is known for its low volumetric shrinkage, high reactivity, and good mechanical properties, which might explain the relatively high stability or resistance to loading of SBU to all dental surfaces independent from the preparation device (54). Another aspect is that SBU and GPB are based on an MDP monomer, which is reported to provide stable adhesion due to ionic bonds formed with the calcium ions of the hydroxyapatite crystals and covalent bonds of the phosphate groups in MDP with the corresponding phosphate groups of hydroxyapatite crystals resulting in insoluble salts. The continuous deposition of successive coats of these salts on the outer surface of the hydroxyapatite crystals is a process known as “nanolayering” and might explain the superior performance of SBU and GPB over OBU, which contains a GPDM-P monomer (glycerophosphate dimethacrylate) instead of MDP (55–58).

Another factor influencing adhesives' performance might be related to photoinitiators. The main photoinitiator used in methacrylate-based materials currently marketed is camphorquinone, a hydrophobic substance that may contribute to phase separation when polymerization occurs in an aqueous medium, leading to decreased longevity and biocompatibility of adhesion (59, 60). SBU and OBU do both contain camphorquinone, but in contrast to OBU, SBU is not based on water and includes additional photoinitiations, which may contribute to more stable adhesion results. The water-based GPB uses diphenylphosphinoxid as an initiator system, probably preventing phase-separation phenomena due to its distinct characteristics in comparison to the hydrophobic character of camphorquinone.

Finally, in view of the results of this study, it appears still necessary either to improve adhesive formulations or to modify

their application protocol on laser-prepared substrates. Air-drying time and pressure, which seem to be material dependent, deserve special attention. Performance of adhesive systems might be improved on Er:YAG laser-prepared enamel and dentin as well as on CO₂ laser-prepared dentin by longer and stronger air-drying. Improvements on CO₂ laser-ablated enamel might be reached by a precedent etching step with highly concentrated phosphoric acid. Future research should also focus on the interaction of dentinal fluid and the specific composition of adhesive materials.

CONCLUSIONS

The results of this study lead to the conclusion that among the three universal adhesives, SBU showed the highest values of continuous marginal adaptation when applied on bur-, 9.3 μm CO₂ laser-, and Er:YAG laser-prepared cavities. This evidenced the capacity of this universal adhesive to deal with bur- and laser-prepared enamel and dentin substrates.

The two universal adhesives GPB and OBU did not provide consistent results on enamel and dentin. Results were highly dependent on the preparation method of enamel and dentin.

In bur-prepared cavities, the three self-etching universal adhesive systems delivered results of marginal adaptation between 80 and 100% CM after loading, on both enamel and dentin margins.

Er:YAG laser may represent a valuable alternative to conventional bur preparation, but only if used in combination with SBU.

The quality of marginal adaptation delivered by the 9.3 μm CO₂ laser was inferior on enamel in comparison to dentin. The specific interaction of this laser with the enamel surface may have adversely affected adhesion to this substrate.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

AUTHOR CONTRIBUTIONS

CA and IK contributed to conception and design of the study. CA performed the laboratory work and organized the data as well as wrote the first draft of the manuscript. ED performed the statistical analysis. TB and ED wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

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REFERENCES

1. Van Meerbeek B, De Munck J, Yoshida Y, Inoue S, Vargas M, Vijay P, et al. Buonocore memorial lecture. Adhesion to enamel and dentin: current status and future challenges. *Oper Dent.* (2003) 28:215–35.
2. Buonocore, MG. A simple method of increasing the adhesion of acrylic filling materials to enamel surfaces. *J Dent Res.* (1955) 34:849–53. doi: 10.1177/00220345550340060801
3. Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. *Dent Mater.* (2011) 27:17–28. doi: 10.1016/j.dental.2010.10.023
4. Pashley DH, Tay FR, Breschi L, Tjaderhane L, Carvalho RM, Carrilho M, et al. State of the art etch-and-rinse adhesives. *Dent Mater.* (2011) 27:1–16. doi: 10.1016/j.dental.2010.10.016
5. McLean DE, Meyers EJ, Guillory VL, Vandewalle KS. Enamel bond strength of new universal adhesive bonding agents. *Oper Dent.* (2015) 40:410–7. doi: 10.2341/13-287-L
6. Nagarkar S, Theis-Mahon N, Perdigo J. Universal dental adhesives: current status, laboratory testing, and clinical performance. *J Biomed Mater Res Part B.* (2019) 107:2121–31. doi: 10.1002/jbm.b.34305
7. Antony Otero C, Di Bella E, Bortolotto T, Krejci I. Influence of 9.3 μm CO₂ and Er:YAG laser preparations on marginal adaptation of adhesive mixed class V composite restorations with one component universal adhesive. *Am J Dent.* (2021) 34:31–38.
8. Bader C, Krejci I. Marginal quality in enamel and dentin after preparation and finishing with an Er:YAG laser. *Am J Dent.* (2006) 19:337–42.
9. Zhang Y, Jiang A. The influence of Er:YAG laser treatment on the shear bond strength of enamel and dentin: a systematic review and meta-analysis. *Quintessence Int.* (2020) 51:8–16. doi: 10.3290/j.qi.a43648
10. Rechmann P, Bartolome N, Kinsel R, Vaderhobli R, Rechmann BMT. Bond strength of etch-and-rinse and self-etch adhesive systems to enamel and dentin irradiated with a novel CO₂ 9.3 μm short-pulsed laser for dental restorative procedures. *Lasers Med Sci.* (2017) 32:1981–93. doi: 10.1007/s10103-017-2302-y
11. Esteves-Oliveira M, Zzell DM, Apel C, Turbino ML, Aranha AC, Eduardo Cde P, et al. Bond strength of self-etching primer to bur cut, Er:Cr:YSGG, and Er:YAG laser-treated dental surfaces. *Photomed Laser Surg.* (2007) 25:373–80. doi: 10.1089/pho.2007.2044
12. Malkoc MA, Tasdemir ST, Ozturk AN, Ozturk B, Berk G. Effects of laser and acid etching and air abrasion on mineral content of dentin. *Lasers Med Sci.* (2011) 26:21–7. doi: 10.1007/s10103-009-0751-7
13. Ramos TM, Ramos-Oliveira TM, Moretto SG, de Freitas PM, Esteves-Oliveira M, de Paula Eduardo C. Microtensile bond strength analysis of adhesive systems to Er:YAG and Er:Cr:YSGG laser-treated dentin. *Lasers Med Sci.* (2014) 29:565–73. doi: 10.1007/s10103-012-1261-6
14. Bader C, Krejci I. Indications and limitations of Er:YAG laser applications in dentistry. *Am J Dent.* (2006) 19, 178–86
15. Rizcalla N, Bader C, Bortolotto T, Krejci I. Improving the efficiency of an Er:YAG laser on enamel and dentin. *Quintessence Int.* (2012) 43:153–60.
16. Peumans M, Kanumilli P, De Munck J, Van Landuyt K, Lambrechts P, Van Meerbeek B. Clinical effectiveness of contemporary adhesives: a systematic review of current clinical trials. *Dent Mater.* (2005) 21:864–81. doi: 10.1016/j.dental.2005.02.003
17. da Silva EM, de Sa Rodrigues CU, de Oliveira Matos MP, de Carvalho TR, dos Santos GB, Amaral CM. Experimental etch-and-rinse adhesive systems containing MMP-inhibitors: physicochemical characterization and resin-dentin bonding stability. *J Dent.* (2015) 43:1491–7. doi: 10.1016/j.jdent.2015.10.004

18. Rosa WL, Piva E, Silva, AF. Bond strength of universal adhesives: a systematic review and meta-analysis. *J Dent.* (2015) 43:765–76. doi: 10.1016/j.jdent.2015.04.003
19. Yamauchi K, Tsujimoto A, Jurado CA, Shimatani Y, Nagura Y, Takamizawa T, et al. Etch-and-rinse vs self-etch mode for dentin bonding effectiveness of universal adhesives. *J Oral Sci.* (2019) 61:549–53. doi: 10.2334/josnuds.18-0433
20. Papadogiannis D, Dimitriadi M, Zafiropoulou M, Gaintantzopoulou MD, Eliades G. Universal Adhesives: setting characteristics and reactivity with dentin. *Materials (Basel).* 12 (2019) 12:1720. doi: 10.3390/ma12101720
21. Foscaldo T, Dos Santos GB, Miragaya LM, Garcia M, Hass, da Silva VEM. Effect of HEMA phosphate as an alternative to phosphoric acid for dentin treatment prior to hybridization with etch-and-rinse adhesive systems. *J Adhes Dent.* (2016) 18:425–434. doi: 10.3290/j.jad.a36891
22. Krejci I, Kuster M, Lutz F. Influence of dentinal fluid and stress on marginal adaptation of resin composites. *J Dent Res.* (1993) 72:490–4. doi: 10.1177/00220345930720020401
23. Bortolotto T, Ferrari M, Onisor I, Tay, Krejci, FI. Marginal adaptation of contemporary dentin bonding agents in enamel and dentin under the simulation of dentinal fluid. *Int Dent S Afr.* (2005) 7:46–56.
24. Bortolotto T, Monaco C, Onisor I, Krejci I. *In vitro* assessment of single-retainer tooth-colored adhesively fixed partial dentures for posterior teeth. *Int J Dent.* (2010) 2010:101095. doi: 10.1155/2010/101095
25. Van Meerbeek B, Van Landuyt K, De Munck J, Hashimoto M, Peumans M, Lambrechts P, et al. Technique-sensitivity of contemporary adhesives. *Dent Mater J.* (2005) 24:1–13. doi: 10.4012/dmj.24.1
26. Marchesi G, Frassetto A, Mazzoni A, Apolonio F, Diolosa M, Cadenaro M, et al. Adhesive performance of a multi-mode adhesive system: 1-year *in vitro* study. *J Dent.* (2014) 42:603–12. doi: 10.1016/j.jdent.2013.12.008
27. Tay FR, Pashley DH, King NM, Carvalho RM, Tsai J, Lai SC, Marquezini L Jr. Aggressiveness of self-etch adhesives on unground enamel. *Oper Dent.* (2004) 29:309–16.
28. Krejci I, Häusler T, Sägesser, Lutz DF. New adhesives in Class V restorations under combined load and simulated dentinal fluid. *Dent Mater.* (1994) 10:331–5. doi: 10.1016/0109-5641(94)90042-6
29. Bortolotto T, Ferrari M, Tay, F, Krejci I. Degradation of thermo-mechanically loaded adhesive Class V restorations after 18 months of water storage. *Am J of Dent.* (2007) 20:83–9.
30. Roebuck EM, Whitters CJ, Saunders, WP. The influence of three Erbium:YAG laser energies on the *in vitro* microleakage of Class V compomer resin restorations. *Int J Paediatr Dent.* (2001) 11:49–56. doi: 10.1046/j.1365-263x.2001.00230.x
31. Braga RR, Ballester RY, Ferracane JL. Factors involved in the development of polymerization shrinkage stress in resin-composites: a systematic review. *Dent Mater.* (2005) 21:962–70. doi: 10.1016/j.dental.2005.04.018
32. Steiner R, Edelhoff D, Stawarczyk B, Dumfahrt H, Lente I. Effect of dentin bonding agents, various resin composites and curing modes on bond strength to human dentin. *Materials (Basel).* (2019) 12:3395. doi: 10.3390/ma12203395
33. Featherstone JD. Modeling the modification depth of carbon dioxide laser-treated dental enamel. *Laser Surg Med.* (1999) 25:335–47. doi: 10.1002/(sici)1096-9101(1999)25:4<335::aid-lsm8>3.0.co;2-f
34. He Z, Chen L, Hu X, Shimada Y, Otsuki M, Tagami J. Mechanical properties and molecular structure analysis of subsurface dentin after Er:YAG laser irradiation. *J Mech Behav Biomed Mater.* (2017) 74:274–82. doi: 10.1016/j.jmbbm.2017.05.036
35. Wilson D, Kent, BE. A new translucent cement for dentistry. The glass ionomer cement. *Br Dent J.* (1972) 132:133–5. doi: 10.1038/sj.bdj.4802810
36. Yoshida Y, Van Meerbeek B, Nakayama Y, Yoshioka M, Snauwaert J, Abe Y, et al. Adhesion to and decalcification of hydroxyapatite by carboxylic acids. *J Dent Res.* (2001) 80:1565–9. doi: 10.1177/00220345010800061701
37. Sezinando A, Serrano ML, Pérez VM, Muñoz RA, Ceballos L, Perdigão J. Chemical adhesion of polyalkenoate-based adhesives to hydroxyapatite. *J Adhes Dent.* (2016) 18:257–65. doi: 10.3290/j.jad.a36222
38. Isolan P, Münchow EA, Basso GR, Pimentel AH, Schwantz JK, da Silva AV, et al. Bond strength of a universal bonding agent and other contemporary dental adhesives applied on enamel, dentin, composite, and porcelain. *App Adhes Sci.* (2014) 2:25. doi: 10.1186/s40563-014-0025-x
39. Thanatvarakorn O, Prasansuttiporn T, Takahashi M, Thittaweerat S, Foxton RM, Ichinose S, et al. Effect of scrubbing technique with mild self-etching adhesives on dentin bond strengths and nanoleakage expression. *J Adhes Dent.* (2016) 18:197–204. doi: 10.3290/j.jad.a36033
40. Tay FR, Pashley DH. Aggressiveness of contemporary self-etching systems. I: Depth of penetration beyond dentin smear layers. *Dent Mater.* (2001) 17:296–308. doi: 10.1016/S0109-5641(00)00087-7
41. Miyazaki M, Tsujimoto A, Tsubota K, Takamizawa T, Kurokawa H, Platt, JA. Important compositional characteristics in the clinical use of adhesive systems. *J Oral Sci.* (2014) 56:1–9. doi: 10.2334/josnuds.56.1
42. Van Landuyt KL, Snauwaert J, De Munck J, Peumans M, Yoshida Y, Poitevin A, et al. Systematic review of the chemical composition of contemporary dental adhesives. *Biomaterials.* (2007) 28:3757–85. doi: 10.1016/j.biomaterials.2007.04.044
43. Moszner N, Salz U, Zimmermann J. Chemical aspects of self-etching enamel-dentin adhesives: a systematic review. *Dent Mater.* (2005) 21:895–910. doi: 10.1016/j.dental.2005.05.001
44. Luque-Martinez V, Perdigão J, Munoz MA, Sezinando A, Reis A, Loguercio, AD. Effects of solvent evaporation time on immediate adhesive properties of universal adhesives to dentin. *Dent Mater.* (2014) 30:1126–35. doi: 10.1016/j.dental.2014.07.002
45. Chen L, Suh, BI. Effect of hydrophilicity on the compatibility between a dual-curing resin cement and one-bottle simplified adhesives. *J Adhes Dent.* (2013) 15:325–31. doi: 10.3290/j.jad.a28604
46. Salz U, Zimmermann J, Zeuner F, Moszner N. Hydrolytic stability of self-etching adhesive systems. *J Adhes Dent.* (2005) 7:107–16. doi: 10.3290/j.jad.a10282
47. Van Landuyt KL, De Munck J, Snauwaert J, Coutinho E, Poitevin A, Yoshida Y, et al. Monomer-solvent phase separation in one-step self-etch adhesives. *J Dent Res.* (2005) 84:183–8. doi: 10.1177/154405910508400214
48. Pashley EL, Zhang Y, Lockwood PE, Rueggeberg FA, Pashley DH. Effects of HEMA on water evaporation from water-HEMA mixtures. *Dent Mater.* (1998) 14:6–10. doi: 10.1016/S0109-5641(98)00003-7
49. Cho BH, Dickens, SH. Effects of the acetone content of single solution dentin bonding agents on the adhesive layer thickness and the microtensile bond strength. *Dent Mater.* (2004) 20:107–15. doi: 10.1016/S0109-5641(03)00071-X
50. Golbari N, Kasraei S, Afrasiabi A, Mostajir E, Mojahedi SM. Effect of diode laser (810 nm) irradiation on marginal microleakage of multi-mode adhesive resins in class V composite restorations. *J Lasers Med Sci.* (2019) 10:275–82. doi: 10.15171/jlms.2019.45
51. Franz, Lettner S, Watts DC, Graf A, Moritz A, Schedle A. Analysis of pre-test failures and bond-strengths of seven adhesive systems to bovine dentine: a nine-year novice/beginner operator study. *Dent Mater.* (2018) 34:1599–609. doi: 10.1016/j.dental.2018.09.009
52. Moosavi H, Forghani M, Managhebi E. Effect of different air-drying time on the microleakage of single-step self-etch adhesives. *Restor Dent Endod.* (2013) 38:73–8. doi: 10.5395/rde.2013.38.2.73
53. Jain M, Shetty S, Mogra S, Shetty VS, Dhakar N. Determination of optimum adhesive thickness using varying degrees of force application with light-cured adhesive and its effect on the shear bond strength of orthodontic brackets: an *in vitro* study. *Orthodontics (Chic.).* (2013) 14:e40–9. doi: 10.11607/ortho.919
54. Cornelio RB, Wikant A, Mjosund H, Kopperud HM, Haasum J, Gedde UW, et al. The influence of bis-EMA vs bis-GMA on the degree of conversion and water susceptibility of experimental composite materials. *Acta Odontol Scand.* (2014) 72:440–7. doi: 10.3109/00016357.2013.856467
55. Cardenas AM, Hass V, Malaquias P, Gutierrez MF, Reis A, Perdigão J, et al. Effect of MDP-containing silane and adhesive used alone or in combination on the long-term bond strength and chemical interaction with lithium disilicate ceramics. *J Adhes Dent.* (2017) 19:203–12. doi: 10.3290/j.jad.a38414
56. Yoshihara K, Yoshida Y, Nagaoka N, Hayakawa S, Okihara T, De Munck J, et al. Adhesive interfacial interaction affected by different carbon-chain monomers. *Dent Mater.* (2013) 29:888–97. doi: 10.1016/j.dental.2013.05.006
57. Elkaffas HH, Hamama H, Mahmoud, SH. Do universal adhesives promote bonding to dentin? A systematic review and meta-analysis. *Restor Dent Endod.* (2018) 43:e29. doi: 10.5395/rde.2018.43.e29

58. Cuevas-Suarez E, Ramos TS, Rodrigues SB, Collares FM, Zanchi CH, Lund RG, et al. Impact of shelf-life simulation on bonding performance of universal adhesive systems. *Dent Mater.* (2019) 35:e204–19. doi: 10.1016/j.dental.2019.05.023
59. Spencer P, Wang Y. Adhesive phase separation at the dentin interface under wet bonding conditions. *J Biomed Mater Res.* (2002) 62:447–56. doi: 10.1002/jbm.10364
60. Ely C, Ottoboni TD, Kumagai RY, Souza NA, Ramos TDS, Arrais CAG, et al. Bond strength of methacrylate-based blends containing elastomeric monomers and alternative initiators after thermomechanical cycling. *J Adhes Dent.* (2019) 21:281–286. doi: 10.3290/j.jad.a42549

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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