The First Step of Writing a Manuscript

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14 Microtensile bond strength to dentin and enamel of **self-etch** vs. **etch**-and-rinse modes of universal **adhesives**.
Cite Cruz J, Sousa B, Coito C, Lopes M, Vargas M, Cavalheiro A.

16 Effect of **adhesive** resin cements on bond strength of ceramic core materials to dentin.
Cite Gundogdu M, Aladag LI.

21 A novel prime-&-rinse mode using MDP and MMPs inhibitors improves the dentin bond durability of **self-etch adhesive**.
Cite Xu J, Li M, Wang W, Wu Z, Wang C, Jin X, Zhang L, Jiang W, Fu B.

15 Bonding of universal **adhesives** to dentine--Old wine in new bottles?
Chen C, Niu LN, Xie H, Zhang ZY, Zhou LQ, Jiao K, Chen JH, Pashley DH, Tay FR.

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2. Materials and methods

2.1. *Specimen preparation*

Following ethical approval by the Ethics Committee of Tokyo Medical and Dental University under protocol number 725, extracted human third molars were collected, and stored in distilled water containing 0.1% thymol solution at 4 °C within a six-month period prior to the experiments. Fifty-six flat dentin surfaces were ground using a model trimmer perpendicular to long axis of the tooth under water lubrication, and then wet-polished using 600-grit SiC paper for 30 s to create a standardized smear layer. Half of the specimens was subjected to smear layer deproteinizing procedure by treating the smear layer-covered dentin surface with 50 ppm HOCl (Comfosy®, Haccpper Advantec Co., Tokyo, Japan) solution for 15 s, and then rinsed with water for 30 s. After air-drying, a reducing agent (*p*-toluenesulfinic acid salt; Accel®, Sun Medical Co. Ltd., Kyoto, Japan) was applied to the HOCl-treated dentin surface for 5 s and air-dried. The remaining half of the specimens was used as controls (without smear layer deproteinizing). The specimens in smear layer deproteinizing and control groups were randomly divided into 4 subgroups (n = 7). One of four kinds of one-step self-etch adhesives; Clearfil™ Bond SE One (SE; Kuraray Noritake Dental Inc., Japan), Scotchbond™ Universal (SU; 3 M ESPE, USA), BeautiBond Multi (BB; Shofu, Japan), and Bond Force (BF; Tokuyama Dental, Japan) was applied to dentin surface according to manufacturers' instructions (Table 1). Subsequently, three increments of resin composite (Clearfil AP-X; Kuraray Noritake Dental Inc., Japan) were built up on the dentin surface with each increment being light cured (830 mW/cm^2; Optilux 501, Kerr, Orange, CA, USA) for 20 s. The specimens were stored in 37 °C water for 24 h.

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2.2. *Microtensile bond strength (μTBS) test*

Each bonded specimen was sectioned parallel to the long axis of the tooth into beams with a bonded surface area of $1.0 \pm 0.1\,mm^2$ using a slow-speed water-cooled diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA). Four beams at the center of each bonded specimen were subjected to μTBS testing in a universal testing machine (EZ test; Shimadzu, Kyoto, Japan) at a crosshead speed of 1 mm/min. The data were analyzed using two-way ANOVA, post-hoc Tukey HSD test, and t-test with the significant level of 0.05. Statistical analysis was carried out using SPSS version 22.0 (SPSS, Chicago, IL, USA).

2.3. *Failure mode analysis*

After the μTBS test, both the dentin and composite surfaces of the fractured specimens underwent a serial dehydration process and were observed using a scanning electron microscope (SEM; JSM-5310LV, JEOL, Tokyo, Japan) for failure mode determination. The predominant failure over 80% of entire surface area was considered and was classified as one of the following; cohesive within dentin, adhesive at dentin/adhesive interface, adhesive at adhesive/resin composite interface, cohesive within resin composite, or mixed (adhesive and cohesive failure occurred). Failure modes were analyzed for statistically significant differences by the non-parametric Pearson chi-square test at a significant level of 0.05.

2.4. *Nanoleakage observation*

Additional specimens prepared as described above, using low-viscosity composite (Protect Liner F; Kuraray Noritake Dental Inc., Tokyo, Japan), were subjected to a silver nitrate staining technique (n = 2 per subgroup). The specimens were cut into two 0.9-mm thick slabs perpendicular to the bonded interface from the center of the tooth. After nail varnish coating, leaving a 1-mm window around the bonded interface, the slabs were immersed in ammoniacal silver nitrate solution for 24 h, rinsed thoroughly with distilled water, and then immersed in photo-developing solution for 8 h under a fluorescent light. Specimen fixation was achieved by processing through Karnovsky's solution, osmium tetroxide, and dehydrated in ascending ethanol series (50–100%) before embedding in epoxy resin [25]. Using an ultramicrotome and a diamond knife (Diatome Ltd., Bienne, Switzerland), 70-nm thick ultrathin sections were prepared and collected on 150-mesh copper grids. The nanoleakage expression of the bonded interface was examined using a TEM operating at 75 kV.

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1. Introduction

Recently, one-step self-etch adhesives with quicker application times and easier handling are being increasingly used in the clinic. Their bond strengths [1,2] and polymerization behavior [3] have improved over time. They are known to form hybridized smear layer on the authentic hybrid layer at the adhesive interface, because they cannot completely remove the smear layer due to their mild acidity [4]. Remnants of the smear layer on the adhesive surface have been purported to adversely affect the dentin bonding performance of self-etching adhesives, because they act as a selective barrier for monomer infiltration [5], giving rise to a physical weak link in the interface [6]. Moreover, their porous characteristics incorporate a certain amount of water [7], which lowers the degree of resin monomer conversion [8,9] and forms nanoleakage in the adhesive layer [10,11].

The dentin smear layer is composed of disorganized organic debris binding mineral particles [12]. Generally, self-etching adhesives can dissolve and remove the mineral phase in the smear layer, but they leave organic debris on the dentin surface, which is not dissolved [13]. Some researchers have demonstrated that treatment with an oxidizing/deproteinizing agent, such as sodium hypochlorite (NaOCl) and hypochlorous acid (HOCl) solutions, can dissolve and remove the organic phase of smear layer, leading to an increased mineral to organic ratio at the smear layer-covered dentin surface [14,15] and thinning of the smear layer [16,17]. Smear layer deproteinizing with HOCl solution, using in combination with a two-step self-etch adhesive (Clearfil SE Bond), can eliminate the hybridized smear layer and prevent nanoleakage formation at the resin–dentin interface [15]. These results indicated that removal of the organic phase of the smear layer would promote further infiltration of resin monomer into the underlying dentin without formation of hybridized smear layer [15]. Additionally, increasing the mineral/organic ratio on dentin surface by smear layer deproteinizing might be advantageous for chemical interaction of acidic functional monomers with hydroxyapatite [18–20]. Regarding NaOCl solution, several researchers have demonstrated that the residual oxidizing effect on NaOCl-treated dentin would affect resin polymerization, leading to a reduction in bond strengths [17,21] and an increase in nanoleakage expression [18,22]. However, these negative effects of NaOCl pretreatment on dentin bonding can be reversed by the subsequent application of reducing/antioxidant agents [21,23]. On the other hand, single pretreatment with HOCl solution had shown to significantly improve bond strengths of self-etching adhesive to caries-affected dentin [16]. Although there were no improvements in bond strengths to normal dentin [24], the quality of hybrid layer was improved as the hybridized smear layer and reticular nanoleakage were eliminated [15].

However, the effects of smear layer deproteinizing with oxidizing agents on bond strengths and nanoleakage expression at the adhesive interface might be dependent upon the type of self-etching adhesive, because of the differences in acidic functional monomers, hydrophilic and hydrophobic monomer compositions, polymerization catalyst, organic solvent etc. There is little information on the effect of smear layer deproteinizing on the dentin interface bonded to one-step self-etch adhesives. Therefore, the aim of this study was to evaluate the effect of smear layer deproteinizing by pretreatment with HOCl solution on dentin bond strengths and nanoleakage expression at the interface using one-step self-etch adhesives.

The null hypothesis was that there was no difference neither in microtensile bond strength nor nanoleakage expression at the adhesive interface of smear layer-deproteinized dentin and no-pretreated smear layer-covered dentin bonded to each one-step self-etch adhesive.

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3. Results

3.1. *Microtensile bond strength (μTBS) test*

The results of the μTBS test are summarized in Table 2. Two-way ANOVA revealed that μTBS was influenced by smear layer deproteinizing ($p < 0.001$) and by type of adhesive ($p < 0.001$). There was a significant interaction between two independent variables ($p = 0.028$). Smear layer deproteinizing with HOCl solution could significantly improve μTBS of SE, SU, and BB ($p < 0.05$), but not for BF ($p > 0.05$).

3.2. *Failure mode analysis*

The percentage of failure modes in each group is summarized in Fig. 1. There were significant differences in failure mode distribution among the groups ($p < 0.05$). For SE, SU, and BB, failure at dentin/adhesive interface or mixed-failure mainly occurred. On the other hand, the BF specimens predominantly failed at the adhesive/resin composite interface. For SE, SU and BF, there were no significant differences in failure mode distribution between the control and smear layer deproteinizing groups ($p > 0.05$). For BB, failure at dentin/adhesive interface significantly decreased in the smear layer deproteinizing group ($p = 0.011$).

3.3. *Nanoleakage evaluation*

In the control groups, TEM micrographs of the resin–dentin interfaces of SE, BB and BF showed the presence of hybridized smear layers, whereas the SU group exhibited only a thin authentic hybrid layer (approximately 0.15 μm) with the absence of a hybridized smear layer. For BB and BF, reticular nanoleakage patterns were observed throughout the hybridized complex, whereas for SE and SU, spotted patterns of nanoleakage were observed at the adhesive interface (Fig. 2A–D)

In the smear layer deproteinizing groups, there was no hybridized smear layer in all the adhesives. Nanoleakage in SE, BB, and BF was observed as a spotted pattern in the authentic hybrid layer, whereas in SU, nanoleakage was hardly exhibited. (Fig. 2E–H)

Table 2 – Mean and standard deviations of microtensile bond strengths (MPa).

Adhesive groups	Pretreatment	
	No pretreatment (control)	HOCl 15 s and Accel® 5 s
SE	54.2 (7.1)^{A,1}	63.2 (9.3)^{a,2}
SU	64.2 (8.0)^{B,1}	72.8 (8.6)^{b,2}
BB	22.2 (2.6)^{C,1}	30.9 (5.0)^{c,2}
BF	29.8 (4.7)^{D,1}	32.1 (7.0)^{c,1}

Significant differences in each column were represented by the different superscript letters. Significant differences in each row were represented by the different superscript numbers.

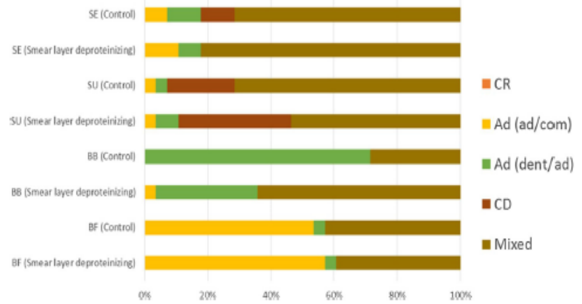

Fig 1 – Bar graph shows percentage of failure modes in each group. Chi-square test revealed significant differences in failure mode distribution among the groups ($p < 0.05$). Comparing the control and smear layer deproteinizing groups, only BB showed significant differences (decrease in adhesive failure at dentin/adhesive interface) ($p = 0.011$), whereas it was not significantly different in other adhesives ($p > 0.05$). The failure mode of BF was predominantly adhesive failure at adhesive/resin composite interface, irrespective of surface pretreatment. CR: cohesive failure within resin composite; Ad (ad/com): adhesive failure at adhesive/resin composite interface; Ad (dent/ad): adhesive failure at dentin/adhesive interface; CD: cohesive failure within dentin; Mixed: mixed failure.

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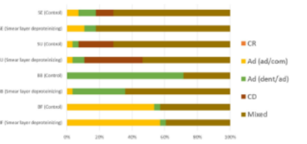

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failure at dentin/adhesive interface or mixed-failure mainly occurred. On the other hand, the BF specimens predominantly failed at the adhesive/resin composite interface. For SE, SU and BF, there were no significant differences in failure mode distribution between the control and smear layer deproteinizing groups ($p > 0.05$). For BB, failure at dentin/adhesive interface significantly decreased in the smear layer deproteinizing group ($p = 0.011$).

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In the control groups, TEM micrographs of the resin–dentin interfaces of SE, BB and BF showed the presence of hybridized smear layers, whereas the SU group exhibited only a thin authentic hybrid layer (approximately 0.15 μm) with the absence of a hybridized smear layer. For BB and BF, reticular nanoleakage patterns were observed throughout the hybridized complex, whereas for SE and SU, spotted patterns of nanoleakage were observed at the adhesive interface (Fig. 2A–D)

In the smear layer deproteinizing groups, there was no hybridized smear layer in all the adhesives. Nanoleakage in SE, BB, and BF was observed as a spotted pattern in the authentic hybrid layer, whereas in SU, nanoleakage was hardly exhibited. (Fig. 2E–H)

4. Discussion

The results of this study showed that smear layer deproteinizing could significantly improve bond strength in SE, SU and BB, eliminate the hybridized smear layer in SE, BB and BF, as well as eliminate reticular nanoleakage in BB and BF. Thus, the null hypothesis can be rejected.

NaOCl solution can effectively dissolve organic substrates from biological materials. NaOCl dissociates to Na^+ and OCl^- and establishes HOCl in water. The proportion of HOCl and the OCl^- in the solution depends on the pH [26,27]. That is, above pH 9, almost all HOCl ionizes to OCl^-, and at an approximate pH of 6, the concentration of HOCl is optimal and its dissociation is minimal [26,28]. The OCl^- ion is the determining factor for proteolytic activity, because OCl^- exerts the chlorination to protein, giving chloramines, which are then broken down into nitrogen-centered radicals and induce the fragmentation of proteins [29]. Additionally, OCl^- ion is capable of infiltrating mineralized collagen because of its small size [30]. On

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Adhesive groups	Pretreatment	
	No pretreatment (control)	HOCl 15 s and Accel® 5 s
SE	54.2 (7.1)[A,1]	63.2 (9.3)[a,2]
SU	64.3 (8.0)[B,1]	72.8 (8.6)[b,2]
BB	22.2 (2.6)[C,1]	30.9 (5.0)[c,2]
BF	29.8 (4.7)[D,1]	32.1 (7.0)[c,1]

Significant differences in each column were represented by the different superscript letters. Significant differences in each row were represented by the different superscript numbers.

the other hand, the "top-down" removal of the organic phase from the mineralized dentin by NaOCl is diffusion-controlled and is both time- and concentration-dependent [30]. When exposed to 2% NaOCl for 4 h, dentinal erosion was not evident by TEM observation, as indicated by the presence of an intact dentin surface, and dentinal tubules were of normal dimension with barely visible lateral branches of the tubules [30]. In this study, a 50 ppm HOCl solution (pH = 7.0) by acidifying NaOCl with HCl was used as a smear layer deproteinizing agent. In this pH condition, most of chlorine exists as HOCl, where OCl^- is minor species [29]. The Cl atom in HOCl behaves as Cl^+, which is a strong electrophile. Upon contacting to collagen, amino groups within the collagen triple helix, which are susceptible to electrophilic attack of Cl^+, are chlorinated and fragmentation occurs [31]. The dissolution of organic phase in the mineralized dentin by HOCl solution would be influenced by diffusion of HOCl molecule from the smear layer-covered dentin surface, which is dependent upon the concentration and the application time. The 15 s treatment of 50 ppm HOCl solution has a comparable organic dissolution ability to dentin surface to the same time treatment of 6% NaOCl solution, in which the mineral to matrix ratio increased at the smear layer-covered dentin surface [15] without the appreciable morphological alterations at smear layer-covered dentin surface on SEM images [16,17]. Presumably, the organic dissolution on smear layer-covered dentin surface by 50 ppm HOCl solution in this study, would be limited in the smear layer and not extend to underlying mineralized collagen due to lower concentration of chlorine (50 ppm) and shorter application time (15 s).

Smear layer deproteinizing by 50 ppm HOCl for 15 or 30 s could reduce reticular nanoleakage formation and eliminate hybridized smear layer at the adhesive interface with two-step self-etch adhesive, Clearfil SE Bond [15]. However, in our pilot study using the one-step self-etch adhesive, SE, the reticular pattern of nanoleakage within the hybrid layer severely increased (not shown). Generally, it is more difficult for one-step self-etch adhesives to polymerize than two-step self-etch adhesives because water/solvent in one-step self-etch adhesives cannot be completely removed from the adhesive layer, which hampers the polymerization process [32]. The results of the pilot study would be due to the interference of monomer polymerization of SE with the remaining oxidizing effect on the HOCl-pretreated dentin surface. Therefore, in this study, application with the reducing agent, Accel®, was undertaken in order to neutralize the oxidizing effect on the HOCl-treated dentin surface. The subsequent application of Accel® could reduce nanoleakage formation at the adhesive interface with SE, compared with the single pretreatment of HOCl solution.

In the no-pretreatment control groups, SE and SU produced hybrid layers of superior quality as the reticular pattern of nanoleakage was absent, but only for SU, whose hybridized smear layer could not be observed. It should be noted that SU created a very thin authentic hybrid layer (approximately 0.15 μm) due to the higher pH of the SU adhesive (pH = 2.7). Generally, the higher the adhesive pH, the lower the demineralization ability of the smear layer and the underlying dentin. Thus, the scrubbing technique is required on the application procedure of SU in order to facilitate the removal of the organic phase of the smear layer as well as dissolution of the mineral phase, leading to elimination of hybridized smear layer [33]. For SE, smear layer deproteinizing could eliminate the hybridized smear layer by the organic dissolubility of HOCl solution. Interestingly, there were no obvious differences in nanoleakage expression within the hybrid layer between the control and smear layer deproteinizing groups in both SE and SU, but smear layer deproteinizing could significantly increase the μTBS of both adhesives to dentin. These results indicate that smear layer deproteinizing could enhance the dentin bonding performance of SE and SU.

For BB and BF in the control groups, there were severe reticular nanoleakage formations within the hybrid layers. This pattern of nanoleakage indicated the area of water retained within the hybrid and adhesive layers, originating from poor monomer infiltration [34,35]. On the other hand, smear layer deproteinizing could improve nanoleakage expression with an absence of reticular pattern nanoleakage, eliminating the hybridized smear layer. These results indicate that dissolution/removal of the organic phase of smear layer could facilitate monomer infiltration into the underlying dentin. Additionally, μTBS of BB to dentin significantly increased with the smear layer deproteinizing. On the other hand, for BF, HOCl and Accel® pretreatment could not improve the μTBS to dentin, in which the specimens predominantly failed at the interface between adhesive/resin composite. In a previous study, it was reported that BF often exhibited a gap between the composite and the adhesive layer at the cavity floor in a Class I cavity [36]. BF contains HEMA/water and utilizes isopropyl alcohol as an organic solvent. HEMA is a hydrophilic monomer and attracts water, which cannot be completely removed from the adhesive layer by air-drying. Moreover, isopropyl alcohol makes evaporation more difficult by air-drying than acetone and ethanol [37]. Therefore, in BF, the oxygen inhibition layer on the light-cured adhesive layer would include larger amounts of residual water/solvents, which would impair co-polymerization with the hydrophobic resin composite. This might be a reason why in the case of BF, improvement of the quality of hybrid layer by smear layer deproteinizing could not lead to significant increase of μTBS value.

Larger amount of nanoleakage can increase fluid penetration within the hybrid layer and facilitate hydrolytic degradation at the adhesive interface over time [38]. Therefore, the reduction of nanoleakage formation with the elimination of hybridized smear layer by the application of deproteinizing agent would lead to an improvement in the long-term durability of dentin bond of one-step self-etch adhesives. In this study, Accel® containing p-toluenesulfinic acid sodium salt, which was introduced as a pretreatment with adhesive root canal sealer after endodontic irrigation with NaOCl, was used to reverse the residual oxidizing effect on HOCl-pretreated dentin. The salt of p-toluenesulfinic acid was reported to accelerate the polymerization of methyl methacrylate [39], therefore pretreatment of Accel® might promote polymerization of the adhesive on dentin surface, leading to improvement of long-term bonding performance of one-step self-etch adhesives to dentin [40]. However, Accel® might lose its polymerization-accelerating ability in this study on HOCl-pretreated dentin due to the neutralization with oxidants via free radical quenching mechanism. Further researches are

necessary about effect of application of deproteinizing agent in combination with polymerization-accelerating reducing agent on long-term stability of dentin bond with one-step self-etch adhesives.

5. Conclusions

Smear layer deproteinizing by HOCl oxidizing solution with subsequent application of Accel® reducing agent could improve the quality of adhesive interface of one-step self-etch adhesives to dentin by increasing dentin bond strength, eliminating the hybridized smear layer and/or preventing reticular nanoleakage formation within the hybrid layer.

REFERENCES

[1] Walter R, Swift Jr EJ, Nagaoka H, Chung Y, Bartholomew W, Braswell KM, et al. Two-year bond strengths of all-in-one adhesives to dentine. J Dent 2012;40:549–55.
[2] Manfroi FB, Marcondes ML, Somacal DC, Borges GA, Junior LH, Spohr AM. Bond strength of a novel one bottle multi-mode adhesive to human dentin after six months of storage. Open Dent J 2016;10:268–77.
[3] Sakano W, Nakajima M, Prasansuttiporn T, Foxton RM, Tagami J. Polymerization behavior within adhesive layer of one- and two-step self-etch adhesives: a micro-Raman spectroscopic study. Dent Mater J 2013;32:992–8.
[4] Hosoya Y, Tay FR, Garcia-Godoy F, Pashley DH. Ultrastructural examination of one-step self-etch adhesive bonded primary sound and caries-affected dentin. Am J Dent 2008;21:368–72.
[5] Pashley DH, Ciucchi B, Sano H, Horner JA. Permeability of dentin to adhesive agents. Quintessence Int 1993;24:618–31.
[6] Koibuchi H, Yasuda N, Nakabayashi N. Bonding to dentin with a self-etching primer: the effect of smear layers. Dent Mater 2001;17:122–6.
[7] Yoshida E, Uno S. Voids formation along the bonding interface between a smeared dentin surface and all-in-one adhesives. Dent Mater J 2004;23:643–9.
[8] Cadenaro M, Antoniolli F, Sauro S, Tay FR, Di Lenarda R, Prati C, et al. Degree of conversion and permeability of dental adhesives. Eur J Oral Sci 2005;113:525–30.
[9] Navarra CO, Cadenaro M, Codan B, Mazzoni A, Sergo V, De-Stefano-Dorigo E, et al. Degree of conversion and interfacial nanoleakage expression of three one-step self-etch adhesives. Eur J Oral Sci 2009;117:463–9.
[10] Mahdan MH, Nakajima M, Foxton RM, Tagami J. Combined effect of smear layer characteristics and hydrostatic pulpal pressure on dentine bond strength of HEMA-free and HEMA-containing adhesives. J Dent 2013;41:861–71.
[11] Shinoda Y, Nakajima M, Hosaka K, Otsuki M, Foxton RM, Tagami J. Effect of smear layer characteristics on dentin bonding durability of HEMA-free and HEMA-containing one-step self-etch adhesives. Dent Mater J 2011;30:501–10.
[12] Spencer P, Wang Y, Walker MP, Swafford JR. Molecular structure of acid-etched dentin smear layers?in situ study. J Dent Res 2001;80:1802–7.
[13] Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater 2011;27:17–28.
[14] Di Renzo M, Ellis TH, Sacher E, Stangel I. A photoacoustic FTIRS study of the chemical modifications of human dentin surfaces: II. Deproteination. Biomaterials 2001;22:793–7.
[15] Thanatvarakorn O, Nakajima M, Prasansuttiporn T, Ichinose S, Foxton RM, Tagami J. Effect of smear layer deproteinizing on resin–dentine interface with self-etch adhesive. J Dent 2014;42:298–304.
[16] Kunawarote S, Nakajima M, Foxton RM, Tagami J. Effect of pretreatment with mildly acidic hypochlorous acid on adhesion to caries-affected dentin using a self-etch adhesive. Eur J Oral Sci 2011;119:86–92.
[17] Taniguchi G, Nakajima M, Hosaka K, Iwamoto N, Ikeda M, Foxton RM, et al. Improving the effect of NaOCl pretreatment on bonding to caries-affected dentin using self-etch adhesives. J Dent 2009;37:769–75.
[18] Kambara K, Nakajima M, Hosaka K, Takahashi M, Thanatvarakorn O, Ichinose S, et al. Effect of smear layer treatment on dentin bond of self-adhesive cements. Dent Mater J 2012;31:980–7.
[19] Mine A, De Munck J, Cardoso MV, Van Landuyt KL, Poitevin A, Van Ende A, et al. Dentin-smear remains at self-etch adhesive interface. Dent Mater 2014;30:1147–53.
[20] Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, et al. Comparative study on adhesive performance of functional monomers. J Dent Res 2004;83:454–8.
[21] Lai SC, Mak YF, Cheung GS, Osorio R, Toledano M, Carvalho RM, et al. Reversal of compromised bonding to oxidized etched dentin. J Dent Res 2001;80:1919–24.
[22] Yiu CK, Garcia-Godoy F, Tay FR, Pashley DH, Imazato S, King NM, et al. A nanoleakage perspective on bonding to oxidized dentin. J Dent Res 2002;81:628–32.
[23] Prasansuttiporn T, Nakajima M, Kunawarote S, Foxton RM, Tagami J. Effect of reducing agents on bond strength to NaOCl-treated dentin. Dent Mater 2011;27:229–34.
[24] Kunawarote S, Nakajima M, Shida K, Kitasako Y, Foxton RM, Tagami J. Effect of dentin pretreatment with mild acidic HOCl solution on microtensile bond strength and surface pH. J Dent 2010;38:261–8.
[25] Ichinose S, Muneta T, Aoki H, Tagami M. TEM observation of seven retrieved total knee joints made of Co–Cr–Mo and Ti–Al–V alloys. Biomed Mater Eng 2003;13:125–34.
[26] Wang L, Bassiri M, Najafi R, Najafi K, Yang J, Khosrovi B, et al. Hypochlorous acid as a potential wound care agent: part I. Stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity. J Burns Wounds 2007;6:e5.
[27] Christensen CE, McNeal SF, Eleazer P. Effect of lowering the pH of sodium hypochlorite on dissolving tissue in vitro. J Endod 2008;34:449–52.
[28] Rutala WA, Weber DJ. Uses of inorganic hypochlorite (bleach) in health-care facilities. Clin Microbiol Rev 1997;10:597–610.
[29] Fukuzaki S. Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. Biocontrol Sci 2006;11:147–57.
[30] Gu LS, Huang XQ, Griffin B, Bergeron BR, Pashley DH, Niu LN, et al. Primum non nocere — the effects of sodium hypochlorite on dentin as used in endodontics. Acta Biomater 2017;61:144–56.
[31] Davies JM, Horwitz DA, Davies KJ. Potential roles of hypochlorous acid and N-chloroamines in collagen breakdown by phagocytic cells in synovitis. Free Radic Biol Med 1993;15:637–43.
[32] Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. J Dent Res 2002;81:472–6.
[33] Thanatvarakorn O, Prasansuttiporn T, Takahashi M, Thittaweerat S, Foxton RM, Ichinose S, et al. Effect of scrubbing technique with mild self-etching adhesives on dentin bond strengths and nanoleakage expression. J Adhes Dent 2016;18:197–204.

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necessary about effect of application of deproteinizing agent in combination with polymerization-accelerating reducing agent on long-term stability of dentin bond with one-step self-etch adhesives.

5. Conclusions

Smear layer deproteinizing by HOCl oxidizing solution with subsequent application of Accel® reducing agent could improve the quality of adhesive interface of one-step self-etch adhesives to dentin by increasing dentin bond strength, eliminating the hybridized smear layer and/or preventing reticular nanoleakage formation within the hybrid layer.

REFERENCES

[1] Walter R, Swift Jr EJ, Nagaoka H, Chung Y, Bartholomew W, Braswell KM, et al. Two-year bond strengths of all-in-one adhesives to dentine. J Dent 2012;40:549–55.
[2] Manfroi FB, Marcondes ML, Somacal DC, Borges GA, Junior LH, Spohr AM. Bond strength of a novel one bottle multi-mode adhesive to human dentin after six months of storage. Open Dent J 2016;10:268–77.
[3] Sakano W, Nakajima M, Prasansuttiporn T, Foxton RM, Tagami J. Polymerization behavior within adhesive layer of one- and two-step self-etch adhesives: a micro-Raman spectroscopic study. Dent Mater J 2013;32:992–8.
[4] Hosoya Y, Tay FR, Garcia-Godoy F, Pashley DH. Ultrastructural examination of one-step self-etch adhesive bonded primary sound and caries-affected dentin. Am J Dent 2008;21:368–72.
[5] Pashley DH, Ciucchi B, Sano H, Horner JA. Permeability of dentin to adhesive agents. Quintessence Int 1993;24:618–31.
[6] Koibuchi H, Yasuda N, Nakabayashi N. Bonding to dentin with a self-etching primer: the effect of smear layers. Dent Mater 2001;17:122–6.
[7] Yoshida E, Uno S. Voids formation along the bonding interface between a smeared dentin surface and all-in-one adhesives. Dent Mater J 2004;23:643–9.
[8] Cadenaro M, Antoniolli F, Sauro S, Tay FR, Di Lenarda R, Prati C, et al. Degree of conversion and permeability of dental adhesives. Eur J Oral Sci 2005;113:525–30.
[9] Navarra CO, Cadenaro M, Codan B, Mazzoni A, Sergo V, De-Stefano-Dorigo E, et al. Degree of conversion and interfacial nanoleakage expression of three one-step self-etch adhesives. Eur J Oral Sci 2009;117:463–9.
[10] Mahdan MH, Nakajima M, Foxton RM, Tagami J. Combined effect of smear layer characteristics and hydrostatic pulpal pressure on dentine bond strength of HEMA-free and HEMA-containing adhesives. J Dent 2013;41:861–71.
[11] Shinoda Y, Nakajima M, Hosaka K, Otsuki M, Foxton RM, Tagami J. Effect of smear layer characteristics on dentin bonding durability of HEMA-free and HEMA-containing one-step self-etch adhesives. Dent Mater J 2011;30:501–10.
[12] Spencer P, Wang Y, Walker MP, Swafford JR. Molecular structure of acid-etched dentin smear layers?in situ study. J Dent Res 2001;80:1802–7.
[13] Van Meerbeek B, Yoshihara K, Yoshida Y, Mine A, De Munck J, Van Landuyt KL. State of the art of self-etch adhesives. Dent Mater 2011;27:17–28.
[14] Di Renzo M, Ellis TH, Sacher E, Stangel I. A photoacoustic FTIRS study of the chemical modifications of human dentin surfaces: II. Deproteination. Biomaterials 2001;22:793–7.
[15] Thanatvarakorn O, Nakajima M, Prasansuttiporn T, Ichinose S, Foxton RM, Tagami J. Effect of smear layer deproteinizing on resin–dentine interface with self-etch adhesive. J Dent 2014;42:298–304.
[16] Kunawarote S, Nakajima M, Foxton RM, Tagami J. Effect of pretreatment with mildly acidic hypochlorous acid on adhesion to caries-affected dentin using a self-etch adhesive. Eur J Oral Sci 2011;119:86–92.
[17] Taniguchi G, Nakajima M, Hosaka K, Iwamoto N, Ikeda M, Foxton RM, et al. Improving the effect of NaOCl pretreatment on bonding to caries-affected dentin using self-etch adhesives. J Dent 2009;37:769–75.
[18] Kambara K, Nakajima M, Hosaka K, Takahashi M, Thanatvarakorn O, Ichinose S, et al. Effect of smear layer treatment on dentin bond of self-adhesive cements. Dent Mater J 2012;31:980–7.
[19] Mine A, De Munck J, Cardoso MV, Van Landuyt KL, Poitevin A, Van Ende A, et al. Dentin-smear remains at self-etch adhesive interface. Dent Mater 2014;30:1147–53.
[20] Yoshida Y, Nagakane K, Fukuda R, Nakayama Y, Okazaki M, Shintani H, et al. Comparative study on adhesive performance of functional monomers. J Dent Res 2004;83:454–8.
[21] Lai SC, Mak YF, Cheung GS, Osorio R, Toledano M, Carvalho RM, et al. Reversal of compromised bonding to oxidized etched dentin. J Dent Res 2001;80:1919–24.
[22] Yiu CK, Garcia-Godoy F, Tay FR, Pashley DH, Imazato S, King NM, et al. A nanoleakage perspective on bonding to oxidized dentin. J Dent Res 2002;81:628–32.
[23] Prasansuttiporn T, Nakajima M, Kunawarote S, Foxton RM, Tagami J. Effect of reducing agents on bond strength to NaOCl-treated dentin. Dent Mater 2011;27:229–34.
[24] Kunawarote S, Nakajima M, Shida K, Kitasako Y, Foxton RM, Tagami J. Effect of dentin pretreatment with mild acidic HOCl solution on microtensile bond strength and surface pH. J Dent 2010;38:261–8.
[25] Ichinose S, Muneta T, Aoki H, Tagami M. TEM observation of seven retrieved total knee joints made of Co–Cr–Mo and Ti–Al–V alloys. Biomed Mater Eng 2003;13:125–34.
[26] Wang L, Bassiri M, Najafi R, Najafi K, Yang J, Khosrovi B, et al. Hypochlorous acid as a potential wound care agent: part I. Stabilized hypochlorous acid: a component of the inorganic armamentarium of innate immunity. J Burns Wounds 2007;6:e5.
[27] Christensen CE, McNeal SF, Eleazer P. Effect of lowering the pH of sodium hypochlorite on dissolving tissue in vitro. J Endod 2008;34:449–52.
[28] Rutala WA, Weber DJ. Uses of inorganic hypochlorite (bleach) in health-care facilities. Clin Microbiol Rev 1997;10:597–610.
[29] Fukuzaki S. Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. Biocontrol Sci 2006;11:147–57.
[30] Gu LS, Huang XQ, Griffin B, Bergeron BR, Pashley DH, Niu LN, et al. Primum non nocere — the effects of sodium hypochlorite on dentin as used in endodontics. Acta Biomater 2017;61:144–56.
[31] Davies JM, Horwitz DA, Davies KJ. Potential roles of hypochlorous acid and N-chloroamines in collagen breakdown by phagocytic cells in synovitis. Free Radic Biol Med 1993;15:637–43.
[32] Tay FR, Pashley DH, Yoshiyama M. Two modes of nanoleakage expression in single-step adhesives. J Dent Res 2002;81:472–6.
[33] Thanatvarakorn O, Prasansuttiporn T, Takahashi M, Thittaweerat S, Foxton RM, Ichinose S, et al. Effect of scrubbing technique with mild self-etching adhesives on dentin bond strengths and nanoleakage expression. J Adhes Dent 2016;18:197–204.

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Conflict of interest statement

The authors declare that there are no conflicts of interest.

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A B S T R A C T

Objectives: This study aimed to investigate deproteinizing effect of sodium-hypochlorite (NaOCl) and mild acidic hypochlorous-acid (HOCl) pretreatment on smear layer-covered dentine and to evaluate their effects on morphological characteristics of resin–dentine interface with self-etch adhesive.

Methods: Human coronal-dentine discs with standardized smear layer were pretreated with 6% NaOCl or 50 ppm HOCl for 15 s or 30 s. Their deproteinizing effects at the treated smear layer-covered dentine surfaces were determined by the measurement of amide:phosphate ratio using ATR-FTIR analysis. In addition, using TEM, micromorphological alterations of hybridized complex and nanoleakage expression were evaluated at the interface of a self-etch adhesive (Clearfil SE Bond) to the pretreated dentine surface with or without subsequent application of a reducing agent (*p*-Toluenesulfinic acid salt; Accel®).

Results: Both pretreatments of NaOCl and HOCl significantly reduced the amide:phosphate ratio as compared with the no-pretreated group ($p < 0.05$), coincident with the elimination of the hybridized smear layer on their bonded interfaces. Nanoleakage within the hybrid layer was found in the no-pretreated and NaOCl-pretreated groups, whereas the subsequent reducing agent application changed the reticular nanoleakage to spotted type. HOCl-pretreated groups showed less nanoleakage expression in a spotted pattern, regardless of reducing agent application.

Conclusions: NaOCl and HOCl solutions could remove the organic component on the smear layer-covered dentine, which could eliminate the hybridized smear layer created by self-etch adhesive, leading to the reduction of nanoleakage expression within hybrid layer.

Clinical significance: Smear layer deproteinizing could modify dentine surface, giving an appropriate substrate for bonding to self-etch adhesive system.

Keywords:
Sodium-hypochlorite
Hypochlorous-acid
Smear layer deproteinizing
Attenuated total reflection Fourier transform infrared
Hybridized smear layer
Nanoleakage

Abstract: In the current trend of materials used for dentin hypersensitivity treatment, calcium–phosphate-containing desensitizers are expected to have advantages in oral environment. A newly formulated desensitizer containing tetracalcium phosphate and dicalcium phosphate anhydrous (CPD-100) was evaluated in comparison to oxalate containing desensitizer (SS) regarding permeability reduction (PR%) by measuring hydraulic conductance on the etched dentin discs *in vitro*. CPD-100 exhibited mean PR% of 91%, which significantly increased to 98% after immersion in artificial saliva (AS) for 4 weeks ($p < 0.001$), while SS showed a significant decrease from 99% to 93% ($p < 0.01$). SEM observation showed newly formed crystallites on CPD-100 treated dentin, which did not exist in SS treated dentin after AS immersion, suggesting that calcium oxalate inhibited formation of new calcium–phosphate minerals. Five-minute acid challenge did not significantly affect PR% of dentin treated by any of the desensitizers. The energy dispersive X-ray spectroscopy (EDS) analysis indicated that the formed layer of CPD-100 were minerals with similar Ca/P ratio to hydroxyapatite. In conclusion, the newly developed calcium–phosphate desensitizer has the potential to exhibit long-term stability in the oral environment, owing to its chemical properties that promote the crystal growth in salivary fluid.

J Biomed Mater Res Part B: Appl Biomater 101B: 303–309, 2013.

Key Words: calcium–phosphate, desensitizer, hypersensitivity, dentin permeability

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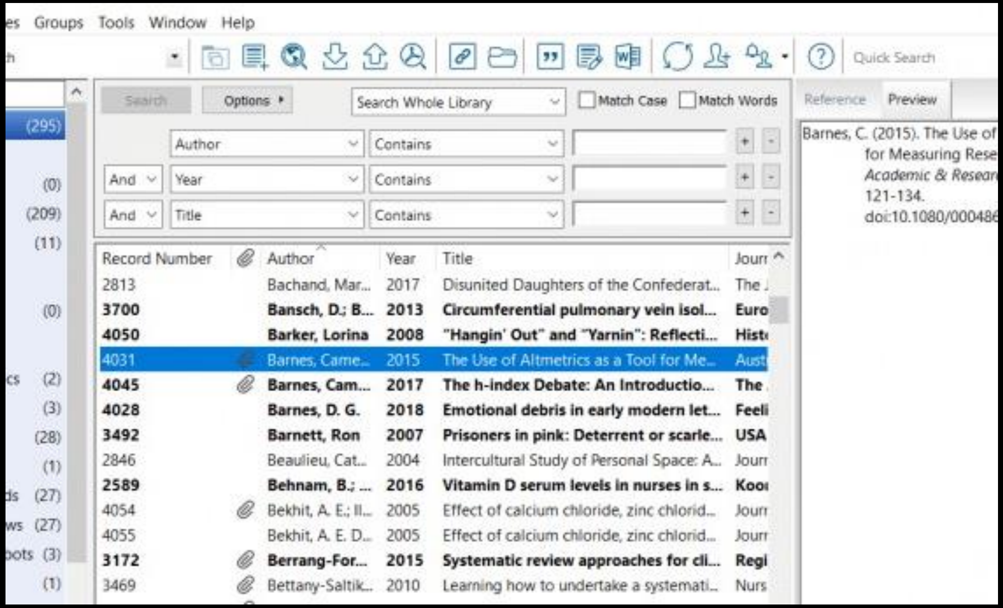
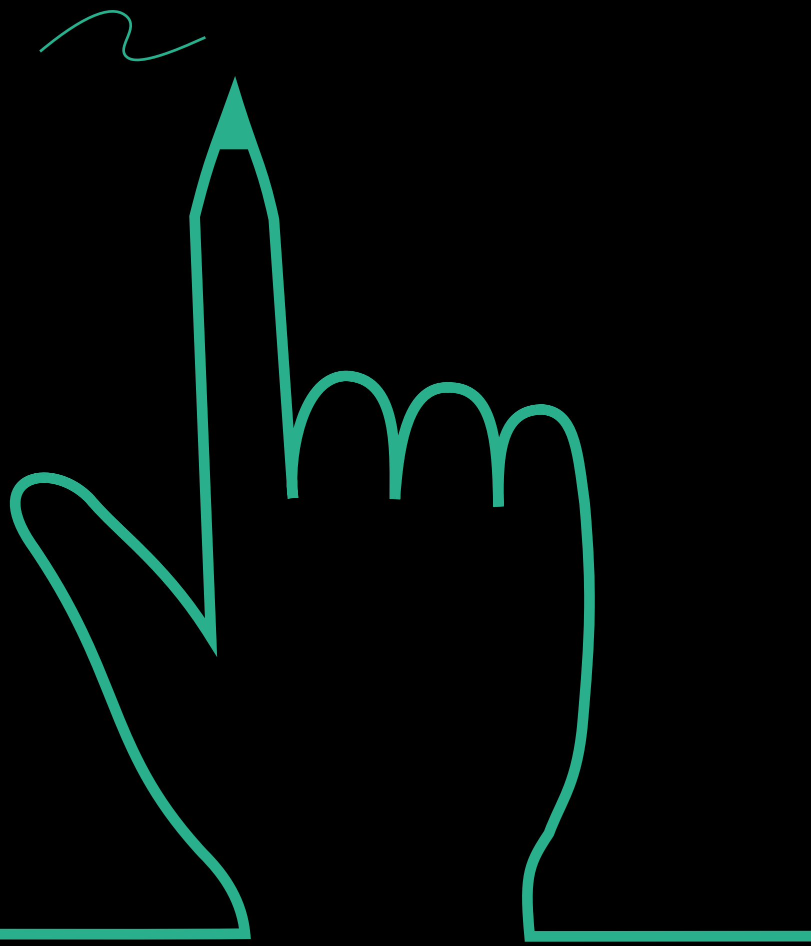


- ➢ Endnote*
- ➢ Sufficient number of references
- ➢ From published articles, books

How to write



Key for writing

Summarize information to your own words.

Put the references having similar findings together.

For beginners

List of frequently used words

Agree

- in agreement with
- in accordance with
- consistent with
- conforming to
- in line with
- as stated in

Disagree

- unlike
- conversely
- controversy
- contradict

Emphasize

- Interestingly
- It should be noted that….
- It is noteworthy that….

Finding

- It was evident….
- It was obvious….
- It was demonstrated…..

Rationale

- This could be attributed to….
- Due to….

Opposite

- However
- On the other hand
- On the contrary

For beginners

List of useful phrases

Introduction

- Previous studies have shown that…..
- There is little information about……
- ……remains unclear and needs to be clarified

Materials and methods

- In order to evaluate…, …..was performed
- …..using the method described by ……
- The data were analyzed using……

Results

- The results of……are summarized in Table 1.
- Fig. 1 exhibits ………
- As shown in Fig. 1,……
- ……… were observed
- Group A shows……..
- Compare with group A, group B……

Discussion

- It is in agreement with….
- It is in accordance with…
- Our findings are conforming to….
- The results of present study are in line with….
- Unlike previous studies,….
- Conversely,…..
- It is in contradiction to previous studies…..
- It is speculated that….
- It is hypothesized that….
- Within the limitation of this study,….
- Further study regarding…..should be performed
- Future study should…..

Final check

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