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Smear layer deproteinization with NaOCl and HOCl: Do application/wash-out times affect dentin bonding of one-step self-etch adhesives?

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This study investigated the influence of application/wash-out times of sodium hypochlorite (NaOCl) and hypochlorous acid (HOCl) on dentin bond strength of one-step self-etch adhesives (1-SEAs). Human coronal dentin discs with a standardized smear layer were pretreated with 6% NaOCl or 100 ppm HOCl for 5 s, 15 s, or 30 s, and washed out with water for 5 s, 15 s or 30 s with or without the application of Clearfil DC Activator (CDA). No pretreatment was used as a control. The discs were bonded with a 1-SEA (Bond Force II or Clearfil Universal Bond Quick) and microtensile bond strength (μTBS) was measured after 24 h. Pretreatment with NaOCl for 15 s and 30 s significantly decreased μTBS ($p<0.05$), irrespective of wash-out time. The application of CDA recovered μTBS but did not outperform the control group. Conversely, pretreatment with HOCl for 15 s and 30 s followed by 30 s wash-out time significantly increased μTBS of 1-SEAs ($p<0.05$), regardless of CDA application.

Keywords: Smear layer deproteinizing, Hypochlorous acid, Sodium hypochlorite, Microtensile bond strength, FTIR

INTRODUCTION

The use of self-etch adhesives (SEAs) is widespread owing to their excellent bonding to tooth substrates, low incidence of postoperative sensitivity, and easy handling. However, their mild acidity compared to phosphoric acid induces an incomplete removal of the smear layer, which is therefore partially incorporated in the adhesive interface[1,2]. The hybridized remnants of the smear debris are considered problematic, because they have no stable connection with the underlying intact dentin, rendering the adhesive interface prone to deterioration in the long term[3].

It has recently been reported that the application of 6% sodium hypochlorite (NaOCl) or 50–200 ppm hypochlorous acid (HOCl) solutions can dissolve the organic phase of the smear layer, thus increasing the mineral-to-organic ratio on the bonding surface and thinning the smear layer[4-10]. In addition, when bonding with SEAs, the deproteinizing pretreatment of dentin surfaces covered with a smear layer can prevent the formation of the hybridized smear layer, and it significantly improves the bonding of SEAs to caries-affected dentin, that is covered with a thicker and collagen-rich smear layer[4,6,8]. However, free radicals are produced during the action of deproteinizing agents, and their presence on the treated surfaces has been reported to adversely affect the polymerization of the adhesives through premature chain termination[11]. To overcome this issue, the subsequent application of antioxidants/reducing agents such as sodium p-toluenesulfinate has been proposed[7,10-14].

NaOCl solutions (2.5%–6%) are widely used in endodontics for debridement, deproteinization, and disinfection[15]. However, the extreme alkalinity of NaOCl (pH 10.8–13.2) makes it possibly toxic and irritating[16,17]. The active component of NaOCl that accounts for deproteinization is OCl^-, whose concentration is pH-dependent. OCl^- remains dissociated at pH levels above 9, whereas at a pH of approximately 6, it may form HOCl[18-20], whose chlorinating and oxidizing actions are stronger than those of OCl^- [21,22]. Therefore, NaOCl solutions mainly contain the less effective OCl^-. Additionally, NaOCl is difficult to wash away from the treated biological surfaces because of its high reactivity with amino acids[23].

In contrast, surface treatment with HOCl leaves significantly fewer chlorine residues than NaOCl[24]. HOCl solutions also have a lower pH than NaOCl and thus exhibit higher antimicrobial and oxidizing

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properties even at considerably lower chlorine concentrations[18,22,25-27]. Additionally, HOCl was shown to be non-irritating and non-sensitizing[20], as opposed to NaOCl. In oral applications, HOCl can be used as a mouth rinse[28], even though its taste is reportedly disagreeable and results in a greater dry tissue sensation than chlorhexidine[29]. Recently, it has also been suggested that HOCl solutions could serve as a therapeutic agent for periodontitis[22] or an endodontic irrigant[30]. In relation to smear layer deproteinization, it has been demonstrated that HOCl has a similar or better deproteinizing effect on smear layer-covered dentin than NaOCl[9]. Moreover, HOCl can be rinsed off the treated surface more easily than NaOCl, which could minimize the negative effect of residual oxidizing radicals on the polymerization of adhesives[31].

The promising effects of HOCl are presumably dependent on its application time and/or wash-out time; however, their influence has not been fully clarified to date. Therefore, the objective of this study was to determine the optimal application/wash-out time of 100 ppm HOCl for smear layer deproteinization. The effect of pretreatment with HOCl on the microtensile bond strength (μTBS) of one-step self-etch adhesives (1-SEAs) to dentin was compared to that of 6% NaOCl. The selected 1-SEAs differed in the content of adhesion-promoting and hydrophilic monomers. Additionally, attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy was used to assess the deproteinizing effects of the NaOCl and HOCl solutions by measuring changes in the amide-to-phosphate ratio on the dentin surfaces. Morphological alterations of the treated surfaces were investigated using a scanning electron microscope (SEM). The null hypothesis was that the application time and wash-out time of the deproteinizing solutions would not affect the μTBS of 1-SEAs to dentin and the dissolution of the organic phase of the dentin smear layer.

MATERIALS AND METHODS

Two 1-SEAs were used in this study, Bond Force II (BF2; Tokuyama Dental, Tsukuba, Japan) and Clearfil Universal Bond Quick (UBQ; Kuraray Noritake Dental, Tokyo, Japan). A sulfinate-containing dual-cure activator (Clearfil Dual Cure Activator; CDA; Kuraray Noritake Dental) was used as a reducing agent. While the instructions for use of BF2 advise against combination with other brands, CDA was used with both 1-SEAs, because Tokuyama Dental does not offer any sulfinate-containing agent and our pilot study confirmed the compatibility of BF2 with CDA. A resin composite (Clearfil AP-X; Kuraray Noritake Dental) was used for build ups of the μTBS specimens. The overview of these materials' composition and application procedure is presented in Table 1. A 6% NaOCl solution (pH 12.2; Jiaen 6%, Yoshida, Tokyo, Japan) and a 100 ppm HOCl solution (pH 6.8) were used as deproteinizing agents. The 100 ppm HOCl solution was prepared by diluting a 500 ppm HOCl solution (Dent Zia; Tokuyama Dental) with water, and its pH was adjusted to 6.8 with 1 M NaOH.

μTBS test

1. Specimen preparation

Two hundred extracted sound human third molars were collected and stored in periodically changed distilled water at 4°C. Their use was approved by the Ethics Committee of Tokyo Medical and Dental University (protocol number 2013-022). Within six months of extraction, occlusal enamel was removed using a model trimmer under water cooling, and the exposed midcoronal

Table 1 Materials used in this study

Material	Batch	Composition	Application procedure
Bond Force II (Tokuyama Dental, Tsukuba, Japan) pH 2.8	130	self-reinforcing phosphoric acid monomer, Bis-GMA, TEGDMA, HEMA, alcohol, water, camphorquinone, sodium fluoride	1. Apply adhesive and wait for 10 s 2. Dry with gentle air for 5 s 3. Light-cure for 10 s
Clearfil Universal Bond Quick (Kuraray Noritake Dental, Tokyo, Japan) pH 2.3	6K0215	10-MDP, Bis-GMA, HEMA, hydrophilic amide monomer, colloidal silica, ethanol, dl-camphorquinone, accelerators, water, sodium fluoride	1. Apply adhesive with rubbing motion (no waiting time) 2. Dry with gentle air for 5 s 3. Light-cure for 10 s
Clearfil DC Activator (Kuraray Noritake Dental)	CH0009	arylsulfinate salt, accelerators, ethanol	1. Apply activator and wait for 5 s 2. Dry with gentle air for 5 s
Clearfil AP-X (Kuraray Noritake Dental)	BR0104	Bis-GMA, TEGDMA, camphoquinone, photoinitiators, pigments, silanated barium glass, silanated silica	1. Apply the resin composite with a maximum thickness of 2 mm 2. Light-cure for 10 s 3. Repeat three times

Bis-GMA: bisphenol A-glycidyl methacrylate, TEGDMA: triethyleneglycol dimethacrylate, HEMA: 2-hydroxyethyl methacrylate, 10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate

dentin surfaces were ground with 600-grit SiC paper under running water for 30 s to produce a standardized smear layer. They were then randomly divided into three groups according to the surface pretreatment conditions: no pretreatment (control group), pretreatment with 6% NaOCl or 100 ppm HOCl. A drop of the deproteinizing solution was gently spread over the entire dentin surface using a disposable microbrush (regular size, Shofu, Kyoto, Japan) for 5 s, 15 s, or 30 s. After the pretreatment, the deproteinized specimens were further divided into four groups according to the wash-out time and application of CDA: 5 s, 15 s or 30 s of washing out without the application of CDA, and 30 s of washing out followed by the application of CDA for 5 s. After the dentin surfaces were air-dried, either of the 1-SEAs (BF2 or UBQ) was applied according to the manufacturer's instructions (Table 1) and light-cured for 10 s (1,000 mW/cm^2; Valo, Ultradent, South Jordan, UT, USA). Lastly, the bonded dentin surfaces were built up with three increments of Clearfil AP-X that were light-cured for 20 s each. The study design is illustrated in Fig. 1.

2. μTBS test

After 24 h of storage in distilled water at 37°C, the specimens were cut in two directions to fabricate beams with a cross-sectional area of 1.0±0.1 mm^2 using a slow-speed diamond saw (Isomet, Buehler, Lake Bluff, IL, USA) under water cooling. Four beams from the central part of the specimens were used, adding up to 16 beams per group. Each beam was glued onto a μTBS testing jig and stressed in tension in a universal testing machine (EZ-SX Test, Shimadzu, Kyoto, Japan) at a crosshead speed of 1 mm/min.

The μTBS data were statistically analyzed using the IBM SPSS Statistics software (version 27.0; IBM, Chicago, IL, USA) at the significance level of 0.05. The Kolmogorov-Smirnov test showed that the distribution of the μTBS data was normal. A three-way ANOVA (variables: application time, wash-out time, and adhesives) was performed separately for each deproteinizing solution (NaOCl and HOCl). Additional three-way ANOVAs (variables: application time, CDA application, and adhesives) were performed to test the effect of CDA. Multiple comparisons between the means were performed using Dunnett's T3 tests.

Fractographic analysis

The dentin and composite fragments of the beams were desiccated, sputter-coated with gold, and observed using the JSM-IT100 SEM (JEOL, Tokyo, Japan). Failure modes were classified as follows: adhesive failure (>80% of the fracture occurred at the dentin-adhesive interface), cohesive failure in dentin (>80% of the fracture occurred in the underlying dentin), cohesive failure in resin (>80% of the fracture occurred in the adhesive and/or the overlying resin composite), or mixed failure (combination of adhesive and cohesive failure, each accounting for <80% of the fracture). Failure modes were statistically analyzed using the non-parametric Pearson's chi-square test at the significance level of 0.05 (IBM SPSS Statistics version 27.0, IBM).

ATR-FTIR spectroscopy

Ninety 2-mm-thick midcoronal dentin discs with a standardized smear layer were prepared as described above. Prior to the pretreatment, control spectra were collected from all the dentin specimens using the FTIR-8300 spectrometer (Shimadzu) in the range of 750–4,000 cm^{-1} at a resolution of 4 cm^{-1} by the co-addition of 64 scans. The specimens were then pretreated with the NaOCl or or HOCl solution and washed-out with water as mentioned above (n=5). The spectra of the pretreated specimens were collected using identical settings. The deproteinizing effect was assessed by comparing the collagen-to-apatite ratio before and after pretreatment. The amide I band at 1,643 cm^{-1} (stretching vibrations of C=O) was selected as representative of collagen, and the ν_3 band at 1,026 cm^{-1} (stretching vibrations of P-O) represented apatite[5,9,32]. The amide-to-phosphate ratios were statistically analyzed using a three-way repeated

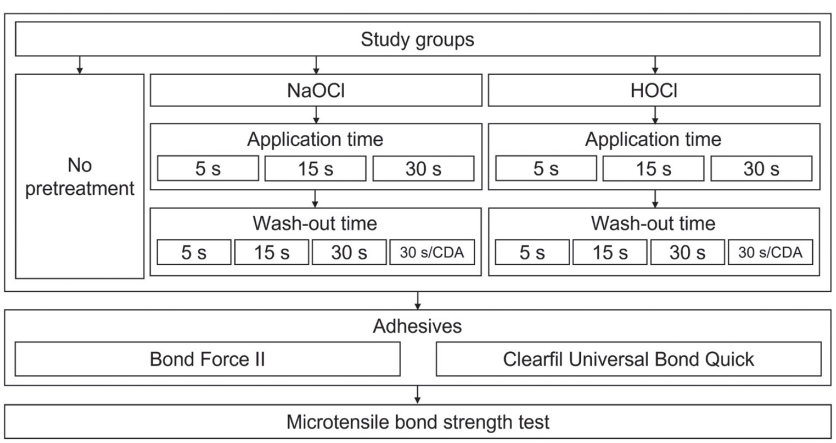

Fig. 1 Study design with the arrangement of groups for the μTBS test. CDA: Clearfil DC Activator

measures ANOVA (variables: deproteinizing agent, application time, and wash-out time), and Tukey's HSD test was used for multiple comparisons at the significance level of 0.05 (IBM SPSS Statistics version 27.0, IBM).

SEM

Thirty-six additional flat dentin surfaces were prepared as described above for the ultrastructural observation of the smear layer-covered dentin surfaces. The specimens were pretreated with the NaOCl or HOCl solution and washed-out with water as mentioned above, and untreated specimens were used as the control (n=2). The specimens were fixed using 2.5% glutaraldehyde for 2 h at 4°C, followed by a 0.1% osmium solution for 2 h at 4°C, and serially dehydrated with ethanol as follows: 50%, 70% and 80% ethanol for 25 min each at 4°C, then 90% and 95% ethanol for 25 min each at room temperature, and finally 100% twice for 25 min. After immersion in hexamethyldisilazane (HMDS) for 10 min, the specimens were dried in a desiccator at room temperature for 24 h[32]. They were then sputter-coated with gold and observed using the JSM-IT100 SEM at a magnification of 5,000×.

RESULTS

μTBS

The three-way ANOVAs revealed that the application time of both NaOCl and HOCl (p<0.001), wash-out time (p<0.001), and type of adhesive (p<0.001) had a significant effect on μTBS. The subsequent application of CDA significantly affected μTBS to NaOCl-pretreated dentin (p<0.001), but did not significantly affect μTBS to HOCl-pretreated dentin (p=0.62). There were significant interactions between the application time of HOCl and wash-out time (p<0.001), between the application time of HOCl and type of adhesive (p=0.007), and between the application time of NaOCl and the subsequent application of CDA (p<0.001). Interactions among the three independent variables were not statistically significant (p>0.05).

The μTBS of UBQ was significantly higher than that of BF2 in all the experimental groups, but the pretreatments had the same effects on both adhesives (Table 2). The pretreatment with NaOCl and HOCl for 5 s did not significantly affect their μTBS (p>0.05). In contrast, the pretreatment with NaOCl for 15 s or 30

Table 2 Means and standard deviations of microtensile bond strengths (MPa, n=16)

Smear layer deproteinization			BF2	UBQ
Agent	Application time	Wash-out time		
No (control)			61.1 (2.0) ^{A,a}	75.4 (3.0) ^{A,b}
NaOCl	5 s	5 s	59.0 (5.3) ^{A,a}	74.0 (3.0) ^{A,b}
		15 s	57.4 (7.0) ^{A,a}	75.5 (3.4) ^{A,b}
		30 s	58.7 (4.1) ^{A,a}	75.2 (3.5) ^{A,b}
		30 s/CDA	59.9 (4.3) ^{A,a}	76.7 (4.8) ^{A,b}
	15 s	5 s	50.8 (5.0) ^{B,a}	62.5 (7.0) ^{B,b}
		15 s	52.1 (4.9) ^{B,a}	66.3 (4.2) ^{B,b}
		30 s	52.6 (5.7) ^{B,a}	68.0 (4.4) ^{B,b}
		30 s/CDA	62.0 (4.1) ^{A,a}	76.2 (3.7) ^{A,b}
	30 s	5 s	46.4 (4.8) ^{B,a}	61.9 (6.35) ^{B,b}
		15 s	51.0 (5.2) ^{B,a}	64.2 (3.8) ^{B,b}
		30 s	52.1 (5.4) ^{B,a}	66.5 (5.7) ^{B,b}
		30 s/CDA	62.9 (5.2) ^{A,a}	75.6 (3.2) ^{A,b}
HOCl	5 s	5 s	61.6 (3.5) ^{A,a}	76.0 (7.9) ^{A,b}
		15 s	61.9 (5.4) ^{A,a}	75.5 (7.5) ^{A,b}
		30 s	61.2 (6.5) ^{A,a}	75.5 (7.3) ^{A,b}
		30 s/CDA	63.0 (7.2) ^{A,a}	75.5 (3.3) ^{A,b}
	15 s	5 s	64.4 (5.3) ^{A,a}	75.4 (8.3) ^{A,b}
		15 s	68.0 (9.8) ^{A,a}	78.1 (8.3) ^{A,b}
		30 s	72.1 (6.0) ^{C,a}	85.8 (6.7) ^{C,b}
		30 s/CDA	73.0 (7.5) ^{C,a}	86.8 (5.0) ^{C,b}
	30 s	5 s	65.6 (5.1) ^{A,a}	77.9 (5.4) ^{A,b}
		15 s	66.1 (7.4) ^{A,a}	81.3 (6.6) ^{A,b}
		30 s	78.4 (7.4) ^{C,a}	85.3 (5.0) ^{C,b}
		30 s/CDA	76.1 (3.6) ^{C,a}	84.8 (3.9) ^{C,b}

Significant differences (p<0.05) are indicated by different superscript upper letters in each column and by different superscript lower letters in each row. BF2: Bond Force II, UBQ: Clearfil Universal Bond Quick, CDA: Clearfil DC Activator

s significantly decreased μTBS compared to untreated dentin (p<0.05), irrespective of the wash-out time (p>0.05). The application of CDA after the wash-out for 30 s recovered the μTBS in groups pretreated with NaOCl for 15 s or 30 s (p<0.05), but there was no significant difference from the control group (p>0.05). The pretreatment with HOCl for 15 s or 30 s did not significantly influence μTBS if washed out for 5 s or 15 s (p>0.05), but the wash-out time of 30 s led to a significant increase in μTBS compared to the control group (p<0.05), regardless of CDA application (p>0.05).

Fractographic analysis

The failure mode distributions in each group are depicted in Fig. 2. The majority of failures were adhesive or mixed, and there were no significant differences among the groups (p=1.00). Representative SEM images of the failures of BF2 and UBQ are presented in Figs. 3 and 4, respectively.

ATR-FTIR

The representative spectra of each experimental group normalized to the ν_3 band (1,026 cm^{-1}) are depicted in Fig. 5. In comparison with the control group, the spectra clearly show a reduction in the amide I band (1,643 cm^{-1}) after the pretreatment with NaOCl or HOCl for 15 s or 30 s. The three-way repeated measures ANOVA revealed that amide-to-phosphate ratios (Table 3) were significantly affected by the deproteinizing agent (p<0.001) and application time (p<0.001), but not by the wash-out time (p=0.762). Interactions between the variables were not significant (p>0.05). The pretreatment with either of the deproteinizing agents for 5 s did not significantly affect the amide-to-phosphate ratio (p>0.05) compared to the control group. However, the extension of the application time to 15 s or 30 s significantly decreased the amide-to-phosphate ratio with both NaOCl and HOCl (p<0.05).

SEM

Representative SEM images of the smear layer-covered dentin surfaces with no pretreatment and after the pretreatment with NaOCl or HOCl are presented in Fig. 6. Without pretreatment, the smear layer was compact and had a uniform texture, grooves produced by the SiC paper were observed (Fig. 6a). The pretreatment with deproteinizing agents did not remove the smear layer on the dentin surface, irrespective of the application/wash-out time (Figs. 6b–g).

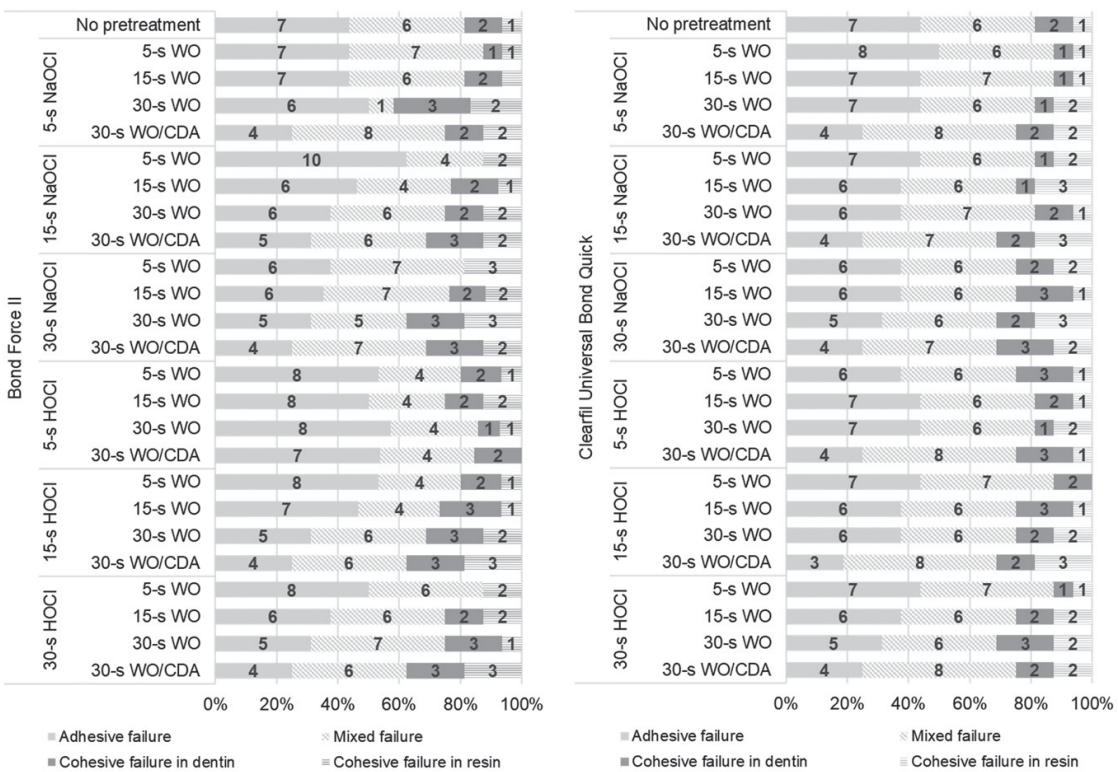

Fig. 2 Distribution of the failure modes in each group.
Adhesive or mixed failures prevailed, and no significant difference was found in their distributions among the groups (p=1.00). The numbers in bars indicate the number of specimens with the respective failure mode. CDA: Clearfil DC Activator, WO: wash-out time

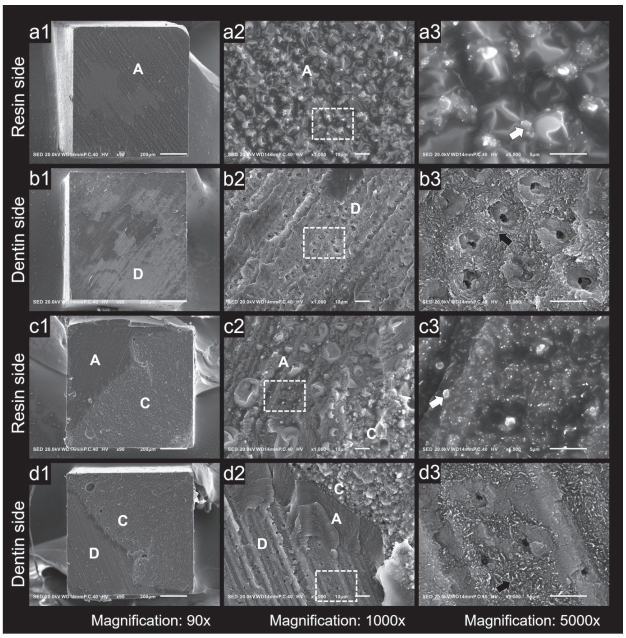

Fig. 3 Representative SEM images of an adhesive failure (a, b) and a mixed failure (c, d) of BF2.
Column 1 presents images at magnification 90×, column 2 at magnification 1,000×, and column 3 at magnification 5,000×. White arrows in a3 and c3 point at a structure that may represent a resin tag, black arrows in b3 and d3 point at intertubular collagen fibrils. A: adhesive, D: dentin, C: resin composite

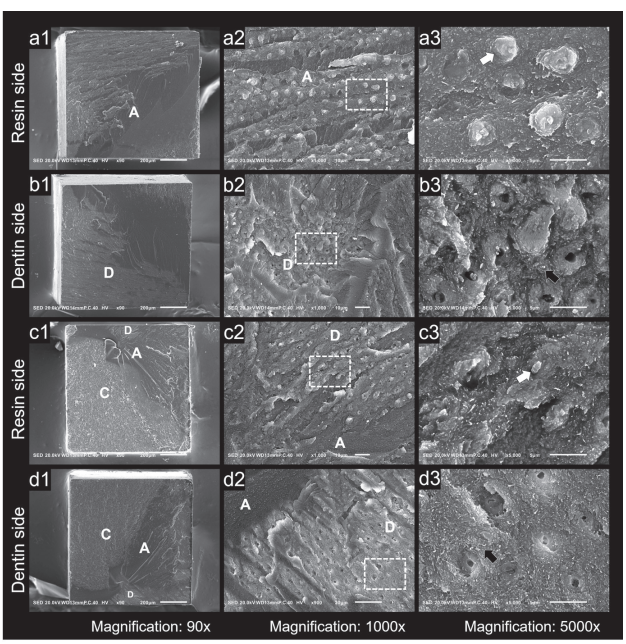

Fig. 4 Representative SEM images of an adhesive failure (a, b) and a mixed failure (c, d) of UBQ.
Please refer to the footnote of Fig. 3 for interpretation.

DISCUSSION

The results revealed that the μTBS of 1-SEAs to dentin decreased significantly if it had been pretreated with NaOCl for 15 s or 30 s, on which the wash-out time had no significant effect. In contrast, the application of HOCl for 15 s or 30 s did not significantly affect μTBS when washed out for 5 s or 15 s, but a significant increase in μTBS was observed when the wash-out time was extended to 30 s. It was also revealed that the deproteinizing effect was significantly affected by the application time and deproteinizing agent, indicating that the 100 ppm HOCl solution was significantly more effective in dissolving organic components of the smear layer than the 6% NaOCl solution. Therefore, the null hypothesis was rejected.

The objective of the smear layer deproteinization concept is to prevent the hybridization of the smear layer by removing its organic phase. This study confirmed that NaOCl and HOCl solutions can dissolve the organic components of the smear layer by oxidation in a time-dependent manner. The application of the solutions for 5 s did not have a significant effect on the amide-to-phosphate ratios on the smear layer-covered dentin, but their application for 15 s and 30 s significantly decreased the ratios regardless of the wash-out time. However, the smear layer was still observed using SEM after the pretreatments, indicating that the dissolution of the organic phase occurred only within the smear layer[6,8,32,33] and that its inorganic phase remained on the bonding surface. This finding agrees with previous studies where no morphological alterations of the smear layer-covered sound dentin surface were observed after the deproteinizing pretreatment[6,8]. The reduction in smear layer thickness was observed only on caries-affected dentin, where the smear layer is thicker and contains more collagen[6,8].

The FTIR analysis also showed that the 100 ppm HOCl solution was more effective than 6% NaOCl. This is likely due to the different pH of the deproteinizing solutions, which affects the proportions of HOCl and OCl^- ions. The 6% NaOCl solution is strongly alkaline and therefore contains very few HOCl molecules, because they ionize into OCl^- at high pH levels[18-20]. The OCl^- ions can disintegrate proteins by chlorination, which leads to the formation of nitrogen-centered radicals and induces the fragmentation of proteins[34,35]. In contrast, the 100 ppm HOCl solution whose pH was adjusted to 6.8 predominantly contained non-dissociated HOCl[34] which exhibits a stronger chlorinating and oxidizing effect than OCl^- [18,22]. The HOCl molecule readily interacts with various biological molecules such as thiols, thioethers, nucleotides, amino groups, and carbohydrates[22]. Additionally, chlorine in HOCl solutions behaves as a cation, which can chlorinate amino acids within the collagen triple helix and cause its fragmentation[36]. This enables HOCl solutions to deproteinize the smear layer even at low chlorine concentrations[21]. The finding that 100 ppm HOCl is more effective in deproteinizing than 6% NaOCl also corroborates a previous report that the

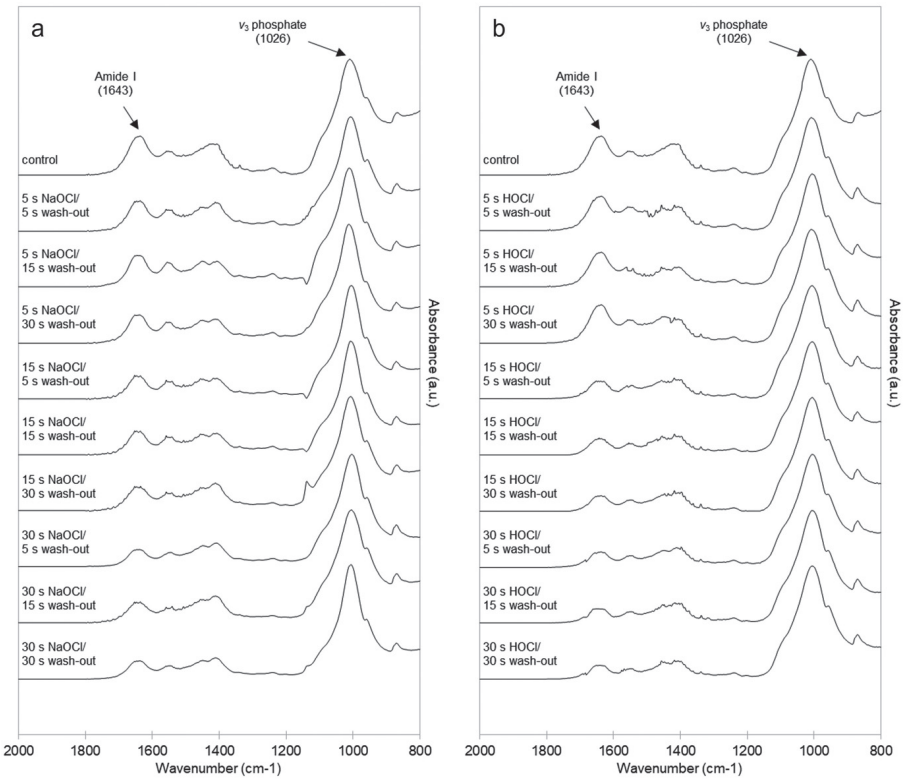

Fig. 5 Representative FTIR spectra acquired on the smear layer-covered dentin after the experimental pretreatments.
The spectra were normalized to the ν_3 phosphate band at 1,026 cm^{-1}, and a decrease in the amide I band at 1,643 cm^{-1} was evident after pretreatments with NaOCl (a) or HOCl (b) for 15 s or 30 s, irrespective of the wash-out time.

Table 3 Means and standard deviations of amide-to-phosphate ratios (n=5)

Agent	Application time	Wash-out time	Amide-to-phosphate ratio
No pretreatment (control)			0.27 (0.05) [a]
NaOCl	5 s	5 s	0.27 (0.04) [a]
		15 s	0.27 (0.01) [a]
		30 s	0.27 (0.04) [a]
	15 s	5 s	0.19 (0.01) [b]
		15 s	0.19 (0.02) [b]
		30 s	0.19 (0.03) [b]
	30 s	5 s	0.18 (0.05) [b]
		15 s	0.18 (0.03) [b]
		30 s	0.18 (0.03) [b]
HOCl	5 s	5 s	0.21 (0.02) [a,b]
		15 s	0.21 (0.03) [a,b]
		30 s	0.21 (0.02) [a,b]
	15 s	5 s	0.16 (0.01) [b]
		15 s	0.16 (0.01) [b]
		30 s	0.16 (0.06) [b]
	30 s	5 s	0.15 (0.00) [b]
		15 s	0.16 (0.03) [b]
		30 s	0.15 (0.01) [b]

Significant differences (p<0.05) are indicated by different superscript letters.

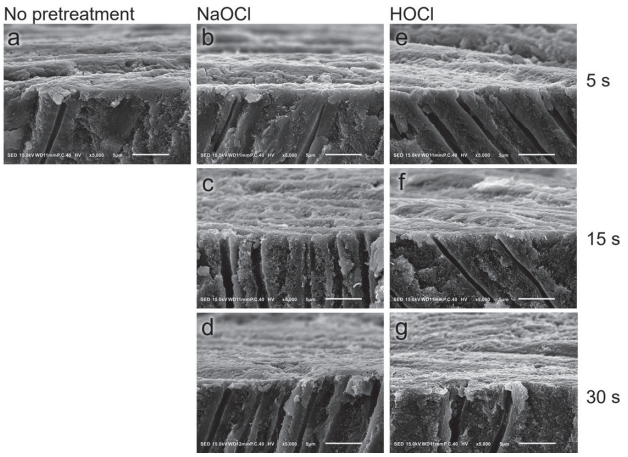

Fig. 6 SEM images of the smear layer-covered dentin surfaces at magnification 5,000×.
In the control group (a), the surface was covered with a uniform and compact smear layer with grooves produced by the SiC paper. In groups deproteinized with NaOCl and HOCl for 5–30 s and washed-out with water for 30 s (b–g), a smear layer of similar characteristics was found on the dentin surfaces. Experimental groups with shorter wash-out time (5 s and 15 s) are not shown, because they did not differ from the groups washed-out for 30 s.

formation of the hybridized smear layer was avoided if HOCl was applied for at least 15 s, whereas 30 s were necessary with NaOCl[9].

Despite the benefits of deproteinization, many studies have reported that the bond strength to dentin pretreated with NaOCl decreased significantly[11,12]. This was attributed to the residues of NaOCl and/or the formation of chloramine-derived radicals in the NaOCl-pretreated dentin, which hinder the polymerization of the adhesives[11,13]. The extent of these adverse effects depends on the diffusion of NaOCl into the substrate and is affected by its concentration and application time[35,37]. The 6% NaOCl treatment significantly decreased the μTBS of UBQ and BF2 when applied for 15 s or 30 s, as opposed to the 5 s application. The extension of the wash-out time slightly increased their μTBS values, but remained significantly lower than the control group. These results indicate that a longer application time enabled the penetration of NaOCl deeper into the substrate and that its remnants or by-products were not completely washed away by rinsing the surface with water for 30 s[23,24,37].

It has been previously revealed that the negative effect of NaOCl can be neutralized by the application of antioxidants or reducing agents, such as sodium p-toluenesulfinate, rosmarinic acid, or sodium ascorbate[7,10,12-14]. CDA is a commercially available dual-cure activator containing an arylsulfinate salt, and mixing it with UBQ enables chemical polymerization upon contact with the overlying resin composite when sufficient light-curing of the adhesive is not achievable[38]. Moreover, it was reported that the degree of conversion of 1-SEAs improved significantly after dentin had been pretreated with a sulfinate agent even when sufficient light energy was delivered[39]. In this study, CDA was used as a reducing agent to neutralize the residual oxidizing effect of NaOCl and HOCl, and its application recovered the compromised μTBS of UBQ and BF2 in the groups pretreated with NaOCl for 15 s or 30 s to the level of the control group. This can be attributed to the enhanced polymerization of the 1-SEAs.

In contrast, HOCl can be washed away more easily than NaOCl, leaving fewer chlorine residues on the treated surfaces[23,24]. Furthermore, chlorine concentration in the 100 ppm HOCl solution was considerably lower compared to the 6% NaOCl solution. Consequently, the pretreatment with HOCl for 15 s or 30 s did not significantly alter the μTBS of UBQ nor BF2 even with a wash-out time of 5 s. Moreover, the extension of the wash-out time to 30 s significantly increased their μTBS values compared to the control group, which can be attributed to the deproteinization, especially avoiding the formation of the hybridized smear layer. These results suggest that the longer wash-out time successfully removed the residues of HOCl and its by-products from the dentin surface, preventing the adverse influence on the polymerization of 1-SEAs. This speculation was also supported by the finding that there was no significant difference in μTBS between the HOCl-pretreated groups washed out for 30 s with and without the subsequent application of CDA. If any oxidizing residues had been present on the bonding surface, the application of CDA would have increased μTBS.

The pretreatments tested in this study had the same effect on both tested 1-SEAs, but the μTBS of UBQ was significantly higher than that of BF2. This may be attributed to the fact that BF2 is based on a self-reinforcing phosphoric acid monomer, while UBQ contains 10-methacryloyloxydecyl dihydrogen phosphate (10-MDP) that is known to form very stable calcium salts, thus promoting adhesion to hard dental tissues[40]. Besides that, UBQ also contains a special polymerization accelerator, which could improve its degree of conversion, and a hydrophilic amide monomer that partly substitutes 2-hydroxyethyl methacrylate (HEMA)[41]. The reduced content of HEMA would presumably have a stronger effect on bonding durability, but only 24 h μTBS was measured herein, which can be seen as a limitation of this study. The lack of aging procedures was caused by the need to test numerous groups, as the objective of this study was to identify the optimal application/wash-out time of NaOCl and HOCl. Therefore, based on the present results, the effect of selected smear layer-deproteinizing protocols on the bonding durability of 1-SEAs to deproteinized dentin should be investigated in follow-up studies. Furthermore, the benefits of smear layer deproteinization were reported to be more marked on caries-affected dentin than on normal dentin, because the smear layer in caries-affected dentin is thicker, more porous, and richer in organic components[6,7,42]. The high antimicrobial activity of HOCl would also be suitable

for cavity disinfection after selective caries removal. Therefore, it is desirable to perform clinical studies on the efficiency of using HOCl for cavity disinfection and smear layer deproteinization in caries treatment.

CONCLUSIONS

Within the limitations of this study, it can be concluded that the 100 ppm HOCl solution exhibited a stronger deproteinizing effect than the 6% NaOCl solution. Furthermore, the HOCl solution could be washed away more easily, leaving fewer oxidative residues on the pretreated dentin surface. Therefore, smear layer deproteinization with NaOCl for 15 s or 30 s significantly decreased the immediate μTBS of 1-SEAs to dentin, whereas pretreatment with HOCl for 15 s or 30 s followed by a wash-out time of 30 s significantly improved their bonding performance.

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REFERENCES

1) Spencer P, Wang Y, Walker MP, Swafford JR. Molecular structure of acid-etched dentin smear layers-in situ study. J Dent Res 2001; 80: 1802-1807.
2) Wang Y, Spencer P. Analysis of acid-treated dentin smear debris and smear layers using confocal raman microspectroscopy. J Biomed Mater Res 2002; 60: 300-308.
3) Tay FR, Sano H, Carvalho R, Pashley EL, Pashley DH. An ultrastructural study of the influence of acidity of self-etching primers and smear layer thickness on bonding to intact dentin. J Adhes Dent 2000; 2: 83-98.
4) Hosaka K, Prasansuttiporn T, Thanatvarakorn O, Kunawarote S, Takahashi M, Foxton RM, *et al*. Smear layer-deproteinization: improving the adhesion of self-etch adhesive systems to caries-affected dentin. Curr Oral Health Rep 2018; 5: 169-177.
5) Hu X, Peng Y, Sum CP, Ling J. Effects of concentrations and exposure times of sodium hypochlorite on dentin deproteination: attenuated total reflection Fourier transform infrared spectroscopy study. J Endod 2010; 36: 2008-2011.
6) 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.
7) Prasansuttiporn T, Thanatvarakorn O, Mamanee T, Hosaka K, Tagami J, Foxton RM, *et al*. Effect of antioxidant/reducing agents on the initial and long-term bonding performance of a self-etch adhesive to caries-affected dentin with and without smear layer-deproteinizing. Int J Adhes Adhes 2020; 102: 102648.
8) 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: 769-775.
9) 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.
10) Thanatvarakorn O, Prasansuttiporn T, Thittaweerat S, Foxton RM, Ichinose S, Tagami J, *et al*. Smear layer-deproteinizing improves bonding of one-step self-etch adhesives to dentin. Dent Mater 2018; 34: 434-441.
11) 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: 1919-1924.
12) 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: 229-234.
13) Monticelli F, Toledano M, Silva AS, Osorio E, Osorio R. Sealing effectiveness of etch-and-rinse vs self-etching adhesives after water aging: influence of acid etching and NaOCl dentin pretreatment. J Adhes Dent 2008; 10: 183-188.
14) Prasansuttiporn T, Thanatvarakorn O, Tagami J, Foxton RM, Nakajima M. Bonding durability of a self-etch adhesive to normal versus smear-layer deproteinized dentin: effect of a reducing agent and plant-extract antioxidant. J Adhes Dent 2017; 19: 253-258.
15) Pamir T, Türkün M, Kaya AD, Sevgican F. Effect of antioxidant on coronal seal of dentin following sodium-hypochlorite and hydrogen-peroxide irrigation. Am J Dent 2006; 19: 348-352.
16) Ballal NV, Das S, Rao BSS, Zehnder M, Mohn D. Chemical, cytotoxic and genotoxic analysis of etidronate in sodium hypochlorite solution. Int Endod J 2019; 52: 1228-1234.
17) Severing AL, Rembe JD, Koester V, Stuermer EK. Safety and efficacy profiles of different commercial sodium hypochlorite/hypochlorous acid solutions (NaClO/HClO): Antimicrobial efficacy, cytotoxic impact and physicochemical parameters in vitro. J Antimicrob Chemother 2019; 74: 365-372.
18) Christensen CE, McNeal SF, Eleazer P. Effect of lowering the pH of sodium hypochlorite on dissolving tissue in vitro. J Endod 2008; 34: 449-452.
19) Ikemura K, Endo T. A review of our development of dental adhesives-effects of radical polymerization initiators and adhesive monomers on adhesion. Dent Mater J 2010; 29: 109-121.
20) 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.
21) Ingle JI. Ingle's endodontics. In: 6th, editor. Hamilton, Ontario, Canada: BC Decker Inc.; 2008. p. 997-998.
22) Mainnemare A, Mégarbane B, Soueidan A, Daniel A, Chapple IL. Hypochlorous acid and taurine-N-monochloramine in periodontal diseases. J Dent Res 2004; 83: 823-831.
23) Abdul-Baki AA. Pitfalls in using sodium hypochlorite as a seed disinfectant in C incorporation studies. Plant Physiol 1974; 53: 768-771.
24) Guentzel JL, Liang Lam K, Callan MA, Emmons SA, Dunham VL. Reduction of bacteria on spinach, lettuce, and surfaces in food service areas using neutral electrolyzed oxidizing water. Food Microbiol 2008; 25: 36-41.
25) Lapenna D, Cuccurullo F. Hypochlorous acid and its pharmacological antagonism: An update picture. Gen Pharmacol 1996; 27: 1145-1147.
26) Ono T, Yamashita K, Murayama T, Sato T. Microbicidal effect of weak acid hypochlorous solution on various microorganisms. Biocontrol Sci 2012; 17: 129-133.
27) Rutala WA, Weber DJ. Uses of inorganic hypochlorite (bleach) in health-care facilities. Clin Microbiol Rev 1997; 10: 597-610.
28) Block MS, Rowan BG. Hypochlorous Acid: A Review. J Oral Maxillofac Surg 2020; 78: 1461-1466.
29) Lafaurie GI, Zaror C, Díaz-Báez D, Castillo DM, De Ávila J,

Trujillo TG, *et al*. Evaluation of substantivity of hypochlorous acid as an antiplaque agent: a randomized controlled trial. Int J Dent Hyg 2018; 16: 527-534.
30) Hsieh SC, Teng NC, Chu CC, Chu YT, Chen CH, Chang LY, *et al*. The antibacterial efficacy and in vivo toxicity of sodium hypochlorite and electrolyzed oxidizing (EO) water-based endodontic irrigating solutions. Materials (Basel) 2020; 13: 260.
31) 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: 261-268.
32) Kusumasari C, Abdou A, Tichy A, Hatayama T, Hosaka K, Foxton RM, *et al*. Effect of smear layer deproteinization with chemo-mechanical caries removal agents on sealing performances of self-etch adhesives. J Dent 2020; 94: 103300.
33) Paing SY, Tichy A, Hosaka K, Nagano D, Nakajima M, Tagami J. Effect of smear layer deproteinization with HOCl solution on the dentin bonding of conventional and resin-modified glass-ionomer cements. Eur J Oral Sci 2020; 128: 255-262.
34) Fukuzaki S. Mechanisms of actions of sodium hypochlorite in cleaning and disinfection processes. Biocontrol Sci 2006; 11: 147-157.
35) 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: 144-156.
36) 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: 637-643.
37) Virdee SS, Farnell DJJ, Silva MA, Camilleri J, Cooper PR, Tomson PL. The influence of irrigant activation, concentration and contact time on sodium hypochlorite penetration into root dentine: An ex vivo experiment. Int Endod J 2020; 53: 986-997.
38) Arrais CA, Giannini M, Rueggeberg FA. Effect of sodium sulfinate salts on the polymerization characteristics of dual-cured resin cement systems exposed to attenuated light-activation. J Dent 2009; 37: 219-227.
39) Hasegawa M, Tichy A, Hosaka K, Kuno Y, Ikeda M, Nozaki K, *et al*. Degree of conversion and dentin bond strength of light-cured multi-mode adhesives pretreated or mixed with sulfinate agents. Dent Mater J 2021; 40: 877-884.
40) Yoshihara K, Nagaoka N, Okihara T, Kuroboshi M, Hayakawa S, Maruo Y, *et al*. Functional monomer impurity affects adhesive performance. Dent Mater 2015; 31: 1493-1501.
41) Kuno Y, Hosaka K, Nakajima M, Ikeda M, Klein Junior CA, Foxton RM, *et al*. Incorporation of a hydrophilic amide monomer into a one-step self-etch adhesive to increase dentin bond strength: Effect of application time. Dent Mater J 2019; 38: 892-899.
42) Nakajima M, Kunawarote S, Prasansuttiporn T, Tagami J. Bonding to caries-affected dentin. The Jpn Dent Sci Rev 2011; 47: 102-114.