Effect of Fluoride in Phosphate Buffer Solution on Bonding to Artificially Carious Enamel

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The purpose of the present study was to evaluate the effect of fluoride on resin bonding to artificially carious enamel. Specimens from demineralized human enamel sections were prepared using two commercially available adhesives (Clearfil SE Bond, Kuraray; Single Bond, 3M) and a composite resin (Clearfil AP-X, Kuraray) according to manufacturers' instructions. They were then immersed in phosphate buffered saline solution with varied fluoride concentrations at 0, 0.1, 0.5, 1, and 10 ppm. After immersion in each solution for one, three, or seven days, microshear bond strength was measured. The bond strengths of both adhesive systems to artificially carious enamel significantly increased after immersion in fluoride-phosphate buffer solution. Based on the findings obtained, we thus proposed not to remove the white enamel lesions for bonding in the clinic. They might be preserved and treated using fluoride applications.

Keywords: Fluoride, Microshear bond strength, Artificially carious enamel

INTRODUCTION

During the last two decades, a plethora of adhesive systems have been developed with a view to producing good adhesion to dental substrates. These significant advances in adhesive dentistry have changed the approaches and attitudes pertaining to cavity preparation. While cavity preparation was once based on the principles proposed by GV Black, it now adopts a more conservative and minimally invasive approach — whereby only carious lesions are removed and the intact tooth structure is preserved[1,2]. This is now regarded as a mainstream treatment for dental caries in the 21st century.

In Minimal Intervention Dentistry, the anchor principle is to delay operative intervention. This is achieved through repeated efforts to remineralize the lesions or prevent further demineralization. Even in cases where surgical intervention is needed, the priority is minimum removal of tooth structure. As such, only the bacterially infected part of a tooth is removed, leaving intact the caries-affected area without bacterial infection. However, several papers have reported that the bond strength of resinous materials to caries-affected dentin or enamel was lower than that to normal tooth strucutres[3-5].

Since the development of fluoride agents in the 1940s, fluoride applications to human enamel are widely used in caries prevention[6-9]. The effectiveness of applied fluoride, either from solution or from dentifrice, and most commonly as sodium fluoride or sodium monofluorophosphate, is increasingly showing results with recent sharp falls in the prevalence of dental caries[10].

Fluoride ion has been shown to play a major role in significantly reducing dental caries[10]. Several *in vitro* studies have been carried out to evaluate the effects of fluoride on caries process, including the dissolution rate of hydroxyapatite[11], the dissolution rate of enamel[12], the remineralization of artificial lesions in enamel[13,14], and the uptake of fluoride into sound and demineralized enamel[15]. These studies have clearly shown that fluoride can both reduce the rate of demineralization and increase the rate of remineralization. However, few studies have evaluated the effect of fluoride on bonded restorations to caries-affected enamel.

In our previous study, we investigated the bonding strength to artificially carious enamel[5]. Bond strength decreased after artificial enamel demineralization, and decrease was gradual with increase of demineralization time. The purpose of this study, therefore, was to evaluate the effects of varied fluoride concentrations on resin bonding to artificially carious human enamel.

MATERIALS AND METHODS

Table 1 lists the materials used in this study and their compositions.

Preparation of enamel specimens

Permission was obtained from our institute for using human teeth in this experiment. To this end, non-carious human molars extracted in the clinic were stored in isotonic sodium chloride solution. Two hundred and sixty enamel sections of 2 mm thickness were then prepared from the buccal surfaces of two hundred and sixty extracted, non-carious human molars with a slow-rotating blade (Isomet, Buehler, Lake Bluff, IL, USA) under running water.

Table 1 Materials used in this study

	Material	Composition
Single Bond (Batch No. 20021008)	Etchant	35% phosphoric acid
	Adhesive	HEMA, Bis-GMA, dimethacrylates, methacrylate, polyalkenoic acid copolymer, photoinitiator, ethanol, water
Clearfil SE Bond (Batch No. 71130)	Primer	MDP, HEMA, hydrophobic dimethacrylate, dl-camphorquinone, aromatic tert-amine, water
	Bond	MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, photoinitiator, aromatic tert-amine, silanated colloidal silica
Clearfil AP-X (Batch No. 01251)		silanated barium glass, silica, colloidal silica, Bis-GMA, TEGDMA, photoinitiator

HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: Bisphenol A diglycidylmethacrylate; MDP: 10-methacryloyloxydecyl dihydrogen phosphate; TEGDMA: triethylene glycol dimethacrylate

Enamel surfaces were polished with 280-grit silicon carbide papers. Then, the enamel slices were immersed in a demineralization solution as previously described by Wefel et al.[16], containing 2.2 mmol/L $CaCl_2$, 2.2 mmol/L NaH_2PO_4, and 50 mmol/L acetic acid adjusted to pH 4.5. The immersion lasted three hours for the intent of creating artificial carious lesions on the surface. To investigate the effect of demineralization after three hours, porosity of the dental tissue was observed using SEM (Fig. 1).

Preparation of bonded specimens
After immersion in demineralization solution, the sections were rinsed under running water for 15 minutes and randomly divided into two groups, treated with either Clearfil SE Bond (Kuraray Medical Inc., Tokyo, Japan) or Single Bond (3M ESPE, St. Paul, USA) according to manufacturers' instructions (Table 2). A cut micro Tygon tube with an internal diameter of approximately 0.7 mm and 0.5 mm height was placed on the enamel surface, into which a resin composite (Clearfil AP-X, shade A3, Kuraray Medical Inc., Tokyo, Japan) was filled. Light curing for 40 seconds was done with a quartz-tungsten-halogen lamp (Optilux 501, Demetron Research Corp./Sybron Dental Specialties, Orange, CA, USA) (Fig. 2). After light curing, the tube was removed and the specimens were immersed in 37 water for 24 hours.

The teeth were then further divided into 26 groups of 10 slices each, according to varied fluoride concentrations of 0.1, 0.5, 1, and 10 ppm in phosphate buffered saline solution (PBS) with varied immersion periods (1, 3, or 7 days). Control specimens were stored in phosphate buffer solution (0 ppm fluoride) for one day.

Microshear bond strength test
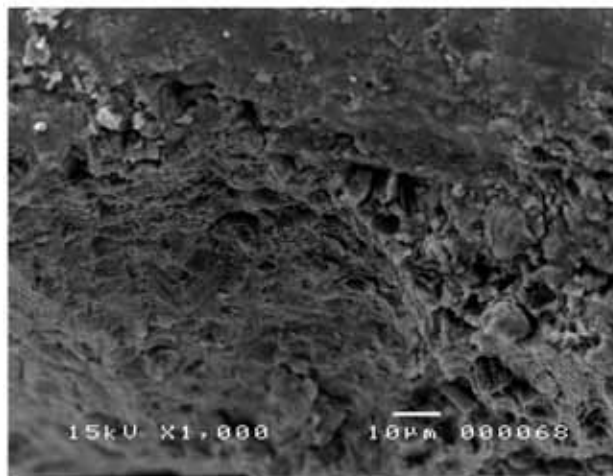After storage in each experimental solution,

Fig. 1 SEM photograph of the surface morphology of enamel after 3 hours' demineralization.

Table 2 Application procedures of two adhesive systems used

	Single Bond	Clearfil SE Bond
(1)	Apply etchant 15 seconds	Apply primer 20 seconds
(2)	Rinse 15 seconds	Dry
(3)	Blot dry with absorbent paper	Apply adhesive
(4)	Apply two coats of adhesive	Light cure 10 seconds
(5)	Light cure 10 seconds	

microshear bond strength of bonded resin to artificially carious enamel was measured[17] using an EZ-Test tensile test apparatus (Shimadzu Co., Kyoto, Japan), as shown in Fig. 3. Microshear bond strength results were statistically analyzed using two-way ANOVA and Tukey Kramer test at 95
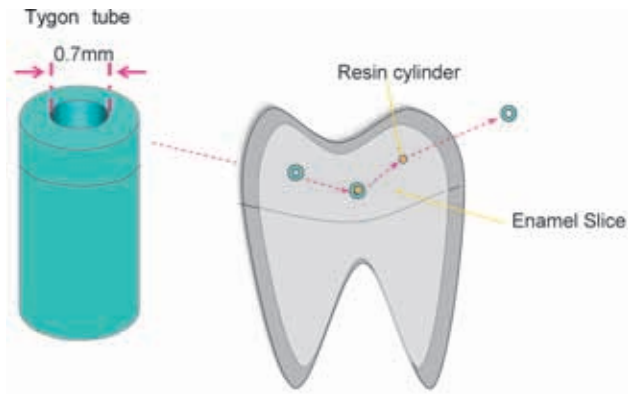
Fig. 2   Specimen preparation for the microshear bond test.   Enamel slices were treated with either Clearfil SE Bond or Single Bond according to manufacturer's instructions.   After the bonding procedure, a micro Tygon tube with an internal diameter of approximately 0.7 mm and 0.5 mm height was placed on the enamel surface, and Clearfil AP-X resin was filled into the tube. After resin was light-cured for 40 seconds, the Tygon tube was removed.
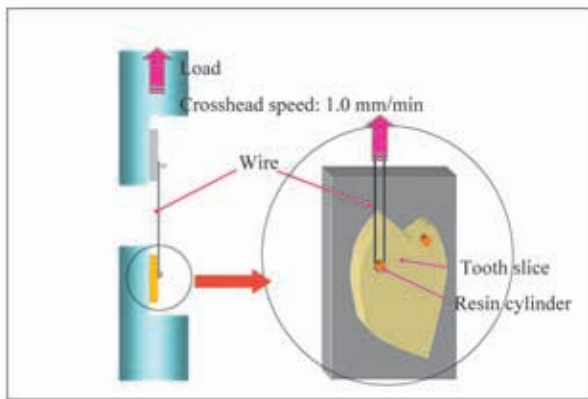

Fig. 3   Schematic illustration of microshear bond test.

level of confidence.

Failure mode analysis
After microshear bond strength test, all the fractured surfaces were inspected to determine the mode of failure using an optical microscope (Olympus OCS Colposcope, Olympus Optical Co. Ltd.) with × 30 magnification.   Fractured specimens were classified into three categories as follows: A 100 adhesive failure; B Mixed failure (adhesive failure in more than 50 of debonded zone); C Mixed failure (cohesive failure in enamel is more than 50 of debonded zone) (Table 4).   In addition, representative samples were also observed using a confocal laser scanning microscope (1LM21, Lasertec Corp., Tokyo, Japan) to confirm the accuracy of the optical inspection. Failure mode results were statistically analyzed using Kruskal Wallis one-way analysis of variance by ranks at 0.05 level of significance.

RESULTS

Microshear bond strength
Table 3 shows the microshear bond strength values of Single Bond and Clearfil SE Bond to artificially carious enamel after immersion in fluoride-PBS solutions for varied experimental periods.   Both factors (fluoride concentration and adhesive material) affected the bond strength and there was a statistically significant interaction between them.

In general, bond strength increased in all the fluoride-PBS solutions as compared to the control for both materials.   For the same fluoride concentration, bond strength gradually increased with the extension of immersion period, such that the highest bond was obtained at 7 days.   Both Clearfil SE Bond and Single Bond achieved the highest bond strength

Table 3   Shear bond strengths (MPa) of two adhesive systems used

		Single Bond		Clearfil SE Bond	
F 0 ppm (Control)	1 day	19.80 ± 4.80	a	20.50 ± 6.20	a
F 0.1 ppm	1 day	23.09 ± 5.39	b	25.27 ± 5.34	b
	3 days	24.00 ± 4.32	b	25.57 ± 3.33	b
	7 days	25.06 ± 5.82	b	26.10 ± 7.74	b
F 0.5 ppm	1 day	26.19 ± 5.32	b	32.71 ± 5.90	c,d
	3 days	28.03 ± 4.87	b,c	33.18 ± 6.02	d
	7 days	32.52 ± 5.72	c	36.03 ± 6.75	d
F 1 ppm	1 day	23.51 ± 6.15	b	27.22 ± 7.65	b,e
	3 days	23.67 ± 4.10	b	33.20 ± 6.98	d
	7 days	27.89 ± 5.26	b,c	33.64 ± 5.93	d
F 10 ppm	1 day	22.83 ± 4.64	b	26.42 ± 5.98	b
	3 days	24.19 ± 5.97	b	31.80 ± 5.28	c,d,e
	7 days	26.84 ± 5.25	b	32.03 ± 4.38	c,d,e

Data are presented as mean ± SD (MPa). N 10.
Different letters indicate statistically significant differences.

Table 4 Failure modes of fractured surfaces of two adhesive systems used

		Single Bond			Clearfil SE Bond		
		A	B	C	A	B	C
F 0 ppm (Control)	1 day	4	6	0	0	9	1
F 0.1 ppm	1 day	4	6	0	1	8	1
	3 days	2	8	0	6	4	0
	7 days	3	7	0	2	8	0
F 0.5 ppm	1 day	3	7	0	7	3	0
	3 days	8	2	0	4	6	0
	7 days	6	4	0	5	5	0
F 1 ppm	1 day	4	5	1	1	8	1
	3 days	2	8	0	3	7	0
	7 days	2	8	0	5	5	0
F 10 ppm	1 day	1	9	0	1	9	0
	3 days	3	7	0	5	5	0
	7 days	5	5	0	4	6	0

A:100 adhesive failure;
B:Mixed failure (adhesive failure in more than 50 of debonded zone);
C:Mixed failure (cohesive failure in enamel in more than 50 of debonded zone).

values after immersion in F 0.5 ppm solution at 7 days. In F 0 ppm and F 0.1 ppm solutions, the bond strengths of Single Bond and Clearfil SE Bond did not show any significant differences. However, in F 0.5ppm, F 1ppm, and F 10 ppm solutions, the bond strength of Clearfil SE Bond was significantly higher than that of Single Bond.

As for the control specimens, they were stored in phosphate buffer solution (0 ppm of fluoride) for only one day. It should thus be clarified that the bond strengths of the control specimens at 1 day could not be considered as lower than the other groups due to the post-polymerization effect of adhesive resins.

Failure mode
Table 4 shows the modes of failure after the bond test. While mixed failure was observed in most cases (adhesive failure in more than 50 of debonded zone), no significant differences in failure mode were found among the adhesive systems or test periods.

DISCUSSION

In our previous study on the bonding strength of resin adhesives to artificially demineralized enamel, it was shown that bond strength decreased with demineralization time for both two-step, self-etching primer adhesive and total-etch, wet-bond adhesive[5]. In the same study[5], the depth of artificial enamel

lesions after immersion in demineralization solution for three hours was approximately 60 μm; moreover, both adhesives showed almost the same bond strength values. Against this background, we employed three hours of demineralization period to create artificial enamel lesions in this study.

Several *in vitro* and *in vivo* studies have been undertaken to investigate the optimal fluoride therapy for dental caries prevention[18,19]. In particular, it has been suggested that there was a relative increase in fluoride uptake by white spot enamel lesions rather than by sound enamel[20]. In addition, the effect of acidic fluoride buffer solution on remineralization was suggested too[20]. Furthermore, Margolis et al.[21] observed that while there was no fluoride uptake in enamel exposed to 1 ppm fluoride aqueous solution, this same concentration of acidic buffer solution was very effective in inhibiting enamel demineralization. Considering these results, we used fluoride-phosphate buffered saline (PBS) solutions with varied fluoride concentrations to investigate the remineralization effect of enamel lesions on bonding in this study.

Results of this study clearly showed that fluoride solutions could improve the shear bond strength of bonded resin to demineralized enamel. It was also found that shear bond strength was significantly influenced by fluoride concentration and immersion period. In a previous study, it has been shown with adequate and sound evidence that fluoride reinforces crystalline apatite within the porous enamel lesions[20]. Fluoride may be incorporated in a soluble (CaF_2) and/or permanently bound (fluoroapatite) form. *In vitro* studies have shown that fluoride applications deposit a reaction product coating of CaF_2 on the anatomical surface of enamel[21,22], and that fluoride is also permanently incorporated into the enamel during application[23-25]. It is highly probable that CaF_2 creates mineral deposition in the porous zone of enamel and increases the microhardness[26], thereby resulting in increased bond strength.

In this study, fluoride permeated through the tooth surface and enamel was remineralized. It should also be highlighted that fluoride from fluoride-releasing adhesives was shown to prevent decrease in dentin bond strength for up to six month[27].

For the same fluoride concentration, bond strength gradually increased with extension in immersion period. Relatively short remineralization periods seemed to provide only partial rehardening of softened enamel[28], while the degree of remineralization would increase gradually in accordance with increase in immersion period. The favorable end result yielded was enhanced bonding.

As for the role of fluoride concentration, both *in*

vitro[29] and *in vivo*[30] studies have been undertaken to examine its effect on inhibition of enamel dissolution and promotion of remineralization. Low intraoral levels of fluoride for a sustained period were found to be beneficial in reducing caries[31]. In this study, both adhesives showed higher bond strength values in 0.5 ppm solution than the other concentration groups. Furthermore, no significant differences in bond strength were observed between 1 ppm and 10 ppm solutions. These results clearly showed that a low fluoride concentration, rather than a high fluoride concentration, could improve the bonding to enamel lesions. Consequently, the results of this study were in agreement with those of previous studies[29,30].

Within the limitations of this *in vitro* study, the bond strength of Clearfil SE Bond was found to be higher than that of Single Bond in 0.5 ppm fluoride solution. Due to the mild etching effect of Clearfil SE Bond self-etch primer, the depth of etched enamel was shallow. As a result, the infiltration of adhesive monomer seemed to occur effectively. At the same time, the penetration of resin into the micropores occurred in tandem with the etching process, producing resin tags and forming micromechanical interlocking[32-34]. The increase of enamel hardness by remineralization with fluoride seemed to be more rapid in Clearfil SE Bond than in Single Bond. This was because Clearfil SE Bond was a "mild" self-etch system, which generally has a pH of around 2 and which produces a shallower enamel etch pattern[35].

In the case of phosphoric acid etching, despite the significant resin tag formation on the enamel surface[36], the approximate depth of resin penetration was reported to be only 3 μm[37,38]. On the other hand, after 15 seconds of phosphoric acid application, the etch depth of enamel ranged from 5 to 10 μm[37,38]. Due to the etching effect, the remaining porous zone in enamel after phosphoric acid application could be deeper than 60 μm. This then poses a greater challenge to the penetration of resin monomer for bonding.

Further, to estimate the bonding in deep cavities that reached dentin, we employed the wet bond method for adhesive application after phosphoric acid etching. Clearly, excess water within the etched zone led to adhesive degradation, thereby resulting in lower bonding strength[39]. While the dry method after etching might seemed to improve bonding, further study is necessary to clarify and confirm this.

Based on the results of this study, we thus suggested that the white spot enamel lesions need not be removed for bonding in the clinic. They might be preserved and treated using fluoride applications. In addition, it was found that bond strength gradually increased with extension of immersion period, such that the highest bond strength was obtained at 7 days in this study. This meant that maintenance of fluoride treatment for a sustained period is important.

In our previous study, we evaluated the bond durability of Clearfil SE Bond and Single Bond to normal human enamel[40]. Leveraging on the results of the current study, the next step forward is to evaluate the effect of fluoride application in enhancing the longevity of enamel bonding.

CONCLUSION

It was concluded that the bond strength of two-step adhesives to artificially carious enamel significantly increased after immersion in fluoride-phosphate buffer solution.

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