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Effect of Single Step Adhesives on the Marginal Permeability of Class V Resin Composites

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Clinical Relevance

According to this study, Clearfil S³ bond showed less restoration leakage compared to the other self-etch adhesives at both enamel and dentin margins.

SUMMARY

This *in vitro* study evaluated the coronal and apical marginal microleakage of four self-etch, seventh generation adhesive systems.

Sixty non-carious human molars were randomly assigned to four groups (n=12). Class V cavities were prepared on the facial or lingual surface of each tooth with coronal margins in enamel and apical margins in cementum (dentin) at the cementoenamel junction. The preparations were restored using four self-etch adhesive systems (*i*Bond, G-Bond, Xeno IV and Clearfil S³ Bond) and a control (no adhesive), followed by insertion of Gradia Direct microfilled hybrid resin composite. The teeth (specimens) were thermocycled 1,000 cycles, immersed in 1% methylene blue dye for 24 hours and invested in acrylic resin. The specimen blocks were then sectioned longitudinally, with dye penetration (microleakage) examined using a 20x binocular microscope. Coronal and apical margins were scored separately for microleakage using a 0-3 ordinal ranking system. Data were analyzed using non-parametric tests at a $p<0.05$ level of significance. A comparison of adhesive and control groups at the coronal and apical margins revealed that significant ($p<.0001$) differences were exhibited. At the coronal margin, Xeno IV revealed significantly less leakage than the other adhesives, with Clearfil S³ Bond exhibiting significantly less leakage at the apical margin. Groups with no adhesive treatment (control) showed significantly greater leakage at both the coronal and apical margin locations. A Wilcoxon signed rank test showed no significant differences at the coronal compared to the apical margins of the four adhesive systems and control tested.

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INTRODUCTION

The evolution of dental bonding agents has progressed rapidly, from adhesion of restorative materials to tooth structure that utilize multiple-step procedures, to the development of improved and "easier to use" single component systems. Currently, seventh generation self-etch systems combine an etchant, primer and adhesive in one container compared to total-etch or etch and rinse systems, whereby separate etchant, primer and adhesive monomers are utilized. Self-etch systems not requiring separate etch and rinse procedures were primarily developed to promote increased dentin substrate adhesion, enhancing marginal integrity, with a reduction and/or elimination of post-treatment sensitivity.[1]

Total-etch systems utilizing 30-40% phosphoric acid are efficient in the removal of smear layer components, causing demineralization of the inorganic enamel surface, which is composed primarily of hydroxyapitite crystals, thus creating microporosities for a micro-mechanical bond.[2-4] Etching dentin by utilizing etch and rinse procedures completely demineralizes the dentin substrate (intertubular and peritubular dentin), while opening the dentinal tubules. With this process, over-conditioning of the organic (collagen) and inorganic (hydroxyapatite) components can occur, causing a collapse and shrinking of the collagenous fibular network due to loss of structural, inorganic support. Following removal of the dentinal hydroxyapatite, incomplete diffusion and penetration of separate adhesive and primer components can occur, producing a resin deficient zone. As a result, exposed collagen fibrils and lack of support by partially infiltrated resin monomers result in a significant reduction in material-tooth structure adhesion.[1,2-4] Material application complexity and operator error (failure to strictly follow manufacturer's instructions) are significant concerns associated with all dental adhesive systems. The total-etch technique, requiring separate etching and rinsing procedures, is still the most effective approach for achieving a strong mechanical bond to the mostly inorganic enamel tooth structure.[2]

Table 1: *Materials Used in This Study*

Material	Manufacturer	Composition*
Xeno IV	Dentsply/Caulk Milford, DE, USA	PENTA, UDMA, methacrylates acetone, water
G-Bond	GC America Alsip, IL, USA	4-META, UDMA, TEGDMA acetone, water
*i*Bond	Heraeus Kulzer Armonk, NY, USA	4-META acetone, glutaral
Clearfil S[3] Bond	Kuraray America, Inc New York, NY, USA	MDP, Bis-GMA, HEMA alcohol, water
Gradia Direct (A3.5)	GC America Alsip, IL, USA	UDMA, Dimethacrylates Fluoro Alumino-silicate glass silica powder, organic filler

**Manufacturer's information*

Bis-GMA: Bis-phenol A diglycidylmethacrylate
4-META: 4-Methacryloxyethyltrimellitate
HEMA: 2-Hydroxyethyl methacrylate
MDP: 10-Methacryloyloxydecyl dihydrogen phosphate
PENTA: Dipentaerythritol pentaacrylate phosphate
TEGDMA: Triethyleneglycol dimethacrylate
UDMA: Urethane dimethacrylate

Self-etch systems composed of aqueous mixtures of phosphoric acid esters and resin monomers partially dissolve the hydroxyapatite constituent of dentin, thus incorporating the smear layer into the demineralized substrate while simultaneously infiltrating the collagen complex with hydrophilic primers and resin monomers.[1-7] Primary clinical advantages associated with self-etch adhesives include the elimination of mixing separate components (decreased manipulation errors) with a reported reduction and/or elimination of post-treatment sensitivity.[1,7]

This *in vitro* study evaluated the microleakage of four single-step, self-etch adhesive systems at the coronal (enamel) and apical (dentin) margins of Class V resin composite restorations.

METHODS AND MATERIALS

Sixty previously extracted non-carious human molars were carefully cleaned of calculus, soft tissue and other debris using a dental curette. The teeth were stored in a 1% chloramine-T (Fisher Chemical, Fair Lawn, NJ, USA) solution consisting of 12% active chlorine diluted in distilled water prior to usage.

Cavity Design

Circular-shaped Class V cavity preparations were cut on the facial or lingual surface at the cementoenamel junction, with coronal margins located in enamel and apical margins located in dentin. The preparations were cut with a #56 carbide bur in a high-speed handpiece cooled with an air-water spray. A 45° bevel was placed on the enamel margin (0.5 mm width) using a #257 diamond bur. Each carbide bur was discarded following preparation of each group of teeth. Preparation dimensions of 3.0 mm x 3.0 mm x 1.5 mm (depth) were measured with a periodontal probe to maintain uniformity.

Restoration Groups

The teeth were randomly assigned to five groups (n=12). All materials were used following manufacturer's instructions (Table 1).

Group 1: Xeno IV

Using a microbrush applicator, Xeno IV was applied to the tooth (enamel and dentin) surfaces and vigorously scrubbed onto the surfaces (15 seconds) for two applications. Excess solvent was removed by gently drying the surfaces with syringe air for at least five seconds. Xeno IV was light polymerized for 10 seconds followed by insertion (1 increment) of the composite restorative (Gradia Direct, shade A3.5).

Group 2: G-Bond

Using a microbrush applicator, G-Bond was applied to the enamel and dentin surfaces, left undisturbed for 10 seconds and dried thoroughly with an air syringe using maximum pressure. G-bond was light polymerized for 10 seconds followed by insertion of Gradia Direct composite.

Group 3: i*Bond*

Using a microbrush applicator, *i*Bond was applied to the cavity preparation starting with the enamel, then the dentin surfaces. The material was left undisturbed for 30 seconds, followed by gentle air drying from an air syringe until a glossy surface was apparent. *i*Bond was light polymerized for 20 seconds, followed by insertion of Gradia Direct composite.

Group 4: Clearfil S³ Bond

Using a microbrush applicator, Clearfil S³ Bond was applied to the enamel and dentin surfaces for 20 seconds, then dried thoroughly with an air syringe using maximum pressure for 5-10 seconds. Clearfil S³ Bond was light polymerized for 10 seconds, followed by insertion of Gradia Direct composite.

Group 5: Control

Prior to insertion of Gradia Direct composite into each cavity preparation, no adhesive agent was applied to the enamel and/or dentin surfaces.

All restorative materials were polymerized with a Schein (Sullivan-Schein, Melville, NY, USA) conventional halogen light. The light had previously been monitored with a radiometer and provided adequate intensity (≥800mW/cm²). The composites were polished with Sof-Lex (3M-ESPE, St Paul, MN, USA) flexible, aluminum oxide disks of decreasing abrasiveness (course to superfine). The teeth were stored in distilled water at room temperature for 7 days prior to leakage assessment.

Assessment of Microleakage

The teeth were thermocycled for 1,000 cycles in separate water baths of 5°C and 55°C, with a dwell time of 60 seconds in each bath and a transfer time of three seconds. The root apices were sealed with utility wax, and the entire tooth surface was coated with two layers of commercial nail varnish to within 1.0 mm of the restoration. The teeth were immersed in a 1% aqueous solution of methylene blue dye for 24 hours at room temperature, thoroughly rinsed to remove excess dye, then invested in clear autopolymerizing resin (Castin' Craft Clear Plastic Casting Resin, ETI, Fields Landing, CA, USA) and labeled. A Buehler Isomet low-speed diamond saw (Buehler Ltd, Evanston, IL, USA) cooled with water, sectioned each tooth block longitudinally through the center of the restoration from the facial to the lingual surface. Two sections were obtained from each block (24 sections per group), yielding dye penetration (microleakage) readings examined at 20x magnification under a Meiji (Meiji-Labax Co, Tokyo, Japan) binocular microscope. The degree of leakage was determined based on an ordinal ranking system (0-3) as follows: 0 degree–no leakage; 1 degree–leakage up to one-half the length of the cavity wall; 2 degree–leakage along the full length of the cavity wall, not including the axial surface; 3 degree–leakage along the full length of the cavity wall, including the axial surface.

Statistical Analysis

The results of dye penetration (leakage) were scored separately for coronal and apical margins for statistical analysis using the Kruskal-Wallis and, if applicable, the Mann-Whitney U non-parametric multiple comparison tests. A Wilcoxon signed rank test was used to compare leakage at the coronal and apical margins of the restoration groups. All data were submitted for statistical analysis at the $p<0.05$ level of significance. The statistical calculations were performed using Statview 5.0 (SAS Institute, Cary, NC, USA).

RESULTS

Table 2 lists the distribution of microleakage scores at the coronal and apical margin locations. The Kruskal-Wallis test revealed a significant ($p<.0001$) difference between the adhesive and control groups at both the coronal and apical margins. Multiple comparison of the five test groups by Mann-Whitney statistically showed three groupings at the coronal margin (Table 3): 1) the restored teeth treated with Xeno IV exhibited significantly less leakage than the other adhesive and control groups, 2) the control group (no adhesive treatment) showed significantly greater leakage than the adhesive groups and 3) the remaining groups (adhesive) clustered together, experiencing intermediate leakage. Results at the apical margin revealed: 1) the restored teeth treated with Clearfil S³ exhibited significantly less leakage than the other groups, 2) The control group, again, showed significantly greater leakage than the adhesive groups and 3) the remaining groups clustered together, experiencing intermediate leakage. A Wilcoxon signed rank test comparing all 120 coronal versus apical surfaces, pair-wise, confirmed no significant ($p=.0759$) differences were exhibited.

Table 2: *Distribution of Microleakage Scores at the Coronal and Apical Margins (n=24)*

Coronal Margin						
Group	**Score**				**Mean**	**Std Dev**
	0	**1**	**2**	**3**		
Xeno IV	9	8	4	3	1.042	1.042
G-Bond	0	9	6	9	2.000	0.885
*i*Bond	1	5	10	8	2.042	0.859
Clearfil S³ Bond	1	12	8	3	1.542	0.779
Control	0	0	0	24	3.000	0.000
Apical Margin						
Group	**Score**				**Mean**	**Std Dev**
	0	**1**	**2**	**3**		
Xeno IV	2	1	1	20	2.625	0.924
G-Bond	1	5	4	14	2.292	0.955
*i*Bond	6	4	1	13	1.875	1.329
Clearfil S³ Bond	13	3	2	6	1.042	1.301
Control	0	0	0	24	3.000	0.000

Table 3: *Statistical Comparison of Microleakage at Coronal and Apical Margins (n=24)*

Coronal Margin		
Group	**Sum Ranks**	**Mean Rank**
Xeno IV[a]	811.000	33.792
G-Bond[cd]	1486.500	61.938
*i*Bond[d]	1519.500	63.313
Clearfil S3[bc]	1115.000	46.458
Control[e]	2328.000	97.000

Using Mann-Whitney multiple comparison test, different letters indicate statistically significant differences

Apical Margin		
Group	**Sum Ranks**	**Mean Rank**
Xeno IV[cd]	1731.500	72.146
G-Bond[c]	1462.500	60.938
*i*Bond[bc]	1290.500	53.771
Clearfil S3[a]	807.500	33.646
Control[e]	1968.000	82.000

Using Mann-Whitney multiple comparison test, different letters indicate statistically significant differences

DISCUSSION

This study evaluated the microleakage of four single component seventh generation self-etch adhesives, all of which demonstrated dye penetration (leakage) at both the coronal and apical margins. The control in this study was a "no adhesive" group containing no adhesive system application. This protocol was considered an appropriate step in that a previous study by Owens, Johnson and Harris[8] tested microleakage using separate self-etch (seventh generation) and total-etch systems. Not wishing to duplicate protocol of that study, the use of a "no adhesive" was therefore adopted as a "negative control."

The restorations treated with the Xeno IV and Clearfil S^3 Bond adhesive systems revealed significantly reduced leakage at the coronal margins compared to the other adhesives, with Clearfil S^3 Bond and *i*Bond adhesives showing significantly less leakage at the apical margins. Also, at the apical margin, Xeno IV showed significantly greater leakage than the other groups, except the control. Overall, Clearfil S^3 Bond revealed superior (not necessarily always significant) results at both margin locations. Explanations for these results include the type of solvent used in the adhesive systems. Instead of acetone, Clearfil S^3 Bond utilizes alcohol as the primer component solvent or "drying agent" for dentin surface conditioning prior to adhesive component attachment. Acetone was the primary solvent in the other adhesive systems. During the bonding process, water remains in the interfibrillar spaces and surrounds the collagen fibrils. In order to achieve optimum attachment of the adhesive monomers, excess water must be removed through either water or water-miscible primer solvents (alcohol or acetone). Alcohol and/or acetone primer solvents dehydrate the water-filled spaces and dry collagen fibrils, chemically producing a higher monomer to water ratio, stiffening the collagen complex, which is conducive to resin monomer attachment. Acetone based solvents promote total dehydration of the collagen components in a very short time, creating conditions that may not be ideal for optimum dentin surface bonding.[9] The Clearfil S^3 Bond formulation includes a proprietary "Molecular Dispersion Technology," enabling a two-phase liquid, hydrophilic/hydrophobic component homogenous state at the molecular level, reportedly resulting in reduction and/or loss of water droplets at the adhesive interface and therein a superior bond.[10] Also, the 10-Methacryloyloxydecyl dihydrogen phosphate (MDP) adhesive monomer molecular structure allows for decalcification and penetration into tooth structure, "creating a chemical bond to calcium." This molecular formulation is purportedly beneficial when bonding to enamel surface substructure; whereby, MDP chemically bonds to hydroxyl apatite, as opposed to a micro-mechanical bond created using total-etch systems (phosphoric acid).[10] In this study, although Clearfil S^3 Bond's performance was good overall, the previously stated claims were not completely justified.

In an *in vitro* microleakage study by Kallenos and others,[9] which compared fifth, sixth and seventh generation adhesives, total-etch was revealed to perform better than self-etch adhesives in Class I composite restorations. According to DeMunck and others,[3] short-term *in vitro* bonding to dentin was relatively ineffective and, "after three months, all classes of adhesives exhibited mechanical and morphological evidence of degradation that resembles *in vivo* aging effects" and "a comparison of contemporary adhesives revealed that the three-step etch-and-rinse adhesives remained the gold standard in terms of durability and only the two-step self-etch adhesives approaching this standard." Limited information is available regarding the *in vivo* clinical durability of recently developed one-step self-etch adhesives. Clinical research conducted by Brackett and others[11] concluded that single component self-etch adhesives performed poorly, with a retention rate of only 50% to 55% after 18 months. In an intra-individual patient-based clinical comparison study, the results revealed that self-etch adhesives showed a faster progressive marginal degradation in non-carious cervical lesions.[12] Also, in a systematic review of current clinical trials involving dental adhesive systems, the results showed that "three-step etch-and-rinse adhesives and two-step self-etch adhesives showed a clinically reliable and predictably good clinical performance and the clinical effectiveness of two-step etch-and rinse adhesives was less favorable, while an inefficient clinical performance was noted for the one-step self-etch adhesives."[13]

Reported advantages of self-etch systems include "simple" application procedures and the reduction and/or elimination of post-treatment sensitivity; however, because of numerous uncontrolled variables encountered during patient treatment, perceived advantages can potentially become disadvantages.[1,14] Although *i*Bond adhesive demonstrated substantially (but not necessarily always significant) lower leakage values than some of the self-etch systems, technique protocols (drying procedures) associated with *i*Bond did prove to be labor intensive and may not be realistic for some practitioners in a clinical setting. The protocols associated with individual adhesive systems often require multiple applications and lengthy waiting periods prior to light polymerization. Although single-step adhesives were developed to reduce the number of steps and, in turn, decrease operator time and error associated with the bonding of resin composite to tooth structure, these systems appear to be somewhat perpetuating past difficulties of prior generation adhesive systems and, according to Peumans and others,[13] "although there is a tendency towards adhesives with simplified application procedures, simplification so far appears to induce loss of effectiveness."

The primary objective of a dental restoration is to create a "perfect" seal, preventing leakage of contaminants contained in the oral environment. Technological advances in materials and techniques have been developed in adhesive dentistry; however, long-term microleakage occurs with all restorations.[2] Microleakage has been defined as the "clinically undetectable" passage of bacteria, fluids, molecules or ions between a cavity wall and the restorative material applied to it and can result from deterioration of the tooth-restoration interface, differences between thermal expansion coefficients of material-tooth tissue or polymerization shrinkage, causing staining, micro-gap formation, recurrent caries and possible pulpal involvement and restoration replacement.[15-18]

Bonding to "dynamic," "living" tooth structure (dentin) can be complicated. Complications include multiple inherent variables: bio-chemical, clinical and methodologic. Several factors associated with self-etch adhesives, directly relating to long-term bonding effectiveness, can cause inadequate hybrid layer formation and, consequently, microleakage at the tooth-restoration margins of composite restorations. These inadequacies can include incomplete alteration and/or removal of smear layer components due to composition (pH, osmolality) and strength of the acidic primer, and inadequate resin film thickness, requiring multiple layering techniques and changes in the monomer/water ratio, resulting in phase separations.[1-3,9,16,19-22] Morphological and histological considerations, and other clinical factors causing inadequate bonding at the material/tooth surface interface, include cavity configuration (C-factor) and dentinal tubule/enamel rod orientation, capillary movement of dentinal tubular fluids, physical characteristics of the restorative material (filler loading, volumetric expansion, modulus of elasticity and polymerization contraction), inadequate margin adaptation of the restorative material during insertion, inappropriate barrier protection (dental rubber dam), tooth location, occlusal stresses/tooth flexure and patient age considerations.[23-31] In this study, since hybrid layer morphology was not evaluated microscopically, the specific nature of restoration failure (microleakage) for each adhesive system is unknown, although several factors were strongly suspected: inefficiency of acidic monomers in alteration of the smear layer for classic hybrid layer formation, cavity C-factor, orientation of dentinal tubules/enamel rods to the cementoenamel junction, use of acetone-based solvent primer systems and post-treatment stresses caused by polymerization contraction.

Microleakage studies provide adequate screening methods, possibly determining clinical success and longevity of adhesive systems.[32-34] Although this study was conducted *in vitro*, which can be a screening apparatus for ensuing *in vivo* studies, previous research indicates that data obtained from *in vitro* microleakage testing may be useful, but not always necessarily reproducible in clinical *in vivo* settings.[35-37] Also, in performing *in vitro* microleakage investigations, obtaining conclusive information can be problematic, since vast differences in research protocols are reported in the dental literature.

The results from this study demonstrate that the "dynamic" nature of the dentin substrate morphology is indeed an important factor and possibly an insurmountable impediment for perfect adhesion of restorative materials to tooth structure. Clinical trials should be performed for carious and non-carious Class V cervical lesions to assess the performance of these new adhesive systems before definite conclusions can be formulated.

CONCLUSIONS

Within the limitations of this *in vitro* study, the following conclusions were drawn:

1) At the coronal margin, the preparations treated with Xeno IV showed significantly less leakage than the other groups.
2) At the apical margin, the preparations treated with Clearfil S³ Bond revealed significantly less leakage than the other groups.
3) At both the coronal and apical margin locations, the control group, comprising preparations without treatment using any adhesive system, showed significantly greater leakage than the adhesive groups.

4) Comparing all coronal versus apical surfaces, no significant differences were encountered.

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