

Influence of ascorbic acid on bonding of peroxide-affected dentin and 4-META/MMA-TBB resin

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Abstract The purpose of this study was to evaluate the tensile bond strength (TBS) to peroxide-exposed dentin. Furthermore, the effect of ascorbic acid (AA) on the bond strength of peroxide-exposed dentin was investigated. Extracted bovine dentin was exposed to 10% carbamide peroxide, 30% hydrogen peroxide, or distilled water for 30 min, then treated with 10% AA (0, 30, 90, and 180 min), and conditioned with 10% citric acid/3% ferric chloride. The polymethyl-methacrylate (PMMA) rod was bonded to the treated bovine dentin with 4-META/MMA-TBB resin. A minidumbbell-shaped bonded specimen was prepared from these bonded assemblies and the TBS was tested. The fractured surfaces were also observed with a scanning

electron microscope. Exposure to peroxide before bonding significantly reduced bond strength. The application of AA to the peroxide-exposed dentin increased bond strength. On the other hand, an adverse effect of AA was found in distilled water-affected dentin. Extended resin fibers were partially seen in the peroxide-exposed dentin. In conclusion, peroxide reduced the bond strength, and the stronger the oxidation, the weaker the obtained bond. Antioxidation with AA recovered the bond strength, and this effect increased the longer the AA was applied.

Keywords Dentin bonding · Antioxidant · Polymerization · Ascorbic acid · Tooth bleaching · Peroxide · 4-META/MMA-TBB resin

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Introduction

Tooth bleaching, e.g., at-home bleaching with carbamide peroxide (CP) and traditional bleaching with hydrogen peroxide (HP), was used for discolored teeth and to satisfy esthetic needs. While tooth bleaching involves colorative esthetics, it does not involve morphological esthetics. Thus, an adhesive material, such as a microfilled resin composite or bonded laminate veneer restoration is sometimes used after tooth bleaching when both colorative and morphological restoration is needed [18].

The bond strength of current adhesive systems was reported to be reduced significantly when bonding is performed immediately after bleaching for both enamel and dentin [2, 15–17, 23, 24, 26, 27]. It was reported that allowing a 1-week period between bleaching and restorative treatment reversed the enamel bond strength [2]. However, this method was not effective for bleached dentin [23, 27]. Thus, the bond strength of, e.g., peroxide and sodium

hypochlorite (NaOCl), to oxidant-exposed dentin has generally been considered to be reduced [3, 14].

It was well known that pretreatment with a combination of NaOCl and phosphoric acid increases the bond strengths with Panavia luting cements (Kuraray Medical) [9, 30]. On the other hand, an inverse effect of the pretreatment with NaOCl and phosphoric acid was obtained when using 4-META/MMA-TBB resin [3, 10, 14]. As shown above, depending on the specific composition of the dentin adhesive, the application of NaOCl upon etching can increase or decrease bond strength [20, 29]. Differing from the many current adhesive systems, 4-META/MMA-TBB resin has some unique characteristics [19]. Therefore, any difference to the current adhesive system may be found at the influence of peroxide on bonding of 4-META/MMA-TBB resin.

The reversal effect of antioxidants, e.g., ascorbic acid (AA), sodium ascorbate, butyl hydroxianizole and sodium thiosulfate on the dentin bond strengths to the oxidant-exposed dentin was recently reported [14–16, 22, 32]. For AA, an adequate concentration was reported to be 10% [22]. However, an adequate period of antioxidant application has not been discussed.

The purposes of this study were to clarify the influence of impairment in the resin bonding to dentin exposed to 10% CP or 30% HP and to investigate the reversal effect of AA on bond strength with both peroxide-exposed and nonexposed dentin. The null hypotheses of this study were that (1) bond strength variation is not dependent on the type of peroxide, (2) AA application time does not affect bond strength, and (3) AA has no effect for increasing the bond strength to dentin untreated with peroxide.

Materials and methods

Specimen preparation and tensile bond testing

Ninety-six extracted bovine teeth, frozen to maintain freshness, were defrosted and cut at the cervix to separate the crown and root. The coronal sides of the cut surfaces were sequentially abraded under a stream of water with SiC paper (#180- up to #600-grit) to prepare the flat dentin surfaces. To standardize the bonding areas, a 3.0×7.0×3.0-mm acrylic frame was affixed to the abraded surface with double-sided tape (Nichiban, Tokyo, Japan).

For the CP groups, the exposed dentin areas of 32 teeth were subjected to a 10% CP gel (Opalescence® Regular, Ultradent Products, South Jordan, UT, USA) for 30 min, rinsed with distilled water for 60 s, and dried. For the HP groups, the exposed areas of 32 teeth were subjected to a 30% HP solution (Wako Pure Chemical, Osaka, Japan) for 30 min, rinsed, and dried in a similar way. For the DW

groups, the exposed areas of 32 teeth were subjected to distilled water for 30 min and dried.

Each dentin specimen was then subjected to 10% AA (Wako Pure Chemical) for 30, 90, or 180 min (30AA, 90AA and 180AA, respectively; each subgroup had eight specimens) and the remaining eight specimens in each group were not treated with AA (0AA). Each specimen was conditioned with 40 µl of a 10:3 solution (10% citric acid/3% ferric chloride) for 15 s, rinsed with distilled water for 30 s, and dried. Immediately after the acid conditioning, the surfaces were bonded to polymethyl-methacrylate (PMMA) square rods (8.0×8.0×8.0 mm) using 4-META/MMA-TBB resin (Super-Bond® C&B, Sun Medical, Moriyama, Japan; also known as C&B Metabond™ in North America, Parkell, Farmingdale, NY, USA) with a brush-dip technique. The bonded specimens were allowed to stand at room temperature for 60 min and were then stored in 37°C water for 24 h. They were then serially sectioned vertically to make 2.0-mm-thick bonded dentin slabs, using a low-speed diamond saw (Isomet™; Buehler, Lake Bluff, IL, USA). Each bonded slab was trimmed to a minidumbbell-shaped test specimen with a 3.0×2.0-mm cross-section at the adhesive interface [1, 11–13] using a diamond point (FG #211 regular, Shofu, Kyoto, Japan) in a high-speed air turbine handpiece with a copious air–water spray. One bovine tooth yielded only one bonded dentin slab. The prepared specimens were affixed to a disposable PMMA jig and were subjected to stress through tension using a universal testing machine (Tensilon RTC-1150-TSD, Orientec, Tokyo, Japan) at a cross-head speed of 0.5 mm/min. The cross-sectional dimensions of the fractured specimens were confirmed with a digital micrometer (Mitutoyo, Tokyo, Japan) after the tensile bond testing and tensile bond strength (TBS) was derived at the bonding area (mm²). When a specimen failed during processing [pretesting failure (PTF)], the TBS was set at 0-MPa [4, 28].

Statistical analysis

The data were recorded and subjected to one-way and two-way ANOVAs after Fisher's protected least significant difference test at the 5% level to determine statistical significance using a commercially available statistical package (StatView® 5.0J, SAS Institute, Cary, NC, USA).

SEM observations

After the tensile bond testing, each fractured specimen was desiccated in the box with silica gel for more than 24 h, placed on an aluminum stub, and sputter-coated with about 15 nm of Au–Pd using a Cool Sputter Coater (SC500A, VG Microtech, East Sussex, UK). The coated specimens were examined under a scanning electron microscope (SEM)

(JSM-6340F, JEOL, Tokyo, Japan) at 15 kV to determine the exact locus of the fracture.

Results

The TBSs of each group are shown in Table 1.

There is a significant difference among the three groups (DW-0AA, CP-0AA, and HP-0AA) when AA was not applied (one-way ANOVA, $p < 0.0001$). For DW-0AA (control), the highest TBS (12.5±5.1 MPa) was obtained among all experimental groups. For CP-0AA, six specimens broke before TBS testing and two had low bond strength. For HP-0AA, all eight specimens broke before testing. Significant differences were found between CP-0AA and DW-0AA, and between HP-0AA and DW-0AA ($p < 0.0001$).

One-way ANOVA revealed that AA application significantly increased the TBS of CP groups ($p = 0.0008$). The AA application group had no PTF. More than 90 min of AA application significantly affected the TBS (0 min vs 30 min: $p = 0.2974$, 0 min vs 90 min: $p = 0.0096$, 0 min vs 180 min: $p < 0.0001$). Especially, 180 min of AA application increased the TBS as no significant difference was found to control DW-0AA ($p = 0.1992$). One-way ANOVA also revealed that AA application significantly increased the TBS of HP groups ($p = 0.0494$). While AA application decreased the number of PTF, the effect of AA on HP was smaller than that of CP. Three PTF was found even if AA was applied for 180 min, and the remaining five specimens obtained very low TBS. One-way ANOVA revealed that AA application significantly decreased the TBS of DW groups ($p = 0.0007$).

SEM photographs of fractured surfaces after the TBS testing are shown in Figs. 1 and 2. DW-0AA (control group) showed a mixture of fractures to be mainly cohesive in the cured resin and partially at the bottom/within the hybrid layer (Fig. 1a). A mixed failure in the resin and adhesive interface was observed in the peroxide-exposed/AA nontreated groups (CP-0AA and HP-0AA) (Fig. 1b,c).

Furthermore, extended resin fibers were partially observed at the fractured surface (Fig. 1c).

A mixed failure in the cured resin and the top of the hybrid layer was observed in CP-180AA (Fig. 2a). A mixture of adhesive failure and cohesive failure in the dentin was observed in DW-180AA (Fig. 2b,c).

Discussion

One of the purposes of this study was to determine the influence of peroxide on bond strength between dentin and 4-META/MMA-TBB resin. The results revealed that the exposure of dentin to peroxide decreased the bond strength, as reported in several previous studies [15, 16, 23, 27] (Table 1).

However, some differences were found between CP and HP. For CP-0, two specimens could be tested for their TBS, and the remaining six were debonded while immersed in water, in spite of how the specimens were prepared. On the other hand, all of the eight bonded specimens were fractured when sliced with the Isomet diamond saw. Even after treatment with AA, CP specimens showed higher bond strength than that of HP specimens. The concentration of peroxide in the 30% HP is approximately ninefold than that in the 10% CP because CP decomposes the two parts of urea and one part of HP. The differences may be due to the concentration of peroxide. In the above results, the first hypothesis was rejected.

In this study, the influence of peroxides on bond strength was larger than the previous reports, which were bonded with the current adhesive systems. Spyrides et al. [23] reported that the shear bond strength of bovine dentin exposed to 35% HP for 30 min to Single Bond/Z100 (3M ESPE) was reduced to 24% compared to the nonexposed control (4.2±2.5 MPa vs 17.4±5.6 MPa). Kaya and Turkun [15] also reported that the shear bond strength of dentin exposed to 35% HP for 10 min reduced by 53% compared to the control (15.3±2.7 MPa vs 28.4±1.7 MPa) when Clearfil SE Bond/Clearfil AP-X (Kuraray Medical) was

Table 1 TBSs of each group (MPa, $n=8$)

AA application (min)	DW			CP				HP			
	Mean±SD	PTF	Statistics	Mean±SD	PTF	Statistics	Mean±SD	PTF	Statistics		
0 min	12.5±5.1	0	a A	0.3±0.7	6	f A	0.0±0.0	8	f A		
30 min	7.4±4.9	0	b, c B	2.7±3.4	0	e, f A, B	0.1±0.1	5	f A		
90 min	5.4±5.8	0	c, d, e B, C	6.5±7.3	0	b, c, d B, C	0.3±0.3	4	f A		
180 min	2.8±2.2	0	d, e, f C	10.1±3.6	0	a, b C	1.7±2.6	3	e, f B		

Different letters express statistically significant differences ($p > 0.05$): Capital letters express differences between the different application time of AA each within the DW, CP, and HP; small letters express differences between each of the 12 groups. PTF Number of pretesting failures

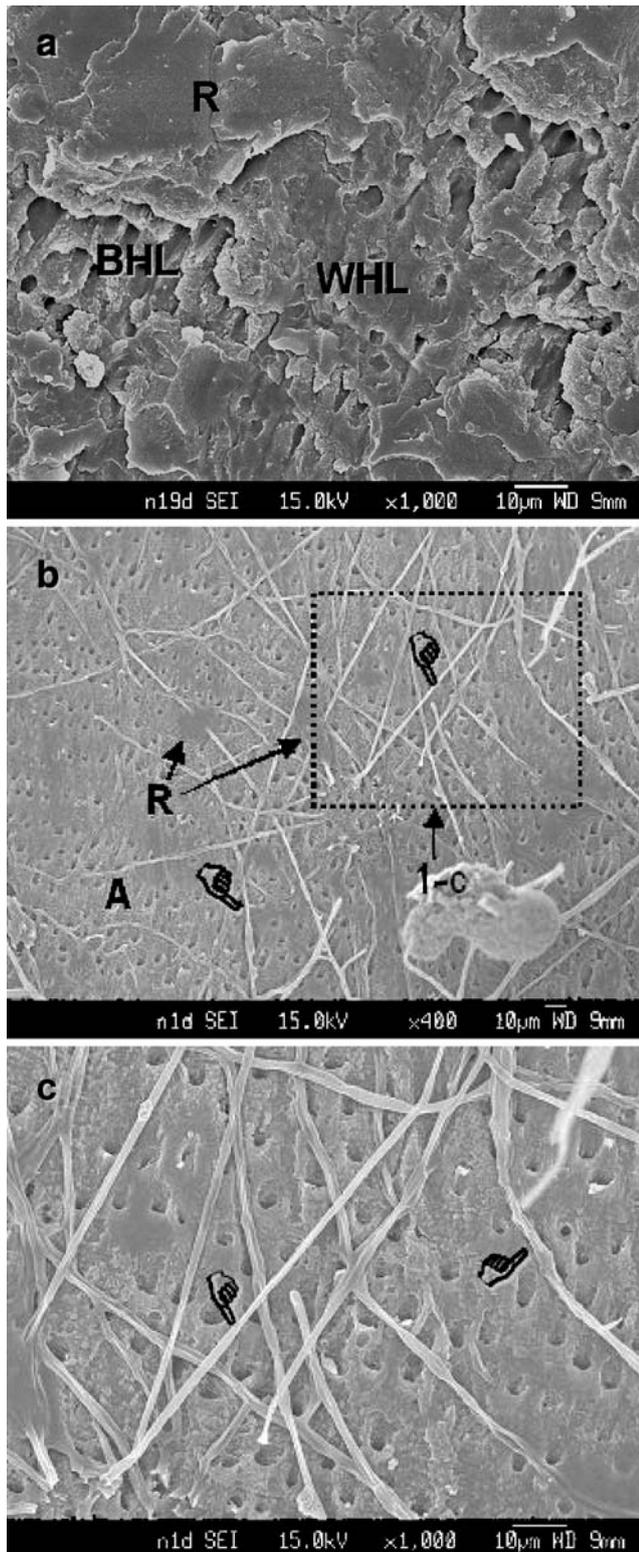


Fig. 1 SEM micrograph of the fractured dentin-side surfaces of bonded specimens (OAA groups). **a** DW-0AA (21.5 MPa; 1,000 \times). Failure in the cured resin (*R*) in almost all parts, at the bottom of the hybrid layer (*BHL*), and partially within the hybrid layer (*WHL*) was observed. **b, c** CP-0AA (pretest failed; 400 \times and 1,000 \times , respectively). Almost all parts show the adhesive failure (*A*) but failure in the resin (*R*) is only partially seen. Extended resin fibers branch out from the failure in the resin (*pointer*). Similar fibers were also partially observed at seven out of eight specimens

The preconditioner of adhesive used in this study includes 3% ferric chloride. The ferric ions (Fe^{3+}) penetrate into the dentin and are not removed, even if the treated surface was sufficiently rinsed [25]. It was well known that ferric chloride contributes to prevent the shrinkage of demineralized collagen and to make the water-soluble protein insoluble [8].

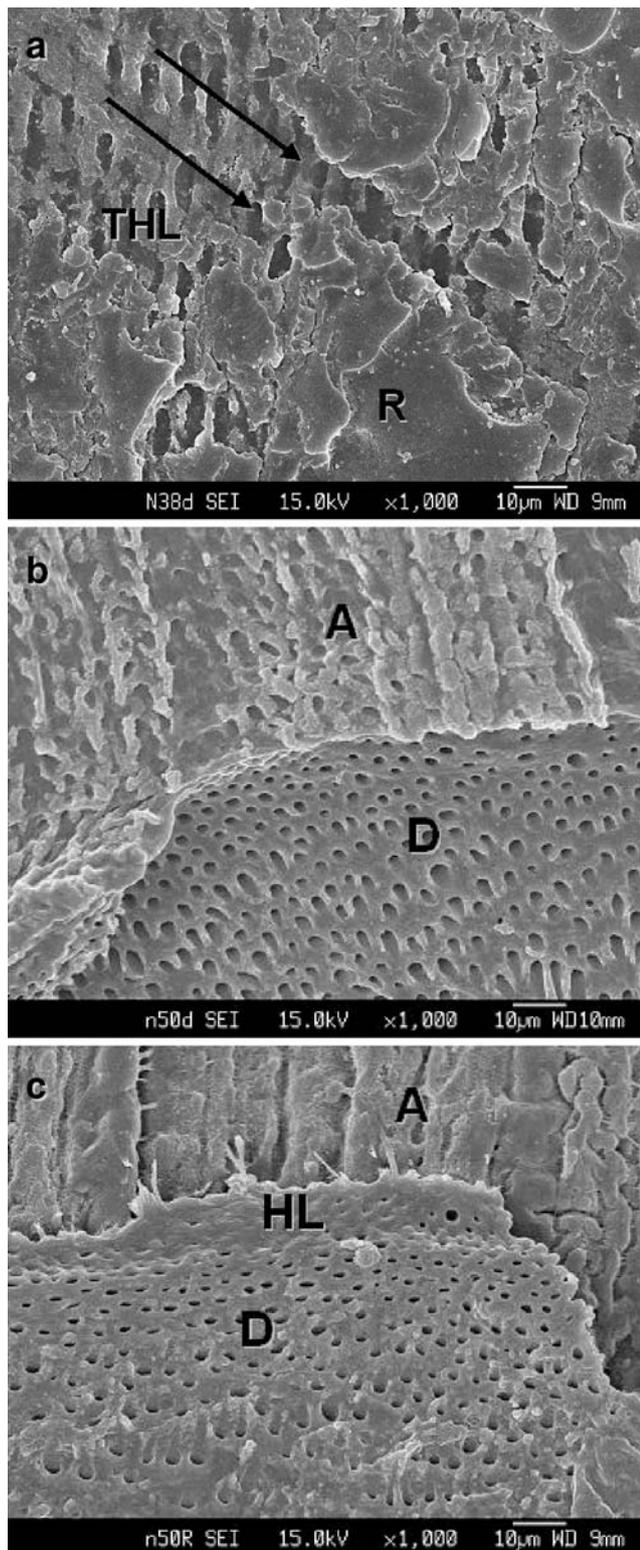
However, in the presence of ferric ion, especially ferric-citrate complexes, peroxides usually generate hydroxyl radical ($\cdot\text{OH}$) directly via the Fenton reaction [5, 31]. The presence of excess radicals may interfere with the polymerization because the resin fibers were extended in the SEM (Fig. 1). Thus, it was found that the combination of Fe^{3+} and peroxide induced the influence on bond strength.

For CP, the longer the AA was applied, the higher was the bond strength obtained. For CP, the reversal effect of AA was found to be significant because there was no significant difference in TBS between DW-0 and CP-180 ($p=0.1992$). These results suggest that excess radical generation from peroxide is interfered by AA [6, 21]. In contrast, the reversal bonding with AA was not realized in the HP group. For HP, the large amount of radicals may be related to changes in the redox cycle [7, 31]. Oxygen-free radicals are generally generated from HP by decomposition. The oxygen radicals generated from HP are more destructive than the liberated oxygen [7]. Based on these results, the second hypothesis that the application period of AA is not affected was rejected.

The application of AA markedly decreased the bond strength in the DW group. These results required the rejection of the last null hypothesis. We can consider two reasons for the low bond strength: oxidation and excessive demineralization. In the presence of both ferric ions and ascorbate, Fe^{3+} is changed to Fe^{2+} , thereby creating ascorbate-free radicals, even if peroxide is not applied. This reaction may inhibit the polymerization of the resin, monomer penetration, or degradation of dentin substrates. In our study, however, the double-etching effect with the 10:3 solution and AA might also be correlated with the reduction in bond strength rather than the oxidation because a fracture of the dentin structure was observed at the failed rod-side surface (Fig. 2c).

This study indicated that peroxide exposure to dentin interfered the bonding of 4-META/MMA-TBB resin.

used. In contrast, all eight specimens failed before testing at 35% HP for 30 min in this study. The differences were probably due to the unique characters of 4-META/MMA-TBB resin, as described below.



◀ **Fig. 2** SEM micrographs of the fractured surface of bonded specimens (180AA groups; original magnification 1,000 \times). **a** Dentin side surface of CP-180AA (11.8 MPa; 1,000 \times). Fractured surface pattern is similar to that of DW-0AA (Fig. 1a). Failure both in the cured resin (*R*) and at the top of the hybrid layer (*THL*) is seen. However, the other site partially shows the fracture of dentin (data not shown). The *arrow* indicates the scratches of SiC paper. **b** Dentin side surface of DW-180AA (2.4 MPa). A mixture of failed adhesive (*A*), dentin (*D*), and hybrid layer (*HL*) is observed. **c** Rod-side surface of the same specimen in Fig. 2b. The failure in dentin was evidentially observed (*D*)

this study design, and more investigations are needed to clarify these phenomenon.

Conclusion

We found that exposure to peroxide and additional treatment with AA on the bonding of 4-META/MMA-TBB resin to dentin results in the following:

1. Peroxide reduced bond strength, and the stronger the oxidation, the weaker the bond.
2. The application of AA recovered bond strength for peroxide-exposed dentin, and this tendency increased the longer the AA was applied.
3. AA had an adverse effect on nonperoxide-exposed dentin.

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Furthermore, it may suggest the deterioration of the bond reduction due to the interaction of ferric chloride and peroxide, and inadequate effect of the additional application of AA. However, we could not prove their suggestion in

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