Effect of experimental acid/base conditioner on microtensile bond strength of 4-META/MMA-TBB resin to dentin after long-term water immersion

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An experimental conditioner (Exp), which was an aqueous solution of 10% ascorbic acid and 5% ferric chloride, was prepared in this study. This study evaluated the effect of Exp on the microtensile bond strength between a self-curing resin and dentin after long-term water immersion. Flat human dentin surfaces were sequentially pretreated with 40% phosphoric acid, 10% sodium hypochlorite, and Exp. Surface pretreatment with an aqueous solution of 10% citric and 3% ferric chloride (10-3) was used as a control. Composite resin rods were bonded to pretreated dentin surfaces using 4-META/MMA-TBB resin. Microtensile bond strengths were evaluated after water immersion at 24 h, 12 months, 24 months, and 36 months. At each immersion period, the bond strength of Exp was significantly higher than that of 10-3. After 36 months, Exp showed no significant decrease in microtensile bond strength, but 10-3 showed significant reductions. Pretreatment with experimental acid/base conditioner markedly improved the bonding durability of 4-META/MMA-TBB resin to human dentin when compared against the conventional 10-3 treatment.

Keywords: Dentin bonding, Ascorbic acid, 4-META/MMA-TBB resin, Surface treatment, Durable bonding

INTRODUCTION

Critical to the longevity of any dental restoration is the bonding quality of luting materials to dentin. A strong and durable bond is required to avoid detachment of indirect restorations, prevent microleakage, secondary caries, and tooth fractures. One indispensable prerequisite for optimum bonding is a contaminant-free dentin surface. However, in clinical situations, dentin is unavoidably and frequently contaminated with saliva, blood, and materials such as temporary cement remnants[1,2], desensitizers[3,4], and medicaments used for root canal treatment[5]. To minimize the unfavorable effects of surface pollutants on adhesive-dentin bonding, it has been recommended to sequentially condition the dentin surface, immediately before luting, with 34%–40% phosphoric acid (H_3PO_4) and 10% sodium hypochlorite (NaOCl) (AD Gel)[6-8].

Dentin bonding systems may contain phosphate or carboxylic acid functional monomers. An example of phosphate-based dentin bonding systems is Panavia Fluoro Cement (Kuraray Co. Ltd., Osaka, Japan), which uses 40% phosphoric acid (Panavia Etching Agent) and 10% NaOCl (AD Gel) to condition exposed dentin surfaces. Pretreatment of dentin with Panavia Etching Agent is designed to remove the smear layer and demineralize the dentin surface. An example of carboxylic acid resins is Super-Bond C&B (Sun Medical Co. Ltd., Moriyama, Japan), which uses the 10-3 liquid (an aqueous solution of 10% citric acid and 3% ferric chloride) for surface pretreatment. With both types of dentin bonding systems, a hybrid layer of resin-reinforced dentin[9-11] is created by the infiltration and impregnation of acidic functional monomers into the subsurface of pretreated, decalcified dentin substrate followed by polymerization *in situ*.

4-META/MMA-TBB resins are composed of 4-methacryloxyethyl trimellitate anhydride (4-META), methyl methacrylate (MMA), and tri-*n*-butylborane derivative (TBB). It was reported that the ferric chloride component of the 10-3 liquid conditioner was the key component in improving the bonding performance of 4-META/MMA-TBB resins to conditioned dentin[12-15]. High initial bond strengths can be achieved by dentin conditioning, but bonding durability is equally critical to long-term clinical success. After dentin conditioning, resin monomer infiltration into the dentinal tubules and collagen fibrils protects and reinforces the exposed collagen fibril network[16]. However, in resin-deficient zone, exposed and unprotected collagen fibrils are susceptible to hydrolytic attack[17]. It has been reported that the tensile bond strength between 4-META/MMA-TBB resin and bovine dentin pretreated with 10-3 solution decreased from 18 MPa to 4 MPa after 2-year immersion in water[18].

When H_3PO_4 was used in conjunction with NaOCl to remove both the inorganic and organic components of dentin, adverse effects stemming from hydrolytic attack seemed to decrease. This was hypothetically attributed to the complete removal of the collagen network from acid-etched dentin surface. Kodama reported that after dentin was pretreated with H_3PO_4 and NaOCl, there was no decrease in bond strength between dentin and Panavia 21 even after 50,000 thermal cycles[19].

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However, NaOCl pretreatment of dentin surfaces has been reported to adversely affect the bonding of 4-META/MMA-TBB resins. It was speculated that residual NaOCl inhibited the polymerization of 4-META/MMA-TBB resins at the bonding interface[20,21]. Ascorbic acid, on the other hand, has been numerously reported to reverse the adverse effects of residual NaOCl on resin-dentin bond strength. For example, application of 10% ascorbic acid or 10% sodium ascorbate completely reversed the significant reductions in bond strength between C&B Metabond (Parkell, Farmingdale, NY, USA) and root dentin caused by 5% NaOCl[22]. This is important because 5% NaOCl is routinely used in endodontic procedures. For the purpose of reversing this detrimental effect of compromised bonding to etched dentin, ascorbic acid is also used in myriad ways as a cleaning solution[23], etchant[24], or polymerization initiator[25].

A previous study demonstrated that 24-h bond strength between 4-META/MMA-TBB resin and dentin conditioned with AD Gel and an experimental conditioner containing 10 wt% ascorbic acid/5 wt% ferric chloride was significantly higher than these groups: 0% ascorbic acid/0% ferric chloride, 5% ferric chloride only, and 10% ascorbic acid only[26]. Although the use of phosphoric acid and NaOCl (AD Gel) resulted in decreased bond strength between 4-META/MMA-TBB resin and dentin, additional conditioning with 10% ascorbic acid and 5% ferric chloride improved the bond strength[26]. It was thus hypothetically concluded that dentin pretreatment with an ascorbic acid/ferric chloride combination helped to completely remove the exposed collagen network and minimize or reverse the decrease in bond strength of carboxylic acid-based resins.

Although favorably high initial bond strength was obtained at 24 h after bonding[26], bond durability and longevity of 4-META/MMA-TBB resins needs further investigation as it was reported that the bond strength of Super-Bond C&B significantly decreased after 10 years[27]. Therefore, the purpose of this study was to evaluate the bond strength of 4-META/MMA-TBB resin after long-term immersion in water, when dentin was conditioned with AD Gel followed by additional conditioning with an experimental aqueous solution of ascorbic acid and ferric chloride.

MATERIALS AND METHODS

Materials

The chemical compositions, manufacturers, and lot numbers of the dentin conditioners and luting agent used in this study are presented in Table 1.

Specimen preparation

Caries-free human third molars were stored in 0.5% chloramine T at 4°C and used within 1 month after extraction. A low-speed saw equipped with a diamond-impregnated disk (Isomet, Buehler Ltd, Lake Bluff, IL, USA) was used to remove the occlusal enamel of each tooth. 320-grit silicon carbide paper was used to create a smear layer on the dentin surface under running water. After rinsing with tap water and air-drying for 15 s, dentin conditioning on the occlusal surfaces of mid-coronal dentin was carried out for each experimental group, 10-3 *versus* Exp, as described in Table 2.

After dentin conditioning, a 4-META/MMA-TBB resin was used to bond a composite resin rod (10 mm diameter, 10 mm length) to the treated dentin specimens. Thirteen bonded specimens from each group were used for microtensile bond strength testing. Two more bonded specimens from each group were used for scanning electron microscopy (SEM) analysis. All bonded specimens were left undisturbed at room temperature for 30 min, and then stored in 37°C water up to a maximum of 36 months.

Microtensile bond strength test

After the bonded specimens were stored in 37°C water for 0 month (24 h), 12 months, 24 months, or 36 months, microtensile bond strengths (micro-TBS) were determined using a table-top testing machine (EZ Test, Shimadzu Corp., Kyoto, Japan) at a crosshead speed of 1.0 mm/min in accordance with manufacturer's specifications.

The mean and standard deviation values of the 13 specimens of each group at each immersion period were calculated. Data were analyzed using two-way analysis of variance (ANOVA) and a *post-hoc* test (Duncan's New Multiple Range Test)[28,29]. Level of statistical significance was set at $\alpha=0.05$.

pH measurement

The pH value of each conditioning agent used in this study was measured using a pH meter (TWIN pH, Horiba Ltd., Kyoto, Japan).

Scanning electron microscopy (SEM)

After immersion in water for 3 years (36 months), 10-3 and Exp specimens were subjected to SEM observation. Bonded specimens were vertically sectioned using a low-speed saw (Isomet, Buehler, Lake Bluff, IL, USA) under running water. They were then demineralized in 6 N HCl (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for 30 s, thoroughly rinsed in water, and immersed in 1% NaOCl (Wako Pure Chemical Industries, Ltd., Osaka, Japan) for 1 h to remove organic elements. The exposed cross-sections of the bonded specimens were sputter-coated with gold (IB-3, Eico Engineering Co. Ltd., Ibaraki, Japan) and observed using SEM (S-3500N, Hitachi Corp., Tokyo, Japan).

RESULTS

Microtensile bond strength

Two-way ANOVA revealed that the factors of dentin conditioner and immersion period had significant effects on micro-TBS. Interaction between these two factors was also significant. The means and standard deviations of bond strength data, as well as Duncan's test results, are presented in Table 3.

Table 1 Dentin conditioners and luting agent used in this study

Material	Composition	Manufacturer	Lot no.
Conditioner			
Panavia EtchingAgent (pH 0.1)	40 wt% phosphoric acid	Kuraray Co. Ltd., Osaka, Japan	0284AL
AD Gel (pH 9.5)	10 wt% sodium hypochlorite	Kuraray Co. Ltd.	00614A
Green Conditioner (10-3) (pH 0.5)	10 wt% citric acid, 3 wt% ferric chloride	Sun Medical Co. Ltd., Moriyama, Japan	FS2
Experimental (pH 0.4)	10 wt% ascorbic acid 5 wt% ferric chloride	Sun Medical Co. Ltd. Katayama Chemical, Osaka, Japan	
Luting agent			
Super-Bond C&B (4-META/MMA-TBB resin)	Initiator: tri-*n*-butylborane derivative	Sun Medical Co. Ltd.	GT73
	Monomer liquid: 5 wt% 4-META/MMA		KE3
	Polymer powder (clear): Pulverized PMMA		GE1

4-META: 4-methacryloyloxyethyl trimellitate anhydride; MMA: methyl methacrylate; PMMA: poly(methyl methacrylate).

Table 2 Dentin conditioning procedures

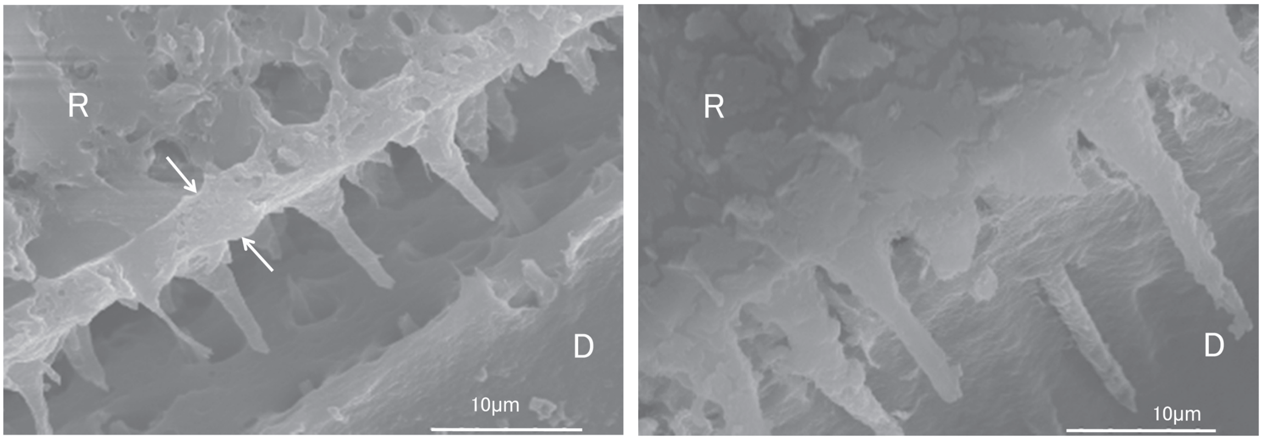
Procedure	10-3	Exp
First treatment	10 wt% citric acid and 3 wt% ferric chloride	40 wt% phosphoric acid
Rinse	10 s 5 s	10 s 5 s
Second treatment	None	10 wt% sodium hypochlorite
Rinse	None	60 s 15 s
Third treatment	None	Experimental conditioner (10 wt% ascorbic acid and 5 wt% ferric chloride aq.)
Rinse	None	5 s 5 s
Adhesive	< 4-META/MMA-TBB resin >	

Table 3 Microtensile bond strength results (SD) expressed in MPa

Group	0 month	12 months	24 months	36 months
10-3	23.9 (8.2)[b]	15.5 (6.1)[c]	17.9 (5.7)[c]	16.2 (7.3)[c]
Exp	36.3 (8.4)[a]	31.5 (4.8)[a]	33.7 (6.5)[a]	31.1 (8.1)[a]

n=13. Values with identical superscript letters are not statistically different (p>0.05).

Mean bond strengths ranged from 15.5 MPa for 10-3 group (12 months) to 36.3 MPa for Exp group (0 month). At each immersion period up to 36 months, the mean bond strength of Exp group was significantly higher than that of 10-3 group. The mean bond strength of 10-3 group was already significantly reduced after 12 months of immersion and then thereafter. For Exp group, there were no significant decreases in mean bond strength even after 36 months of water immersion.



(a) (b)

Fig. 1 (a) SEM cross-sectional view of the resin-dentin interface of 10-3-treated specimen after 36 months of water immersion (×1,000), where R: resin; D: dentin; Arrows indicate the hybrid layer.
(b) SEM cross-sectional view of the resin-dentin interface of Exp-treated specimen after 36 months of water immersion (×1,000), where R: resin; D: dentin. No hybrid layer was observed.

Hybrid layer observation by SEM

SEM images of the cross-sections of 10-3 and Exp specimens after 36-month immersion in water are shown in Figs. 1(a) and 1(b). A hybrid layer could be seen in the 10-3 specimen (Fig. 1(a)). Cracks and voids were also observed at the interface between the adhesive resin and hybrid layer (Fig. 1(a)). In stark contrast, neither a hybrid layer nor cracks were seen in the Exp specimen (Fig. 1(b)).

DISCUSSION

Oxygen inhibits resin polymerization. It was reported that NaOCl liberated oxygen which caused strong inhibition of resin polymerization at the interface, thereby weakening the bond of 4-META/MMA-TBB resin to dentin[20]. However, ascorbic acid —an antioxidant and natural reducing agent— naturalized and reduced NaOCl in their chemical reaction[21], thereby reversing the compromised bond strength[21]. Thus, ascorbic acid is widely recommended to reverse the adverse effects of residual NaOCl on resin-dentin bond strength after AD Gel conditioning[21].

While NaOCl inhibits resin polymerization, ferric ions adsorbed on dentin surfaces initiate and accelerate the polymerization of methyl methacrylate (MMA)[13-15]. Ferric ions also aid to reduce cure shrinkage[14] and subdue the denaturing effect of dentin collagen exposed to a pretreatment acid[12]. In the present study, ferric chloride improved the bonding between resin and dentin even though NaOCl had completely removed the exposed collagen network. In our previous study, maximum bond strength of 4-META/MMA-TBB resin to collagen-depleted dentin was not obtained in the absence of ferric chloride, even when ascorbic acid was used[26]. Taken together, our results confirmed that ferric chloride accelerated the polymerization of 4-META/MMA-TBB resin.

Adhesive monomer infiltration into demineralized dentin is key to creating a resin-impregnated hybrid layer[9-11], composed of collagen fibrils exposed by demineralization. Collagen fibrils that are completely coated by resin are protected from degradation. However, the mechanical properties of incompletely resin-coated collagen fibrils within the hybrid layer could be weakened by hydrolysis[17]. It has been reported that the bond strength of Super-Bond C&B to bovine dentin that was conditioned with 10-3 decreased after long-term water immersion[18]. However, treatment with AD Gel dissolved and removed the exposed dentinal collagen[6]. In Fig. 1(b), no hybrid layer was observed in the Exp specimen. This could be a reason why the bond strength of Exp group was significantly higher than 10-3 group at each immersion period up to 36 months, since AD Gel application in Exp group removed the threat of unprotected collagen fibrils being subject to hydrolytic attack.

Cracks and voids were seen on the SEM image of 10-3 group (Fig. 1(a)), suggesting that the coupling between the adhesive and top of the hybrid layer was the weakest link in the bonded assembly. However, no such findings were observed in the Exp group after 36 months of water immersion (Fig. 1(b)). This might explain why the Exp specimens showed no significant decrease in bond strength even after 36 months of water immersion. Together with the bond strength results of this study, it was clearly shown that additional conditioning with 10 wt% ascorbic acid and 5 wt% ferric chloride after AD Gel application minimized the decrease in bond strength between carboxylic acid-based resins and dentin after long-term immersion in water.

Ascorbic acid is a strong reducing agent that readily reacts with dissolved oxygen in aqueous solutions. Nonetheless, ascorbic acid has a longer shelf life in acidic solutions than in neutral or alkaline solutions. The 5 wt% ferric chloride solution used in this experiment was acidic. With due consideration to the shelf life of ascorbic acid, it would be reasonable and expedient to use ascorbic acid with ferric chloride as a conditioner liquid on dentin.

CONCLUSION

Compared to the bond strength of 10-3-treated dentin, human dentin sequentially treated with phosphoric acid, NaOCl, and an experimental conditioner of 10 wt% ascorbic acid with 5 wt% ferric chloride showed significantly improved bond strength and durability with 4-META/MMA-TBB resin, even after long-term immersion in water.

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REFERENCES

1) Ribeiro JC, Coelho PG, Janal MN, Silva NR, Monteiro AJ, Fernandes CA. The influence of temporary cements on dental adhesive systems for luting cementation. J Dent 2011; 39: 255-262.
2) Takimoto M, Ishii R, Iino M, Shimizu Y, Tsujimoto A, Takamizawa T, Ando S, Miyazaki M. Influence of temporary cement contamination on the surface free energy and dentine bond strength of self-adhesive cement. J Dent 2012; 40: 131-138.
3) Camps J, Pizant, S, Dejou J, Franquin JC. Effects of desensitizing agents on human dentin permeability. Am J Dent 1998; 11: 286-290.
4) Soeno K, Taira Y, Matsumura H, Atsuta M. Effect of desensitizers on bond strength of adhesive luting agents to dentin. J Oral Rehabil 2001; 28: 1122-1128.
5) Soeno K, Taira Y, Atsuta M. Influence of formaline cresol on bond strength of adhesive luting agents to dentin. J Oral Rehabil 2000; 27: 623-628.
6) Fujita E, Takada Y, Kato T, Kondo Y, Suzuki K, Yamashita A. Effect of dentin treatment on adhesion of adhesive composite resin to dentin — Dissolution of collagen. Adhesive Dentistry 1990; 8: 227-235.
7) Prati C, Chersoni S, Pashley DH. Effect of removal of surface collagen fibrils on resin-dentin bonding. Den Mater 1999; 15: 323-331.
8) Perdigao J, Lopes M, Geraldeli S, Lopes GC, Garcia-Godoy F. Effect of a sodium hypochlorite gel on dentin bonding. Dent Mater 2000; 16: 311-323.
9) Nakabayashi N, Kojima K, Masuhara E. The promotion of adhesion by the infiltration of monomers into tooth substrates. J Biomed Mater Res 1982; 16: 265-273.
10) Van Meerbeek B, Inokoshi S, Braem M, Lambrechts P, Vanherle G. Morphological aspects of the resin-dentin interdiffusion zone with different dentin adhesive systems. J Dent Res 1992; 71: 1530-1540.
11) Pashley DH, Carvalho RM. Dentin permeability and dentin adhesion. J Dent 1997; 25: 355-372.
12) Nakabayashi N. Bonding of restorative materials to dentine: the present status in Japan. Int Dent J 1985; 35: 145-154.
13) Kadoma Y, Imai Y. Effect of ferric salts on polymerization of MMA by TBBO in the presence of a collagen sheet — A model to study the mechanism of adhesion of MMA resin to dentin. J Jpn Dent Mater 1988; 7: 817-823.
14) Imai Y, Kadoma Y, Kojima K, Akimoto T, Ikakura K, Ohta T. Importance of polymerization initiator systems and interfacial initiation of polymerization in adhesive bonding of resin to dentin. J Dent Res 1991; 70: 1088-1091.
15) Kamigaito M, Ando T, Sawamoto M. Metal-catalyzed living radical polymerization. Chem Rev 2001; 101: 3689-3746.
16) Takarada K, Kojima M, Ishihara K, Nakabayashi N. Durability of bonding between 4-META/MMA-TBB resin to dentin pretreated with 10-3 — The effect of 10-3 pretreating peroid and subsequent glutaraldehyde treatment. J Jpn Dent Mater 1990; 9: 831-840.
17) Burrow MF, Satoh M, Tagami J. Dentin bond durability after three years using a dentin bonding agent with and without priming. Dent Mater 1996; 12: 302-307.
18) Kiyomura M. Bonding strength to bovine dentin with 4-META/MMA-TBB resin: Long-term stability and influence of water. J Jpn Soc Dent Mater 1987; 6: 860-872.
19) Kodama T. A study on dentin bonding —Effect of surface pretreatment on adhesion to dentin. Adhes Dent 1997; 15: 1-20.
20) Nikaido T, Takano Y, Sasafuchi Y, Burrow MF, Tagami J. Bond strengths to endodontically treated teeth. Am J Dent 1999; 12: 177-180.
21) Kataoka H, Yoshioka T, Suda H, Imai Y. Effect of sodium hypochlorite on adhesion of 4-META/MMA-TBB resin to dentin. Jpn J Conserv Dent 1999; 42: 241-247.
22) Morris MD, Lee KW, Agee KA, Bouillaguet S, Pashley DH. Effects of sodium hypochlorite and RC-Prep on bond strengths of resin cement to endodontics surfaces. J Endod 2001, 27: 753-757.
23) Jedrychowski JR, Caputo AA, Prola J. Influence of a ferric chloride mordant solution on resin-dentin retention. J Dent Res 1981; 60: 134-138.
24) Code JE, Schumacher GE, Antonucci JM. Ascorbic acid as an etchant-conditioner for resin bonding to dentin. In: Shalaby SW, Ikada Y, Langer R, Williams J, editors. ACS Symposium 540, Polymers of biological and biomedical significance. Washington, DC: Am Chem Soc; 1994; p. 147-156.
25) Antonucci JM, Grams CL, Termini DJ. New initiator systems for dental resins based on ascorbic acid. J Dent Res 1979; 58: 1887-1899.
26) Soeno K, Taira Y, Matsumura H, Atsuta M, Suzuki, S. Adhesion of 4-META/MMA-TBB resin to collagen-depleted dentin —Effect of conditioner with ascorbic acid/ferric chloride. Dent Mater J 2004; 23: 100-105.
27) Aoki K, Kitasako Y, Ichinose S, Burrow MF, Ariyoshi M, Nikaido T, Tagami J. Ten-year observation of dentin bonding durability of 4-META/MMA-TBB resin cement — a SEM and TEM study. Dent Mater J 2011; 30: 438-447.
28) Duncan DB. Multiple range and multiple F tests. Biometrics 1955; 11: 1-42.
29) Duncan DB. Multiple range tests for correlated and heteroscedastic means. Biometrics 1957; 13: 164-176.