Bonding Durability of a Self-etch Adhesive to Normal Versus Smear-layer Deproteinized Dentin: Effect of a Reducing Agent and Plant-extract Antioxidant

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Purpose: To evaluate the effect of a reducing agent and plant-extract antioxidant on the bonding durability of a self-etch adhesive to normal and NaOCl-treated, smear-layer–deproteinized dentin.

Materials and Methods: Flat smear-layer–covered dentin surfaces from 60 extracted human molars were prepared by removing the occlusal enamel. The teeth were divided into two groups with or without NaOCl-deproteinizing treatment for 30 s, and further divided into three subgroups as follows: no application of antioxidant, application of Accel (p-toluenesulfinic acid sodium salt solution) for 5 s, or application of rosmarinic acid solution for 5 s. All treated dentin surfaces were bonded with a two-step self-etch adhesive (Clearfil SE Bond) and restored with composite (Clearfil AP-X). The bonded teeth were sectioned into a hourglass-shaped sticks with a composite-dentin bonded interface area of 1.0 mm^2. After storage in artificial saliva for 24 h or 1 year, the specimens were subjected to the microtensile bond strength test (n = 15). Data were statistically analyzed with three-way ANOVA, Tukey's post-hoc test, and the t-test ($p < 0.05$).

Results: Without an antioxidant, 1-year storage significantly reduced the bond strengths of the self-etch adhesive to normal and smear-layer–deproteinized dentin compared with those after 24-h storage ($p < 0.05$). Application of Accel and rosmarinic acid restored the compromised initial bond strengths to smear-layer–deproteinized dentin ($p < 0.05$), and prevented long-term deterioration of bond strengths to both normal and smear-layer–deproteinized dentin ($p > 0.05$).

Conclusion: Application of Accel and rosmarinic acid improved bonding durability of the self-etch adhesive to both normal and smear-layer–deproteinized dentin.

Keywords: self-etch adhesive, dentin bonding durability, anti-oxidant/reducing agent, cross linker, MMP-inhibitor, smear-layer deproteinizing.

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Sodium hypochlorite (NaOCl) is normally used as a chemical irrigant during endodontic procedures[16] due to its antibacterial properties and the ability to dissolve organic tissue. When NaOCl solution is applied to smear-layer–covered dentin, the smear layer is deproteinized and thinned by dissolution of the organic phase.[48] Therefore, smear layer deproteinizing can increase the mineral:organic ratio on the dentin surface[48] and improve the affinity of hydrophobic materials to dentin by removing hydrated collagen debris in the smear layer.[25] Self-etch adhesives can partially demineralize the smear-layer–covered dentin subsurface due to its mild acidity, but are not able to dissolve the organic phase of the smear layer. The remaining organic phase of the smear layer can give rise to a resin-impregnated smear layer (so-called hybridized smear layer) on the hybrid layer, which is likely to act as a selective barrier for monomer penetration into dentin substrate.[39] Therefore, deproteinizing the smear layer could improve the quality of

the resin-dentin interface of self-etch adhesives by facilitating the penetration of self-etch adhesive into the dentin subsurface and eliminating the hybridized smear layer.[48] Smear-layer deproteinizing could produce an appropriate substrate for the dentin bonding of self-etch adhesives.[48] Some researchers have demonstrated that pretreatment with deproteinizing agents, such as NaOCl and hypochlorous acid (HOCl) solutions, can improve bond strengths of self-etch adhesives to caries-affected dentin with a thick, organic-rich smear layer.[27,36,46]

On the other hand, other studies have demonstrated that NaOCl-treated dentin compromised the initial bond strengths.[18,35,37] This is thought to be due to the remnants of NaOCl and its by-products, which exhibited a negative effect on the polymerization of dental adhesives.[37] The compromised bond strength to NaOCl-deproteinized dentin surfaces could be reversed by application of antioxidants before the adhesive procedure,[26,29,50,51] because antioxidants can interact with the oxidized by-products of NaOCl,[35] resulting in neutralization of the oxidizing effect of a NaOCl-deproteinized dentin surface.[8,21,43]

Many plant-extract products are known to be antioxidants.[11,15] Additionally, some of them are reported to be collagen cross linkers and/or matrix metalloproteinase (MMP) inhibitors.[42] Their intermolecular crosslinks with the collagen matrix enhance the mechanical stability of collagen matrix, which can prevent collagen degradation in the hybrid layer and maintain long-term dentin bonding stability.[24] MMPs are a group of 23 human enzymes capable of degrading all extracellular matrix components. Among other enzymes, human dentin contains collagenase (MMP-8), gelatinases (MMP-2,-9), and enamelysin (MMP-20),[31,44,45] which can degrade exposed dentin collagen fibrils within the hybrid layer, leading to the loss of hybrid layer integrity and the reduction of composite-dentin bond strength over time.[9,33] Many studies have demonstrated that MMP inhibitors, such as chlorhexidine, can prevent self-degradation of the composite-dentin interface and improve the durability of the hybrid layer.[9,10,22,41] Rosmarinic acid is a polyphenolic flavonoid extracted from rosemary, which has cross-linking and MMP-inhibitory abilities,[42] as well as a high antioxidizing capacity.[2,23] The application of rosmarinic acid solution can improve the compromised initial bond strength of self-etch adhesives to NaOCl-deproteinized smear-layer–covered dentin.[40] However, there are few studies on the bonding durability of self-etch adhesives to NaOCl-deproteinized smear-layer–covered dentin.

Accel (Sun Medical; Kyoto, Japan) contains p-toluenesulfinic acid sodium salt (a reducing agent)[30,49] and was introduced as a pretreatment with adhesive root canal sealer after endodontic irrigation with NaOCl. p-toluenesulfinic acid sodium salt can also restore the bond strength of self-etch adhesives to NaOCl-deproteinized smear-layer–covered dentin.[40] p-toluenesulfinic acid sodium salt has long been used as an accelerator for the polymerization of composites.[3,5,6] It is possible that the application of Accel affects the polymerization behavior of adhesives and prevents long-term deterioration of the adhesive interface with dentin.

These various effects among different reducing/antioxidant agents might provide different bonding durability of self-etch adhesives between normal and NaOCl-deproteinized smear-layer–covered dentin, but little information is available. Thus, the purpose of this study was to evaluate the effect of Accel and rosmarinic acid on the long-term microtensile bond strength of a two-step self-etch adhesive (Clearfil SE Bond) to normal and NaOCl-deproteinized smear-layer–covered dentin. The null hypothesis tested was that application of these antioxidants does not improve the bonding durability of a two-step self-etch adhesive to normal and NaOCl-deproteinized smear-layer–covered dentin.

MATERIALS AND METHODS

Specimen Preparation

Sixty extracted, noncarious human third molars which were stored frozen were used in this study, according to a protocol approved by the Human Research Ethics Committee, Tokyo Medical and Dental University, Tokyo, Japan. Under water lubrication, the occlusal enamel was ground down perpendicular to the long axis of the tooth to expose a flat surface of sound dentin. Then, the occlusal dentin surface was polished with 600-grit silicon carbide paper for 30 s under running water to create a standardized smear layer. The teeth were randomly divided into two groups: without or with NaOCl-deproteinizing treatment for 30 s. After rinsing with water for 10 s and air drying, the dentin surfaces were further divided into three subgroups as follows: no application of antioxidant, application of Accel (which contains p-toluenesulfinic acid sodium salt solution) for 5 s, and application of 100 μM rosmarinic acid in 5% ethanol for 5 s.

After air drying, a two-step self-etch adhesive (Clearfil SE Bond, Kuraray Noritake; Tokyo, Japan) was applied to all the dentin surfaces according to the manufacturer's instructions (Table 1), and composite (Clearfil AP-X, Kuraray Noritake) was built up in three 1.5-mm increments. Each increment was light cured for 20 s with a light-curing unit (Optilux 501, Kerr; Orange, CA, USA).

Microtensile Bond Strength (μTBS) Test

After water storage at 37°C for 24 h, the bonded teeth were vertically sectioned into three 0.7-mm-thick slabs using a low-speed diamond saw (IsoMet Low Speed Saw, Buehler; Lake Bluff, IL, USA) under water lubrication. Using a cylindrical fine diamond bur (Intensiv SA, Swiss Dental; Zurich, Switzerland) with a high-speed handpiece under water spray, each slab was hand trimmed to an hourglass shape, with the bonded interface at the isthmus having the dimensions 0.7 × 1.4 mm^2. The thickness and width of the bonded interface was measured using a digital micrometer (Digimatic Solar, Mitutoyo; Tokyo, Japan). Three slabs were prepared from each tooth; hence, ten teeth from each group yielded 30 specimens for bond strength evaluation. The specimens in each group were randomly divided into two groups for storage in artificial saliva for 24 h or 1 year (fresh artificial saliva was replenished every week). After the

Table 1 Materials used

Material	Manufacturer	Batch number	Composition
Accel	Sun Medical; Kyoto, Japan	MM2F	p-toluenesulfinic acid sodium salt, ethanol, water
Clearfil SE Bond	Kuraray Noritake; Tokyo, Japan	01042A	Primer: 10-MDP, HEMA, hydrophilic aliphatic dimethacrylate, N,N-diethanol-p-toluidine, CQ, water Bond: bis-GMA, HEMA, DMA, 10-MDP, toluidine, silanated silica, CQ Bonding procedure: apply primer for 20 s; gently air dry with compressed air spray; apply bond; light cure for 10 s
Clearfil AP-X	Kuraray Noritake	1016AB	Bis-GMA, TEG-DMA, silanated barium glass filler, silanated silica filler, silanated colloidal silica, CQ, initiators, accelerators, pigments

Abbreviations: 10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; bisGMA: 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl]propane; TEG-DMA: triethyleneglycol dimethacrylate; CQ: camphorquinone.

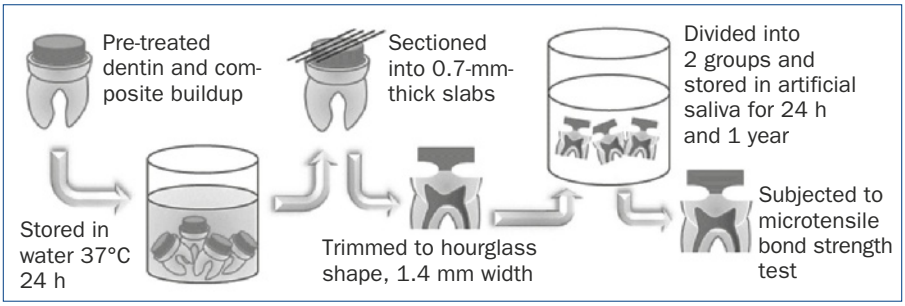

Fig 1 Schematic illustrating sample preparation.

designated storage period, all specimens were attached to a universal testing machine (EZ Test, Shimadzu; Kyoto, Japan) with cyanoacrylate glue (Zapit, DVA; Anaheim, CA, USA), and were subjected to the microtensile bond test at a crosshead speed of 1 mm/min[40] (Fig 1).

Failure Mode Analysis

After bond strength testing, the dentin sides of the specimens were observed using a stereomicroscope (Nikon SMZ1000, Nikon; Kanagawa, Japan) at 120X magnification to determine the failure mode. Failure modes were classified according to one of four types: 1. mixed failure (adhesive failure between composite and dentin, as well as cohesive failure in the bonding agent and/or dentin); 2. adhesive failure (80%–100% of the failure occurred between composite and dentin); 3. cohesive failure in dentin (80%–100% of the failure occurred in the underlying dentin); 4. cohesive failure in composite (80%–100% of the failure occurred in the adhesive composite and/or overlying composite).

Statistical Analysis

Microtensile bond strength data were statistically analyzed using three-way ANOVA. Tukey's test and the t-test were used as post-hoc tests. Failure modes were analyzed using the nonparametric Pearson's chi-squared test. All statistical analyses were performed at a confidence level of 95% using SPSS v 22.0 (IBM; Armonk, NY, USA).

RESULTS

μTBS Test

The microtensile bond strength results are summarized in Table 2. There were no premature failures during specimen preparation in this study. Three-way ANOVA revealed that the type of dentin surface ($p < 0.001$), antioxidant application ($p < 0.001$), and storage period ($p < 0.001$) statistically significantly influenced bond strength. Significant interactions were observed between the type of dentin surface and antioxidant application ($p < 0.001$) and between antioxidant application and storage period ($p = 0.002$), but no interaction was found between type of dentin surface and storage period ($p = 0.168$). Tukey's multiple comparisons revealed that for 24-h bond strength, smear-layer–deproteinized dentin exhibited statistically significantly lower bond strength than did normal dentin ($p < 0.05$). The application of Accel and rosmarinic acid to normal dentin did not show a negative effect on bond strength ($p > 0.05$), and both antioxidants restored compromised initial bond strengths to smear-layer–deproteinized dentin ($p < 0.05$). After 1-year storage in artificial saliva, the t-test revealed that microtensile bond strengths to normal dentin and smear-layer–deproteinized dentin significantly decreased compared with bond strengths after 24-h storage ($p < 0.05$). However, the application of Accel and rosmarinic acid did not significantly decrease the bond strengths to either normal or smear-layer–deproteinized dentin ($p > 0.05$).

Table 2 Microtensile bond strength (MPa) means and standard deviations (n = 15)

Time		24 h	1 year
Normal dentin	No treatment	55.2 (4.1)^{A1}	45.8 (4.0)^{a2}
	Accel	57.4 (5.2)^{A1}	55.8 (5.2)^{b1}
	Rosmarinic acid	54.8 (3.9)^{A1}	52.6 (4.7)^{b1}
Smear-layer–deproteinized dentin	No treatment	43.0 (4.4)^{B1}	38.5 (5.7)^{c2}
	Accel	53.4 (5.6)^{A1}	51.6 (5.3)^{b1}
	Rosmarinic acid	54.0 (3.9)^{A1}	53.1 (4.4)^{b1}

Different letters indicate a significant difference in each row; different numbers indicate a significant difference in each column ($p < 0.05$).

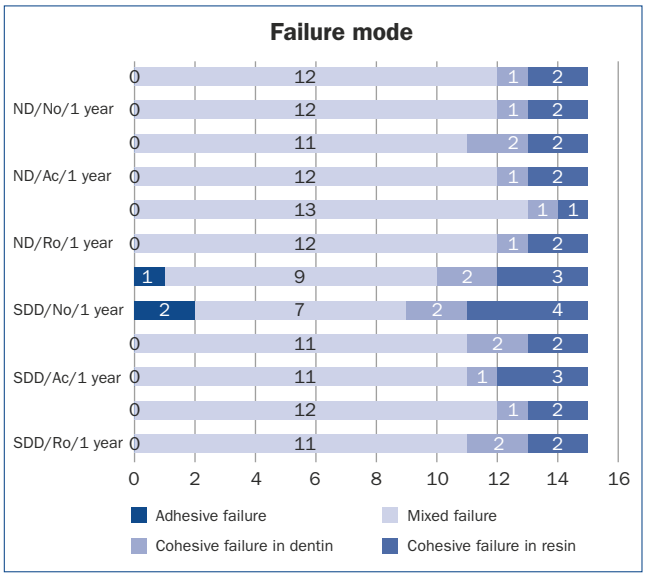

Fig 2 Failure mode of composite-dentin bond in each group (n = 15). Numbers in each bar refer to the number of each failure type in the given group. ND: normal dentin; SDD: smear-layer–deproteinized dentin; N: no antioxidant; Ac: Accel; Ro: rosmarinic acid.

Failure Mode Analysis

The failure modes are summarized in Fig 2. In all groups, the predominant failure mode was mixed. There were no significant differences in failure modes between the experimental groups ($p = 0.859$).

DISCUSSION

The results of this study showed that the bond strength of a two-step self-etch adhesive (Clearfil SE Bond) to 30-s NaOCl-treated dentin was lower than to that of normal dentin. This result is in agreement with previous studies.[29,37,50] As NaOCl is a potent biological oxidant,[17] the breakdown of this molecule yields reactive, residual free radicals, which could compete with the propagating vinyl free radicals generated during light activation of the resin adhesive. As a result, polymerization could be incomplete due to premature chain termination,[29] leading to a reduction in dentin bond strength. Although previous studies have reported that following 30-s application of NaOCl solution, SEM showed that smear layer remained on the dentin surface,[46] and the organic phase was found to be reduced on the smear-layer–deproteinized dentin surface.[27] Additionally, a 30-s application of NaOCl solution was reported to eliminate the hybridized smear layer at the adhesive interface with Clearfil SE Bond, but increase nanoleakage in the hybrid layer.[48] This increase in nanoleakage could also be due to impaired polymerization of resin monomers by a residual oxidizing effect of NaOCl.[48] Increased nanoleakage facilitates hydrolytic degradation at the adhesive interface over time.[38] Nevertheless, 1-year storage caused a similar reduction (10%–17%) in the bond strengths to normal and smear-layer–deproteinized dentin. This might be due to elimination of hybridized smear layer at the adhesive interface with smear-layer–deproteinized dentin, because hybridized smear layer is regarded as a weak link at the interface.[47]

The application of Accel and rosmarinic acid restored initial bond strength to smear-layer–deproteinized dentin. Some researchers have demonstrated that applying an antioxidant/reducing agent (eg, sodium ascorbate, sodium thiosulphate solution) reversed the negative effect on dentin bonding by NaOCl.[26,29,50] Antioxidants possess three main mechanisms to control oxidation: free-radical chain breaking, metal chelating, and free-radical quenching. In the latter, antioxidants react with oxidants to neutralize unpaired electrons and form stable products, which limits the activity of oxidants.[4] In this scenario, an antioxidant can provide the redox potential to oxidized dentin substrate, leading to the optimal polymerization of composite.[51]

Additionally, the application of Accel and rosmarinic acid improved the long-term bond strength to normal and smear-layer–deproteinized dentin. Thus, the null hypothesis can be rejected. Rosmarinic acid consists mainly of rosemary extracts.[23] Among 72 species of herbs and their solvent extraction in oil systems or oil-in-water emulsions, rosemary possesses the best antioxidant activity.[12-14] Rosmarinic acid (a-o-caffeoyl-3,4-dihydroxyphenyllactic acid) is a diphenolic compound[34] that contains two catechol (1,2-dyhydroxybenzene) rings (Fig 3), contributing to rosmarinic acid's polarity. The antioxidant activity of rosmarinic acid can be attributed to the ability of catechol to form an intermolecular hydrogen bond between free hydrogen of its hydroxyl and phenoxyl radicals, improving its radical stability. The advantage of rosmarinic acid is the presence of four phenolic hydrogens (-OH) which contribute to controlling free radical oxidation.[4] The -OH group in phenol acts as a chain-breaking antioxidant because it scavenges reactive radicals. The resulting radicals tend to be poorly reactive because of electron delocalization into the aromatic ring, so that the reactive radical is replaced by one of limited reactivity.[7]

Moreover, rosmarinic acid has cross-linking and MMP-inhibitory properties. The cross-linking effect of phenolic flavonoids, such as rosmarinic acid, is attributed to its interaction with proline-rich proteins, such as collagen.[19] A natural cross linker may improve dentin bonding, because the cross-linked collagen matrix exhibits increased mechanical properties and resistance to proteolytic degradation.[20,24] MMPs are important proteolytic tools in the extracellular collagen matrix, and activation of MMPs (collagenases) results in an excessive degradation of the extracellular matrix component.[1] Apart from cross linking, inhibition of MMP-2 and -9 proteolytic activity may retard caries progression and increase the durability of composite-dentin bonds.[32] The use of an MMP inhibitor, such as chlorhexidine, offers promise for increasing the durability of composite-dentin bonds.[9,22,41] In restorative dentistry, rosmarinic acid with cross-linking and MMP-inhibitory effects would be a useful agent for improving the longevity of bonding of self-etch adhesives to normal and smear-layer–deproteinized dentin, in addition to its antioxidant ability.

Accel contains a reducing agent, p-toluenesulfinic acid sodium salt. Therefore, the application of Accel can neutralize the residual oxidizing effects on smear-layer–deproteinized dentin surface by a redox reaction, which has been shown to restore compromised initial bond strength[40] and reduce nanoleakage in the hybrid layer.[48] Additionally, the salt of p-toluenesulfinic acid is an effective accelerator for the polymerization of methyl methacrylate,[6] enhancing conversion rates of composite polymerization[37] and increasing bond strengths.[5] In this study, the application of Accel also improved long-term bond strengths to normal and smear-layer–deproteinized dentin. The residual monomers at the adhesive interface could form nanoleakage pathways and accelerate hydrolytic degradation, decreasing the composite-dentin bond strength over time. Remnants of the Accel component at the dentin surface might increase the degree of conversion of the adhesive agent, leading to an improvement in dentin bonding stability. On the other hand, in contrast to the situation on normal dentin, residual Accel components on the smear-layer–deproteinized dentin surface may be reduced due to its redox reaction with oxidized by-products of NaOCl. Further research is required on the effect of Accel on the degree of conversion of the adhesive in normal and smear-layer–deproteinized dentin.

Based on the present study, Accel or p-toluenesulfinic acid sodium salt solution showed the potential to improve the bonding durability of the tested self-etch adhesive to both normal and smear-layer–deproteinized dentin due to its ability to accelerate polymerization. Further, the application of rosmarinic acid with cross-linking and MMP-inhibitor abilities could contribute to maintaining long-term bonding stability to normal and smear-layer–deproteinized dentin. Application of these agents on the dentin surface is appropriate for increasing the composite-dentin bond durability in the clinical situation. However, additional steps during the bonding procedure prolong chairside time and might introduce a greater number of variables. Moreover, the effect of eliminating the hybridized smear layer by smear layer-deproteinizing with NaOCl on long-term dentin bond strengths of the tested self-etch adhesive is unclear. Recently, a mildly acidic HOCl solution has been introduced as a smear layer-deproteinizing agent, with a less negative influence on the dentin bonding ability of self-etch adhesives. This is related to the fact that HOCl has oxidizing and deproteinizing abilities at a low concentration, and leaves less residue on the treated surface after rinsing with water.[27,28] Further studies involving TEM observation are necessary to evaluate long-term morphological alteration at the composite-dentin interface with smear-layer deproteinizing, in order to improve this method.

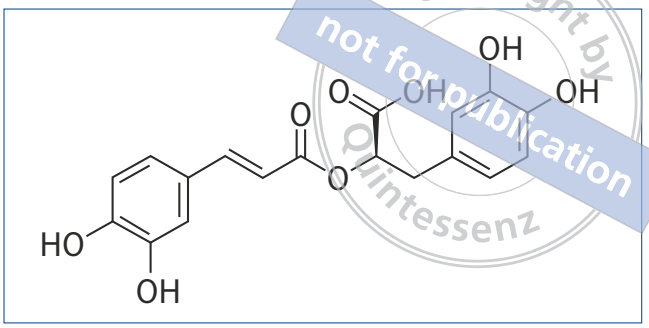

Fig 3 Molecule of rosmarinic acid containing four phenolic hydrogens.

CONCLUSION

The application of Accel and rosmarinic acid on smear-layer–deproteinized dentin reversed the negative effect of NaOCl deproteinizing on the microtensile bond strengths of a two-step self-etch adhesive (Clearfil SE Bond). Furthermore, the application of Accel and rosmarinic acid improved the bonding durability to both normal and smear-layer–deproteinized dentin.

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Clinical relevance: The application of Accel or rosmarinic acid prior to self-etch adhesive could improve the durability of the composite-dentin bond. Thus, composite restorations made using this technique could have a longer service life than those placed using the conventional bonding technique.