The effect of the dentin preparation with an ultrasonic abrasion on the microtensile bond strength of self-etch adhesive systems

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Purpose: This study evaluated the effect of the dentin preparation with an ultrasonic abrasion on the microtensile bond strengths (μTBS) of two-step and one-step self-etch adhesive systems to dentin.
Materials and Methods: A two-step self-etch adhesive, Clearfil SE Bond (Kuraray Medical) and a one-step self-etch adhesive, Clearfil S^3 Bond (Kuraray Medical) were applied on dentin prepared with a diamond-coated round working tip, Excavus (EX, Satelec) activated through ultrasonic frequency oscillations, with a regular grit diamond bur (DB) in a turbine handpiece, with a steel bur (SB) in a micromotor handpiece, or with a #600 grid silicon carbide abrasive paper (SiC). Then, a photo-cured composite was placed and polymerized. The μTBS was measured after storage in water for 24 hours at 37˚C. The data were statistically analyzed using a two-way ANOVA and Dunnett's C test at 95% level of confidence. The prepared surfaces and their crosscut surfaces were observed using a scanning electron microscope (SEM).
Results: For Clearfil SE Bond, the μTBS of EX was higher than that of DB and there were no differences among EX, SB, and SiC groups. For Clearfil S^3 Bond, the μTBSs of EX and DB were lower than those of SB and SiC. The μTBS values of Clearfil SE Bond were significantly higher than Clearfil S^3 Bond, irrespective of the dentin preparation methods. The two-way ANOVA analysis revealed that there was a significant interaction between the dentin preparation methods and the adhesive systems ($p<0.05$). Bond strength was influenced by the dentin preparation method ($p<0.05$) and by the adhesive systems ($p<0.05$). SEM observation of the dentin surfaces prepared with EX showed the thicker and uneven smear layers, compared with SB and SiC.
Conclusion: The μTBS to dentin prepared using an ultrasonic abrasion with EX, when Clearfil SE Bond was applied, was similar to SB and SiC groups and higher than DB group. However, when Clearfil S^3 Bond was applied, the μTBS to dentin prepared with EX was statistically lower than SB and SiC groups and were similar to DB group. The mean μTBSs of Clearfil SE Bond were higher than those of Clearfil S^3 when using the same dentin surface preparation. **(Int Chin J Dent 2010; 10: 7-15.)**
Key Words: cavity preparation, microtensile bond strength, self-etch adhesive system, smear layer

Introduction

The trend in dentin adhesives has shifted from etch and rinse bonding systems[1,2] to self-etch adhesive systems.[3,4] The less technique sensitiveness[5-9] of self-etch adhesives systems is due to the elimination of both rinsing and drying steps and the requirement to keep the dentin "wet". The mild acidity of self-etch adhesives can reduce the dentin permeability after self-etching priming, which is effective in preventing postoperative sensitivity.[10] Self-etch adhesive systems can be classified into two groups; two-step self-etch adhesive systems and one-step self-etch adhesive systems. It has been reported that the performance of one-step self-etch systems showed lower bond strengths compared with two-step self-etch adhesives.[2,11]

Clinically, after mechanical preparation of the cavity with a dental instrument, an amorphous layer of organic and inorganic debris, so-called "smear layer", is formed on the surface.[12] It is well known that the quantity and quality of the smear layer widely vary depending on the manner in which they were created.[13,14] Differences in smear layers prepared with different instruments affected the bond strengths of resins to dentin.[4,15,16] Toida reported that the smear layer should be removed or modified with conditioners such as acidic solutions in order to obtain good adhesion to dentin.[17] Etch and rinse bonding systems remove entirely the smear layer and also smear plugs, while self-etch adhesives partially demineralize the smear layer and incorporate its remnants into the hybrid layer due to their less aggressiveness. Therefore, it has been speculated that the effectiveness of self-etch adhesive systems might be affected by the smear layer thickness, density, or quality.[18-21]

High dentin bond strengths have been reported in many *in vitro* studies for several self-etch adhesive systems.[3,10,22-25] Although most *in vitro* bonding studies used silicon carbide abrasive papers (SiC) for the dentin surface preparation, steel, carbide, diamond burs, or air-driven abrasive particles are clinically used for cutting the hard tooth tissues. Thus, the researches about the influence of different preparation on the interaction between adhesive and substrates[20,23,26,27] are clinically relevant.

Cavity preparation methods, including laser,[11] air abrasion,[28] and sonic removal of tooth structure, have been developed in an attempt to provide less uncomfortable dental treatment and further preserve tooth structure. Recently, a new cavity preparation method using a modified ultrasonic-scalar handpiece equipped with a diamond-coated working tip activated through ultrasonic frequency oscillations has been introduced, because ultrasonic tips can be ideal for creating small-volume cavities for adhesive conservative dentistry and allow direct access to the lesion without damaging the adjacent tooth or nearby healthy tissue. Nevertheless, bond strength of dental adhesives to the dentin prepared with this instrument is not well understood and it is unclear how it cuts the dentin surface morphologically. Therefore, information on the effects of the dentin preparation with this ultrasonic abrasion method on the bond strength, comparing with conventional preparation methods, is clinically required.

The purpose of this study was to evaluate the effect of an ultrasonic abrasion and different dentin preparation methods on microtensile bond strength (µTBS) using two commercially available self-etch adhesive systems (a two-step and a one-step self-etch adhesive). The null hypotheses to be tested were that the methods for dentin preparation do not affect the µTBS of self-etch adhesives to dentin; and that the adhesive materials do not affect the µTBS to dentin.

Materials and Methods

The research design of this study was subjected to the guideline of the Ethical Committee of Tokyo Medical and Dental University, Faculty of Dentistry. Twenty-four caries-free extracted human third molars were used within one month of extraction and stored frozen until use. The occlusal enamel was removed perpendicular to the long axis of the tooth using a low speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under running water to obtain flat mid-coronal dentin surfaces. Each dentin surface was ground with a wet #600 grit silicon carbide paper (SiC, Marumoto Struers, Tokyo, Japan) with 20 strokes of 15 cm length by hand pressure for the creation of a standard smear layer. All the surfaces were randomly divided into four groups of six teeth according to the four surface preparation methods (Table 1). In EX group, the dentin surfaces were prepared with a diamond-coated working tip Excavus (EX, satelec, Bordeaurx, France) in an ultrasonic generator (Suprasson P5 Newtron, Satelec). In DB group, the dentin surfaces were prepared with a regular grit diamond bur (DB, M340, J. Morita, Kyoto, Japan) mounted to an air turbine handpiece (Super Torque Lux2 640B Kavo, Biberach, Germany) at between 100,000 and 120,000 rpm. In SB group, the dentin surfaces were prepared with a round type steel bur (SB, 016, J. Morita) mounted to a contra angle micromotor handpiece (INTRAmatic LUX2 10LN, Kavo) at 2,000 rpm. The dentin surfaces had been marked passes with each equipment under copious air-water spray until entire surface was covered with uniform scratches (EX, DB, and SB groups). SiC group had a control surface prepared with #600 grit SiC as a baseline surface without any further preparation (control group). The same operator performed these preparations.

After dentin surface preparation, a black plastic ring with 8 mm in internal diameter and 2 mm in height was placed on the prepared surface and fix with a wax. A two-step self-etch adhesive, Clearfil SE Bond (Kuraray Medical, Tokyo, Japan) or a one-step self-etching adhesive, Clearfil S^3 Bond (Kuraray Medical) was applied to all the diversely prepared dentin surfaces strictly following the manufacturer's instructions (Table 1). Details regarding the selected adhesives, such as the chemical composition and application mode of the materials tested in this study are also summarized in Table 1. After adhesive procedures, a resin composite, Clearfil AP-X (Kuraray Medical) was injected to the plastic rings as a bulk and was light-cured for 40 s at 600 mW/cm^2 using a halogen light-curing unit (XL3000, 3M-ESPE, St. Paul, MN, USA).

Table 1. Adhesive systems used in this study.

Adhesive system	Composition	pH	Batch No.	Bonding procedures
Clearfil SE Bond (Kuraray Medical, Tokyo, Japan)	Primer: MDP, HEMA, Multifunctional methacrylate, Photoinitiator, Water Bond: MDP, HEMA, Multifunctional methacrylate, Microfiller, Photoinitiator	2.0	011383	Apply the primer for 20 s. Gently air blow. Apply the bonding agent and light cure for 10 s.
Clearfil S^3 Bond (Kuraray Medical)	MDP, HEMA, Multifunctional methacrylate, Microfiller, Photoinitiator, Ethanol, Water	2.7	011183	Apply the adhesive to dentin for 20 s. Air dry for 5 s to evaporate the solvent. Light cure for 20 s.

MDP: 10-methacryloyloxydecyl dihydrogenphosphate; HEMA: 2-hydroxyethyl methacrylate

Microtensile bond test

After storage in water at 37˚C for 24 hours, each specimen was vertically crosscut and re-crosscut to approximately 0.7 mm x 0.7mm using a low speed diamond saw (Isomet) under water cooling to obtain the beam-shape specimens. The final width and thickness of the bonded areas were measured using a digital caliper (Mitsutoyo, Tokyo, Japan). The specimens were then attached to a testing device (Bencor-Multi-T, Danville Engineering, San Ramon, CA, USA) with a cyanoacrylate adhesive, Model Repair II Blue (Dentsply-Sankin, Ohtawara, Japan), which in turn, were placed in a table-top material tester, EZ-Test (Shimadzu, Kyoto, Japan) for microtensile bond testing at a crosshead speed of 1 mm/minute (n=30).[29] After the microtensile bond strengths were measured, all of the specimens were inspected visually and microscopically (x20, Dentcraft Dent-Optics DX, Yoshida, Tokyo, Japan) to determine the modes of failure.

Statistical analysis

The μTBS data were analyzed by using a two-way ANOVA to test the effects of the preparation by the dental instruments and the adhesive systems on bond strength. The interaction between the two factors was also analyzed. Dunnett's C test was used for a post-hoc multiple comparison test at the 95% level of confidence.

SEM observation

Eight extracted third molars were used for a scanning electron microscope (SEM) observation of the prepared dentin surfaces before and after the application of adhesives. Flat dentin disks with a thickness of approximately 1.5 mm were cut perpendicular to the long axis of the teeth by a diamond saw (Isomet). All the dentin surfaces were prepared in the same manner as the specimens for microtensile bond test of each group (EX, DB, SB, and SiC). Subsequently, four disks were used for SEM observation of the dentin surfaces after the treatment with the adhesives. The surfaces were treated with either the primer of Clearfil SE Bond or Clearfil S^3 Bond. For Clearfil SE Bond group, the primer was applied on the surface for 20 s and then rinsed with acetone. For Clearfil S^3

Bond group, the adhesive was applied for 20 s and then rinsed with acetone to remove adhesives without light curing. All the disks were fixed in 10% neutral buffered formalin for 12 hours. All fixed specimens were washed in running tap water and dehydrated in ascending grades of ethanol (50, 60, 70, 85, 95 and 99.99%) for 15 minutes respectively, and sputter coated with gold. The coated specimens were observed with an SEM (JSM-5310, JEOL, Akishima, Japan).

Results

The results of μTBS of each experimental group are shown in Table 2. The two-way ANOVA analysis revealed that there was a significant interaction between the methods of the dentin preparation methods and the adhesive systems ($p<0.05$). Bond strength was influenced by the dentin preparation method ($p<0.05$) and by the adhesive system ($p<0.05$).

In specimens bonded with Clearfil SE Bond, there were no statistically significant differences among EX (76.8±10.1 MPa), SB (80.0±12.3 MPa), and SiC (82.7±13.8 MPa), although they were significant higher than DB (67.8±13.1 MPa) ($p<0.05$). In specimens bonded with Clearfil S^3 Bond, the μTBS values of SiC (58.1±10.8 MPa) and SB (54.8±20.5 MPa) were higher than those of DB (44.0±17.5 MPa) and EX (43.2±17.6MPa). The μTBS values of Clearfil SE Bond were significantly higher than Clearfil S^3 Bond, irrespective of the dentin preparation methods.

When visually inspected using light microscopic examination (x20), the representative micromorphology of the failure patterns of Clearfil SE Bond group was mixed with a failure of interfacial and partially cohesive failure in dentin. In Clearfil S^3 Bond group, the frequent failure pattern was interfacial failure or a failure between dentin and adhesive. The difference of the failure mode was not found among EX, DB, SB, and SiC groups with the same adhesives.

Table 2. Microtensile bond strengths of self-etch adhesive systems for different preparation methods.

Instruments used	Clearfil SE Bond	Clearfil S^3 Bond
EX	76.8 ± 10.1^a	43.2 ± 17.6^c
DB	67.8±13.1	44.0 ± 17.5^c
SB	80.0 ± 12.3^a	54.8 ± 20.5^b
SiC	82.7 ± 13.8^a	58.1 ± 10.8^b

Values are mean±S.D. (MPa, n=30). EX: Excavus (Satelec); DB: a diamond bur; SB: a steel bur; SiC: #600 grit silicon abrasive carbide paper. Same superscript letters indicate statistically no significant differences in the same column ($p>0.05$).

The SEM images of the dentin surfaces prepared with different methods are shown in Fig. 1. In Fig. 1a, EX demonstrated a dense smear layer and dentinal tubules were not observed. In Fig. 1b, the surfaces prepared with DB showed the relatively coarser grooves. The SEM image of dentin surface prepared with SB was covered with visible smear layer and the dentinal tubules were not seen (Fig. 1c). The SEM image of SiC showed that a lot of scratches left by the abrasive paper, and the flat surface prepared with SiC was completely covered with smear layer (Fig. 1d). The dentinal tubules occluded with smear plugs were also observed over the entire surface in Fig. 1d. The results of SEM observation, prepared dentin surfaces of DB and EX showed that the grooves left by the burs were coarser than SiC and SB groups (Fig. 1). The dentin prepared with EX presented an irregular surface, compared to dentin prepared with SiC.

SEM images of the crosscut surfaces of the prepared dentin with different methods are shown in Fig. 2, which revealed the thickness of the smear layer. The thickness of the smear layers of EX and DB appeared to be thicker than those of SB and SiC (Fig. 2a-d). The thickness of the smear layer prepared by EX was about 8 µm (Fig. 2a). In the DB, the defect of dentin was observed beneath the smear layer (arrow, Fig. 2b).

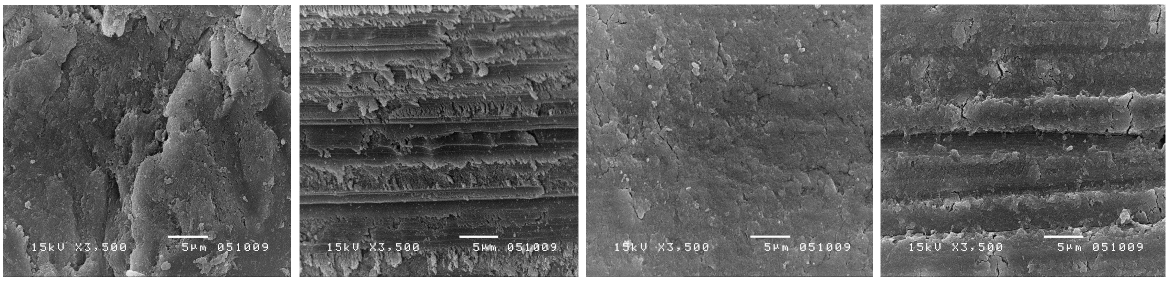

Fig. 1. a b c d
SEM images of the prepared dentin surfaces of each group. a: EX group; b: DB group; c: SB group; d: SiC group (original magnification 3,500x; bar=5 µm). Dentin surface of EX group (d) is rough and irregular.

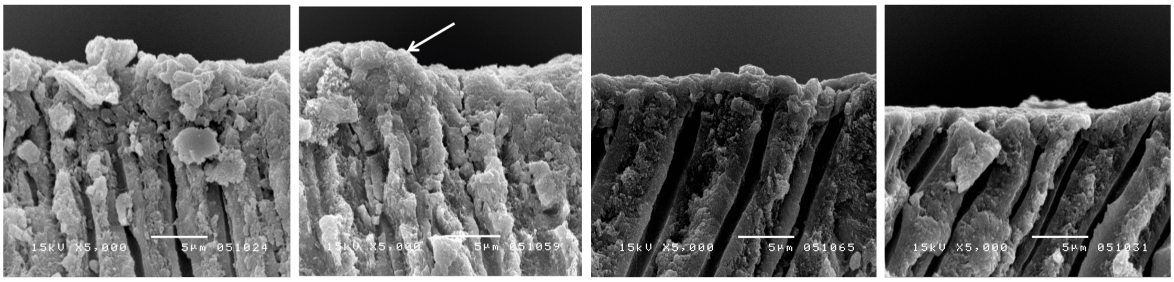

Fig. 2. a b c d
SEM images of cross cut surfaces of the prepared dentin. a: EX group; b: DB group (An arrow shows the defect of dentin beneath the smear layer.); c: SB group; d: SiC group (original magnification 5,000x; bar=5 µm). The thickness of smear layers of EX and DB appeared to be thicker than those of SB and SiC.

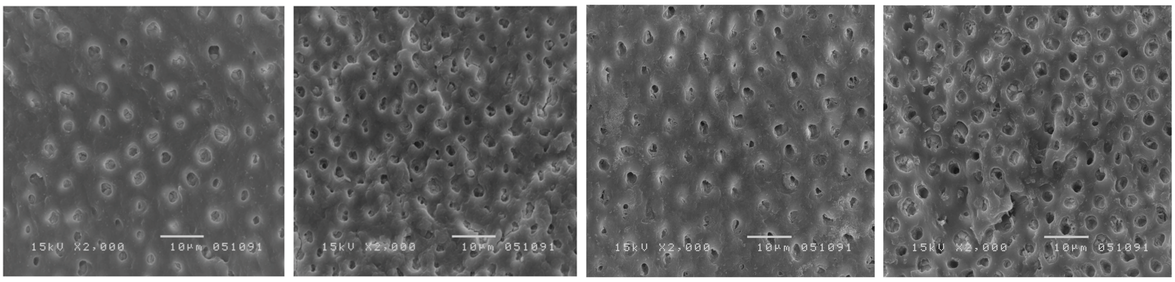

Fig. 3. a b c d
SEM images of dentin surfaces treated with Clearfil SE Bond. a: EX group; b: DB group; c: SB group; d: SiC group (original magnification 2,000x; bar=10 µm). In EX and DB groups (a and b), the smear plugs were partially removed, the intertubular dentin and the peritubular dentin were slightly etched and the edges of the dentinal tubules were clearly observed. For the SB and SiC groups (C, D), the smear layer and the smear plugs were not observed.

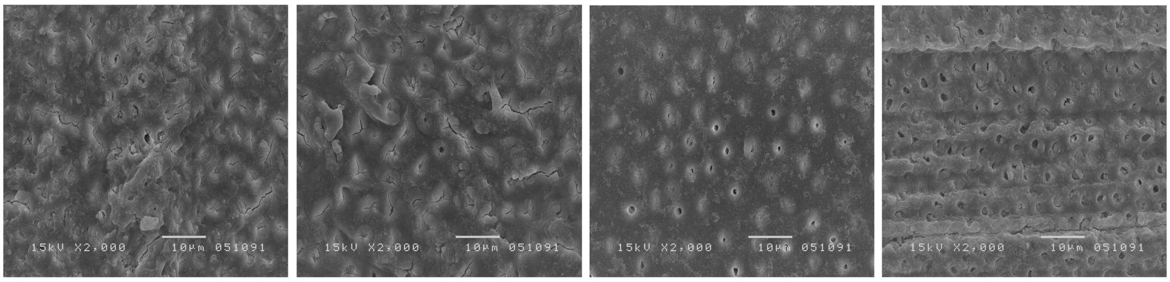

Fig. 4. a b c d
SEM images of dentin surfaces treated with Clearfil S^3 Bond. a: EX group; b: DB group; c: SB group; d: SiC group (original magnification 2000x; bar=10 µm). In EX and DB groups (a and b), the dentin surface remained covered with a great amount of smear layer. In SB group (c), the tubules orifices were evident but not enlarged and occluded with residual smear plugs. In SiC group (d), residual smear plugs were found still within the dentinal tubules although much of the peritubular dentin matrix was removed.

The SEM images of the top view of the prepared dentin surfaces treated with self-etching primer of Clearfil SE Bond and Clearfil S^3 Bond are shown in Figs. 3 and 4. Distinct zones which appeared to alter in specimens treated with Clearfil SE Bond were observed in EX and DB groups (Figs. 3a and b). In some area, the smear plugs were removed and the intertubular dentin and the peritubular dentin were slightly etched and the edges of the dentinal tubules were clearly observed. In other area, the residual smear layer and smear plugs were observed. The residual smear layer and smear plugs could be seen in all groups, and they appeared to be more conspicuous in the surfaces prepared with DB than with EX (Figs. 3a and b). In SB treated with Clearfil SE Bond group and SiC trated with Clearfil SE Bond group, the smear layer and the smear plugs were not observed, the intertubular dentin and the peritubular dentin of the tubule orifices were slightly etched, and the edges of dentinal tubules were clearly observed (Figs. 3c and d). In EX treated with Clearfil S^3 Bond and DB treated with Clearfil S^3 Bond groups, the dentin surface remained covered with a great amount of smear layer (Figs. 4a and b). In SB trated with Clearfil S^3 Bond group, the tubules orifices were evident but not enlarged and occluded with residual smear plugs (Fig. 4c). In SiC treated with Clearfil S^3 Bond group, residual smear plugs were found within the dentinal tubules although much of the peritubular dentin matrix was removed (Fig. 4d).

Discussion

In this study, it was shown that different dentin preparation methods significantly affect the microtensile bond strengths of both adhesive materials ($p<0.05$), and that μTBSs of Clearfil SE Bond and Clearfil S^3 Bond were significantly different when bonded to dentin irrespective of surface preparation methods. Therefore, the results require rejection of the null hypotheses that the dentin preparation methods do not affect the μTBS of self-etch adhesives to dentin; and that self-etch adhesive systems do not affected the μTBS to dentin.

The specimens prepared with SiC showed the highest bond strength in each adhesive group. The SiC abrasive paper created a reasonably flat substrate with fewer grooves and irregularities than other dentin preparation methods (Fig. 1d). Since thin and permeable smear layer is a better substrate for self-etch adhesives which have a mild acidity,[27] the smear layer generated by SiC had higher permeability and was probably easily dissolved and incorporated by the self-etching primers, possibly providing a strong hybrid layer and therefore showing the high bond strengths. Generally, an abrasive paper can provide a uniform smear layer on the flat dentin compared with rotary cutting instruments.

On the other hand, the specimens prepared with DB showed the lowest bond strength in each adhesive group. The diamond bur produced a rough surface (Fig. 1b). This irregularity produced by coarse diamond particles of the bur might be one of the reasons for the lowest bond strengths. The deep grooves created by a diamond bur can cause uneven distribution of smear debris with thicker accumulation at the base of the undulations.[30] This regional variation in smear layer thickness may have contributed to the uneven penetration of the acidic resin monomers through the smear layer, thereby compromising the bond strength of DB-prepared dentin which was applied with each adhesive. Moreover, high speed burs may induce more thermal and mechanical stress, compared to a steel bur with low speed, which affects underlying dentin. The dentin prepared with high-speed burs may also create micro cracks. The results in this study are in agreement with the previous studies showing the negative effect of surface preparation with a diamond bur on bond strength.[3,31] Ogata reported that a coarse diamond bur operated at high speed reduced the bond strength of two-step self-etch adhesive systems, due to inadequate infiltration of these self-etching primers into the prepared dentin surfaces covered with a thick smear layer.[23,26] Inoue et al.[32] reported that a certain amount of smear layer, produced with the regular-grit diamond bur, possibly decreased the bond strength of a one-step adhesive to the cut dentin.

Infiltration of a high concentration of acidic resin monomers through the smear layer into the underlying mineralized dentin matrix is difficult to accomplish.[32] It is well known that the quality and the quantity of the smear layer vary widely according to the way it is created.[13,14,23,26] It has been reported that the thick smear layer would be difficult substrate to achieve high bond strength.[22] Watanabe et al.[4] reported that the bond strength and the rate of monomer diffusion varied with the grit size of abrasive papers, which created different smear layer thickness. Ogata reported that the bond strengths of self-etching systems to dentin could be affected by differences in the quantity of residual smear layer because of the weak acidity of self-etching primer.[24]

The µTBS to dentin prepared using an ultrasonic abrasion with EX, when Clearfil SE Bond was applied, were similar to SB and SiC groups and higher than DB group. However, when Clearfil S^3 Bond was applied, the µTBS of EX group was statistically lower than SB and SiC group and was similar to DB group. These results suggested that the thickness of smear layer created by EX (approximately 8 µm, Fig. 2a) could be affected by the acidity of the self-etching primer of Clearfil SE Bond and Clearfil S^3 Bond (pH was 2.0 and 2.7 respectively by manufacturer's instruction). Fig. 3 revealed that the primer of Clearfil SE Bond seemed to be more effective for removing the smear layer and demineralizing the dentin surfaces prepared by EX because of lower pH, compared to Clearfil S^3 Bond because of lower pH. Figure 4 indicated that the monomers of Clearfil S^3 Bond were not able to infiltrate enough through the thick smear layer produced by EX, resulting lower bond strengths. Since both Clearfil SE Bond and Clearfil S^3 Bond are mild self-etch adhesives, they incorporate the smear layer and smear plugs into the hybridized complex and form a thin hybrid layer. Clearfil S^3 Bond has a higher pH and milder acidity, compared to Clearfil SE Bond and is expected to cause less demineralization of dentin. Consequently, the difference in pH of adhesives might affect the result of this study.

Neutralization of the acidic monomers by the buffering components in the thick smear layer could have accounted for the demineralization and penetration of adhesive monomers of self-etch adhesives into underlying dentin.[33] Since the acidity of self-etch adhesive systems is relatively mild, one of the current concerns is the buffering and obstructing potential of the smear layer related to differences in its thickness and nature.[24] The irregularities produced by EX might be another reason for the statistically significant lower bond strengths in both Clearfil SE Bond and Clearfil S^3 Bond bonded specimens. Thorough air-blowing for Clearfil S^3 Bond may lead to minimal thickness of adhesive at the top of the groove left by the preparations.[25] The low resin concentration could result in the resin composites displacing the oxygen-inhibited layers from the hybrid layer surface.[34] These factors may cause the reduction of the bond strength of Clearfil S^3 Bond. Further studies are required to evaluate the effect of the preparation methods with an ultrasonic abrasion on the durability of resin-dentin bond of self-etch adhesives systems.

The ultrasonic abrasion technique has not gained wide acceptance due to the time-consuming effect, and poor visibility caused by the abrasive slurry and maintenance of difficulty. Furthermore, caries and resilient restorative materials such as gold alloy could not be removed effectively with this technique.[35] Since Excavus (EX) which was equipped with a ultra-sonic unit that operate between 18,000 and 45,000 Cps.,[36] it would be very useful due to not only high precise cut but also less noise and vibration in the clinical situation.

Within the limitations of this study, it is concluded that: 1) the µTBS to dentin prepared using an ultrasonic abrasion with EX, when Clearfil SE Bond was applied, were similar to SB and SiC groups and higher than DB group. However, when Clearfil S^3 Bond was applied, the μTBS to dentin prepared with EX was statistically lower than SB and SiC group and were similar to DB group; and 2) the μTBS of Clearfil SE Bond was higher than that of Clearfil S^3 Bond when using the same dentin surface preparation.

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