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Title

Microleakage of Class II composite restorations bonded with universal adhesives containing different functional monomers: an in vitro study

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Abstract

Background: This in vitro study aimed to evaluate and compare the microleakage of Class II composite restorations bonded with universal adhesives containing different functional monomers, with particular emphasis on a chemically cured universal adhesive applied using different etching strategies.

Methods: Standardized Class II cavities were prepared in 120 extracted human molars and randomly allocated into 12 groups ($n = 10$) according to the adhesive system and etching strategy. The adhesives evaluated included a three-step etch-and-rinse system, a two-step self-etch system, and three universal adhesives applied in total-etch, selective-etch, and self-etch modes, including a chemically cured universal adhesive. All cavities were restored with a nanohybrid resin composite and subjected to 5000 thermocycles between 5 °C and 55 °C. Microleakage at occlusal and gingival margins was assessed using 0.5% basic fuchsin dye penetration under stereomicroscopy. Data were analyzed using non-parametric statistical tests ($\alpha = 0.05$).

Results: Significant differences in microleakage were observed among the groups ($p < 0.001$). The three-step etch-and-rinse adhesive demonstrated the lowest microleakage values at both occlusal and gingival margins. Universal adhesives applied in self-etch mode exhibited significantly higher microleakage, particularly at the gingival margin. The chemically cured universal adhesive showed comparable marginal sealing only when combined with phosphoric acid etching.

Conclusions: Within the limitations of this in vitro study, multi-step adhesive systems and universal adhesives used with total-etch or selective-etch strategies provided superior marginal sealing compared with single-step self-etch applications. These findings highlight the clinical importance of etching strategy selection in Class II composite restorations, particularly at gingival margins where light attenuation may compromise polymerization in deep proximal boxes.

Keywords: Class II composite restoration; Dental adhesion; Etching strategy; Functional monomer; Microleakage; Chemically cured adhesive; Universal adhesive

Background

The growing emphasis on aesthetic and minimally invasive dentistry has expanded the use of composite resin-based restorative materials [1,2]. Advances in adhesive technology, combined with improvements in mechanical and optical properties, have markedly enhanced the clinical performance of resin composites [1]. Nevertheless, inadequate marginal adaptation and subsequent microleakage remain among the most frequent causes of restoration failure [3,4].

Microleakage is defined as the passage of bacteria, fluids, and molecules along the tooth-restoration interface and has been associated with marginal discoloration, postoperative sensitivity, secondary caries, and pulpal pathology [5,6]. The main contributing factors include insufficient adhesion between restorative materials and tooth tissues, mismatched thermal expansion coefficients, and polymerization shrinkage stresses.

Preventing microleakage at the tooth–restoration interface is critical for the long-term success of composite restorations [7].

Universal or multimode adhesives were developed to simplify bonding procedures, allowing application in total-etch, self-etch, or selective-etch modes. Their reduced technique sensitivity and clinical convenience have led to widespread clinical use, as supported by in vitro and systematic investigations [8,9]. Recent clinical data further indicate comparable performance when specific protocols are followed [10].

Universal adhesives commonly contain functional monomers such as 10-MDP (10-methacryloyloxydecyl dihydrogen phosphate), which chemically interact with hydroxyapatite by forming stable and hydrolytically resistant MDP-Ca salts, thereby improving immediate and long-term bond durability [11]. Tokuyama Universal Bond (TUB) differs from conventional light-curing systems by incorporating 3D-SR monomers and the MTU-6 adhesive-promoting monomer, enabling chemical interaction with both tooth substrates and metal oxides [12,13].

In addition, it employs a chemically cured mechanism based on proprietary BoSE technology, in which a borate initiator generates borane radicals in the presence of peroxide as a co-initiator. This system has been reported to demonstrate higher catalytic activity than traditional benzoyl peroxide/amine redox initiators under strongly acidic conditions, and recent evidence suggests that borate initiators may achieve higher degrees of conversion than camphorquinone-based photoinitiators [14]. Furthermore, TUB integrates γ -methacryloxypropyl

triethoxysilane (γ -MPTES), functioning as a built-in silane coupling agent [15,16].

Despite promising outcomes with universal adhesives, limited evidence exists regarding the microleakage behavior of chemically cured systems [14,17]. This issue is clinically relevant because light attenuation in deep proximal boxes or at gingival margins of Class II cavities may compromise polymerization of light-activated adhesives, potentially reducing their bonding effectiveness [5,18]. In such situations, chemically cured systems may theoretically provide an advantage, yet their sealing ability remains insufficiently documented, particularly under different etching strategies. Clinical studies have reported generally comparable outcomes between self-cure and light-cure adhesives, although slightly higher early failure rates have been observed for self-cure formulations [17].

Accordingly, this *in vitro* study aimed to evaluate and compare the microleakage of Class II composite restorations bonded with universal adhesives containing different functional monomers, including a chemically cured universal adhesive applied using total-etch, self-etch, and selective-etch strategies. The null hypothesis was that restorations bonded with a chemically cured universal adhesive would exhibit microleakage scores comparable to those bonded with light-curing universal adhesives.

Methods

This in vitro study was approved by the Clinical Research Ethics Committee of Bolu Abant Izzet Baysal University (Approval No: 2020/274). A total of 120 extracted human mandibular molars from adult patients, free of caries and restorations (periodontal extraction), were cleaned and stored in distilled water at 4 °C for no longer than three months until testing to prevent dehydration or structural changes. Standardized Class II cavities were prepared under water cooling using a 2.0 mm diamond bur (M&A Diatech, Heerbrugg, Switzerland) mounted on a high-speed handpiece; a new bur was used after every four preparations. All preparations were performed by a single operator. Occlusal cavities measured 3 mm buccolingually and 2 mm in depth; the proximal box measured 4 mm buccolingually and 2 mm mesiodistally at the cemento-enamel junction. The gingival cavosurface margin of the proximal box was located at the cemento-enamel junction (CEJ), representing a transitional enamel-dentin interface. Dimensions were verified with a Williams periodontal probe.

Specimens were randomly allocated by simple randomization into 12 groups (n=10) according to adhesive system and etching strategy: OptiBond FL (total-etch); Clearfil SE Bond 2 (selective-etch; self-etch); Gluma Bond Universal (total-etch; selective-etch; self-etch); Futura Bond U (total-etch; selective-etch; self-etch); Tokuyama Universal Bond (total-etch; selective-etch; self-etch). Adhesives, manufacturers, principal components, and application modes are summarized in Table 1. A circumferential matrix (Automatrix MT, Dentsply, Milford, DE, USA) was placed. For total-etch and selective-etch groups, 37% phosphoric acid

(Scotchbond Etchant, 3M ESPE, St. Paul, MN, USA) was applied, rinsed, and gently air-dried. Adhesives were applied per manufacturers' instructions; light-cured systems were polymerized with an LED unit (BluLex GT1200, Monitex, Taiwan; 1200 mW/cm²).

All cavities were restored with a nanohybrid resin composite (Grandio SO, shade A2; VOCO, Cuxhaven, Germany) in 2 mm increments, each cured for 20 s with the same light source. Restorations were finished/polished under water using fine-grit diamond burs and polishing rubbers (Identoflex, Kerr). All restorative procedures were performed by a single operator to ensure standardization.

Specimens were stored in distilled water at 37 °C for 24 h and subjected to thermocycling (5000 cycles between 5 °C and 55 °C; 30 s dwell; 5 s transfer) in a dedicated device (SD Mechatronik, Germany). The storage solution was not refreshed during this period. Root apices were sealed with wax; all external tooth surfaces except for a 1 mm rim around restoration margins were covered with three layers of nail varnish. Samples were immersed in 0.5% basic fuchsin for 24 h. A 0.5% basic fuchsin solution was used as the tracer dye because dyes used in microleakage studies differ in molecular size and physicochemical properties, which influence their ability to penetrate microscopic gaps and porosities. Basic fuchsin is among the dyes commonly used in dye penetration techniques for microleakage evaluation [19]. The specimens were then rinsed, embedded in acrylic resin (Meliodent, Heraeus Kulzer, Germany), and sectioned mesiodistally through the center of the

restoration using a precision cutting machine (Isomet, Buehler, Lake Bluff, IL, USA) under water cooling.

Dye penetration at occlusal and gingival margins was examined under a stereomicroscope (SMZ 800, Nikon, Tokyo, Japan) at 20× magnification. Microleakage was scored using widely adopted criteria (0–3) (Table 2; [7,18,20]). Two calibrated, blinded examiners independently scored all sections (Cohen's $\kappa = 0.85$); discrepancies were resolved by consensus.

The sample size ($n = 10$ per group) was determined based on an a priori power analysis (GPower 3.1, Heinrich-Heine-Universität, Düsseldorf, Germany) to detect a medium effect size ($f = 0.30$) with a significance level of $\alpha = 0.05$ and a power of 0.80. The expected effect size ($f = 0.30$) was selected to represent a medium effect size according to Cohen's conventions for power analysis. Based on this analysis, a minimum of 8 specimens per group was required; therefore, 10 teeth per group were included to compensate for potential specimen loss.

Statistical analysis

Statistical analysis was performed using NCSS 2007 (Kaysville, UT, USA). Normality of data distribution was assessed with the Shapiro-Wilk test. Given the ordinal, non-normal distribution of microleakage scores, results were expressed as median (minimum–maximum). Group comparisons were conducted with the Kruskal-Wallis test, and pairwise comparisons were performed using the Dunn-Bonferroni post-hoc test with adjustment for multiple comparisons. The significance level was set at $\alpha = 0.05$, with p -values < 0.05 considered statistically significant. In

addition to p-values, effect sizes (eta-squared, η^2) were calculated for Kruskal-Wallis tests to assess the magnitude of differences among groups.

Results

Microleakage at the gingival wall

Microleakage values for the gingival wall are summarized in Table 3 and illustrated in Figure 1. Significant differences were observed among adhesive groups (Kruskal-Wallis, $H = 50.65$, $p < 0.001$; $\eta^2 = 0.37$). The lowest gingival microleakage values were recorded in the OFL group (median 0.5, 0–1). Universal adhesives applied in self-etch mode generally exhibited higher gingival microleakage values compared with total-etch and selective-etch applications. The highest gingival microleakage was observed in the FBU-SE group, while GBU-SE and TUB-SE also showed significantly higher values compared with other groups. Within the TUB adhesive, the self-etch mode resulted in greater gingival microleakage than the total-etch and selective-etch modes.

Microleakage at the occlusal wall

Occlusal microleakage scores are presented in Table 4 and Figure 2. Significant differences were detected among groups (Kruskal-Wallis, $H = 46.92$, $p < 0.001$; $\eta^2 = 0.31$). The lowest occlusal microleakage values were observed in the OFL and CSB-SEL groups (median 0, 0–1). Universal adhesives applied in self-etch mode generally demonstrated higher occlusal microleakage values compared with total-etch and selective-etch strategies. The highest occlusal microleakage scores were

recorded in the GBU-SE, FBU-SE, and TUB-SE groups. Within the TUB adhesive, the self-etch mode showed higher occlusal microleakage than the total-etch and selective-etch modes.

Representative stereomicroscope images

Representative stereomicroscope images illustrating the different microleakage scores used in the evaluation criteria are shown in Figure 3.

Discussion

The present study evaluated the microleakage performance of universal adhesives containing different functional monomers when applied in total-etch, selective-etch, and self-etch modes. The null hypothesis was rejected, as the chemically cured universal adhesive (TUB), particularly when applied in self-etch mode, showed higher microleakage values compared with the other adhesive systems.

An important aspect of the present study is the evaluation of a chemically cured universal adhesive applied under different etching strategies. While numerous studies have investigated light-curing universal adhesives, limited information is available regarding the marginal sealing behavior of chemically cured systems. This issue is clinically relevant in deep proximal boxes where light attenuation may compromise polymerization of light-activated adhesives. Therefore, evaluating the performance of a chemically cured universal adhesive under different etching protocols provides additional insight into the clinical behavior and bonding performance of contemporary adhesive

systems [14,17].

Universal adhesives allow application with different etching strategies (total-etch, selective-etch, and self-etch), and their bonding performance varies depending on their chemical composition and functional monomers [16,21,22]. Self-etch adhesives simultaneously demineralize and infiltrate dental tissues; therefore, the quality of the hybrid layer depends largely on effective monomer-substrate interactions [23,24].

These adhesives commonly include functional monomers such as HEMA, GPDM, and 10-MDP, which influence bonding performance through chemical interaction and hydrophilicity [25]. Among these, 10-MDP has been extensively studied for its ability to form stable, hydrolytically resistant salts with calcium, thereby improving long-term bond durability [26]. Hydrophilic components such as HEMA may increase water sorption and permeability of simplified adhesive systems, which may affect the stability of the adhesive interface [23]. Moreover, differences in polymerization mechanisms between redox-based chemically cured systems and conventional camphorquinone-initiated photopolymerization may further influence the formation and durability of the adhesive layer [14]. Higher microleakage values were observed at gingival margins compared with occlusal margins, which is consistent with previous reports and is attributed to thinner enamel and the presence of dentin or cementum substrates at the gingival region [27-30].

The literature remains inconsistent regarding which bonding approach most effectively minimizes microleakage. While some authors have

suggested favorable performance of self-etch adhesives in Class II cavities [31], others have reported superior marginal sealing with etch-and-rinse or total-etch strategies [18,32,33]. Santini et al. [34] reported no significant differences between self-etch and total-etch adhesives in Class V restorations. In the present study, OFL, a three-step total-etch adhesive, demonstrated the lowest microleakage values among all tested adhesive systems. It should be noted that the number of experimental groups was not structurally balanced across adhesives because each material was evaluated according to its clinically recommended application modes. OptiBond FL represents a conventional three-step etch-and-rinse adhesive and therefore was assessed only in total-etch mode, whereas the universal adhesives were evaluated using all applicable etching strategies. Accordingly, comparisons across adhesive systems should be interpreted in the context of these inherent differences in material design and application concept.

Mechanistically, OFL incorporates GPDM, which, despite forming weaker calcium ionic bonds than 10-MDP [15,35], benefits from phosphoric acid etching in etch-and-rinse protocols. This approach enhances micromechanical retention through resin tag formation, which may play a critical role in durable bonding [16]. This suggests that effective substrate conditioning can compensate for differences in chemical bonding potential.

Consistent with previous studies, one-step self-etch adhesives exhibited inferior sealing performance compared with two-step systems [36-38]. In the present study, self-etch applications of GBU, FBU, and TUB

showed significantly higher microleakage than CSB ($p < 0.001$). The relatively high microleakage observed for Futura Bond U in self-etch mode may also be related to the hydrophilic nature of simplified one-step universal adhesives, which can increase water sorption and permeability of the adhesive layer and potentially compromise interfacial stability. CSB's favorable performance is likely attributed to the nano-layering ability of 10-MDP [39] and the presence of colloidal silica nanofillers, which strengthen the adhesive matrix and reduce polymerization shrinkage [1,2]. Selective enamel etching did not provide additional benefits for CSB, in agreement with previous findings [40]. No significant differences were observed between OFL (total-etch) and the CSB groups (CSB-SEL and CSB-SE).

Dye penetration was selected as the assessment method due to its sensitivity and practicality, although its inherent limitations, including two-dimensional evaluation and examiner subjectivity, are well recognized [41-43]. To minimize bias, microleakage was independently assessed by two blinded and calibrated examiners, with high inter-examiner agreement ($\kappa = 0.85$), supporting the internal validity of the findings.

With respect to TUB, its formulation includes 3D-SR monomers, MTU-6, BoSE initiator technology, and γ -methacryloxypropyl triethoxysilane, enabling chemical interaction and enhanced polymerization under acidic conditions [14]. In the present study, TUB applied in self-etch mode exhibited the highest microleakage values (median = 2), whereas total-etch (median = 0.5) and selective-etch (median = 1) applications

significantly improved marginal sealing ($p < 0.001$). These findings suggest that adequate substrate conditioning may be more critical for marginal sealing than the curing mechanism itself. This observation is consistent with recent laboratory and clinical studies reporting protocol-dependent performance differences between chemically cured and light-curing universal adhesives [10,14,17].

Nevertheless, these findings should be interpreted with caution because the present study did not include interfacial morphology evaluation, polymerization depth analysis, or degree of conversion measurements. Therefore, the clinical performance of universal adhesives should not be interpreted solely based on their curing mechanism, but rather in conjunction with the selected etching strategy and substrate conditions. Despite the valuable findings of the present study, certain limitations should be acknowledged. This was an *in vitro* investigation, and the results cannot be directly extrapolated to clinical conditions. Functional stresses such as mastication, thermal fluctuations, pH changes, and biofilm activity were not simulated. In addition, dye penetration analysis provides a two-dimensional and semi-quantitative evaluation of marginal leakage and does not allow assessment of nanoleakage pathways or interfacial degradation mechanisms. Accordingly, these results should be interpreted as an indicator of marginal sealing rather than a direct predictor of long-term clinical performance. Only microleakage was assessed; additional analyses such as nanoleakage, bond strength testing, and interfacial morphology evaluation were not performed. Although the ordinal scoring system used in the present study is widely

accepted for microleakage evaluation, quantitative image-analysis methods may provide more precise measurements of dye penetration depth. Furthermore, only one representative material per category was tested, which may limit the generalizability of the findings. In addition, long-term degradation mechanisms such as hydrolysis of functional monomers were not investigated.

Conclusions

Within the limitations of this in vitro study, the findings indicate that both adhesive formulation and etching strategy significantly influence the marginal sealing performance of Class II composite restorations. Simplified one-step universal adhesives demonstrated inferior sealing when applied in self-etch mode, particularly at gingival margins, whereas multi-step etch-and-rinse strategies provided more consistent marginal adaptation. The chemically cured universal adhesive showed improved performance when combined with total-etch or selective-etch protocols. These findings emphasize that appropriate etching strategy selection may be more critical than the adhesive curing mechanism itself in optimizing marginal sealing under clinically relevant conditions.

Declarations

Abbreviations

10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate

4-MET: 4-methacryloxyethyl trimellitate

ANOVA: Analysis of variance

Bis-GMA: Bisphenol A-glycidyl methacrylate

CSB: Clearfil SE Bond 2

ET: Etch-and-rinse

FBU: Futura Bond U

GBU: Gluma Bond Universal

GPDM: Glycerol phosphate dimethacrylate

HEMA: 2-hydroxyethyl methacrylate

LED: Light-emitting diode

MDP: Methacryloyloxydecyl dihydrogen phosphate

SE: Self-etch

SEL: Selective enamel etching

TE: Total-etch

TEGDMA: Triethylene glycol dimethacrylate

TUB: Tokuyama Universal Bond

UDMA: Urethane dimethacrylate

γ -MPTES: γ -methacryloxypropyl triethoxysilane

Ethics approval and consent to participate

This in vitro study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Clinical Research Ethics Committee of Bolu Abant Izzet Baysal University (Approval No: 2020/274). As the study used extracted human teeth obtained in accordance with institutional and national regulations, informed consent to participate was waived by the Ethics Committee, as

no living human participants or identifiable personal data were involved.

Consent for publication

Not applicable.

Availability of data and materials

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

FAB conceived and planned the study and oversaw its overall execution. ED performed the restorative procedures on the specimens and carried out the experimental procedures. Microscopic evaluation and data analysis were conducted independently by FAB and BBCO in a double-blinded manner. ED and BBCO prepared the initial draft of the manuscript. FAB critically reviewed the manuscript, made final revisions, and approved the final version. All authors read and approved the final manuscript.

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Table 1. Adhesive systems evaluated and their manufacturers, principal components, and application protocols

Adhesive system (group code)	Manufacturer (City, Country)	Principal components	Application protocol (per manufacturer)
OptiBond FL (OFL-TE) - three-step etch-and-rinse	Kerr, Orange, CA, USA	37% phosphoric acid; HEMA; GPDM; Bis-GMA; UDMA; silica fillers	Etch enamel 30 s, dentin 15 s; rinse; gently air-dry; apply primer 15 s, air-dry; apply adhesive, light-cure 30 s
Clearfil SE Bond 2 (CSB-SE / CSB-SEL) - two-step self-etch	Kuraray Noritake, Tokyo, Japan	10-MDP; HEMA; Bis-GMA; hydrophilic/hydrophobic monomers	SE: Apply primer 20 s, air-dry; apply bond, light-cure 10 s. SEL: Enamel etch 15 s, rinse, dry; then SE protocol

Gluma	Kulzer GmbH,	10-MDP;	4-MET;	Apply 20 s; air-
Bond	Hanau,	HEMA; UDMA; ethanol;	dry 5 s; light-	
Universal	Germany	silica nanofillers	cure 10 s (with	
(GBU-TE /			or without prior	
GBU-SEL /			etching,	
GBU-SE) -			depending on	
universal			mode)	
Futura	VOCO GmbH,	Bis-GMA;	HEMA;	Apply 20 s; air-
Bond U	Cuxhaven,	UDMA; acidic adhesive	dry; light-cure	
(FBU-TE /	Germany	monomers; nanofillers	10 s (etching	
FBU-SEL /			depends on	
FBU-SE) -			mode)	
universal				
Tokuyama	Tokuyama	Monomer A: MTU-6,	Mix equal	
Universal	Dental, Tokyo,	3D-SR monomer, Bis-	drops of A and	
Bond (TUB-	Japan	GMA, TEGDMA, γ -	B; apply 20 s;	
TE / TUB-		MPTES (silane).	air-dry 5 s; self-	
SEL / TUB-		Monomer B: borate	cures (no light-	
SE) -		initiator, peroxide	curing). For	
chemically			TE/SEL, etch	
cured			enamel with	
universal			phosphoric	
(two-			acid before	
			bonding	

component

)

OFL = OptiBond FL; CSB = Clearfil SE Bond 2; GBU = Gluma Bond Universal; FBU = Futura Bond U; TUB = Tokuyama Universal Bond; TE = total-etch; SEL = selective-etch; SE = self-etch. Bis-GMA = bisphenol A-glycidyl methacrylate; UDMA = urethane dimethacrylate; HEMA = 2-hydroxyethyl methacrylate; MDP = 10-methacryloyloxydecyl dihydrogen phosphate; GPDM = glycerol phosphate dimethacrylate; 4-MET = 4-methacryloxyethyl trimellitate; TEGDMA = triethylene glycol dimethacrylate; γ -MPTES = γ -methacryloxypropyl triethoxysilane.

Table 2. Microleakage scoring criteria used for evaluation

Score	Criteria description
0	No dye penetration at occlusal or gingival margins
1	Dye penetration up to one-third of the cavity depth along the cavity wall

2	Dye penetration extending beyond one-third but not reaching the axial wall
3	Dye penetration reaching and/or passing along the axial wall

Microleakage was scored according to widely adopted criteria reported in previous studies [7,18,20]. Two blinded examiners independently evaluated all sections under stereomicroscopy, and discrepancies were resolved by consensus.

Table 3. Gingival microleakage scores at the gingival wall of Class II restorations (n = 10 per group)

Group	Score (0/1/2/3)	distribution	Median (Min-Max)
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OFL-TE	5/5/0/0	0.5 (0-1) ^a
CSB-SEL	4/5/1/0	1 (0-2) ^a
CSB-SE	1/6/3/0	1 (0-2) ^a
GBU-TE	3/4/3/0	1 (0-2) ^a
GBU-SEL	2/6/2/0	1 (0-2) ^a
GBU-SE	0/1/5/4	2 (1-3) ^b
FBU-TE	2/6/2/0	1 (0-2) ^a
FBU-SEL	3/5/2/0	1 (0-2) ^a
FBU-SE	0/1/3/6	3 (1-3) ^c
TUB-TE	5/3/1/1	0.5 (0-3) ^a
TUB-SEL	2/4/3/1	1 (0-3) ^a
TUB-SE	0/2/4/4	2 (1-3) ^b

Values are presented as median (minimum-maximum). Score distribution indicates the number of specimens receiving scores 0, 1, 2, and 3. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn-Bonferroni post-hoc comparisons. Different superscript letters indicate statistically significant differences among groups ($p < 0.05$). Overall comparison showed a statistically significant difference among groups ($H = 50.65$; $p < 0.001$; $\eta^2 = 0.37$).

Table 4. Occlusal microleakage scores at the occlusal wall of Class II restorations (n = 10 per group)

Group	Score distribution (0/1/2/3)	Median (Min-Max)
OFL-TE	8/2/0/0	0 (0-1) ^a
CSB-SEL	7/3/0/0	0 (0-1) ^a
CSB-SE	5/5/0/0	0.5 (0-1) ^a
GBU-TE	7/3/0/0	0 (0-1) ^a
GBU-SEL	6/3/1/0	0 (0-2) ^a
GBU-SE	0/2/6/2	2 (1-3) ^b
FBU-TE	6/4/0/0	0 (0-1) ^a
FBU-SEL	7/2/1/0	0 (0-2) ^a
FBU-SE	0/4/4/2	2 (1-3) ^b
TUB-TE	5/5/0/0	0.5 (0-1) ^a
TUB-SEL	5/4/1/0	0.5 (0-2) ^a
TUB-SE	2/4/3/1	1 (0-3) ^b

Values are presented as median (minimum-maximum). Score distribution indicates the number of specimens receiving scores 0, 1, 2, and 3. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn-Bonferroni post-hoc comparisons. Different superscript letters indicate statistically significant differences among groups ($p < 0.05$). Overall comparison showed a statistically significant difference among groups ($H = 46.92$; $p < 0.001$; $\eta^2 = 0.31$).

Figure Legends

Figure 1. Gingival wall microleakage scores (median, minimum-maximum) for all adhesive groups. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn-Bonferroni post-hoc comparisons ($p < 0.001$).

OFL, OptiBond FL; CSB, Clearfil SE Bond; GBU, Gluma Bond Universal; FBU, Futura Bond U; TUB, Tokuyama Universal Bond; TE, total-etch; SEL, selective-etch; SE, self-etch.

Figure 2. Occlusal wall microleakage scores (median, minimum-maximum) for all adhesive groups. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn-Bonferroni post-hoc comparisons ($p < 0.001$).

OFL, OptiBond FL; CSB, Clearfil SE Bond; GBU, Gluma Bond Universal; FBU, Futura Bond U; TUB, Tokuyama Universal Bond; TE, total-etch; SEL, selective-etch; SE, self-etch.

Figure 3. Representative stereomicroscope images illustrating different levels of microleakage observed in the present study. The images represent typical examples used to illustrate the microleakage scoring criteria.

(A) Score 0 – no dye penetration at the tooth–restoration interface.

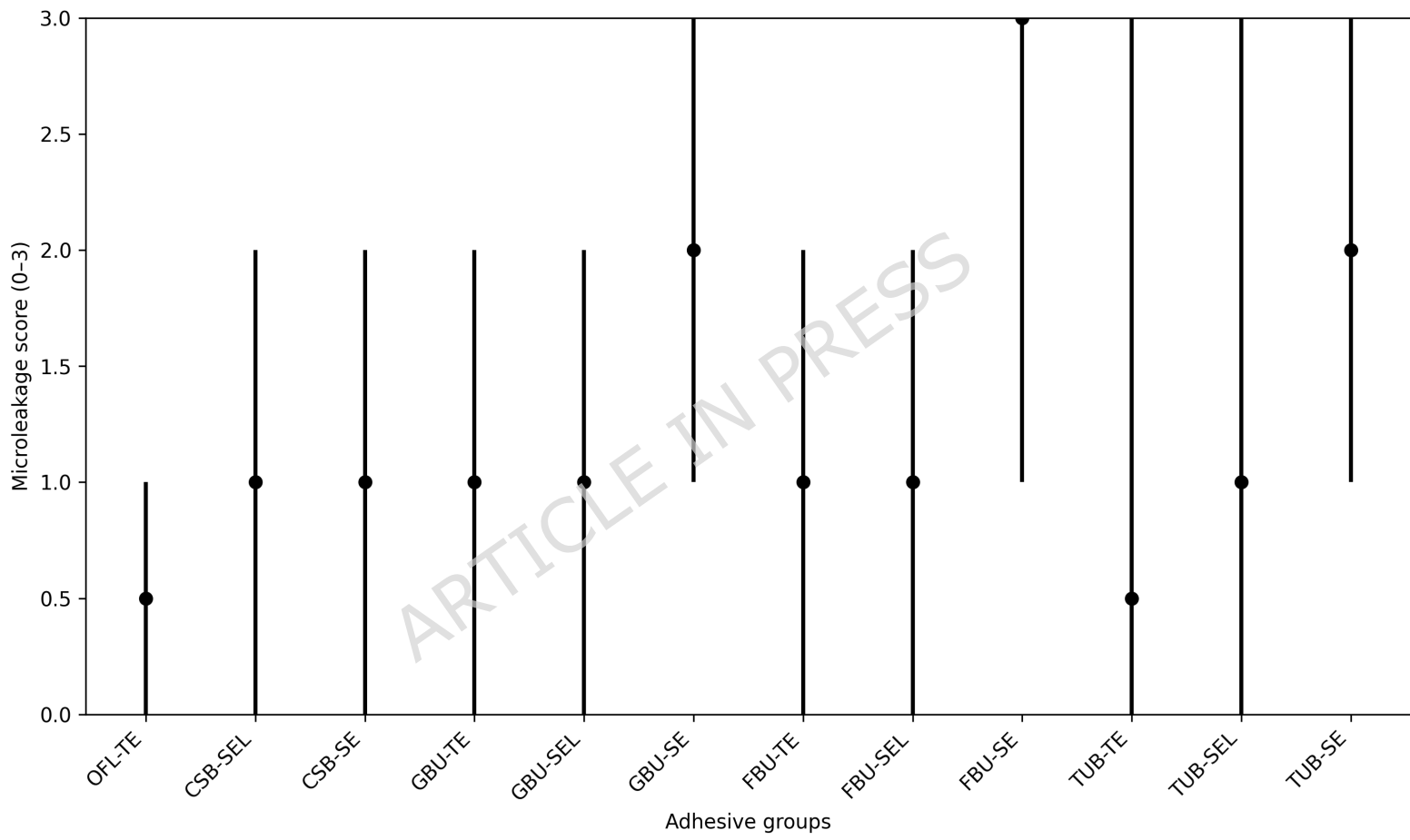
(B) Score 1 – dye penetration extending up to one-third of the cavity wall.

(C) Score 2–3 – dye penetration extending beyond one-third of the cavity wall and approaching or reaching the axial wall.

Images were obtained under stereomicroscopy at $\times 20$ magnification.

Scale bar = 1 mm.

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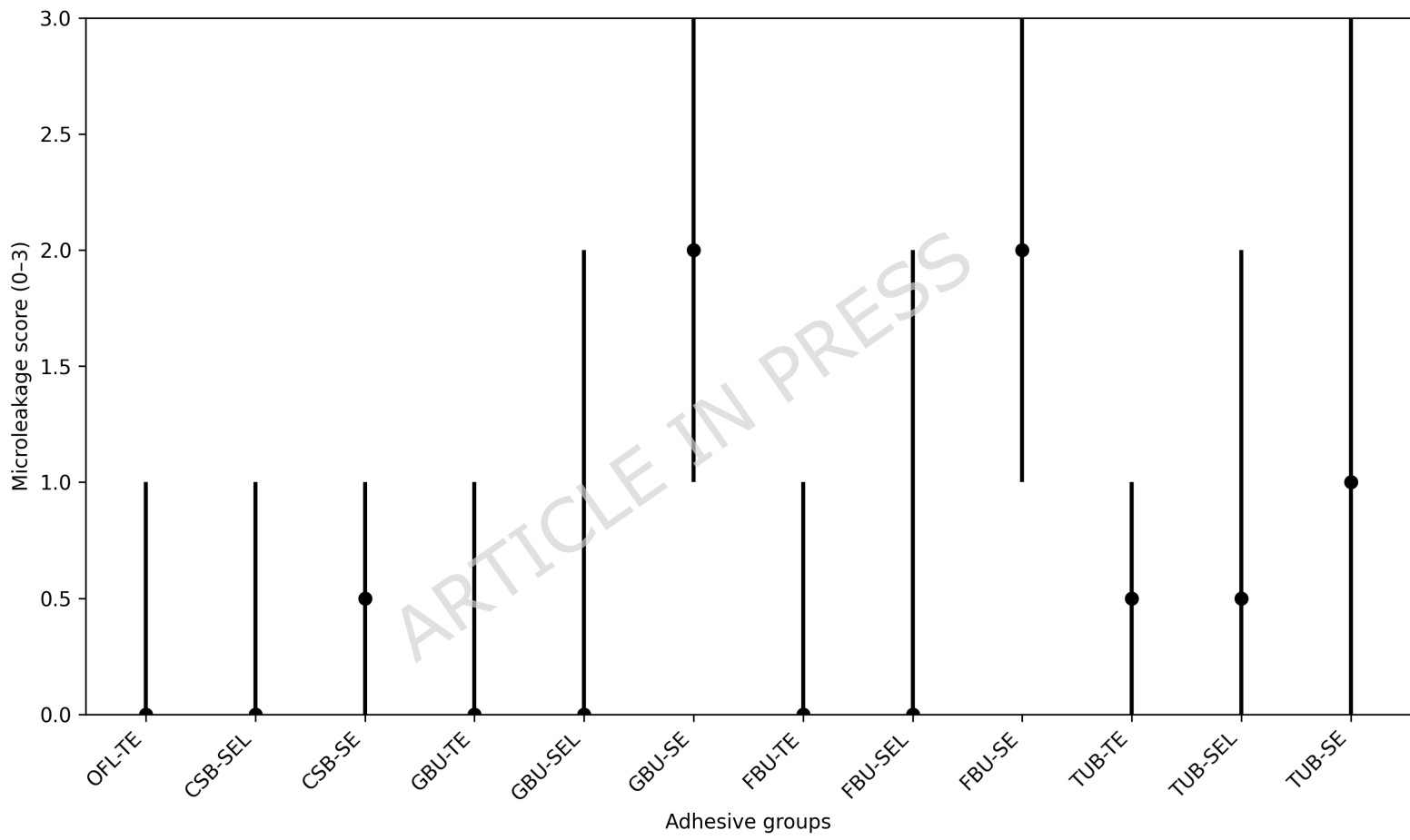




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Clearfil SE Bond 2 (CSB-SE / CSB-SEL) - two-step self-etch	Kuraray Noritake, Tokyo, Japan	10-MDP; HEMA; Bis-GMA; hydrophilic/hydrophobic monomers	SE: Apply primer 20 s, air-dry; apply bond, light-cure 10 s. SEL: Enamel etch 15 s, rinse, dry; then SE protocol

Gluma	Kulzer GmbH,	10-MDP;	4-MET;	Apply 20 s; air-
Bond	Hanau,	HEMA; UDMA; ethanol;	dry 5 s; light-	
Universal	Germany	silica nanofillers	cure 10 s (with	
(GBU-TE /			or without prior	
GBU-SEL /			etching,	
GBU-SE) -			depending on	
universal			mode)	
Futura	VOCO GmbH,	Bis-GMA;	HEMA;	Apply 20 s; air-
Bond U	Cuxhaven,	UDMA; acidic adhesive	dry; light-cure	
(FBU-TE /	Germany	monomers; nanofillers	10 s (etching	
FBU-SEL /			depends on	
FBU-SE) -			mode)	
universal				
Tokuyama	Tokuyama	Monomer A: MTU-6,	Mix equal	
Universal	Dental, Tokyo,	3D-SR monomer, Bis-	drops of A and	
Bond (TUB-	Japan	GMA, TEGDMA, γ -	B; apply 20 s;	
TE / TUB-		MPTES (silane).	air-dry 5 s; self-	
SEL / TUB-		Monomer B: borate	cures (no light-	
SE) -		initiator, peroxide	curing). For	
chemically			TE/SEL, etch	
cured			enamel with	
universal			phosphoric	
(two-			acid before	
			bonding	

component

)

OFL = OptiBond FL; CSB = Clearfil SE Bond 2; GBU = Gluma Bond Universal; FBU = Futura Bond U; TUB = Tokuyama Universal Bond; TE = total-etch; SEL = selective-etch; SE = self-etch. Bis-GMA = bisphenol A-glycidyl methacrylate; UDMA = urethane dimethacrylate; HEMA = 2-hydroxyethyl methacrylate; MDP = 10-methacryloyloxydecyl dihydrogen phosphate; GPDM = glycerol phosphate dimethacrylate; 4-MET = 4-methacryloxyethyl trimellitate; TEGDMA = triethylene glycol dimethacrylate; γ -MPTES = γ -methacryloxypropyl triethoxysilane.

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Microleakage was scored according to widely adopted criteria reported in previous studies [7,18,20]. Two blinded examiners independently evaluated all sections under stereomicroscopy, and discrepancies were resolved by consensus.

Table 3. Gingival microleakage scores at the gingival wall of Class II restorations (n = 10 per group)

Group	Score (0/1/2/3)	distribution	Median (Min-Max)
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CSB-SEL	4/5/1/0		1 (0-2) ^a
CSB-SE	1/6/3/0		1 (0-2) ^a
GBU-TE	3/4/3/0		1 (0-2) ^a
GBU-SEL	2/6/2/0		1 (0-2) ^a
GBU-SE	0/1/5/4		2 (1-3) ^b
FBU-TE	2/6/2/0		1 (0-2) ^a
FBU-SEL	3/5/2/0		1 (0-2) ^a
FBU-SE	0/1/3/6		3 (1-3) ^c
TUB-TE	5/3/1/1		0.5 (0-3) ^a
TUB-SEL	2/4/3/1		1 (0-3) ^a
TUB-SE	0/2/4/4		2 (1-3) ^b

Values are presented as median (minimum-maximum). Score distribution indicates the number of specimens receiving scores 0, 1, 2, and 3. Statistical analysis was performed using the Kruskal-Wallis test followed by Dunn-Bonferroni post-hoc comparisons. Different superscript letters indicate statistically significant differences among groups ($p < 0.05$). Overall comparison showed a statistically significant difference among groups ($H = 50.65$; $p < 0.001$; $\eta^2 = 0.37$).

Table 4. Occlusal microleakage scores at the occlusal wall of Class II restorations (n = 10 per group)

Group	Score distribution (0/1/2/3)	Median (Min-Max)
OFL-TE	8/2/0/0	0 (0-1) ^a
CSB-SEL	7/3/0/0	0 (0-1) ^a
CSB-SE	5/5/0/0	0.5 (0-1) ^a
GBU-TE	7/3/0/0	0 (0-1) ^a
GBU-SEL	6/3/1/0	0 (0-2) ^a
GBU-SE	0/2/6/2	2 (1-3) ^b
FBU-TE	6/4/0/0	0 (0-1) ^a
FBU-SEL	7/2/1/0	0 (0-2) ^a
FBU-SE	0/4/4/2	2 (1-3) ^b
TUB-TE	5/5/0/0	0.5 (0-1) ^a
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