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The Effect of Occlusal Loading on the Microleakage of Class V Restorations

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Clinical Relevance

The occlusal stress generated in the cervical region during normal function and parafunction may increase microleakage or detoriate the margins of Class V restorations. This study suggests that self-etch adhesives achieve marginal sealing equal to total-etch adhesives under occlusal loading.

SUMMARY

Objective: This *in vitro* study evaluated the microleakage of Class V cavities restored with a resin composite and different adhesive systems after occlusal loading.

Methods & Materials: Standardized box-shaped Class V cavities were prepared on the buccal side of 135 non-carious human premolars. The teeth were randomly divided into three groups of 45 premolars each and restored as follows: Group 1–two-step total-etch adhesive (Single Bond, 3M) + resin composite (Supreme, 3M ESPE); Group 2–two-step self-etch adhesive (Clearfil SE, Kuraray) + resin composite and Group 3-one-step self-etch adhesive (Xeno III, Dentsply) + resin composite. The restorations were finished with aluminum oxide discs (Sof-Lex, 3M). Fifteen teeth in each group received 10,000 x 100 N and 250 N occlusal loads, respectively, and the remaining 15 teeth served as the control. The premolars were immersed in 2% methylene blue for 24 hours. The dye penetration was examined under a stereomicroscope, and the results were statistically analyzed by Kruskal Wallis, Mann-Whitney U and Wilcoxon Signed Rank tests to determine differences between the groups.

Results: Gingival margins showed more dye penetration than occlusal margins in all the tested groups ($p<0.05$). In all the tested adhesive sys-

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tems, 100 N occlusal loading did not change dye penetration; however, Groups 1 and 2 exhibited better marginal sealing than Group 3 at the enamel margins under 250 N occlusal loading.

Conclusion: Within the limitations of this *in vitro* study, it may be concluded that enamel margins provided better marginal sealing than dentin/cementum margins and the two-step self-etch adhesive exhibited better marginal sealing than an all-in-one adhesive at the enamel margins under 250 N occlusal loading.

INTRODUCTION

At the gingival margins, Class V cavities are extended to enamel towards the occlusal and dentin. Dentin and enamel are different bonding substrates. Dentin includes fewer minerals but more organic and water content than enamel. Because of this, the bonding of resins to dentin is far more difficult and less predictable than bonding to enamel. A cohesive bond to dentin is achieved by diffusion of hydrophilic resins into and around the collagen fibers of etched intertubular dentin. Complete penetration into the entire depth of the demineralized zone is necessary to prevent bacterial microleakage and recurrent caries.[1]

Dentin adhesives and resin-based composites have been widely used because of the increasing demand for esthetic restorations in daily clinical dentistry. Dentin adhesives employ two different means to achieve micromechanical retention between resin and dentin. The first method, the total etch or etch and rinse technique, attempts to remove the smear layer completely with acid etching and rinsing. The second approach, the self-etch technique, incorporates the smear layer as a bonding substrate.[2]

Contemporary self-etch adhesives have been developed by replacing the separate acid conditioning step with increased concentrations of acidic resin monomers. Two-step self-etch primers combine etching and priming into a single step. Recently introduced one-step two-component self-etch adhesives contain two liquids, both of which are applied to both substrates after mixing.[3-4]

Teeth are subjected to heavy occlusal stresses during normal function and parafunction. It has been suggested that occlusal loads cause the tooth to flex. As the tooth flexes, tensile and shear stresses are generated in the cervical region of the tooth.[5] This stress, which is generated in the cervical region, may increase microleakage or deteoriate the margins of Class V restorations.

A clinical trial is the most effective method for assessing the quality of restorations.[6] However, it takes too long to obtain long-term clinical data, and the products are often superseded during that time.[6] Thermocycling and occlusal loading have been used in *in vitro* studies to simulate the "aging" effects that restorative materials are subjected to in the mouth. Thermal cycling has been used in order to simulate changing intraoral temperature conditions, while occlusal loading or mechanical cycling simulates mastication.[7-8]

This *in vitro* study evaluated the effect of occlusal loading under 100 N and 250 N on the microleakage of Class V cavities restored with different adhesive systems and a resin composite.

METHODS AND MATERIALS

A total of 135 extracted human premolars, without decay, cracks or previous restorations, and which were scaled and cleaned with slurry of pumice flour, were used in this study. Standard Class V cavities (3 mm wide, 3 mm high, 1.5 mm in depth) were prepared with a high speed handpiece at the cemento-enamel junction on the buccal surfaces of premolars. The occlusal margins were cut in enamel and the cervical margins in

Table 1: *Materials Used*

Groups	Type	Adhesive System
Single Bond (3M, St Paul, MN, USA)	Two-step total-etch	Acid: 37% phosphoric acid Adeziv:Bis-GMA; HEMA; PPA; CQ; ethanol; water
Clearfil SE Bond (Kuraray Co Ltd, Osaka, Japan)	Two-step self-etch	Primer: 10-MDP; HEMA; Hydrophilic dimethacrylate; N, N-diethanol p-toluidine; water Bonding: 10-MDP; Bis-GMA; HEMA; hydrophobic methacrylate; CQ; N, N-diethanol p-toluidine; silanated colloidal silica
Xeno III (Dentsply, Kontstanz, Germany)	One-step self-etch	Liquid A: HEMA; Purified water; Ethanol; 2, 6-Di-tert-butyl hydroxy toluene (BHT); Nanofiller Liquid B: Tetramethacryloxyethyl pyrophosphate (Pyro-EMA); Pentamethacryloxyethyl cyclophosphazen mono fluoride (PEM-F); Urethane dimethacrylate (UDMA); 2, 6-Di-tert-butyl-p-hydroxytoluene (BHT); Camphorquinone; p-Dimethylamino ethyl benzoate (EPD)

cementum. All the prepared teeth were randomly divided into three equal groups of 45 teeth, each differing by the adhesive system used: Single Bond (3M ESPE Dental Products, St Paul, MN, USA), Clearfil SE Bond (Kuraray Co Ltd, Osaka, Japan) and Xeno III (Dentsply, Kontstanz, Germany). All the materials were applied according to the manufacturers' instructions (Table 1). After dentin treatment, all the cavities were restored with a nanofill composite (Filtek Supreme, 3M ESPE) and polymerized for 40 seconds using an Elipar Frelight II (Dentsply) light curing unit. The restorations were finished with aluminum oxide discs (SofLex, 3M ESPE) and the teeth were thermocycled for 500 cycles between 5°C and 55°C.

All surfaces, except for the restorations and 1 mm from the margins were coated with two layers of nail varnish. The teeth were embedded in cylindric aluminum molds in acrylic resin up to 2 mm apical to the cervical wall of the restoration. Then, the groups were subdivided into three subgroups. Fifteen teeth in the first subgroup were used as the control (no occlusal loading), the 15 teeth in each of the other subgroups were loaded with 100 N and 250 N with a mechanical loading device (Figure 1), respectively. The adjustable parameters were duration (500 milliseconds), relaxation time (190-200 millisecond) and number of cycles (10,000), tip of the diameter (4 mm) and cycles/seconds (1.73/seconds).

After occlusal loading, all of the teeth were immersed in a 2% methylene blue dye solution for 24 hours. The teeth were then rinsed under tap water and dried. They were sectioned faciolingually using a low-speed saw (Mecatome T201A, Presi, Grenoble, France). Sections were assessed for dye penetration with a stereo-microscope (Nikon Eclips E600, Tokyo, Japan) at 20x magnification. Two investigators blindly scored all interfaces and the mean score was recorded. Dye penetration at the composite/tooth interface was scored for both the occlusal and cervical margins on a nonparametric scale from 0 to 3 (Table 2): 0=no dye penetration; 1=dye penetration of less than half of the axial wall; 2=dye penetration of more than half of the axial wall; 3=dye penetration spreading along the axial wall.

Statistical analyses were performed using the Kruskal-Wallis test. Post-hoc comparisons were done with the Mann-Whitney U test to determine differences between the groups. Comparison of the enamel and cementum margins of the groups was performed using the Wilcoxon Signed Rank test.

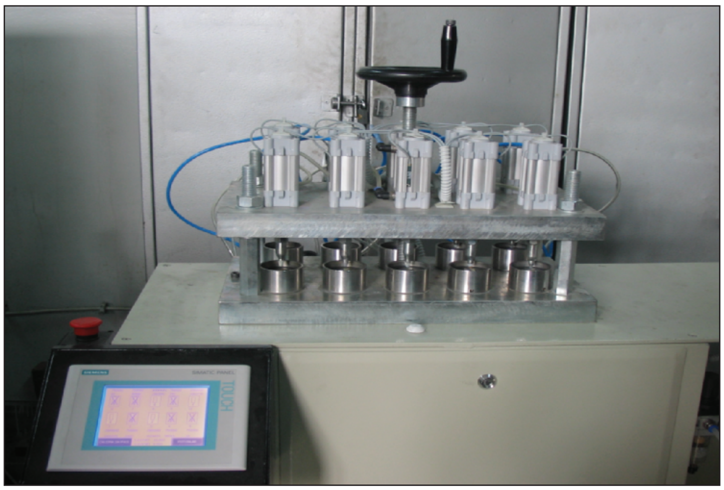

Figure 1. *The mechanical loading device.*

Table 2: *Criteria for Dye Penetration Scores*

Score	Degree of Dye Penetration
0	No dye penetration
1	Dye penetration less than half the axial wall
2	Dye penetration more than half the axial wall
3	Dye penetration spreading along the axial wall

Table 3: *Microleakage Scores Obtained From the Non-loaded Specimens*

	Enamel Margins				Cementum Margins			
Adhesive Systems	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Single Bond	8	6	1	0	3	7	2	3
Clearfil SE Bond	10	5	0	0	9	0	1	5
Xeno III	5	10	0	0	6	5	1	3

Table 4: *Microleakage Scores Obtained From 100 N Occlusally Loaded Specimens*

	Enamel Margins				Cementum Margins			
Adhesive Systems	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Single Bond	8	7	0	0	4	8	0	3
Clearfil SE Bond	6	8	1	0	3	5	1	6
Xeno III	4	11	0	0	5	5	1	4

RESULTS

The microleakage scores obtained from the control, 100 N and 250 N occlusal loading groups are given in Tables 3, 4 and 5, respectively. Representative microleakage photogaphs (20x) of groups are presented in Figures 2 through 4.

The cementum margins showed more dye penetration than enamel margins in all the tested groups ($p<0.05$). When the adhesive systems were compared, in the control and 100 N occlusal loading groups, the microleakage scores had no statistically significant difference at both the

Table 5: *Microleakage Scores Obtained From 250 N Occlusally Loaded Specimens*

	Enamel Margins				Cementum Margins			
Adhesive Systems	**0**	**1**	**2**	**3**	**0**	**1**	**2**	**3**
Single Bond	5	10	0	0	1	6	4	4
Clearfil SE Bond	10	4	0	1	5	3	0	7
Xeno III	1	12	2	0	0	7	4	4

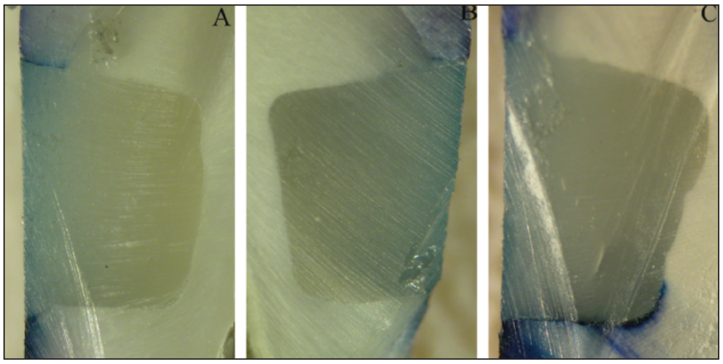

Figure 2. *Representative microleakage photographs of non-occlusally loaded Single Bond (A), Clearfil SE Bond (B) and Xeno III (C) groups.*

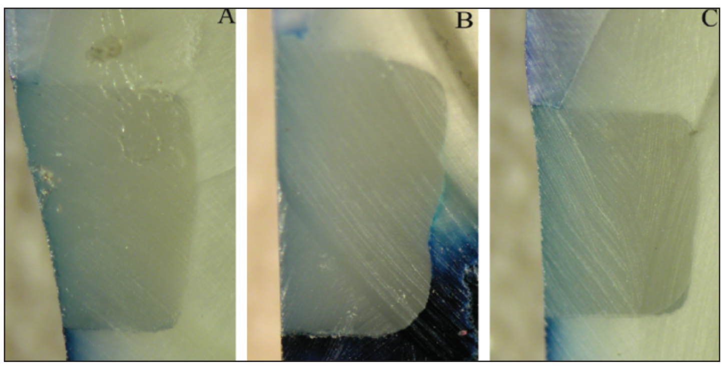

Figure 3. *Representative microleakage photographs of 100 N occlusally loaded Single Bond (A), Clearfil SE Bond (B) and Xeno III (C) groups.*

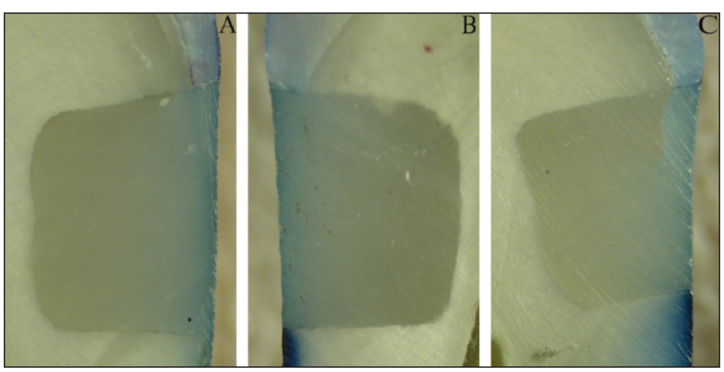

Figure 4. *Representative microleakage photographs of 250 N occlusally loaded Single Bond (A), Clearfil SE Bond (B) and Xeno III (C) groups.*

enamel and cementum margins ($p>0.05$). In the occlusally loaded groups with 250 N, there were no statistically significant differences between the cementum margins ($p>0.05$), but there were statistically significant differences between the enamel margins of Group 1 (Single Bond) and Group 3 (Xeno III) ($p=0.032$) and Group 2 (Clearfil SE Bond) and Group 3 (Xeno III) ($p=0.001$) (Table 6). When the adhesive system compared to itself, no statistically significant difference was observed between the control and the 100 N and 250 N loading groups both in the enamel and cementum margins with mechanical loading ($p>0.05$) (Table 7).

DISCUSSION

Clinical trials remain the gold standard for evaluating the performance of dental materials, but they must also take into consideration that the products under investigation may become obsolete by the time useful clinical data are collected. Thus, preclinical screening via laboratory tests is still an important tool for the evaluation of dentin adhesives.[9] Clinicians and researchers use microleakage as a measure for assessing the performance of restorative materials in the oral environment. Different techniques are used for microleakage evaluation, but the most frequently employed method is the migration of dye along the tooth/restoration interface.[10] Dye penetration measured on sections of restored teeth is the most common technique for evaluating microleakage at the tooth restoration interface.[11-12] Although this method is simple, economic and fast, the subjectivity of reading the specimens has been noted as being a shortcoming related to this methodology.[12] In this study, the mean score of the two investigators was recorded and an *in vitro* model was chosen to:

1. standardize the model.
2. obtain "ideal" adhesion conditions.
3. allow thermocycling, simulating stress caused by thermal variations.[13]
4. allow mechanical cycling, simulating stress caused by occlusal loads.

In the current study, higher leakage was detected in dentin when compared to enamel. This finding is in agreement with some authors, who used different combinations of dentin bonding agents and resin-based composites in both Class II and Class V restorations[1,14-17] but contradicted others.[16,18-21] These differences necessitate careful inspection of the testing methodologies.

The higher leakage scores detected in dentin compared to enamel can be related to the composition of these two tissues. Bonding to enamel is a relatively simple process without major technical requirements or difficulties. On the other hand, bonding to dentin

Table 6: *Kruskal-Wallis Test Results of the Comparison of the Adhesive System*

	Loading	Adhesive Systems	n	Mean Rank	Median	Chi-square	Asymp Sig
Enamel Margins	Control	Single Bond	15	23.00	0	3.056	0.217
		Clearfil SE Bond	15	19.33	0		
		Xeno III	15	26.67	1		
	100 N	Single Bond	15	19.77	0	2.076	0.354
		Clearfil SE Bond	15	23.60	1		
		Xeno III	15	25.63	1		
	250 N	Single Bond	15	22.83	1	1.994	0.002
		Clearfil SE Bond	15	15.67	1		
		Xeno III	15	30.50	1		
Cementum Margins	Control	Single Bond	15	25.67	1	1.070	0.586
		Clearfil SE Bond	15	21.20	0		
		Xeno III	15	22.13	1		
	100 N	Single Bond	15	21.00	1	1.498	0.473
		Clearfil SE Bond	15	26.17	1		
		Xeno III	15	21.83	1		
	250 N	Single Bond	15	23.23	2	0.232	0.890
		Clearfil SE Bond	15	21.80	1		
		Xeno III	15	23.97	2		

Table 7: *Kruskal-Wallis Test Results of the Comparison of the Occlusal Loading*

	Adhesive Systems	Loading	n	Mean Rank	Median	Chi-square	Asymp Sig
Enamel Margins	Single Bond	Control	15	22.07	0	1.248	0.536
		100 N	15	21.27	0		
		250 N	15	25.67	1		
	Clearfil SE Bond	Control	15	20.83	0	3.294	0.193
		100 N	15	27.33	1		
		250 N	15	20.83	1		
	Xeno III	Control	15	19.83	1	5.415	0.067
		100 N	15	21.27	1		
		250 N	15	27.90	1		
Cementum Margins	Single Bond	Control	15	22.20	1	3.205	0.201
		100 N	15	19.43	1		
		250 N	15	27.37	2		
	Clearfil SE Bond	Control	15	19.33	0	2.050	0.359
		100 N	15	25.33	1		
		250 N	15	24.33	1		
	Xeno III	Control	15	19.17	1	4.401	0.111
		100 N	15	21.43	1		
		250 N	15	28.40	2		

presents a much greater challenge. Several factors account for these differences between enamel and dentin bonding; whereas, enamel is a highly mineralized tissue composed of more than 90% (by volume) hydroxyapatite. Dentin contains a substantial proportion of water and organic materials; it presents a moist surface that impairs the bonding mechanism.[22]

Despite the continuing evaluation of adhesive systems, no currently available adhesive technique has been able to produce predictable results when the preparation margins are located in dentin.[23-25] Contraction stresses generated during placement of a composite restoration contribute significantly to early marginal leakage, especially in dentin.[14] The lower bond strength obtained in dentin is not strong enough to counteract the stress developed during polymerization shrinkage, which impairs sealing capacity.[26] The conventional Class V cavity employed in this study represents a great challenge to the adhesive systems used, due to the high C-factor.[23, 27-28]

In this study, three different adhesive systems (total-etch, two-step self-etch and one-step self-etch) were used, because these adhesives create a hybrid layer with different mechanisms via treatment of the smear layer produced during cavity preparation.[29]

Although most self-etch adhesives bonded well to cut enamel prior to functional and thermal stresses, they were significantly less effective after fatigue testing. It is known from earlier reports that the micromorphological interaction of etch and rinse adhesives extends deeper into enamel. On the other hand, it is also known that self-etching systems provide a network of intercrystaline retention, leading to a large bonding surface.[30-32] In this study, when no occlusal loading or 100 N occlusal loading was applied, there was no statistically significant difference between the etch & rinse system and the self-etching systems, either two-step or one-step self-etch adhesives. But when the restorations were occlusally loaded with 250 N, there were statistically significant differences in the enamel margins of the total-etch and one-step self-etch adhesives. This finding is in agreement with Frankenberger and Tay,[33] who also said that there were significant differences between the ability of the two-step self-etch and one-step self-etch adhesive to withstand stresses generated via fatigue testing. The difference between two-step and one-step self-etching systems should not be related to the different ways that they interact with enamel. The difference is probably attributed to the fact that one-step self-etch adhesives are more susceptible to water absorption. In the absence of a coupling hydrophobic bonding agent, one-step self-etch adhesives behave as permeable membranes after polymerization.[34-35] This may expedite dye penetration between partially demineralized enamel and restorative material, plasticizing and eventually weakening the bonded enamel interfaces.

In all the materials tested, there was no significant difference between mechanical loading, even with 100 or 250 N. The reason for this finding could be the loading direction. In the current study, an axial load of 100 and 250 N was applied to the specimens. It has been suggested that occlusal loads cause the tooth to flex, particularly during lateral excursions. As the tooth flexes, tensile and shear stresses are generated in the cervical region of the tooth.[30] Rees[37] concluded that varying the position of the occlusal load produced marked variations in the stresses found in cervical enamel. Loads that mimic the loading produced during lateral excursions of the mandible produced higher stresses.

The performance of an adhesive system is more dependent upon the operator or application protocol than on its chemical origin or classified generation.[38-46] Self-etching systems are generally considered less technique-sensitive when compared to systems that utilize separate acid conditioning and rinsing steps. They seem to eliminate factors, such as overetching, overdrying and overwetting.[2] Giachetti and others[43] reported on detailed total-etch adhesives, especially those sensitive to operator skill and those that demonstrate efficacy only when used by the expert operator. On the other hand, self-etch adhesive systems showed little sensitivity to operator skill. Giachetti and others[43] concluded that self-etch two-step bonding adhesive was less technique-sensitive in obtaining a reliable seal with dentin. According to their study, total-etch and self-etch adhesives showed similar microleakage results both at the enamel and dentin margins, even after mechanical loading.

CONCLUSIONS

Within the limitations of this *in vitro* study, it may be concluded that:

- all of the the tested adhesive systems provided better sealing at enamel margins than at cementum margins.
- 100 N occlusal loading did not change dye penetration in any of the adhesive systems tested (marginal integrity).
- when 250 N occlusal loading was applied, the two-step self-etch adhesive exhibited better marginal quality than the all-in-one adhesive at the enamel margins.

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