Effect of Smear Layers Created by Different Burs on Durability of Self-Etching Adhesive Bond to Dentin of Primary Teeth

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Aim: *The aim of this study was to evaluate the effects of a smear layer generated by a high-speed diamond or carbide bur on the durability of microtensile bond strength (µTBS) of a self-etching adhesive to primary dentin.* ***Study Design:*** *Flat occlusal dentin surfaces of 105 human primary molars were exposed using 600 grit silicon carbide paper before being divided into 2 groups for further grinding with either a high-speed diamond or carbide bur. Ten prepared dentin surfaces treated by each bur were evaluated for the characteristics of the smear layer using a scanning electron microscope (SEM). Seventy-five specimens from each bur-prepared group were applied with a 2-step self-etching adhesive (Clearfil SE Bond®) then built up with a resin composite. Each bonded specimen was sectioned into a 1-mm thick slab and trimmed to a dumbbell shape with a cross-sectional area of approximately 1 mm². All slabs were divided into 3 groups (n=25) according to 3 storage times of 24 hrs, 3 months, and 6 months, in distilled water at 37°C. After storage, the µTBS was determined using a universal testing machine. All fracture specimens were prepared for observation of failure modes. Ten bonded specimens of each bur group were prepared for observation of the resin-dentin interface using an SEM. Smear-layer thickness, µTBS, and failure mode distributions were statistically analyzed.* ***Results:*** *The high speed carbide bur created a significantly thinner smear layer than the diamond bur ($p < 0.05$). Dentin surfaces treated with a high-speed carbide bur generally obtained significantly higher µTBS than the diamond bur group ($p < 0.05$). The µTBS gradually decreased over time such that specimens stored for 6 months had significantly lower bond strength than those stored for 24 hrs ($p < 0.05$). Self-etching adhesive created a hybrid layer of the same thickness when prepared with either a carbide bur or diamond bur, but the carbide bur group had longer and more resin tags.* ***Conclusion:*** *High-speed carbide bur groups had a higher µTBS than diamond bur groups for all storage times, and bond strengths decreased over time in both substrate groups. The use of a carbide bur produced a thinner smear layer and therefore is recommended when using this 2-step self-etching adhesive to bond the resin composite to primary dentin.*

Key words*: smear layers, hybrid layer, bond strength, self-etching adhesive, burs, primary dentin*

INTRODUCTION

Nowadays, minimal intervention is an important consideration in restorative dentistry. The development of new dentin bonding systems has brought about great improvement in adhesive dentistry and also changed the philosophy behind cavity preparation. Many factors should be considered in the selection of restorative materials for various situations involving primary teeth and the bond strength of dentin is one such factor.

Resin composite is a material of choice for restoration in primary teeth. In 1955, Buonocore introduced the acid-etching technique for enamel surfaces for bonding[1]. Currently, adhesive systems can generally be divided into 2 types depending on approaches used on the smear layer created by instruments during cavity preparation. One is a total-etching adhesive which is basically divided into a separate etch and rinse phase. The other is a self-etching adhesive that is composed of non-rinse acidic monomers which condition and prime enamel and dentin simultaneously. This system dissolves a smear layer and partially demineralizes the underlying dentin surface. Since this system is not rinsed off, the dissolved smear layer is incorporated in the bonding process[2]. The self-etching system allows the simplification of

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restorative procedures saves time and makes the technique less sensitive[3]. This system demonstrates many advantages especially in children, whose behavior makes restorative treatment difficult and requires less time than adults. Furthermore, the total-etching system may cause excessive etching of the dentin which then produces weak bonding to collagen fibers at the bottom of the demineralized dentin that is not completely impregnated by the resin monomer[4]. So the self-etching system allows for simultaneous demineralization and resin infiltration, which should lead to an optimally infiltrated hybrid layer[5]. In addition, it may have the potential to promote less postoperative sensitivity[6] because it has demineralized the smear layer in the bonding process leaving residual smear plugs that cause less dentinal fluid flow than the total-etching system[7]. A very rough or thick smear layer may interfere with the diffusion of self–etching primer into the underlying dentin, or may buffer acidic primer, making the pH too high to demineralize the underlying dentin[8]. This may influence the strength of the bond as well. Several studies have examined the effects of the smear layer's characteristics on the bond strength of self–etching adhesives[8,9]. While bonding to permanent teeth has been studied extensively, few studies have addressed self-etching bonding to primary teeth[10,11,12]. Preparing the dentin surface with various instruments (e.g. diamond bur, carbide bur, stainless steel bur, air-driven abrasive particles) creates quantitatively and qualitatively different smear layers[8,13]. As the use of phosphoric acid-etching (total-etching system) removes the smear layer and smear plugs, the self–etching system is less aggressive and partially demineralizes the smear layer and incorporates its remnants into the hybrid layer[8]. As a result, different rotary instruments influence the interaction between substrate and adhesive.

The purposes of this study were to evaluate the effect of a high speed diamond bur and high speed carbide bur on the microtensile bond strength in short and long-term water storage and observe the micro-morphology of the smear layer and resin-dentin interface in primary dentin under SEM.

MATERIALS AND METHOD

This experiment was reviewed and approved by the Mahidol University Institutional Review Board.

One hundred and five extracted, sound human primary molars were used in this study. The inclusion criteria of selected teeth were that they not have carious lesions and be free of crack lines, restoration and enamel defects. The teeth were stored at 4ºC in normal saline solution for no longer than 3 months following extraction. The occlusal enamel surface was removed by trimming and wet ground parallel to the occlusal plane using 180, 320 and 600 grit silicon carbide paper in order to expose a flat dentin surface. The prepared teeth were further randomly assigned to 3 groups: twenty teeth were employed in evaluation of the smear layer's characteristics; seventy-five teeth were used in evaluation of microtensile bond strength; and ten teeth were used in evaluation of the resin-dentin interface.

Evaluation of the characteristics of the smear layer under SEM

Twenty teeth were randomly divided into 2 groups (n = 10) according to the types of burs used for preparation of the dentin surfaces: ten teeth were prepared with a cylindrical medium grit diamond bur (Intensive Swiss Dental Product, Montagola, Switzerland) and the remaining teeth were prepared with a carbide bur (Kerr, Ontario, Canada) using constant pressure at high speed (320,000-350,000 rpm) under running water for 20 strokes. A new bur was used for each pair of specimens. Calibrated manual pressure of 300 g was applied during the preparation of the sample's surface.

A transversal groove of 1 mm. depth was prepared with a carborundum disc on the opposite side of the prepared dentin surface and was then carefully fractured by applying bending force onto the groove.

The specimens were immersed in 10% formalin buffer for 24 hours. After fixation, the specimens were dehydrated for 15 minutes in a series of ascending grades of ethanol (50%, 60%, 70%, 85%, 95% 99.99%) twice[14]. After the ethanol series, the specimens were dried in a dessicator for 24 hours and mounted on aluminum stubs with carbon cement and colloidal silver paint. The specimens were sputter-coated with gold and observed under a scanning electron microscope (SM 5410 LV, JEOL Co., Tokyo, Japan). The dentin surface preparation was created by the same operator.

Evaluation of micro-tensile bond strength

The teeth were longitudinally sectioned in a buccal to lingual direction with a low speed diamond saw into two halves and randomly divided into 2 groups (n = 75) according to the type of bur used for dentin surface preparation.

After preparation of the dentin surface, the samples were bonded with Clearfil SE Bond® (Kuraray, Osaka, Japan) following the manufacturer's instructions. Resin composite (Clearfil AP-X®, Kuraray, Osaka, Japan) was built up on the bonded surface with an incremental technique to achieve a 6 mm-thick block with light-cured for 40 sec. (all materials shown in table 1). Specimens were serially sectioned approximately 1 mm thick, parallel to the long axis of the tooth using a low-speed diamond saw under running water. The slabs were trimmed and shaped into a dumbbell-shape with a superfine diamond bur under water coolant. The cross-sectioned area at resin-dentin interface was approximately 1 mm^2. The samples were further randomly divided to 3 groups (n = 25) according to storage time (24-hour, 3-month, 6-month water storage time).

The trimmed specimens were attached to a testing apparatus of a universal testing machine with a cyanoacrylate adhesive. Tensile forces were applied at a crosshead speed of 1mm/min with cell load size of 50 Newtons. Failure modes were observed under the scanning electron microscope. The failure modes were classified into 4 groups which are adhesive failure, cohesive failure in adhesive resin, cohesive failure in bonding and cohesive failure in the dentin. The modes of failures of each category were recorded in percentages.

Evaluation of resin-dentin interface under SEM

The teeth were longitudinally sectioned in a buccal to lingual direction with a low speed diamond saw into two halves and randomly divided into 2 groups (n = 10) according to the type of bur used for dentin surface preparation and restoration. All of the specimens were stored in distilled water at room temperature for 24 hours. Then specimens were sectioned parallel to the long axis of the tooth with a low-speed diamond saw. The two pieces of

Table 1. Adhesive system and application mode

Adhesive	Classification	Manufacturer	Batch No.	Composition	Application mode
Clearfil SE Bond®	Two-step self-etching adhesive	Kuraray Osaka Japan	081164 #1970-TH	**1) PRIMER (self-etching primer)** Principle ingredients: 10-ethacryloyloxydecyl dihydrogen phosphate (MDP) 2-Hydroxyethyl methacrylate (HEMA) Hydrophilic dimethacrylate dl-Camphorquinone N,N-Diethanol-p-toluidine Water **2) BOND (bonding agent)** Principle ingredients: 10-ethacryloyloxydecyl dihydrogen phosphate (MDP) Bis-phenol A diglycidylmethacrylate (Bis-GMA) 2-Hydroxyethyl methacrylate (HEMA) Hydrophobic dimethacrylate dl-Camphorquinone N,N-Diethanol-p-toluidine Silanated colloidal silica	1. apply primer 20s 2. dry with mild air flow 3. apply bond 4. gentle air flow 5. light cure 10s

the specimens were used for evaluation of the micromorphology of the resin-dentin interface under SEM. Specimens were rinsed under tap water and embedded in epoxy resin. After setting, the specimens were polished with wet silicon carbide paper with 600, 1,000, 1,200, and 2,500 grit, and diamond paste of grain 6, 3, 1 and 0.25 µm respectively. The specimens were etched for 3 seconds with 10% H_3PO_4 and rinsed with de-ionized water for 15 seconds followed by immersion in 5.25% sodium hypochlorite solution for 10 minutes[14]. The specimens were rinsed and kept in a dessicator for 24 hours then were sputter-coated with gold and observed under a scanning electron microscope.

Statistical analysis

1. Evaluation of the smear layer.

   Mean and standard deviations of thickness of the smear layer were calculated for each group. A Mann-Whitney U test was used to test differences in mean thickness of the smear layer between test groups.

2. Evaluation of micro-tensile bond strength.

   Mean and standard deviations of the microtensile bond strengths measured in Megapascals (MPa) were calculated for each group. A One-Way ANOVA was used to test the difference in means of the bond strength between groups. A Dunnett T3 test was used to test the difference in means of bond strength between groups according to different storage times. An independent sample T-test was used to test differences in means of bond strength between different groups of bur type. The distribution of modes of failure was compared with a Kruskal-Wallis test. Calculations were performed with SPSS version 18 at the significance level of 0.05 in all statistical tests.

RESULTS

Smear layer characteristics

The smear layer created by two different bur types (diamond vs. carbide bur) are shown in figure 1.

Surface characteristics: The electron micrographs of dentin surfaces prepared with a diamond bur demonstrated flat surfaces covered with a visible smear layer. The group prepared with the carbide bur showed fewer irregularities and smear plugs that occluded dentinal tubules than the group with the diamond bur.

Smear layer thickness: The thickness of the smear layer produced by the carbide bur (2.11±0.89µm) was significantly less than that created by the diamond bur (3.39±1.14µm), ($p=0.019$) as shown in table 2.

The smear plugs: The smear plug in the dentinal tubules demonstrated various degrees of depth depending on the type of bur used. The carbide bur created smear plugs while it occluded more shallowly into the dentinal tubules than the diamond bur.

Table 2. Means and standard deviations of dentin smear layer thickness produced by different burs (n=10).

Type of bur	Smear layer thickness (µm)
Diamond bur	$3.39±1.14^{A}$
Carbide bur	$2.11± 0.89^{B}$

Different superscript letters indicate statistical difference ($p<0.05$)

Microtensile bond strength

During the experimental phase of this study, no specimen was fractured during storage. The means of microtensile bond strength and standard deviations for each group are presented in table 3.

Microtensile bond strength was affected by bur type ($p<0.001$) and storage time ($p<0.001$) although the interaction between both factors was not statistically significant ($p=0.732$)

Figure 1. Electron micrographs (×3,500) of dentin surfaces prepared with diamond bur (A) and carbide bur (B) The arrows in the small picture (×2,000) indicate the dentinal tubule with smear plug.

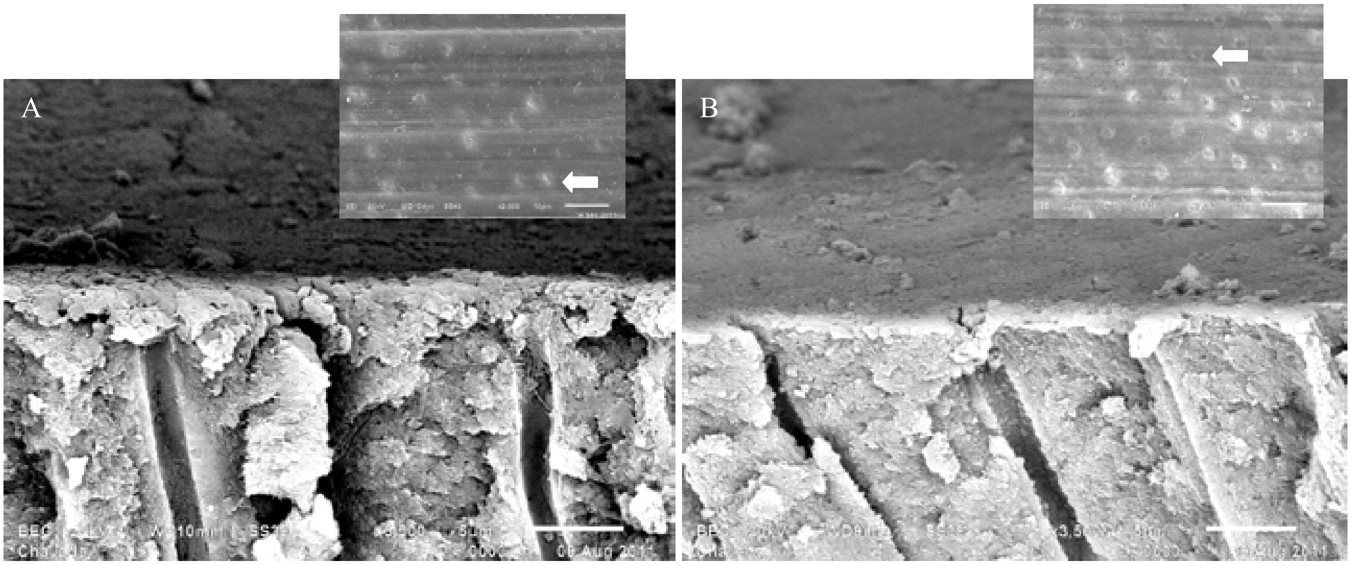


Table 3. Microtensile bond strength (MPa:Mean±SD) (n=25).

Diamond Bur			Carbide Bur		
Time	Premature failure	Microtensile bond strength (MPa:Mean±SD)	Time	Premature failure	Microtensile bond strength (MPa:Mean±SD)
24 hours	0	14.52±5.06 ^{A,a}	24 hours	0	20.04±6.33 ^{A,b}
3 months	0	11.82±4.38 ^{A,B,a}	**3 months**	0	15.68±7.32 ^{A,B,b}
6 months	0	9.87±5.60 ^{B,a}	**6 months**	0	15.35±6.77 ^{B,b}

For each vertical column: value with different caption letters indicate statistically different (p<0.05) when compared with different storage time. For each horizontal row: value with different lower case letters indicate statistically different (p<0.05) between two type of burs.

The effect of bur type: The use of a carbide bur to prepare dentin surfaces demonstrated significantly higher microtensile bond strength to primary dentin than the diamond bur when the data were analyzed at the same storage time.

The highest microtensile bond strength to dentin was established when using the carbide bur to prepare dentin surfaces which were then stored in distilled water for 24 hours (20.04±6.33 MPa). The lowest microtensile bond strength was found in the diamond bur-prepared group stored in distilled water for 6 months (9.87±5.60 MPa).

The effect of storage time: The group stored in distilled water at 37°C for a duration of 24 hours exhibited significantly higher microtensile bond strength than did that stored for 6 months prepared with both types of bur. No significant differences of micro- tensile bond strength were detected when compared with 3 months storage with both types of bur.

Failure modes: This study showed no preload failure. The great majority of specimens in each group demonstrated failure at the resin-dentin interface or adhesive failure (figure 2). The data analysis with a Kruskal-Wallis test found no statistically significant differences among failure mode distributions among the groups.

Evaluation of resin-dentin interface under SEM

The penetration of the resin into the dentinal tubules and the formation of a resin-dentin inter-diffusion area or hybrid layer were observed in both groups. The thickness of the hybrid layers in both groups were approximately 0.5-1.0 µm. We found that the group with dentin prepared with the diamond bur had minimal formation of resin tags while the dentin group prepared with the carbide bur had more and longer resin tags as shown in figure 3.

Figure 2. Failure mode distributions (%) of each group.

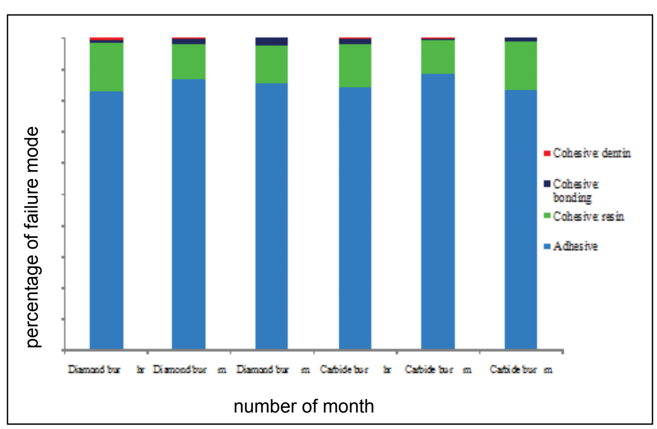
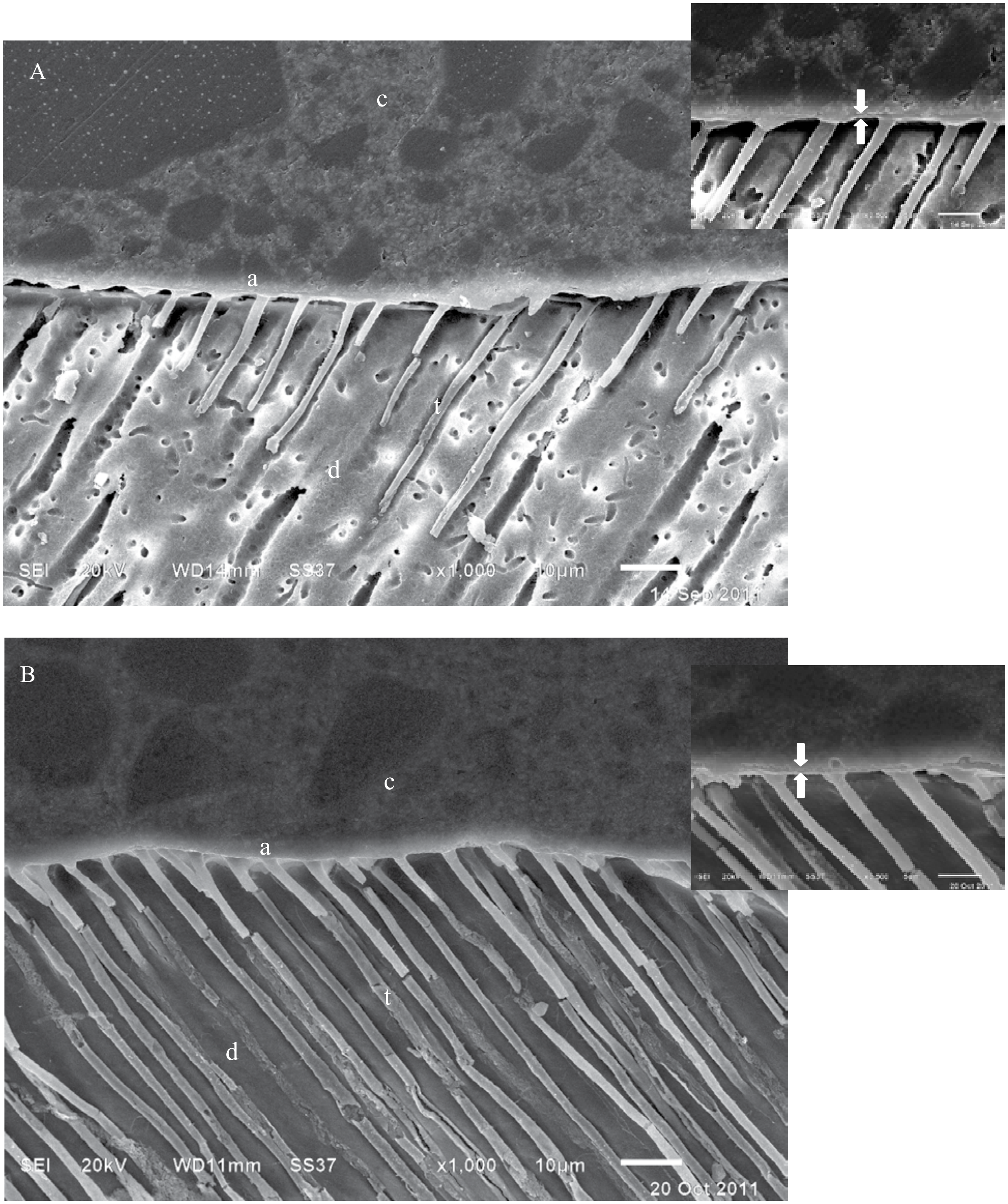


Figure 3. SEM image (×1,000) of the resin-dentin interface of dentin prepared with diamond bur (A) and carbide bur (B), The arrows in the small picture (×3,500) indicate thickness of the hybrid layer, a=adhesive, d=dentin, t=resin tags, c=resin composite.



DISCUSSION

We were interested in self-etch adhesives because the application time is shorter and the technique-sensitivity less than total-etch adhesives. When compared with all-in-one adhesives, self-etch adhesives produce more durable[15]. In this study we used Clearfil SE Bond®, the mild self-etching system, because it has been shown to be one of the most reliable adhesive systems and demonstrated high bond strength values in numerous studies[16,17] and also remains the gold standard in bond durability[15].

In this study, we found that dentin prepared with a diamond bur created a significantly thicker smear layer than that with the carbide bur ($P<0.05$). Similar results have been reported in other studies[16,18]. This might be due to the fact that the carbide bur used blades to cut rather than the abrasive cutting of the diamond bur. Blade cutting produces a new surface, and therefore creates less debris[19]. The smear layer on ground dentin surfaces performed like a barrier for resin infiltration during bonding. While phosphoric acid-etching removes the smear layer and smear plugs, the self–etching system is less aggressive and partially demineralizes the smear layer and incorporates its remnants into the hybrid layer[8]. Consequently, the effectiveness of self–etching adhesive systems might be affected by the smear layer's characteristics. Our study demonstrated that dentin surfaces prepared with a carbide bur demonstrated significantly higher microtensile bond strength than those prepared with a diamond bur for all storage times ($p<0.05$). Because of the effect of smear layers, the carbide bur produced thinner, smoother and fewer smear plugs than the diamond bur. This finding emphasizes the importance of using a bur that creates a thin smear layer when applying the self-etching adhesive system for bond restoration. These results are in agreement with the reports in other studies[15, 18] but differ from the study of Suttabanasuk *et al.*[20] They found that dentin surfaces prepared with a carbide bur showed lower microtensile bond strength than the diamond bur. We found that the highest microtensile bond strength of SE was 20.04±6.33 MPa, but the results of previous studies were 18.72±7.59MPa[21], 30.20±8.60MPa[22], 29.29±13.10 MPa[23] and 27.68±13.26 MPa[24], respectively. We consider that the differences between the studies mentioned previously might be due to differences in study design.

The durability of bond between adhesive and dentin is a critically important factor for the longevity of bond restoration. In our study, the microtensile bond strength of SE gradually decreased over time, and significantly reached the lowest bond strength after six months ($p<0.05$) with both bur types. These findings are similar to those of Burrow *et al*[25] and Reis *et al*[26] who reported a decrease in bond strength after long-term storage in water, but differ from the study of Sano *et al*[27] who found no significant difference in bond strength over one year. These differences might be due to differences in study design.

The storage condition influenced the long-term durability of dentin bonding[28]. In this study, distilled water was used as a storage medium which was changed every week and the specimens were reduced to 1 mm^2 cross-sectional area for microtensile bond testing prior to storage in distilled water to maximize hydrolysis at the interface of bonding resin and hybrid layer over time which led to a decrease in bond strength over time. The reason for this is similar to that found in the study of Shono *et al.*[29] They found that a smaller cross-sectional area of the resin-dentin interface, as used for microtensile bond strength testing, could accelerate the deterioration of bond strength over time.

Non-impregnated resin infiltration at the base of the hybrid layer might be a menace to adhesion integrity over time. Even if the self-etching adhesive system simultaneously etches and primes dentin, a discrepancy between the depth of demineralization and the depth of resin infiltration might occur[5], however, it could signal degradation over time due to hydrolytic and enzymatic degradation of collagen matrix[30]. Pashley *et al*[31] found that degradation can take place over time in the absence of bacteria via host-derived matrix metalloproteinase enzymes in dentin matrix that can be activated by low pH.

Concerning the mode of failures, most of the failure modes observed in this study were adhesive failures for all groups. Our results are similar to those of the study of Sardella *et al*[22] These findings reflect a normal characteristic of the microtensile test which induces stress to concentrate in the bonded area and then gives back a more reliable result than conventional approaches such as the shear and tensile bond strength test[32].

At the resin-dentin interface, SEM examination also demonstrated that both dentin surfaces prepared with the diamond bur and carbide bur had similar hybrid layer thickness but it was observed that the carbide bur group showed more and longer formation of resin tags than did the diamond bur group. Since the dentin prepared with a diamond bur created a thicker smear layer than that prepared with the carbide bur, thicker smear layers reflected an increased number of close dentinal tubules after self-etching primer treatment and also produced fewer and shorter resin tags. Nakornchai *et al*[21] and Nogueira *et al*[33] reported no correlation between the hybrid layer thickness. In our study, there was no difference in hybrid layer thickness between bur types but there was a difference in the amount and length of resin tags which might affect the microtensile bond strength.

CONCLUSION

The micro-morphology of primary dentin surfaces prepared with a diamond bur demonstrated more irregularities, occluded dentinal tubules, and significantly thicker smear layer than those prepared with a carbide bur.

The two-step self-etching adhesive system, Clearfil SE Bond®, showed significantly higher microtensile bond strength at the bonded primary dentin surface prepared with the carbide bur than that with the diamond bur in every storage time. In addition, the bond strength values gradually decreased over time.

Primary dentin surfaces prepared with two types of bur did not differ in hybrid layer thickness but did differ in the amount and length of resin tags. More and longer resin tags were found when primary dentin surfaces were prepared with the carbide bur.

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