The purpose of this study was to evaluate ascorbic acid (AS) and ferric chloride (FE) for bonding 4-META/MMA-TBB resin to dentin that had been treated with NaClO. An experimental dentin conditioner consisting of 10%AS and 5%Fe (10AS-5FE) and three controls (10AS-0FE, 0AS-5FE, and 0AS-0FE) were prepared. Ascorbic acid neutralizes NaClO. The flattened dentin surfaces were modified sequentially with phosphoric acid etchant, NaClO agent, and the experimental conditioner, then each surface was bonded to a stainless steel rod with 4-META/MMA-TBB resin. The Super-Bond C&B (10-3/SB) system was also used. 24-hour tensile bond strengths were determined. The bonding system using 10AS-5FE conditioner showed significantly high bond strength compared to 10AS-0FE, 0AS-5FE, and 0AS-0FE. No significant differences were observed between 10AS-5FE and 10-3/SB. Microphotographs suggested that no hybrid layer formed in the 10AS-5FE group. Although the use of phosphoric acid and NaClO resulted in decreased bond strength between 4-META/MMA-TBB resin and dentin, additional conditioning with ascorbic acid and ferric chloride improved the bond strength.

Key words: Ascorbic acid, 4-META/MMA-TBB resin, Dentin bonding

INTRODUCTION

Although dentin bonding systems have been improved with the development of suitable conditionings, maximum bond strength is not obtained when contaminants such as temporary cements\textsuperscript{1}), desensitizers\textsuperscript{2-4}) and medicaments for root canal treatment\textsuperscript{5}) exist on the dentin surface. A consistent dentin bonding is essential to the success of restorative treatments.

An attempt to improve adhesion has been made on adhered surfaces that were cleaned sequentially with 34-40 wt% phosphoric acid and 10 wt% NaClO\textsuperscript{6-8}). Since both inorganic and organic components are removed from the surface exposing the mineralized dentin substrate, the adverse effects of contaminants are slight\textsuperscript{6}) after this conditioning (AD gel method).

Generally, adhesive resins are classified according to the functional monomer contained. The representative phosphoric resin (Panavia Fluoro Cement, Kuraray Co. Ltd., Osaka, Japan) showed bond strength of 11.5±3.0 MPa to dentin conditioned with the AD gel method\textsuperscript{9)}, while a carboxylic resin (4-META/MMA-TBB resin) formed a hybrid layer\textsuperscript{3-11)} at the interface with bond strength of 14 MPa - 18 MPa when the dentin was conditioned with 10 wt% citric acid and 3 wt% ferric chloride (10-3 liquid). The 4-META/MMA-TBB resin and the 10-3 liquid are commercially available as Super-Bond C&B (Sun Medical Co. Ltd., Moriyama, Japan) or C&B Metabond (Parkell, Farmingdale, NY, U.S.A.), and ferric chloride is reported to be a key element in this dentin bonding system\textsuperscript{12-15}). However, no attempts were made to bond the carboxylic type resin to dentin surfaces whose collagen had been removed during pretreatment of that substrate.

The bond strength was adversely affected when the dentin was irrigated with NaClO, suggesting that NaClO inhibits the polymerization of resin at the bonding interface\textsuperscript{16}). It was demonstrated that the bond strength was reduced in the bonding between 4-META/MMA-TBB resin and dentin treated with NaClO\textsuperscript{17}). Thus, the bonding between carboxylic resin and dentin conditioned with AD gel has not been established yet.

Ascorbic acid, a water-soluble vitamin, neutralizes and reduces NaClO. It has been shown that a 5% solution of NaClO produced significant reductions in the bond strength of C&B Metabond to dentin that could be reversed by applying 10% ascorbic acid to counteract the NaClO\textsuperscript{18}). It is also used as an accelerator component in formulating initiator systems\textsuperscript{19)}, and is compared to ethylenediaminetetraacetic acid (EDTA) as a cleansing solution\textsuperscript{20}).

Taking these facts into consideration, it was hypothesized that the dentin conditioner consisting of ascorbic acid and ferric chloride may minimize the adverse effect of NaClO and improve the bond strength of 4-META/MMA-TBB resin to dentin.
conditioned with the AD gel method. The aim of this study is to investigate the efficacy of the novel dentin conditioner for bonding of carboxylic resin to collagen-depleted dentin.

**MATERIALS & METHODS**

**Specimen preparation**

The conditioners and luting agents used in this study are summarized in Table 1. An aqueous solution of an experimental conditioner was prepared by dissolving 0-20 wt% ascorbic acid (AS) and 0-10 wt% ferric chloride (FE) into room temperature distilled water. The labial surfaces of 90 bovine teeth were flattened using a model trimmer (Y-230, Yoshida Dental Distribution Co. Ltd., Tokyo, Japan). To expose dentin, they were ground with silicon carbide paper (Marumoto Struers, Tokyo, Japan) in a two step series of #400 and #600 under water irrigation. The teeth were then rinsed with tap water and air-dried for 15 seconds.

Table 2 shows a diagram of how the dentin surfaces were conditioned and bonded in each experimental group. A piece of adhesive masking tape (50 μm thick) with a 5 mm diameter hole was attached to each dentin surface to define the bonding area and the thickness of luting materials. The dentin specimen and a sandblasted stainless steel rod (10 mm in diameter, 30 mm long) were joined with the respective luting agent. 80 teeth were randomly selected and divided into 8 groups of 10 each for tensile testing, and a further 10 bonded specimens were prepared for scanning electron microscopy. All of the bonded specimens were left undisturbed at room temperature for 30 minutes, and stored in water at 37 °C for 24 hours.

**Tensile testing**

Tensile bond strengths (TBS) were determined by means of a universal testing machine (Autograph AGS-10kNG, Shimadzu Corp., Kyoto, Japan) at a crosshead speed of 2.0 mm/minute. The means and standard deviations of 10 specimens were calculated. The data was analysed by analysis of variance (ANOVA) and a post hoc test (Duncan’s new multiple range test) for which significant levels were set at P=0.05 (n=10).
BONDING OF RESIN TO COLLAGEN-DEPLETED DENTIN

Table 2 Bonding procedures

<table>
<thead>
<tr>
<th>First treatment</th>
<th>PA/AD/SB</th>
<th>PA/AD/AS-FE*/SB</th>
<th>PA/AD/10-3/SB</th>
<th>10-3/SB</th>
<th>PA/AD/ED/Panavia FC</th>
<th>All-Bond</th>
</tr>
</thead>
<tbody>
<tr>
<td>Washing</td>
<td>PA</td>
<td>PA</td>
<td>PA</td>
<td>10-3</td>
<td>PA</td>
<td>All-Etch</td>
</tr>
<tr>
<td></td>
<td>15 seconds</td>
<td>15 seconds</td>
<td>15 seconds</td>
<td>30 seconds</td>
<td>15 seconds</td>
<td>30 seconds</td>
</tr>
<tr>
<td>Washing</td>
<td>AD Gel</td>
<td>AD Gel</td>
<td>AD Gel</td>
<td>AD Gel</td>
<td>ED Primer (A/B)</td>
<td>Primer (A/B)</td>
</tr>
<tr>
<td></td>
<td>60 seconds</td>
<td>60 seconds</td>
<td>60 seconds</td>
<td>60 seconds</td>
<td>No washing</td>
<td>60 seconds</td>
</tr>
<tr>
<td>Washing</td>
<td>PA/AD/AS-FE*</td>
<td>10-3</td>
<td>ED Primer (A/B)</td>
<td>60 seconds</td>
<td>No washing</td>
<td>60 seconds</td>
</tr>
<tr>
<td>Bonding</td>
<td>PA/AD/AS-FE*</td>
<td>10-3</td>
<td>ED Primer (A/B)</td>
<td>60 seconds</td>
<td>No washing</td>
<td>60 seconds</td>
</tr>
<tr>
<td></td>
<td>Super-Bond C&amp;B (4-META/MMA-TBB resin)</td>
<td>Panavia FC</td>
<td>Oxyguard II</td>
<td>C&amp;B Cement</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*AS-FE, 0-20 wt% ascorbic acid (AS) and 0-10 wt% ferric chloride (FE)

RESULTS

Tensile testing

The results of TBS are illustrated in Fig. 1 according to the concentrations of ascorbic acid and ferric chloride. The highest bond strength was observed when the conditioner contained 10 wt% AS with 5 wt% FE (designated as 10AS-5FE).

One-way ANOVA run on the tensile testing results revealed that the bond strength was significantly influenced by the type of bonding procedure ($F=87.6, P=0.0001$). Average bond strengths, standard deviations, and Duncan groupings are summarized in Table 3. The average bond strengths varied from 2.2±0.7 MPa to 10.9±1.9 MPa. No significant

Scanning electron microscopy

Five 10-3/SB specimens and five PA/AD/AS-FE/SB specimens were prepared for SEM evaluation. The bonded specimens were cut vertically with a low speed saw (Isomet, Buehler, Lake Bluff, IL, U.S.A.). They were immersed in 6N HCl (Waco Pure Chemical Industries, Ltd., Osaka, Japan) for 30 seconds to dissolve the mineral components, then immersed in 10 wt% NaClO (Waco Pure Chemical Industries, Ltd., Osaka, Japan) for 60 minutes to remove the organic components from the bonded specimen. After sputter-coating with gold (IB-3, Eico Engineering Co. Ltd., Mito, Japan), cross sectional views of the dentin-resin interface were observed with a scanning electron microscope (SEM) (S-3500N, Hitachi Corp., Tokyo, Japan). The most representative cross-sectional views of both the 10-3/SB and PA/AD/AS-FE/SB group were photographed.

Vertical bars indicate standard deviation.

Fig. 1 Tensile bond strengths between 4-META/MMA-TBB resin and dentin treated with phosphoric acid etchant, NaClO agent, and experimental conditioner containing ascorbic acid and ferric chloride.

(a) AS-FE conditioner containing 0, 5, 10, or 20 wt% ascorbic acid with 5 wt% ferric chloride.

(b) AS-FE conditioner containing 0, 3, 5, or 10 wt% ferric chloride with 10 wt% ascorbic acid.
Table 3  Tensile bond strength results

<table>
<thead>
<tr>
<th>Bonding procedures</th>
<th>Mean (SD) (MPa)</th>
<th>Grouping*****</th>
</tr>
</thead>
<tbody>
<tr>
<td>PA/AD/0AS-0FE*/SB</td>
<td>2.2 (0.7)</td>
<td>a</td>
</tr>
<tr>
<td>PA/AD/0AS-5FE***/SB</td>
<td>2.3 (0.5)</td>
<td>a</td>
</tr>
<tr>
<td>PA/AD/10AS-0FE****/SB</td>
<td>3.3 (1.4)</td>
<td>a</td>
</tr>
<tr>
<td>PA/AD/10AS-5FE*****/SB</td>
<td>10.9 (1.9)</td>
<td>b</td>
</tr>
<tr>
<td>PA/AD/10-3/SB</td>
<td>2.5 (0.7)</td>
<td>a</td>
</tr>
<tr>
<td>10-3/SB</td>
<td>10.4 (2.2)</td>
<td>b</td>
</tr>
<tr>
<td>PA/AD/ED/Panavia FC</td>
<td>10.5 (1.7)</td>
<td>b</td>
</tr>
<tr>
<td>All-Bond 2</td>
<td>3.5 (0.9)</td>
<td>a</td>
</tr>
</tbody>
</table>

*0AS-0FE, No ascorbic acid and no ferric chloride
**0AS-5FE, 5 wt% ferric chloride without ascorbic acid
***10AS-0FE, 10 wt% ascorbic acid without ferric chloride
****10AS-5FE, 10 wt% ascorbic acid with 5 wt% ferric chloride
*****Identical letters indicate that the values are not statistically different (p>0.05).

Fig. 2 Scanning electron micrographs.
(a) Cross-sectional view of the resin-dentin interface in the specimen bonded with 10-3/SB (1000×). [R: 4-META/MMA-TBB resin, D: dentin, H: hybrid layer]
(b) Cross-sectional view of the resin-dentin interface in the specimen bonded with PA/AD/10AS-5FE/SB (1000×). [R: 4-META/MMA-TBB resin, D: dentin]

Scanning electron microscopy
The scanning electron microphotographs are shown in Fig. 2. The dentin surface was seen to be at a lower level than the resin surface. A hybrid layer was observed in the cross sectional view of the specimen bonded with 10-3/SB (Fig. 2-a), while no structure resembling a hybrid layer was observed at the resin-dentin interface in the specimen bonded with PA/AD/10AS-5FE/SB (Fig. 2-b).

DISCUSSION
The results of this study suggested that a dentin conditioner containing both ascorbic acid and ferric chloride improved adhesive bonding between carboxylic resin and dentin conditioned with phosphoric acid and NaClO. The conditioner was designed to bond the carboxylic type resin to dentin surfaces whose collagen had been removed during pretreatment of that substrate. This bonding system proposed a novel dentin bonding of carboxylic resin when dentin surface consists of the structure without a hybrid layer.

The bond strength between the 4-META/MMA-TBB resin and bovine dentin pretreated with 10-3 solution decreased from 18 MPa to 4 MPa after immersion in water for a period of two years[30]. The region where the collagen fibers were incompletely enveloped by the resin was susceptible to hydrolytic attack[31]; the resin protected the collagen exposed on...
the dentin surface. Little collagen was exposed on the dentin surface after treatment with the AD gel method. No hybrid layer was observed in Figure 2-b. Taking these findings into consideration, it can be speculated that the hydrolytic attack is minimized in the PA/AD/10AS-5FE group. Therefore, it is assumed that a consistent dentin bonding can be contributed to the success of restorative treatments.

The tensile bond strength was evaluated using bovine dentin in this study. The use of bovine dentin is considered acceptable, since no significant differences in the bond strengths as well as microleakage behavior between bovine and human dentin have been reported.

Comparison between groups 10-3/SB and PA/AD/10-3/SB showed that the application of the AD gel method (PA/AD) resulted in decreased bond strength. This agrees with the finding that an endodontic cleaning with NaClO weakened dentin bonding in the Super-Bond system. The decreased bond strength may be attributed to the destruction of collagen fibers by AD gel, which has a NaClO concentration of 10%. 10-3 conditioning may not have enough collagen to infiltrate resin monomer into the demineralized dentin by AD gel conditioning. However, it was reported that the bond strength for 10-3 liquid recovered when the dentin surface was conditioned with ascorbic acid after being treated with 6% NaClO. Further study is desired to evaluate the about effect of the concentration of NaClO.

Even when ferric chloride was used, the maximum bond strength was not obtained in the absence of ascorbic acid (groups PA/AD/0AS-5FE/SB). NaClO is neutralized and reduced in the chemical reaction with ascorbic acid. It is therefore concluded that our hypothesis that the undesirable effects of NaClO were eliminated by the use of ascorbic acid was correct. However, the group PA/AD/10AS-0FE/SB indicated that bond strength is not improved with ascorbic acid alone.

Regarding the role of the ferric ion, it was shown that the polymerization of MMA was accelerated by ferric ions adsorbed onto dentin. When the initiation of polymerization starts at the dentin-resin interface, the adverse effect of polymerization shrinkage can be minimized. The ferric ion may have another role in terms of stabilizing dentin collagen during acid conditioning. However, the exposed collagen had already been removed from the dentin surface before the ferric chloride was applied in this study. Accordingly, we theorized that ferric chloride accelerated the polymerization of the resins.

Although ascorbic acid reacts with oxygen in aqueous solution, it has a better shelf life in acidulous solution than neutral or alkaline solutions. The 5 wt% ferric chloride solution used in this experiment was acidulous. Ascorbic acid acts as an effective antioxidant, such that it would be reasonable from the perspective of shelf life to use ascorbic acid with ferric chloride as a conditioner liquid. It was found that both agents exhibited effective function individually when dentin was treated with AD gel. However, their synergetic effect was not confirmed, as it was not evaluated in this study.

In this study, it was confirmed that this dentin conditioner improved the bond strength between collagen-depleted dentin and carboxylic resin. However, further investigation is promising to assess long-term durability, and to reduce the number of clinical steps in this novel dentin conditioning system.

**CONCLUSIONS**

A novel dentin conditioned with 10 wt% ascorbic acid and 5 wt% ferric chloride improved the bonding between 4-META/MMA-TBB resin and dentin conditioned with phosphoric acid etching and subsequent NaClO treatment. This conditioner effectively facilitated the bond between carboxylic resins and collagen-depleted dentin.

**REFERENCES**