Microleakage in Class II Composite Restorations Bonded with Self-Etch, One-Step, One-Component Adhesive System: A CLSM Study

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ORAL SURGERY

Microleakage in Class II Composite Restorations Bonded with Self-Etch, One-Step, One-Component Adhesive System: A CLSM Study

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ABSTRACT

Objectives: **The purpose of this study was to compare the effect of self-etch, one-step, one-component adhesive system to etch and rinse adhesive system on the microleakage of class II restorations located in dentin using confocal laser scanning microscope.**

Materials and Methods: **52 upper permanent premolar teeth were used and two class II cavities (3x1.5 mm) with gingival margins ended 1mm below CEJ were prepared and filled in each tooth. Two adhesive systems: self-etch, one-step, one-component adhesive system (G Bond, GC, Japan), etch and rinse adhesive system (Adper Single Bond 2, 3M ESPE, USA) and two composite materials: nanocomposite (Filtek Z350, 3M ESPE, USA), microhybrid composite (Filtek Z250, 3M ESPE, USA) were used and applied in this study according to the manufacturer's instructions. The 104 cavities were divided randomly into four groups (n = 26). Half of the groups were restored with Filtek Z350 (3M ESPE, USA) and the other half with Filtek Z250 (3M ESPE, USA). Furthermore, half of the groups were bonded with G Bond (GC, Japan) and the other half with Adper Single Bond 2 (3M ESPE, USA). The specimens were thermocycled between 5° to 55℃ with 30 second dwell time for 500 cycles. The samples were then immersed in 0.5% Rhodamine B dye for 10 hours and sectioned longitudinally. Dye penetration at the gingival margin was quantified in millimeters under confocal laser scanning microscopy (CLSM) at 10x magnification. Data were analyzed using Two-way ANOVA and results with $P < 0.05$ were considered statistically significant.**

Results: **No significant difference ($P > 0.05$) in dye penetration was discovered between self-etch, one-step, one-component adhesive system (G Bond) and etch and rinse adhesive system (Adper Single Bond 2).**

Conclusion: **Self-etch, one-step, one-component adhesive system shown efficacy in reducing class II gingival microleakage at least as good as those of etch and rinse adhesive systems.**

KEY WORDS

G bond, CLSM, class II, microleakage, rhodamine B, nanocomposite, self etch.

INTRODUCTION

Good adhesion between dentin and restorative resins is of primary importance in clinical practice. With regard to that, the search for improved adhesive and resin composite materials has received considerable interest in the recent years. Due to the improved properties of adhesive materials, resin-based composite restorations have been made more reliable and long-standing[1].

New approaches to bonding restorative materials to tooth substrates without phosphoric acid etching, such as self-etching systems, have recently been introduced. These simplified systems aim to reduce the sensitivity of techniques by reducing the number of clinical steps involved[2]. As a result of this, their popularity is increasing.

Unfortunately, the first evaluations of the sixth (6^{th}) generation system showed sufficient bond to conditioned dentin but, the bond to enamel was less effective[1]. This may be due to insufficient etching to enamel. In addition, they are available in two bottles by which one drop of liquid from each will be mixed together before application to the tooth structure. This can cause error to occur due to unequal ratio of liquid mixed or mishandling of the bottles. For this, some manufacturers introduced the 7^{th} generation one bottle adhesive systems to simplify the procedure even more.

One of the advantages of using single-step, self-etch, one-component adhesives is it can prevent discrepancies occurring between the depth of etching and resin monomer penetration. This is because the single-step, self-etch, one-component adhesive systems form a continuous layer by simultaneous demineralization with acidic monomers, followed by resin monomer penetration into the dentin substrate[2].

Although there have been reports regarding the performance of total-etch and self-etch (5^{th} and 6^{th} generations) adhesive systems, studies and reported data on the capability of the newly introduced self-etch, one-step, one-component adhesive system (7^{th} generation) in sealing the margins of restorations in class II cavities is very limited. Moreover,

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Table 1. Description of study groups

Groups	Composite	Adhesive	Sample size
1	Z250 (3M ESPE, USA)	Adper Single Bond 2 (3M ESPE, USA)	26
2	Filtek Z250 (3M ESPE, USA)	G-BOND (GC, Japan)	26
3	Filtek Z350 (3M ESPE,USA)	Adper Single Bond 2 (3M ESPE, USA)	26
4	Filtek Z350 (3M ESPE, USA)	G-BOND (GC, Japan)	26

26: total number of cavities per group sample

most of the previously conducted studies on self etch adhesives tested these materials on Class V preparations[3-5].

Araujo Fde *et al*. (2006)[6] and Fruits *et al*. (2006)[7] reported that major marginal microleakage occurs on the gingival surfaces located in dentin. Up to date, data regarding microleakage of self-etch, one-step, one-component adhesive (7th generation) compared to total etch adhesive systems in class II restorations is still limited. Therefore, it is important to determine the effect of self-etch, one-step, one-component adhesive materials on the gingival surface microleakage in class II restorations. This raises the question whether self-etch, one-step, one-component, adhesive systems and nanofilled composites are efficient in preventing or reducing microleakage in class II cavities

Microleakage testing conducted by previous studies using normal low-resolution optical microscopy use fluorescent dyes since these dyes present a very visible, strong color. In our previous study, [8] the microleakage of nanocomposites restorations were not affected by the different finishing techniques. Dye penetration was quantified in millimeters under stereomicroscope at 50X magnification, which were made separately for the occlusal and cervical margins[8]. However, by the introduction of confocal laser scanning microscope, microleakage testing starts to rely on the fluorescence criteria of the dye rather than the color, which give us a new advantage over conventional microscopy. Confocal laser scanning microscope offers several other advantages over conventional optical microscopy. This includes the ability to control depth of field, elimination or reduction of background information away from the focal plane (that leads to image degradation) and the capability to collect serial optical sections from thick specimens. This technique generates significant improvement in resolution, lying somewhat between that of conventional light microscope and scanning electron microscope (SEM). Moreover, this type of microscopy enables high-resolution images to be made of samples with minimum requirements for specimen preparation[9]. Therefore, CLSM may provide more accurate detection of microleakage[10]. For all these reasons, this technology was utilized for microleakage testing of the current study.

Therefore, the purpose of this *in vitro* experimental study is to evaluate the efficacy of self-etch, one-step, one-component adhesive systems in reducing the microleakage of class II composite restorations, in relation to the conventional systems using confocal laser scanning microscope (CLSM). However, the research hypothesis was that self-etch, one-step; one-component adhesive system has lower microleakage as compared to the total etch system in class II restorations.

MATERIALS AND METHODS

This randomized single blinded in vitro experimental study was carried out in the Craniofacial Science Laboratory at the School of Dental Sciences, Universiti Sains Malaysia (USM). Extracted human upper permanent premolar teeth, which have been extracted due to orthodontic treatment or periodontal disease, were used in this study. Only intact human upper permanent premolar teeth were used in the study sample. Ethical clearance was obtained from Research and Ethics Committee of Universiti Sains Malaysia, USM. Furthermore, informed consent was obtained from the patients and their participation was voluntary. PS software[11] was used in this study to calculate the sample size based on comparing two means. As a result 23 cavities were needed in each study group. With anticipation of 5% tooth broken during sectioning (10% of the cavities), the sample for each group was decided to be 26 cavities. Therefore, 52 teeth (104 cavities) were used in this study.

The teeth were collected and examined under light microscope (Zeiss Axiostar Plus, Germany) following previous inclusion criteria, at 10x magnification. The examined teeth were cleaned using ultrasonic scaler and stored in a 0.2% thymol solution until used for cavity preparation.

Cavity Preparation

Two vertical slut cavities were made on the mesial and distal surfaces of each tooth with rounded angles. The cavities were prepared 1 mm below the cemento-enamel junction with no enamel remaining in the gingival wall. The cavities were approximately 3 mm in width and 1.5 mm in depth. The cavities were prepared using diamond burs (Meisinger US-No 1557, Germany) with a diameter of 1mm, at high speed with water cooling. The bur was replaced after every 10 preparations. The periodontal probe was used to measure the dimensions of the cavities. Hand instrument was used to finish the approximal boxes to a cavo-surface margin of 90° angle. The same operator performed all cavity preparations and restorations. The prepared teeth were stored in distilled water until restored. After the needed sample sizes were reached, block randomization method was used to divide the prepared cavities into four groups.

(n = 26) as shown in table 1.

Restorative procedure

All the cavities were filled following the manufacturer instructions. The incremental technique and A2 shade were used in this study. For all groups, transparent matrix was used to reestablish the proximal surface of the restorations. The matrix was tightened and held by finger pressure against the gingival margin of the cavity so that the preparations were not overfilled at the gingival margin. Prior to starting each new restoration, the light source intensity was measured with a radiometer (CURE RITE Dentsply, USA). After the whole cavity was filled, the matrix band was removed and two additional curing of 40 seconds each were made to direct the light to the buccal and the lingual surface of the teeth. Immediately, the restorations were polished with Sof-lex disc systems (3M ESPE, USA) in decreasing granulation to simulate the clinical situation.

After all the restorative procedures were completed, the 104 restoration were immersed, and kept in distilled water at room temperature for one week. All specimens were thermocycled between 5-55℃ for 500 cycles, with a dwell time of 30 seconds. Distilled water was used for thermocycling process in all water baths. After thermocycling, the teeth were dried and the apices of the samples were sealed with sticky wax. The teeth were covered with two coats of nail varnish except for the restorations and a 1-mm rim of the tooth structure around each restoration. Then teeth were kept in distilled water for 24 hours to provide rehydration that might have occurred during nail varnish application and drying.

Dye Immersion, Sectioning

The teeth were subsequently immersed in 0.5% rhodamine B fluorescent dye solution at room temperature for 10 hours. To prepare 0.5% rhodamine B solution, 0.5 g of dye powder were dissolved in 100 ml of deionized water and the pH was modified by adding 0.05 M NaOH to the solution until the pH was 6.78. After that, the teeth were then rinsed carefully with distilled water and blot dried. The teeth were then sectioned longitudinally under copious distilled water using a diamond disc (Exakt hard material cutter, Germany). The samples were positioned to ensure that both mesial and distal boxes are sectioned into two halves.

Dye Penetration Measurements

The samples were analyzed using confocal microscope (LeicaSP2, Germany) with an argon laser at a 488/543 nanometer (nm) excitation wavelength. A 10 micrometer confocal slit with 515 nm long pass barrier filter were used and the samples were analyzed at 10x magnification (Figure 1). The microleakage measurement was determined by evaluating the presence of fluorescent dye penetrating from the cavosurface margin towards the axial wall in the gingival wall. The section with higher penetration of dye material was used for the microleakage measurements and the mesial and distal cavities microleakage was scored separately

To test for dye penetration measurements reproducibility, repeated measurements after two weeks were performed for 20% of the subjects.

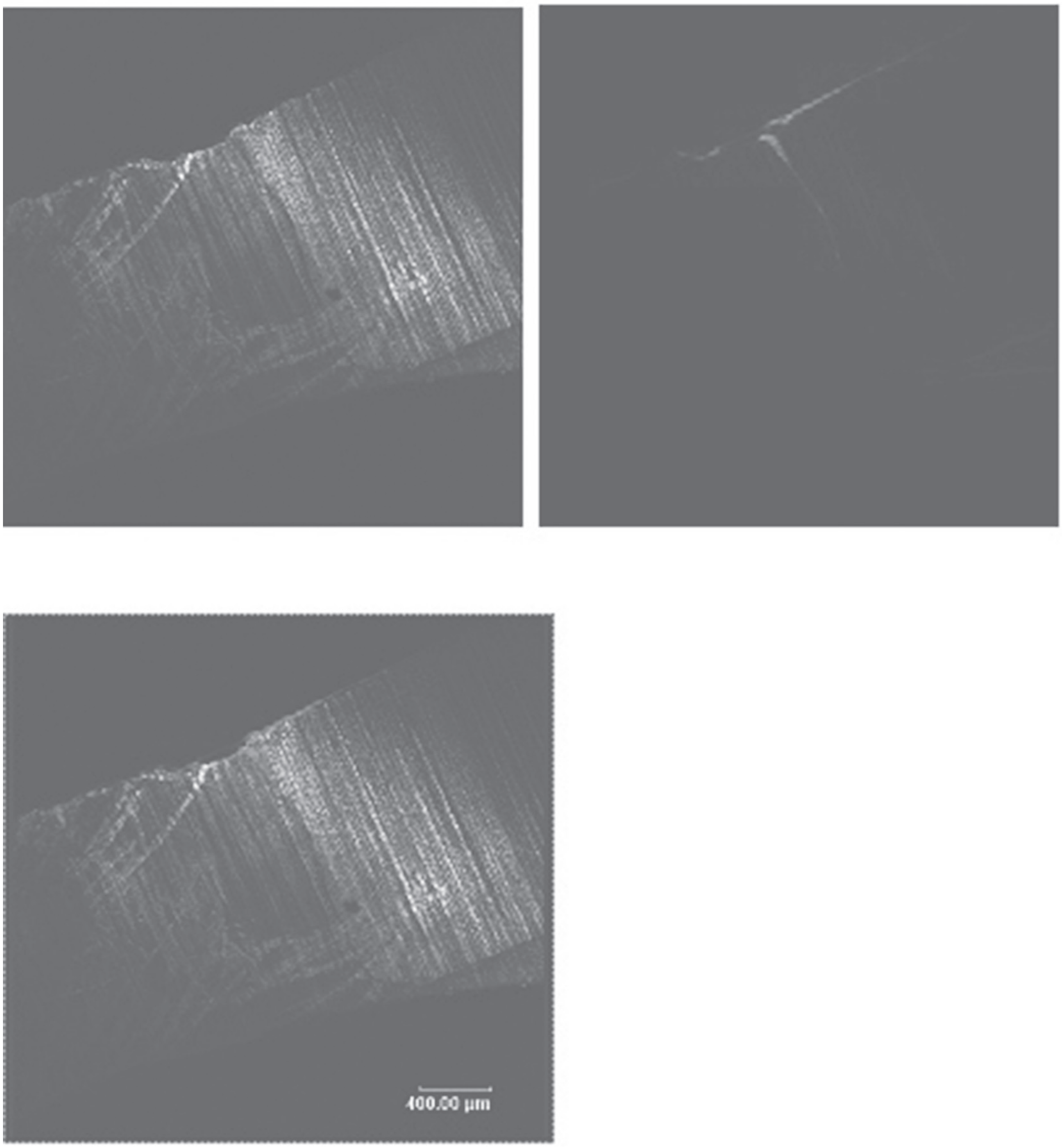

Figure 1. CLSM image of microleakage for restoration viewed at 10x magnification

Table 2. Reproducibility of dye penetration measurements

Measurements (mm)	Means difference (SD)	95% confidence interval of the difference		*t* statistic[a]	*P* value	ICC
		Lower	upper			
Amounts of dye penetration	-0.0808 (.065)	-0.020	0.005	-1.266	0.208	0.994

[a]Independent *t* test

Table 3. Comparison of microleakage between the G Bond adhesive system (n = 52) and Adper single bond 2 adhesive system (n = 52)

Adhesive system	Microleakage (mm)		F statistic[b]	*P* value[b]
	Mean (SD)	Adj. Mean[a] (95% CI)		
Adper single bond 2	0.55 (0.70)	0.55 (0.39; 0.71)		
G Bond	0.35 (0.39)	0.35 (0.19; 0.51)	3.26 (1, 101)	.07

[a] Adjusted for filling material

[b] Two-Way ANOVA

The data were entered and analyzed using a commercially available statistical software package, SPSS 12.0.1 for Windows. Two-Way ANOVA statistical analysis was used to compare the different groups. $P < 0.05$ was considered statistically significant.

RESULTS

Reproducibility of measurements

The differences between repeated measurements were tested for significance using paired *t* test. Paired *t* test showed no significant differences ($P > 0.05$) between repeated measurements for any of the measurements (Table 2). The degree of reproducibility of measurements, i.e. intra class correlation coefficient (ICC) was calculated. ICC was nearly 0.994 which suggest that the measurements were almost identical or with negligible errors.

The first and third groups were compared with the second and fourth groups. As a result, the measurements of 52 samples of self-etch, one-step, one-component adhesive systems (G Bond) were compared with the measurements of 52 samples of etch and rinse adhesive system (Adper single bond 2).

The statistical analysis of microleakage between the self-etch, one-step, one component (G Bond) adhesive system and the etch and rinse (Adper single bond 2) adhesive system showed no significant differences ($P > 0.05$) between the two groups. Table 3 represents the results of the statistical analysis of the study with $P > 0.05$.

DISCUSSION

The hypothesis tested in this experiment was that self-etch, one-step, one-component adhesive systems are better in reducing microleakage than etch and rinse systems. The microleakage mean of G Bond was lower than Adper single bond 2 but the results showed no statistical significant difference between the use of the two different adhesives ($P > 0.05$). Therefore, the previously stated hypothesis was rejected. The results obtained in the current study matched with the results of a few previous studies.(Ernst (2004)[12] stated that in the cervical cementum margins of class II restorations, self-etching adhesives seems to work as good as the total-etch adhesives.

In addition, Brackett *et al*. (2004)[4] reported that no significant differences in gingival marginal leakage were found between self-etch and etch and rinse adhesive systems in class V restorations centered on the cemento-enamel junction (CEJ). Casagrande *et al*. (2005)[12] reported that self-etch and etch and rinse adhesive systems showed similar performance at cervical margins of primary teeth and no statistical difference was found between the sealing capabilities of these adhesives. Burrow and Tyas (2007)[13] reported that the one-year results of G-Bond showed similar outcomes to other recent studies that have investigated the use of self-etching priming adhesives (5th generation) for the restoration of non carious cervical lesions. Moreover, Abo *et al*. (2004)[14] reported that there is no difference in apical leakage in class V cavities between the all-in-one adhesive i-Bond (7th generation) with that of Clearfil SE (5th generation) adhesive system.

For class II restorations, etch and rinse adhesives as well as self-etching adhesives are the focus of scientists. However, one has to keep in mind that being superior was not the initial aim of the self-etching adhesives. Initially, they were developed with intention for it to be more safe and easier to handle. These later characteristics might lead to superior results in the hand of dentists who are not well practiced in the use of etch and rinse technique[15].

One of the most recent all-in-one systems introduced is G-Bond (GC Corporation, Japan). Its chemical component is a little unusual as it does not contain 2-hydroxyethyl methacrylate (HEMA). In addition, the application time is extremely short and a strong air blast is needed to evaporate the solvent and spread the resin to a very thin layer. Therefore, the constituents and method of application of G-Bond are somewhat different from many other self-etching adhesive systems[13].

G-Bond is considered as a 'mild-etch' adhesive system that does not contain HEMA. Bonding mechanism of this system is achieved by the inclusion of two functional monomers, namely 4-methacryloxyethyl trimellitic acid (4-MET) and a phosphate ester. The exclusion of HEMA makes this adhesive ideal for use in a dental practice where the staff or a patient may have a known sensitivity to HEMA[13].

'Mild' self-etch adhesives (pH of around 2) dissolve the dentin surface only partially, so that a substantial number of hydroxyapatite crystals remain within the hybrid layer. Specific carboxyl or phosphate groups of functional monomers can then chemically interact with this residual hydroxyapatite. This two-fold bonding mechanism (*i.e.*, micro-mechanical and chemical bonding) is believed to be advantageous in terms of restoration durability. It has a micro-mechanical bonding component that may in particular provide resistance to abrupt de-bonding stress. The chemical interaction may result in bonds that better resist hydrolytic break-down and thus keep the restoration margins sealed for a longer period[15].

The air-blowing may also be beneficial at the level of the hybrid layer, as strong air-blowing results in better solvent (acetone-ater) removal. Less solvent in the hybrid layer means more resin and also a better polymerization conversion rate, and thus produced a stronger and more impermeable hybrid layer[15].

Although a great diversity in laboratory testing of adhesive systems and lack of standardization of testing methodologies exist, good correlation still exists between laboratory and clinical effectiveness. However, because of an additional chemical bonding potential to hydroxyapatite, the mild self etch approach may be the most promising in terms of durable bonding to dental hard tissue[2].

CONCLUSIONS

Under the conditions set up for this study, several conclusions can be drawn:

* The self-etch, one-step, one-component adhesive system (G Bond) performed as good as etch and rinse system (Adper single bond 2) in reducing microleakage of class II composite restorations with gingival margins ended in dentin.
* No material was able to totally eliminate microleakage in class II composite restorations with gingival margin ended in dentine.
* CLSM was a useful tool for microleakage evaluation.

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