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The Effects of Dentin Adhesives and Liner Materials on the Microleakage of Class II Resin Composite Restorations in Primary and Permanent Teeth

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Purpose: *To compare the occlusal and gingival microleakage of Class-II composite restorations utilizing etch-and-rinse and self-etch adhesives and different liner materials in primary and permanent teeth. Study design: Standardized class-II cavities were prepared in freshly-extracted sound primary and permanent molars (n=80/each), with all cavosurface margins involving enamel. The main experimental groups were; A. Single Bond 2/primary teeth; B. Adper SE Plus/primary teeth; C. Single Bond 2/permanent teeth; and D. Adper SE Plus/permanent teeth. Each group comprised 4 subgroups (n=10/each) with respect to the liner material employed (n=10/subgroup): 1. Fuji VII; 2. Fuji Triage; 3. Filtek Supreme XT Flowable Composite, and 4. No liner. All teeth were restored with Filtek Supreme XT Universal Nanofilled Composite. Following thermocycling and immersion in basic fuchsin, the extent of microleakage was measured on crown sections using image analysis. The data were analyzed with Wilcoxon Signed Ranks Test, Mann-Whitney U-Test and Kruskal-Wallis One-Way ANOVA at $\alpha=0.05$.* ***Results:*** *In both primary and permanent teeth the use of etch-and-rinse adhesive resulted in similar occlusal and gingival microleakage values ($p>0.05$). As for the self-etch adhesive, similar results were observed ($p>0.05$) with the exception of significantly less occlusal leakage in the Fuji Triage VII and Fuji Triage subgroups of primary teeth than those of permanent teeth ($p<0.05$). When the effects of liner material and the type of adhesive were disregarded, significantly more gingival microleakage was observed in primary teeth than in permanent teeth ($p<0.01$), while the occlusal microleakage values were similar ($p>0.05$). Irrespective of the tooth type and adhesive material, comparison of subgroups containing a liner material with those without one revealed no significant differences for both occlusal and gingival microleakage values ($p>0.05$).* ***Conclusions:*** *Occlusal microleakage was similar in both primary and permanent teeth, while a lesser extent of gingival seal was observed in primary teeth. Overall, placement of a liner material did not improve resistance to microleakage.*

Keywords: *microleakage, quantitative, etch-and-rinse adhesives, self-etch adhesives*

INTRODUCTION

Despite considerable advances in the field of restorative biomaterials, microleakage under restorations remains to be a major problem in daily clinical practice.[1] In particular, resin-based composites still suffer from the gap at the tooth/restorative interface resulting from polymerization shrinkage. Such gaps may lead to postoperative sensitivity and[1] marginal deterioration, as well as microleakage-related problems such as recurrent caries[2] and pulp injury.[3] Several materials and techniques that have been suggested to reduce the microleakage include acid-etching of enamel, incremental build-up of the composite resin and the use of liner materials.

Flowable composites have been initially recommended as a liner material under hybrid or packable resin composites due to their low viscosity, increased elasticity, and wettability.[4] These materials possess specific flow characteristics and relatively lower filler content compared to those of hybrid resin composites.[5] Encouraging laboratory results in reducing microleakage have been reported with the use of flowable composites.[6-8]

Conventional glass ionomer cements have also been recommended as liner materials, owing to their ability to release fluoride, self-adhere to tooth structures, and biocompatibility with the pulp tissue.[9,10] However, problems associated with their handling and setting time do no not always render these materials practical under a variety of clinical scenarios including the child patient. More recently, light cured glass ionomer cements with lower viscosity and increased wettability have been suggested to overcome those

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Table 1. Material compositions and their respective application modes.

Product	Composition		Application
Fuji VII (GC Corporation, Tokyo, Japan)	BisGMA, UDMA, triethylene glycol, fluorosilicate glass, silicon dioxide, pigments, initiators		Apply to the cavity, light cure for 20 s.
Fuji Triage (GC Corporation, Tokyo, Japan)	Aluminofluoro-silicate glass, Pigment Trace, Polyacrylic acid, Distilled water, Polybase carboxylic acid		Apply to the cavity, wait 3-4 minutes
Filtek Supreme XT Flowable Restorative (3M ESPE, St. Paul, USA)	Zirconia/silica filler, UDMA, Bis-GMA, TEG-DMA, and water		Apply to the cavity, light cure for 20 s.
3M Scothbond Etchant (3M ESPE, St. Paul, USA)	35% phosphoric acid, colloidal silica		Apply to enamel for 30 s, to dentin for 15 s, wash for 10 s, gently air dry
Single Bond 2 (3M ESPE; Seefeld, Germany)	Bis-GMA, HEMA, water, ethanol, Polyalkenoic acid copolymer, photoinitiator.		Apply and leave for 20 s, dry gently 2-5 s, light cure for 10 s.
	Primer	**Adhesive**	
Adper SE Plus (3M ESPE, St. Paul, USA)	MDP, HEMA, Hydrophilic Dimethacrylate	MDP, bis-GMA, HEMA, silanated colloidal silica	Apply primer and leave for 20 s, gently air dry, apply adhesive, light cure for 10 s.
Filtek Supreme XT Universal Restorative (3M ESPE, St. Paul, USA)	Bis-GMA, TEGDMA, UDMA, bisphenol A, polyethylene glycol diether dimethacrylate, Silica nanofillers, Zirconia/silica nanoclusters		Incrementally apply to the cavity, light cure each increment for 20 s.

disadvantages and reduce microleakage under restorations, especially in the gingival region.[11]

At present, the application of dental adhesives is an indispensable step for resin composite restorations, as the survival of resin-based restorations is largely bound to the effective sealing ability of the adhesive system used.[12] While many studies have reported varying levels of microleakage associated with the use etch-and-rinse and self-etch adhesives in permanent teeth,[13,14] little, if any comparative data exists with regard to their sealing properties on primary teeth. Owing to the well-documented structural and chemical differences in enamel and dentin of primary and permanent teeth,[15,16] it is reasonable to assume that adhesive resins may show different effects on primary teeth.

For almost every adhesive system available today, the manufacturers' "instructions for use" seldom specifies differences for application in primary or permanent teeth. It may seem that a majority of the restorative materials is primarily designed to be used in permanent teeth. Therefore, the results of microleakage experiments on permanent teeth should not be directly applied to primary teeth and separate experimental testings should, indeed, be carried out on primary teeth before recommending for clinical use.[17] Based on these considerations, the present study aimed to evaluate the microleakage of Class-II resin composite restorations in primary and permanent teeth bonded with different adhesive systems and liner materials.

MATERIALS AND METHOD

Intact, freshly-extracted human primary second molars and permanent third molars were used. Following removal of tissue remnants with a hand instrument, the teeth were cleaned with a rubber cup and slurry of pumice, and investigated under a stereomicroscope at 20X for surface cracks or developmental defects. Selected teeth (80 primary molars and 80 permanent molars) were stored in 0.2% thymol in normal saline solution before use (a maximum of 1 month). Class-II cavity preparations were made by one operator using a high-speed handpiece with air-water spray and a # 1090 diamond fissure bur (Diatech Dental AG, Heerbrugg, Switzerland). All cavosurface margins were beveled (approximately 1 mm) using the same bur. New burs were used after every ten preparations. For the purpose of standardization, the occlusal part of the preparation measured 3 mm in depth and 2 mm in buccolingual width, and the proximal margins were placed 1 mm above the cementoenamel junction (CEJ). The depth of the box from cavosurface margin to the axial wall was 3 mm and the buccolingual width was 3.0 mm. Buccal and lingual walls of the preparations were approximately parallel and connected to the gingival wall with rounded line angles. Following cavity preparation, the root apices and the furcation regions were sealed with dental wax in order to prevent leakage into the pulp.

The materials used in the study are presented in Table 1. All restorative procedures were carried out in accordance with the manufacturers' instructions. The present study had a 2 (adhesives) X 2 (primary/permanent tooth) X 4 (liner materials) design. Accordingly, etch-and-rinse (Single Bond 2: 3M ESPE, Seefeld, Germany) and self-etch (Adper SE Plus; 3M ESPE, St. Paul, USA) adhesives were used. The four main groups of the study were as follows: **A:** Single Bond 2, primary teeth, **B:** Adper SE Plus, primary teeth, **C:** Single Bond 2, permanent teeth, **D:** Adper SE Plus, permanent teeth. Each group comprised 4 subgroups of liner materials (n=10): **1.** Fuji VII (GC Corporation, Tokyo, Japan); **2.** Fuji Triage (GC Corporation, Tokyo, Japan); **3.** Filtek Supreme XT Flowable Restorative (3M ESPE, St. Paul, USA); **4.** No liner (Table 2). All liner materials were applied on both pulpal floor and axial wall with a maximum thickness of 1 mm.

Table 2. Study groups

Group	Subgroup	n	Teeth	Base
Acid etch + Single Bond 2 + Filtek Supreme XT Universal Restorative	A1	10	Primary	Fuji VII
	A2	10		Fuji Triage
	A3	10		Filtek Supreme XT Flowable
	A4	10		No liner
	C1	10	Permanent	Fuji VII
	C2	10		Fuji Triage
	C3	10		Filtek Supreme XT Flowable
	C4	10		No liner
Adper SE Plus + Filtek Supreme XT Universal Restorative	B1	10	Primary	Fuji VII
	B2	10		Fuji Triage
	B3	10		Filtek Supreme XT Flowable
	B4	10		No liner
	D1	10	Permanent	Fuji VII
	D2	10		Fuji Triage
	D3	10		Filtek Supreme XT Flowable
	D4	10		No liner

In subgroups A3, B3, C3 and D3, Filtek Supreme XT Flowable Restorative was applied following application of the respective adhesive system. The application of the glass-ionomer cements (Fuji VII and Fuji Triage) was followed by the adhesives. All teeth were restored with a nanofilled composite material (Filtek Supreme XT Universal Restorative, 3M ESPE, St. Paul, U.S.A.), using incremental technique. A Tofflemire matrix retainer with a metal band was utilized during application of the composite resin. A quartz-tungsten-halogen curing unit with a light intensity of 500 mW/cm^2 (Hilux 200 Curing Light, Benlioğlu Dental Inc., Ankara, Turkey) was used for photopolymerization. The light intensity of the curing unit was checked before applications in each subgroup. Polishing of the specimens was accomplished with a series of Sof-Lex disks (3M Dental Products, St. Paul, U.S.A.).

Following storage in distilled water at 37^o C for one week, the specimens were thermocycled for 1000 cycles between 5 and 55^o C with a dwell time of 15 and a transfer time of 30 seconds. Two coats of nail varnish was applied 1 mm short of the margins to be exposed to dye. Specimens were then immersed in 0.5% basic fuchsin solution (Wako Pure Chemical Industry; Osaka, Japan) at 37^0 C for 24 hours. Thereafter, the specimens were thoroughly rinsed with distilled water, air dried and embedded in epoxy resin (Struers; Copenhagen, Denmark). A slow-speed, water-cooled diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) was used to obtain 5 sections of 0,5 mm thickness from each tooth.[18] A digital photograph of each section was obtained at 20X under a stereomicroscope (Olympus; Tokyo, Japan), and images were transferred to a Macintosh PowerPC workstation. Image analysis software (ImageJ for MacOSX; V.1.34, National Institutes of Health; Bethesda, MD, USA) was used to measure the extent of occlusal and gingival dye penetration in millimeters along the enamel/restorative interface. One calibrated operator, blinded to treatment groups, made the measurements. The microleakage value for each specimen, and thereafter for each tooth and subgroup was calculated as mean ± SD. The obtained data were analyzed with Wilcoxon Signed Ranks Test, Mann-Whitney U-Test and Kruskal-Wallis One-Way ANOVA where $\alpha=0.05$.

RESULTS

The microleakage values (mm) were presented in Table 3 as mean±SD. The occlusal and gingival microleakage values did not differ significantly among the test groups ($p>0.05$). Although the extent of gingival microleakage was greater in groups A, B and C, the differences were only significant in subgroups A1, A2, A3, A4, B2, and C3 ($p<0.05$). In Group D, all subgroups showed greater occlusal microleakage. However, the differences were not significant ($p>0.05$).

In primary teeth, pairwise comparisons among subgroups with regard to the effects of etch-and-rinse and self-etch adhesives (e.g. A1 vs. B1) showed that gingival microleakage was significantly greater in groups A1, A2 and A4 (etch-and-rinse) than in B1, B2 and B4 (self-etch) ($p<0.05$, $p<0.01$ and $p<0.01$, respectively). When the same comparisons were made for permanent teeth (e.g. subgroups C1 vs. D1), the amount of occlusal microleakage was significantly greater in D1 and D4 (self-etch) than in C1 and C4 (etch-and-rinse) ($p<0.05$ and $p<0.01$, respectively). All remaining pairwise comparisons revealed statistically insignificant differences with respect to occlusal and gingival microleakage ($p>0.05$).

When the etch-and-rinse adhesive was used, the primary teeth (subgroups of A) did not differ significantly from permanent teeth (subgroups of C) with respect to occlusal and gingival microleakage ($p>0.05$). When the self-etch adhesive was employed, respective comparisons (subgroups of B vs. D) showed that occlusal microleakage was significantly less in the Fuji Triage VII and Fuji Triage subgroups of primary teeth (B1 and B2) than those of permanent teeth (D1 and D2) ($p<0.05$). All remaining pairwise comparisons revealed statistically insignificant differences for both occlusal and gingival microleakage values ($p>0.05$).

In the experimental subgroups without a liner material (A4, B4, C4, D4), the etch-and-rinse adhesive (subgroups A4 and C4) showed significantly less occlusal microleakage than the self-etch adhesive (subgroups B4 and D4) in both primary and permanent teeth ($p<0.001$). However, the gingival microleakage did not differ among these subgroups ($p>0.05$).

Table 3. Microleakage results obtained in the study

Restoration Procedure	Subgroup	Teeth	Liner	Microleakage (mean ± SD) (mm)	
				Occlusal	Gingival
Acid Etch + Single Bond 2 + Filtek Supreme XT Universal Restorative	A1	Primary	Fuji VII	0,4556 ± 0,3179	1,4660 ± 0,2257
	A2		Fuji Triage	0,4391 ± 0,1477	1,2371 ± 0,1340
	A3		Filtek Supreme XT Flowable	0,2173 ± 0,1046	0,9793 ± 0,1423
	A4		No liner	0,1007 ± 0,0380	1,0920 ± 0,1618
	C1	Permanent	Fuji VII	0,2923 ± 0,2150	0,6320 ± 0,1591
	C2		Fuji Triage	0,3313 ± 0,1751	0,7480 ± 0,2144
	C3		Filtek Supreme XT Flowable	0,1810 ± 0,0550	0,9243 ± 0,2145
	C4		No liner	0,1357 ± 0,0444	0,5693 ± 0,0342
Adper SE Plus + Filtek Supreme XT Universal Restorative	B1	Primary	Fuji VII	0,2780 ± 0,0938	0,3044 ± 0,0652
	B2		Fuji Triage	0,2816 ± 0,1105	0,4692 ± 0,1032
	B3		Filtek Supreme XT Flowable	0,3240 ± 0,0701	0,5156 ± 0,1652
	B4		No liner	0,3843 ± 0,1175	0,5337 ± 0,1352
	D1	Permanent	Fuji VII	0,7872 ± 0,3936	0,5808 ± 0,0269
	D2		Fuji Triage	0,7843 ± 0,2433	0,4583 ± 0,1681
	D3		Filtek Supreme XT Flowable	0,4844 ± 0,2324	0,2156 ± 0,0548
	D4		No liner	0,8033 ± 0,0572	0,3127 ± 0,0537

In each row, values marked bold indicate significant differences in subgroups with respect to occlusal and gingival microleakage (p<0.05).

When the effects of liner material and the type of adhesive were disregarded (A4 and B4 vs. C4 and D4), significantly greater gingival microleakage was observed in primary teeth than in permanent teeth ($p<0.01$). However, the differences for occlusal microleakage were insignificant ($p>0.05$). Irrespective of the tooth type and adhesive material, comparison of subgroups containing a liner material (A1, A2, A3 + B1, B2, B3 + C1, C2, C3 + D1, D2, D3) with those without one (A4 + B4 + C4 + D4) revealed no significant differences for both occlusal and gingival microleakage (all $p>0.05$).

DISCUSSION

This factorial-design *in vitro* study aimed to evaluate the effects of type of adhesive, tooth, and liner material on the occlusal and gingival microleakage of Class-II resin composite restorations in primary and permanent teeth. Microleakage tests are one of the commonly used techniques to evaluate the sealing performance of restorative materials and adhesive systems.[7, 14, 17, 19] Conventionally, these tests comprise a subjective scoring system which categorizes the amount of penetrated dye along the restorative/tooth interface. In contrast to this qualitative method, the present study used image analysis in combination with the dye penetration technique. A relative merit of this objective approach compared with conventional subjective scoring systems is that, there was no need for scoring by separate evaluators, consensus scoring in borderline cases, as well as statistical procedures with regard to inter-examiner reliability.

In general, studies on Class-II composite resins have reported significantly greater microleakage values at the gingival than at the occlusal region.[6,8,20] Maintaining a good access to the proximal box and controlling moisture at the gingival floor may be problematic during a Class-II adhesive restoration. In addition, due to the structural differences between enamel, dentin and cementum, gingival margins that are located in cementum/dentin are the sources of major marginal leakage.[19,21] Because these two structures do not offer same conditions for adhesion of resin composites, microleakage at the cemento dentinal margins becomes one of the most important causes of failure in Class-II composite restorations.[19] In the present study, however, no significant difference was found between occlusal and gingival microleakage among the study groups. Presumably, a contributing factor to this finding is the proximal box margins that were placed 1 mm above the CEJ, i.e., margins that were surrounded by enamel. Araujo *et al* [20] have also reported significantly less gingival microleakage in Class-II cavities where the gingival margins were prepared in enamel. Another contributory factor could be the beveling of enamel margins, which may be more effective in minimizing microleakage than the type of adhesive used.[17] This is particularly important in the presence of the outer aprismatic layer of enamel,[22] which in its unground state may adversely influence the adhesion.[23,24] The prismless enamel layer is more frequently seen in primary teeth,[25] and is 3 to 9 times thicker (16-45μm),[22] compared to that of permanent teeth (<5μm).[25] It has also been demonstrated that the frequency of the prismless enamel tends to be higher in the cervical region.[26] Thus, beveling the cavosurface margins is helpful. The additional benefit of this treatment is that it provides a greater marginal surface to compensate for polymerization shrinkage, which could help reduce microleakage.[27] It also improves the bonding effectiveness of self-etch adhesives in cavities whose margins are placed in enamel.[24]

In the present study, a greater extent of gingival microleakage was observed in all subgroups of A, B and C. However, the differences were significant in only 6 subgroups (A1, A2, A3, A4, B2, C3). Five of these were primary teeth subgroups, with 4 of them belonging to Group A (etch-and-rinse) and 1 belonging to Group B (self-etch). The remaining was a self-etch adhesive subgroup

in which the flowable resin was used as liner on permanent teeth (C3). These findings demonstrate the lesser extent of gingival seal in primary teeth with the tested adhesives,[17] and their differential effects with regard to tooth type.

With the exception of subgroup A3, the gingival microleakage was significantly greater in primary teeth subgroups of Group A (etch-and-rinse), compared to those of Group B (self-etch) where flowable composite was used as liner. Only two *in vitro* studies have investigated the effects of flowable liners on the microleakage of primary tooth composite restorations. Despite their encouraging results, those studies were carried out in Class-I[28] and Class-V[29] cavities, necessitating cautious interpretation of the results. When the etch-and-rinse permanent teeth subgroups were compared to those of the self-etch subgroups, it was found that the use of self-etch adhesive resulted in more occlusal leakage in subgroups where self-the cure glass-ionomer and no liner were used, respectively.

The occlusal and gingival microleakage did not differ with the use of etch-and-rinse adhesive in primary and permanent teeth. The lack of comparative studies related to the effect of etch-and-rinse adhesives on the microleakage of composite restorations in primary and permanent teeth makes the interpretation of this result impossible. As regards to the use of self-etch adhesive in primary and permanent teeth, significantly greater occlusal microleakage was observed in 2 permanent teeth subgroups (D1 and D2), where self-cure or light-cured glass ionomer cements were used as liner materials, respectively.

When no liner material was used, the application of self-etch adhesive resulted in significantly greater occlusal microleakage in both primary and permanent teeth. This finding could be explained by lower resin–enamel bond strength of all-in-one self-etch adhesives on ground and unground enamel.[30,31] The self-etch adhesive used in the present study is classified as "strong" due to its very low pH (<1).[23] All-in-one adhesives like Adper SE Plus have demonstrated lower bond strength to both enamel and dentin than one-bottle etch-and-rinse systems, such as Single-Bond.[32,33] The microtensile bond strength tests have also shown superior results with the use of etch-and-rinse adhesives, regardless of the presence or absence of prior enamel preparation.[24,34,35] Hence, selective etching of enamel with phosphoric acid is still considered the best option for effective and durable bond with the use of self-etch adhesives.[12,36,37]

In the absence of a liner material, application of the tested adhesives resulted in significantly greater gingival microleakage in primary teeth. The mineral content of primary tooth enamel might be responsible for this observation. In comparison to their permanent analogues, primary tooth enamel shows decreased mineralization.[15] Primary teeth also differ in the chemical composition of dentin, which is less dense and less mineralized.[38] These variations in composition and morphology have been reflected by lower bond strengths and increased microleakage in studies on primary tooth enamel and dentin.[38-41]

The use of a liner material and the choice of adhesive system did not result in significantly less occlusal or gingival microleakage in primary and permanent teeth. This finding is in contrast with earlier studies, which reported beneficial effects of using flowable composites,[6,7,42] as well as self- and light-cured glass–ionomers[28,43,44] as liners to reduce gingival or occlusal microleakage in Class-I, II and V resin composite restorations. However, the role of cavity configuration factor, C-factor, should be considered when comparing the results in these cavity types. In high C-factor cavities (e.g. in Class-I and -II cavities), light-induced polymerization of resin composite results in debonding of composite material on one or more walls as the shrinkage forces cannot be relieved by resin flow.[45] The resultant marginal gaps leads to microleakage.[14,46]

The rationale behind using flowable resin composites as liners is mainly related to their decreased viscosity, which helps them to flow easily onto all prepared surfaces. This was achieved by reducing the filler content and allowing the increased resin to lower the viscosity of the mixture.[1] It has been assumed that these materials might act as a flexible intermediate layer to help relieve the stresses during polymerization shrinkage of the restorative resin that could lead to less marginal leakage.[1,6] However, researchers have shown that flowable resin composites exhibit more volumetric shrinkage than traditional composites because they have less filler load and relatively increased resin content.[46] Results of other in vitro studies reported increased microleakage with the use of flowable liners.[8,47] As regards to the use of glass-ionomer liner under Class-II composite resin restorations, Aboushala *et al* [48] have reported no significant reduction in microleakage unless the liner was carried out to the cavosurface margin. However, no such attempt was made which could be an explanation for the results obtained.

As with the present study, all laboratory research protocols possess their own limitations. Although the thermal stresses of the oral environment were simulated to a certain extent, not all variables such as mechanical cycling, enamel rod orientation, pH of the oral cavity, diet characteristics etc. could be reproduced.[17]

CONCLUSIONS

Within the limitations of the present study, following conclusions were drawn:

1. Occlusal and gingival microleakage does not differ with the type of tooth, adhesive and liner material.
2. While occlusal microleakage was almost similar in both types of teeth, a lesser extent of gingival seal was observed in primary teeth with the tested adhesives.
3. When no liner material is used, significantly less occlusal microleakage occurs in both primary and permanent teeth with the use of the tested etch-and-rinse adhesive.
4. When no liner material is used, the tested adhesives lead to more gingival microleakage in primary teeth.
5. With the tested adhesives, the use of a liner material does not help reduce the occlusal or gingival microleakage in primary and permanent teeth.

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