Original Article

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Effect of modified smear layer on the bond strength of all-in-one adhesives to dentin

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Objectives: To evaluate the effect of modified smear layer using EDTA, NaOCl solutions with and without agitating application to solution on the bond strength of all-in-one adhesives to dentin.

Materials and Methods: One hundred and two non-carious third molars were cut perpendicular to the long axis at 2 mm. above CEJ. The standardized smear layer on dentin surface was created by polishing with 600-grit carbide paper. Then, all specimens were randomly divided to 6 groups of 15 teeth: 1) Distilled water, 2) Distilled water with agitation, 3) 17% EDTA, 4) 17% EDTA with agitation, 5) 1% NaOCl, and 6) 1% NaOCl with agitation. One ml of each solution was applied on the dentin surface for 60 s. Smear layer characteristic was observed under SEM. Three all-in-one adhesives (SE-ONE: SE, G-ænial bond: GB, Optibond all-in-one: OP) were applied following the instructions. All specimens were restored with resin composite (Z250XT). The micro-tensile bond strength test and failure mode analysis were investigated. Smear layer characteristic was observed in each experimental group of two teeth using SEM.

Results: Modified smear layer using both solutions significantly increased the bond strength in SE and OP groups with $p < 0.05$. Agitation application could produce significantly higher bond strength in GB and SE ($p < 0.05$). The clean dentin surface with some erosion and opening of dentinal tubules were found when modified with EDTA. While, NaOCl exhibited the reduction in smear layer thickness and dense smear layer.

Conclusion: Modified smear layer with EDTA and NaOCl could gain the bond strength of some all-in-one adhesives (SE, OP). The agitation application to pretreated solutions improved the bonding performance of some all-in-one adhesives (SE, GB)

Keywords: modified smear layer, agitation, all-in-one adhesives, micro-tensile bond strength, EDTA, sodium hypochlorite.

How to cite:

Introduction

Recently, all-in-one adhesives have been developed to reduce clinical application step, making their friendly use and reducing technique sensitivity. In restorative dentistry, when removing dental caries and preparation of the cavity, the debris called smear layer will cover the dentin substrate and may interfere the proper impregnation of resin monomers into the dentin substrate. This problem does not occur in etch and rinse system, but self-etch adhesives does, especially in low acidity type of self-etch adhesives.[1]

The acidity of all-in-one adhesives affects the capability of dissolving inorganic content of dentin substrate. Mild and ultra-mild one-step self-etch adhesives could decalcify dentin in shallow depth of resin penetration and partially dissolve smear layer.[2] This adhesive layer is

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included in the hybrid layer called hybridized smear layer or resin-smear complex. This layer seems to be a weak point of the adhesion. Previous study revealed that nanoleakage occurred mostly in this area.[3] There was a reduction in bond strength of self-etch adhesives on smear layer-covered dentin compared with smear layer free one.[4]

The quality of adhesives depends on many factors, the enhancing of resin impregnation could gain the better bond quality and durability. The modification of smear layer, a method to increase the bond quality, could help the reduction of a thick smear layer and enhance the resin penetration into dentin substrate. In endodontics, EDTA and NaOCl are used to remove smear layer in root canal system. EDTA can dissolve inorganic content of smear layer,[5] whereas NaOCl deproteinize the smear layer, increasing in wettability of resin impregnation[6]. For restorative dentistry, there are a controversy about the effect of NaOCl on the bonding performance of adhesives. Some previous studies showed the reduced bond strength of adhesives on NaOCl-treated dentin surface.[6,7] On the other hands, no significant effect on the bonding performance was illustrated.[8,9] From our knowledge and comprehension, there are less information about the effect of EDTA and NaOCl solution on the bonding performance of all-in-one adhesives. Therefore, the purpose of this study was to evaluate the effect of modified smear layer using EDTA and NaOCl solutions with and without agitating application to solutions on the bond strength of all-in-one adhesives to dentin. The null two hypotheses were 1) there was no significant difference in micro-tensile bond strength of all-in-one adhesives among pretreatment irrigating solutions. 2) there was no significant difference in micro-tensile bond strength between the agitation and no agitation techniques to all pretreatment irrigating solutions.

Materials & Methods

One hundred and two extracted non-carious human third molars, stored in 0.1% thymol solution at 4 °C, were cut horizontally at cervical 1/3 of the crown, 2 mm above the CEJ to expose a middle dentin with a low speed diamond saw (Isomet; Buehler, Evanston, IL, USA) under water lubricant. The cutting surface of all specimens were polished with 600-grit silicon-carbide paper under running water for 30 s each, creating a standardized smear layer. Then, the specimens were randomly divided into 6 experimental groups of 15 teeth as below.

1. Pretreatment with distilled water (control)
2. Pretreatment with distilled water + agitation (control)
3. Pretreatment with 17%EDTA
4. Pretreatment with 17%EDTA+agitation
5. Pretreatment with 1%NaOCl
6. Pretreatment with 1%NaOCl + agitation

The dentin surfaces in each group were pretreated as above mentioned with 1 ml of solution for 60 seconds. For the agitation application, the solution was agitated with micro-brush on cutting surface of dentin for 60 seconds. After that, all specimens were rinsed with 5 ml of distilled water for 30 seconds and air blown with dental triple syringe for 5 seconds. The pH of 17% EDTA and 1% NaOCl solutions were measured by pH meter (Model Oricon research 710A plus, Thermo Oricon, USA) prior to application.

Fifteen teeth in each experimental group (five teeth in each adhesive) were bonded with three adhesives; SE-ONE (Kuraray Noritake, Okayama, Japan), G-ænial Bond (GC, Tokyo, Japan) and Optibond all-in-one (Kerr, CA, USA) as the manufacturers' instructions. Then, a resin composite (Filtek™ Z250 XT shade A2, 3M ESPE, MN, USA) was placed on the treated dentin surface with 2 mm incremental layer in total of

4 mm height. The dentin surface area for placing a resin composite was 6×6 mm^2. The LED light curing unit (Bluephase N, Ivoclar Vivadent AG, Liechtenstein, Germany) was used for 20 second in each layer to completely polymerize the resin composite, which was covered all resin composite surface during polymerization. The light intensity of the curing unit was monitored by a light meter (light meter 200, Rolence enterprice Inc., Taoyuan, Taiwan) at 1,000 mW/cm^2 before used. After that, all specimens were stored in distilled water at 37 °C for 24 hours.

SEM Analysis of the Smear Layers

Two teeth in each experimental group were used to evaluate the effect of pretreatment solutions and agitation application on the smear layer covering dentin surface by SEM. Mid-coronal dentin disks were obtained in 2 mm thickness. A transverse groove in 1 mm depth was created on the apical side using a flame-shaped, superfine grit diamond burs (Diamond Point FG, Shofu, Kyoto,Japan) with an average diamond particle size of 30 µ. The dentin surface on the coronal side of disks was treated as mentioned above. The dentin disks were fixed in 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.4 for 12 h at 4°C, then rinsed with 20 ml of 0.2 M sodium cacodylate buffer at pH 7.4 for 1 h. Then, all dentin disks were rinsed in distilled water for 1 min, dehydrated in ascending grades of ethanol: 25% for 20 min; 50% for 20 min; 75% for 20 min; 95% for 30 min; and 100% for 60 min. After that, the disks were immersed in hexamethyldisilazane (HMDS) (Sigma Chemical Co., St Louis, MO, USA) for 10 min and then left drying in desiccator for 24 h. Ultimately, the disks were fractured into 2 halves and sputter coated with gold-palladium and observed under SEM (JSM-6610LV, JEOL LTD, Tokyo, Japan).

Table 1 Materials used in this study

Materials	Compositions	pH	Application procedure (Manufacturer's instruction)			
			Apply	Leave undisturbed	Air blow	Light cure
SE-ONE LOT 5T0034 (Kuraray Noritake, Okayama, Japan)	10-MDP, HEMA, Bis-GMA, hydrophobic aliphatic methacrylate, hydrophilic aliphatic dimethacrylate. water, ethanol, sodium fluoride, CQ, Initiators	2.3	10 s	-	5 s	10 s
G-ænial Bond LOT 1502071 (GC, Tokyo, Japan)	4-MET, TEGDMA, phosphoric ester monomer, acetone, water	1.5	20 s	10 s	5 s (maximum pressure)	10 s
Optibond all-in-one LOT 548742 (Kerr, CA, USA)	GPDM, GDM, HEMA, Bis-GMA, water, ethanol, acetone, silica filler, CQ, sodium hexafluorosilicate, ytterbium fluoride	2.5	20 s (scrubbing twice)	-	5 s	10 s

Abbreviations: HEMA, 2-hydroxyethyl methacrylate; TEGDMA, triethylene glycol dimethacrylate; Bis-GMA, bisphenol A glycerolate dimethacrylate; CQ, camphoroquinone; 10-MDP, 10-methacryloloxydecyl dihydrogen phosphate; GPDM,glycerolphosphatedimethacrylate; GDM, glycerol dimethacrylate; 4-MET, 4-methacryloxy ethyltrimellitic acid.

Micro-tensile bond strength test

The micro-tensile bond strength was measured at 24 hours water storage. A 1x1 mm^2, rectangular beam were prepared with low speed diamond saw (Isomet; Buehler, Evanston, IL, USA) under water lubrication. Four beams in each tooth with cross sectional areas of 0.9 mm^2 were collected to measure the bond strength. The beams obtained with peripheral enamel or with remaining dentin thickness less than 2 mm were excluded from the μTBS test. Total of 20 beams (n=20) in each experimental groups were obtained and subjected to micro-tensile bond strength test using the universal testing machine (EZ-S Shidmazu, Shidmazu Corp, Kyoto Japan) at a crosshead speed of 1 mm/min. Fracture specimens were firstly observed under a stereoscopic zoom microscope (Nikon SMZ1000, Nikon; Kanagawa, Japan) at 120X magnification and then under a scanning electron microscopy (SEM) at magnification of 80, 200 and 500. Failure mode was investigated and classified into 4 types

Type 1: Adhesive failure (>80% failure occurred at interface of resin dentin bond.)

Type 2: Mixed failure (Mixed with adhesive failure at the resin/ dentin interface and cohesive failure in resin and/or dentin.)

Type 3: Cohesive failure in dentin (>80% of the failure occurred in the underlying dentin.)

Type 4: Cohesive failure in resin (>80% of the failure occurred in the adhesive resin and/or overlying composite.)

Statistical analysis

The means and standard deviations of micro-tensile bond strength test (MPa) were analyzed by Kolmogorov Smirnov test (K-S test) to determine the distribution of the data and Levene's test was also used to test the homogeneity of variance. All micro-tensile bond strength values were analyzed using 3-way ANOVA (Independent variable: adhesive, irrigating solution, agitation). Multiple comparisons with Tukey's test were performed to compare among all independent variables. Failure modes were statistically analyzed by nonparametric Pearson chi-square test. All statistical analysis were analyzed using PASW® statistics 18 software (SPSS Inc., Chicago, IL, USA) at a 0.05 level of significance.

Results

The smear layer characteristic under SEM micrographs are shown in Figure 1-6. Pretreated with EDTA (Figure3) showed clean dentin surface, smear layer was removed and peritubular dentin was partially demineralized, while NaOCl-treated smear layer (Figure5) was thin and dense. The agitation application to solutions on dentin surface affected the characteristic of smear layer. The distilled water with agitation (Figure 2) was more effective to clean the superficial smear layer. The smear layer was looser and thinner than no agitation groups. Whereas, EDTA solution with agitation application (Figure 4) caused partial decalcification on dentin surface, demonstrating better chelating reaction of EDTA to dissolve mineral content on pretreated dentin surface. The opening of dentinal tubules and partially dissolved peritubular dentin have been noticed. The smear layer characteristic in NaOCl with agitation was thin and dense. However, no opening of dentinal tubules and the decalcification have been noticed

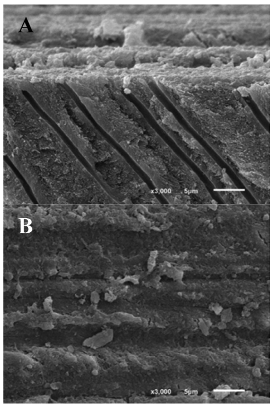

Figure 1 SEM images of distilled water pretreated group showed irregular and rough smear layer in cross cut view (A) and top view (B)

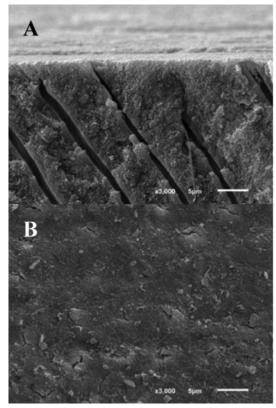

Figure 2 SEM images of distilled water with agitating application showed smooth and thin smear layer in cross cut (A) and top view (B)

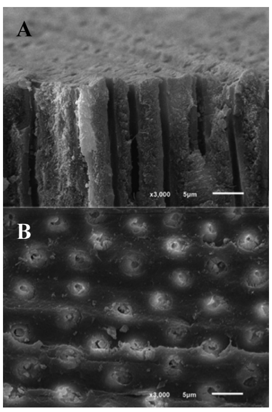

Figure 3 SEM images of EDTA pretreated group showed smear layer removal with partially demineralized peritubular dentin in cross cut (A) and top view (B)

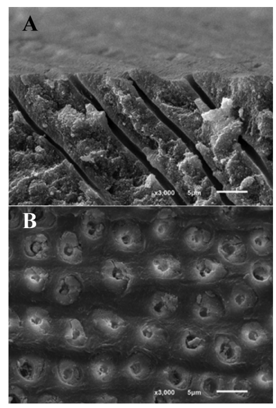

Figure 4 SEM images of EDTA with agitating application showed opening of dentinal tubules and demineralized peritubular dentin in cross cut (A) and top view (B)

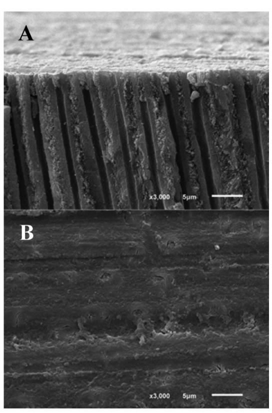

Figure 5 SEM images of NaOCl pretreated group showed dense smear layer and smear plug in cross cut (A) and top view (B)

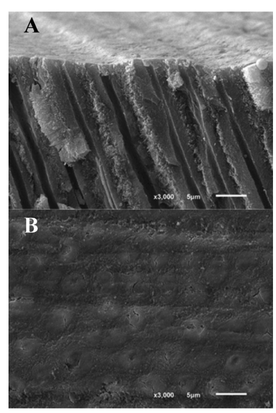

Figure 6 SEM images of NaOCl with agitating application showed smooth smear layer and smear plug with no opening of dentinal tubules in cross cut (A) and top view (B)

For micro-tensile bond strength test, three way ANOVA and multiple comparisons with Tukey's test revealed that the pretreatment solutions (distilled water, EDTA, NaOCl) and adhesives (SE, GB, OP) affected the bond strengths with interaction among these independent variables (p = 0.00). The mean and standard deviation of micro-tensile bond strength of each group are shown in Table 2. When comparing among the adhesives in any pretreatment solution with or without agitation application, Optibond All-in-one in group pretreated with NaOCl and agitation showed the significant highest micro-tensile bond strength (54.93±7.51 MPa), while G-ænial Bond in group pretreated with distilled water and agitation produced the lowest micro-tensile bond strength (27.37±5.30 MPa).

Consideration to the pretreatment solutions with or without agitation application in any adhesives, the pretreated dentin with 17%EDTA and 1%NaOCl solutions increased significantly micro-tensile bond strength of SE and OP comparing to distilled water treated dentin. However, there were no significant differences in bond strength of GB among the pretreatment solutions (p > 0.05). Moreover, the agitation application in pretreatment solution could increase significantly the micro-tensile bond strength of SE and GB. Nevertheless, this significant increased bond strength could not be noticed when Optibond All-in-one was applied either with agitation or without agitation

In failure mode, the fracture sites were observed to determine the failure pattern of adhesives under SEM at magnification of 80 and 200. Figure 7 and 8 demonstrated the distribution and representative SEM images of each failure pattern among groups.

Table 2 The mean and standard deviation (MPa) of micro-tensile bond strength of pretreatment groups with distilled water, EDTA and NaOCl either with or without agitation. (n=20)

Pretreatment solutions	All-in-one adhesives	No Agitation		Agitation	
Distilled water	1. SE	28.21±5.93	Aa	36.50±7.22	Ab
	2. GB	27.37±5.30	Aa	32.66±6.52	Ab
	3. OP	35.36±6.43	Ba	35.22±7.65	Aa
17% EDTA	4. SE	40.29±6.24	Ba	54.55±7.85	Bb
	5. GB	27.76±6.58	Aa	33.07±6.67	Ab
	6. OP	50.57±7.46	Ca	49.64±7.61	Ba
1% NaOCl	7. SE	34.97±4.65	Ba	39.74±5.13	Ab
	8. GB	27.85±6.08	Aa	33.19±6.00	Ab
	9. OP	52.58±7.86	Ca	54.93±7.51	Ba

The different capital letter indicated significant difference in each column and solution (p<0.05), whereas the different small letter indicated significant difference in each row (p<0.05)

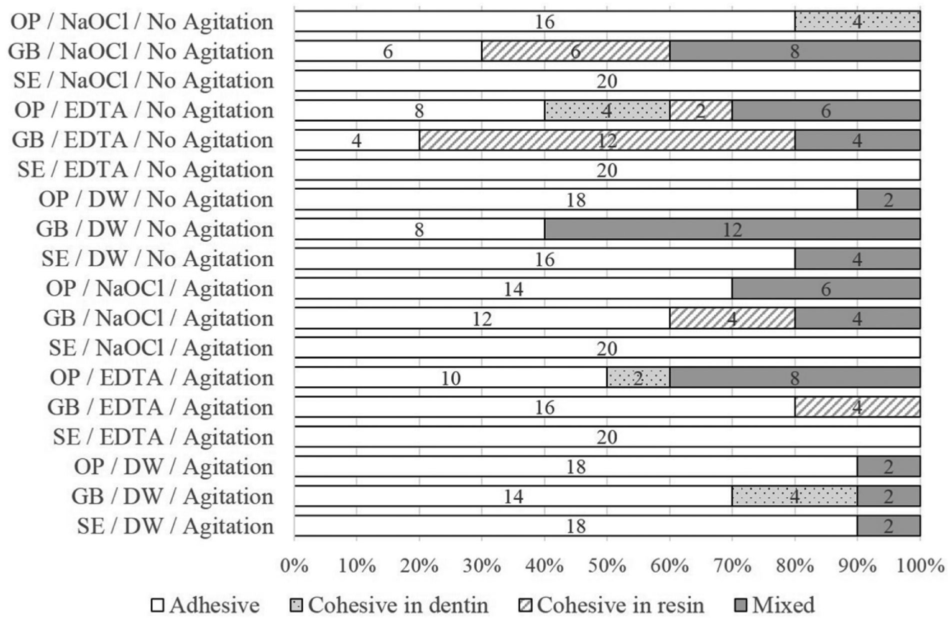

Figure 7 Failure mode distribution

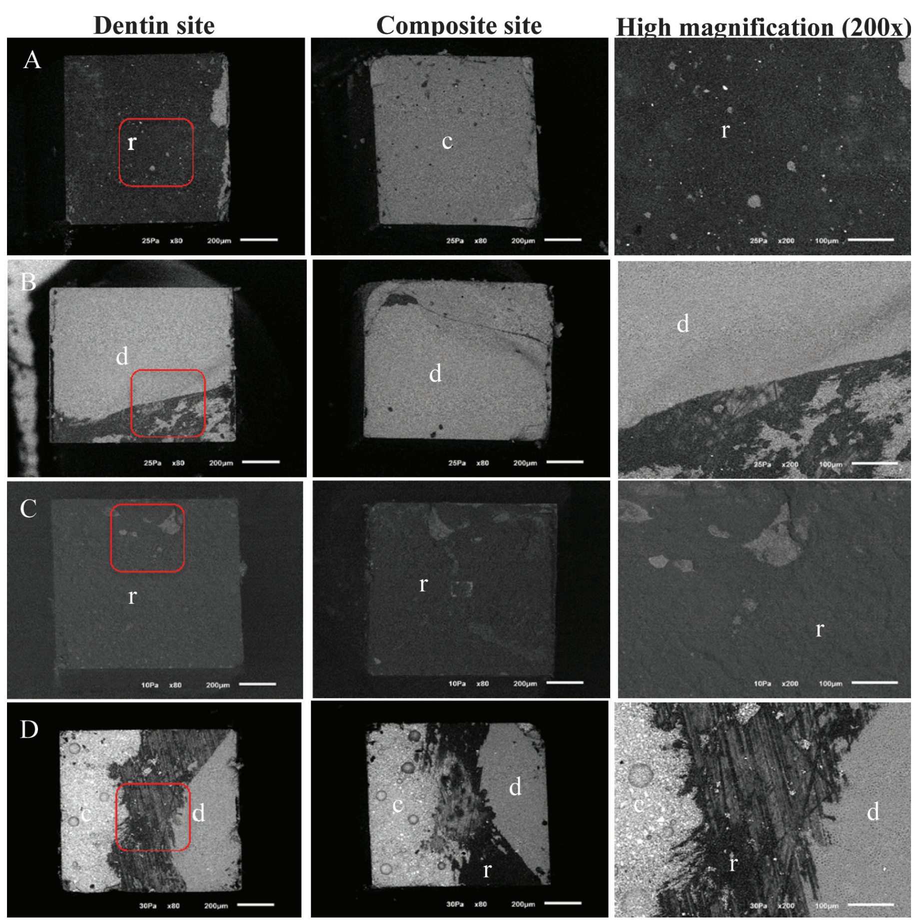

Figure 8 The representative SEM micrographs of each fracture site in failure mode analysis. Dentin site was on the left. Composite site was in the middle. The high magnification (200X) on the dentin site (red square) was shown in the right side; Adhesive failure (A), Cohesive failure in dentin (B), Cohesive failure in resin (C) and mixed failure (D). d; dentin, r; adhesive resin and c; resin composite

Non-parametric analysis with Pearson chi-square test of homogeneity of proportion was performed to analyze the distribution of fractographic. There was significant difference among all groups ($p<0.05$). The most failure mode was the adhesive failure between treated dentin and adhesive. Noticeably, the cohesive failure within adhesive resin was found in only G-ænial Bond. On the other hands, the cohesive failure in dentin was mostly found in Optibond All-in-one.

Discussion

From our results, the pretreated dentin substrate with EDTA, NaOCl solutions significantly increased the bond strengths of some all-in-one adhesives (SE, OP). In consequence, the hypothesis that there was no significant difference in micro-tensile bond strength of all-in-one adhesives among pretreatment irrigation solutions has been partially accepted. This result was consistent with the previous studies that pretreated with EDTA and NaOCl could improve the bond strength of self-etch adhesives.[8,9]

Theoretically, the smear layer is composed of inorganic and organic contents. The modified smear layer with NaOCl and EDTA enhanced the penetration of acidic monomers into underlying dentin.[5,10,11] EDTA, mild etching effect, partially demineralized the dentin and removed some smear plugs (Figure 3). Therefore, it enhanced the infiltration and the chemical reaction of the resin monomers to the underlying dentin substrate.[12] The concentration, pH and contact time of EDTA affected on the chelating mechanism.[13,14,15] The application of 17% EDTA for 60 s, pH=7.45, showed an optimal for removing smear layer and facilitated the bond strength. Even though the pH of 17% EDTA, mild basic solutions, may increase the surface pH and neutralize the reaction of acidic functional monomers to dentin substrate, but there was no negative effect in our study. The rinsing off with distilled water following EDTA treatment could reduce the surface pH, reducing the neutralized reaction of acidic monomer. The application of 1% NaOCl also enhanced the immediate bond strength of some all-in-one adhesives. This 1% NaOCl could deproteinize the organic smear layer and increase wetting ability of dentin substrate.[16] Also, pretreatment with NaOCl decreased an amide:phosphate ratio without altered in carbonate:phosphate ratio in FTIR spectroscopy.[6] This indicated that NaOCl decreased only organic phase of smear layer and dentin substrate , but had no effect on the chemical adhesion with inorganic content of dentin substrate. However, our result was contrary to Lai et al., which showed that application of 5.25%NaOCl for 60 s had negative effect on bond strength when 5.25% NaOCl for 1 min was applied.[17] While, our study used 1%NaOCl for 60 s (pH 12.63) to modify smear layer following with distilled water rinsing. SEM revealed a thin and dense smear layer without opening of dentinal tubules (Figure 5). It seems that the oxidizing reaction of 1%NaOCl deproteinized only the denatured collagen on superficial smear layer. Previous study stated the prolong contact time and high concentration of NaOCl could hamper the bond strength of self-etch adhesives[18] as the residual chloramine-derived free radicals, which this residue could cause the premature termination of monomers propagation. Moreover, the increase of surface pH from high concentration of NaOCl could buffer the acidity of resin monomers to demineralize on dentin substrate. However, the rinsing with distilled water after 1%NaOCl application in our study might reduce the residual chloramine-derived free radical. This would decrease the effect of residual chloramine-derived free radicals on the adhesion of adhesives. Therefore, the use of 1%NaOCl for 60 s with or without agitation in our study would positively effect on the bond strength of one-step self-etch adhesives. The concentration and contact time of NaOCl application in our study might be optimized to facilitate the bond strength without adverse effect from residual chloramine-derived free radicals. Our result was consistent with Thanatvarakorn O et al. [19] Smear layer deproteinizing could improve the bond strength of one-step self-etch adhesives, eliminating hybridized smear layer and preventing reticular nanoleakage formation in resin–dentin bonding interface.

Acidity of self-etch adhesives would also affect on the bond strength by the penetration of acidic monomer through the smear layer. The more acid contains, the more penetration is[1]. The micro-tensile bond strength of G-ænial bond showed no significant difference among the pretreatment with EDTA, NaOCl and distilled water. This result may be due to the pH of G-ænial bond (pH=1.5), which is more acidity than SE-ONE (pH=2.3) and Optibond All-in-one (pH=2.5). The strong acidity of self-etch adhesive could dissolve the thick smear layer to underlying dentin rather than the weak one. G-ænial bond, the intermediate one-step self-etch adhesives, could resolve the smear layer and infiltrate into underlying dentin whether the pretreated solutions to dentin substrate were used or not.[20,21] Moreover, the thickness of smear layer had an influence on the penetration ability of mild self-etch adhesives to the demineralized dentin.[22,23] Therefore, the modification of the smear layer by various pretreatment solutions would not alter the bond strength of G-ænial bond.

The agitation application to solutions on dentin provided significant increased the bond strength of SE-ONE and G-ænial bond. The agitation to solutions would facilitate a fresh EDTA and NaOCl to react with smear layer and dentin substrate. It also transferred the energy to solutions. Even in distilled water, agitated motion would provide cleaner and thinner smear layer (Figure 2, 4, 6). In mild self-etch adhesive, the reduction of smear layer thickness would create better the resin penetration of adhesive to dentin substrate.[24] However, no any research have been performed on the effect of the reduction of smear layer thickness to the chemical reaction of functional monomer in self-etch adhesive on dentin. Thus, further study should be established to comprehend this effect.

However, Optibond All-in-one showed no significant difference between agitation and no agitation application to pretreated solutions (EDTA, NaOCl, distilled water). The manufacturer's instruction of Optibond All-in-one suggested applying the adhesive with scrubbing motion for 20 s twice. Scrubbing or agitation of all-in-one adhesives effectively removed smear layer and chasing water,[25] refreshed the resin monomers to interact with dentin substrate[26] and enhanced the reaction of resin monomers. Agitation to adhesives could gain both the immediate and long term bond strength.[27] Therefore, the hypothesis that there was no significant difference in micro-tensile bond strength between the agitation and no agitation techniques to all pretreatment irrigating solutions has been partially accepted.

There are many factors influenced the bond strength of adhesives such as type of cross linking monomers[28], degree of conversion[29], remaining solvent[30,31] and type of fillers in adhesives[32]. The statistical analysis showed the pretreatment solutions and adhesives impacted on the micro-tensile bond strength. The chemical components of all-in-one adhesives were obviously distinct such as the functional monomers. In our study, SE-ONE produced higher bond strength than G-ænial bond. The composition of SE-ONE is 10-MDP, which is effective to bond with hydroxyapatite. On the other hand, the bonding potential of 4-MET, functional monomer of G-ænial bond, was substantially lower than 10-MDP[33]. Moreover, GPDM in Optibond All-in-one composes of an acidic phosphate group for demineralizing the tooth structure and for chemical interaction to the calcium ions within the tooth structure. Its two methacrylate functional groups can copolymerize with other methacrylate monomers. This provides the increase in crosslinking density and enhances mechanical strength for the polymerized adhesive.[34] Therefore, Optibond All-in-one could produce the highest bond strength on any modified dentin.

Conclusions

Within the limitation of this study, the modification of the smear layer by pretreatment with NaOCl and / or EDTA combined with agitation application could improve the bond strengths of some all-in-one adhesives. The pretreated solutions altered the characteristic of smear layer and dentin substrate. However, this study evaluated only the immediate bond strength. Therefore, the long-term durability should be further investigated.

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