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Effect of accelerated aging on the bonding performance of fluoridated adhesive resins

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The purpose of this study was to investigate the dentin bond durability of a one-step, fluoride-containing, glass ionomer-based adhesive system, Reactmer Bond (RB), and that of a two-step, fluoride-containing, self-etch adhesive system, Clearfil Protect Bond (CPB). Enamel was removed from the occlusal surfaces of teeth, and flat dentin surfaces were entirely covered with a composite resin following the application of an adhesive material (n=10). After specimens were sectioned into rectangular sticks of 0.87±0.03 mm^2, the sticks were randomly assigned into two accelerated aging time period groups: 1 week or 1 year. Microtensile bond strengths were determined. Bond strength of RB increased significantly after 1 year (1 week=27.80±10.57 MPa *versus* 1 year=36.93±14.38 MPa) (p<0.05). In contrast, there was no significant difference in bond strength between the two time periods for CPB (1 week=51.74±17.8 MPa *versus* 1 year=56.03±18.85 MPa) (p>0.05). Both fluoride-containing adhesives seemed to demonstrate reliable bonding performance after 1 year of accelerated aging in water.

Keywords: Adhesion, Bond durability, Glass ionomer adhesive, Microtensile bond strength, Self-etch adhesive

INTRODUCTION

Strong and durable adhesion to the tooth substrate is critical to the long-term clinical success of direct and indirect resin-bonded restorations. The teeth in the oral cavity are constantly subjected to temperature changes, chewing loads, and chemical attacks by acids and enzymes that may cause degradation in the bonding interface of a restored tooth. This results in marginal discoloration, poor marginal adaptation, marginal leakage, post-operative sensitivity, and loss of retention of the restoration[1,2]. Studies have confirmed that resin-dentin bonds formed by contemporary hydrophilic dentin adhesives deteriorate over time[3-9].

Adhesion to the tooth substrate is based on complete infiltration and subsequent *in situ* polymerization of resin monomers into demineralized collagen fibrils exposed by acid etching[10]. The result is micromechanical interlocking of the resin with the tooth structure. However, incomplete resin infiltration leaves an exposed demineralized dentin zone at the base of the hybrid layer[11-13]. Collagen fibrils within this zone cannot be protected against denaturation challenges. These denuded collagen matrices are also filled with water, which serves as a functional medium for the hydrolysis of resin matrices by endogenous and exogenous collagenolytic enzymes, which then promotes microleakage around the resin restoration[14]. Unfortunately, complete replacement of lost apatite by resin within the intrafibrillar spaces has never been demonstrated. Nevertheless, it was found that fluoride-containing adhesives exhibited efficacy in inhibiting secondary caries, which arises from microleakage at the tooth-restoration interface, by enhancing bond durability with remineralization of caries-affected dentin preserved beneath resin composite restorations[15,16].

There are different kinds of fluoride-containing adhesives available in the dental market. Clearfil Protect Bond (CPB) is a two-step, self-etch primer adhesive system that contains an antibacterial monomer (12-methacryloyloxydodecylpyridinium bromide (MDPB)) in the primer and sodium fluoride in the adhesive, which releases fluoride ions[17]. Reactmer Bond (RB) is a glass ionomer-based, tri-curable, all-in-one adhesive which contains fluoroaluminosilicate glass (FASG) and pre-reacted glass (F-PRG) particles that release fluoride[18].

Since fluoride contributes to prevention of caries associated with adhesive materials, we speculated that fluoride-containing adhesive materials might also contribute to the long-term durability of composite resin restorations by preserving bond durability with remineralization of dentin over time. Therefore, the purpose of this *in vitro* study was to investigate the bond durability performances of a fluoride-containing self-etch adhesive and a fluoride-containing glass ionomer-based adhesive to dentin after 1 year of accelerated aging in water.

MATERIALS AND METHODS

Twenty extracted, intact, human mandibular molars were used in the present study. The teeth were collected with patients' informed consent under a protocol reviewed and approved by the Ethics Committee of the Faculty of Dentistry, Selcuk University. The teeth were stored at 4°C in 0.5% chloramine water and used within 1 month following extraction.

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Enamel was removed from the occlusal surfaces of teeth to create flat dentin surfaces by using a low-speed diamond saw (Isomet, Buehler Ltd., Lake Bluff, IL, USA) under running water. The occlusal dentin surfaces were ground by hand using 600-grit silicon carbide papers under running water for 60 s to produce standardized smear layers. Teeth were randomly divided into two test groups (n=10) according to adhesive material. An adhesive material, CPB or RB, was applied to the dentin surfaces according to the manufacturer's directions. Table 1 lists the adhesive materials used in this study and their application procedures.

Adhesive-applied dentin surfaces were covered with a composite resin corresponding to the adhesive material used (Reactmer Paste, Shofu Inc., Japan or Clearfil AP-X, Kuraray Noritake Dental, Japan). The resin composite was cured in two increments to produce a 4-mm-thick block on the bonding surface. Restored specimens were stored in distilled water at 37°C for 1 week. Roots of the teeth were removed 2 mm below the cementoenamel junction using a slow-speed diamond saw under copious water spray. Then, each tooth specimen was sectioned parallel to the long axis into rectangular sticks of 0.87±0.03 mm^2, of which the upper part was resin composite and the lower part was dentin (Fig. 1). Fifteen to twenty test sticks were prepared from each tooth. Test sticks were randomly divided into two accelerated aging time period groups: 1 week and 1 year (n=75).

Specimens' storage water was changed daily until microtensile testing. For bond strength testing, each stick was attached to a testing apparatus (Bencor Multi-T, Danville Engineering Co., Danville, CA, USA) using a cyanoacrylate adhesive (Zapit, DVA, Corona, CA, USA) and then subjected to a tensile force at a crosshead speed of 1 mm/min. Microtensile bond strengths were determined in MPa, which was derived by dividing the applied force (N) at the time of fracture by the bonding area (mm^2).

After microtensile bond strength (μTBS) testing, the fracture mode of each stick was determined by examination under a dissecting microscope at 10× magnification (SZ-PT, Olympus, Tokyo, Japan). Failure modes at the fractured interface were classified as follows: a–adhesive failure between dentin and resin; c–cohesive failure in resin; d–cohesive failure in dentin; m–mixed failure (including adhesive failure between dentin and resin and cohesive failure in resin or dentin)[19]. Table 2 lists the failure modes of each test group and the numbers of sticks which showed pre-test failure.

Two-way ANOVA was used to determine the relationship between adhesive material and accelerated aging time period. Comparisons of μTBS values of the same adhesive material between two time periods were carried out using paired t-test. Independent t-test was used to determine differences in μTBS among the different test groups. Statistical analysis was performed using a software package (SPSS 10.0 for Windows,

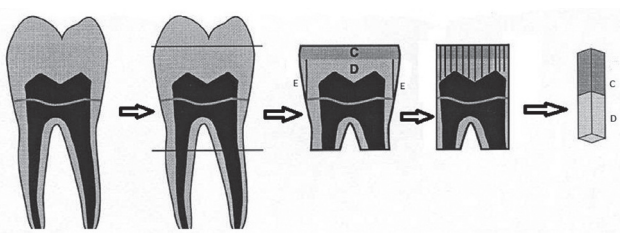

Fig. 1 Preparation of specimens for the aging regime and microtensile bond strength test.
D: Dentin; C: Composite; E: Enamel.

Table 1 Compositions, lot numbers, application procedures, and manufacturers of bonding systems used in the study

Adhesive Materials	Composition	Lot No.	Application Procedures	Manufacturers
Self-etch System Clearfil Protect Bond	Primer: MDPB, MDP, HEMA, water, hydrophilic, dimethacrylate, photoinitiators	0012A	Apply primer, leave for 20 s, mild air-blow, apply bond, air-blow, light-cure for 10 s	Kuraray Noritake Dental, Japan
	Bond: MDP, HEMA, Bis-GMA, silinated colloidal silica, surface treated NaF	0020A		
Glass-ionomer System Reactmer Bond	Bond A : FASG, F-PRG, water, acetone, new initiators	080309	Mix Bond A and B, apply adhesive, leave for 20 s, air-blow, light-cure for 20 s	Shofu Inc, Japan
	Bond B : 4-AET, UDMA, 2-HEMA, photo-initiator	080309		

Abbrevations: MDPB: 12-methacryloyloxydodecyl pyridinium bromide; MDP: 10-methacryloxydecyl dihydrogen phosphate; HEMA: 2-hydroxyethyl methacrylate; Bis-GMA: bisphenol-A-glycidyl methacrylate; NaF: sodium fluorite; F-PRG, Full-reaction type pre-reacted glass ionomer filler; 4-AET, 4-acryloxyethyltrimellitate; 4-AETA, 4-acryloxyethyltrimellitate anhydride; methacrylate; UDMA, urethane dimethacrylate

Table 2 Mean bond strength and standard deviation values and failure modes of specimens

Study Groups	*n*	Microtensile Bond Strength [MPa] Mean (SD)	Failures Modes				PF
			a	m	c	d	
Reactmer Bond (1 w)	71	27.80 (10.57)	54	19	2	0	2
Reactmer Bond (1 y)	71	36.93 (14.38)	49	23	3	0	1
Clearfil Protect Bond (1 w)	75	51.74 (17.80)	68	5	0	2	0
Clearfil Protect Bond (1 y)	75	56.03 (18.85)	50	10	1	14	0

a: Adhesive failure, m: Mixed failure, c: Cohesive failure in resin, d: Cohesive failure in dentin, PF: Pre-testing failure

SPSS Inc., Chicago, IL, USA). Pre-test failures and cohesive failures in the resin were not included in mean μTBS calculation.

RESULTS

Table 2 and Fig. 2 show the mean μTBS values of the test groups of this study. Two-way ANOVA revealed significant differences in μTBS between the adhesives (p<0.05) and between the time periods (p<0.05). However, there was no significant interaction between adhesive material and accelerated aging time period (p=0.083). Bond strength values of CPB to dentin were higher than those of RB after 1 week and 1 year (p<0.05). However, the bond strength value of RB became significantly increased after 1 year when compared with that after 1 week (27.8±10.6 MPa *versus* 36.9±14.4 MPa respectively) (p<0.05). In contrast, there was no significant difference in μTBS between the two time periods for CPB (51.7±17.8 MPa and 56.0±18.9 MPa respectively) (p>0.05).

For both RB and CPB, predominant failure mode after 1 week was adhesive failure between dentin and resin at 72 and 90% respectively. After 1 year, adhesive failure rate reduced by 7% for RB and 24% for CPB.

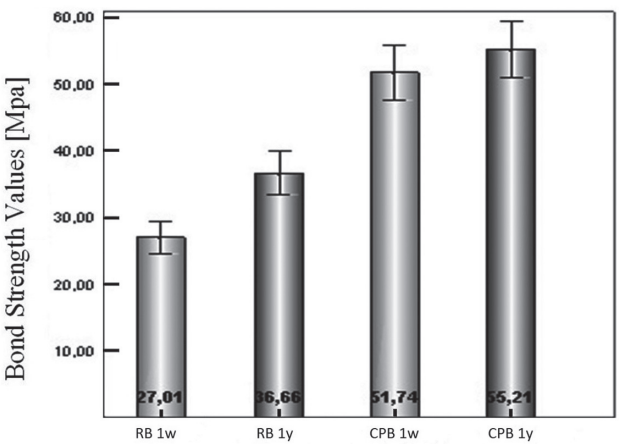

Fig. 2 Mean bond strength and standard deviation values of the test groups.

DISCUSSION

Bond durability between dentin and adhesive materials has been subjected to long-term *in vitro* evaluation in numerous studies[5,8,20-22]. In these studies, bond deterioration after water storage was the result of degradation of interfacial components, such as denaturation of collagen and/or elution of degraded or insufficiently cured resin[5,23]. Most degradation processes were diffusion rate-dependent, and that the length of diffusion path was as important as the length of diffusion time. Although most adhesives had varied degrees of decreased bond strength after long-term water storage, some studies demonstrated that incorporating fluoride into adhesives improved the stability of resin-dentin bonds[24-27].

In the present study, small μTBS sticks were used for artificial aging. Changes in bond strength to dentin of two fluoride-containing adhesive systems were assessed after 1 week and 1 year of direct water storage. Storing tiny μTBS sticks was considered as a form of accelerated aging[28]. We assumed that accelerated aging would give an overall picture of bond durability in an aggressively wet environment. Our results confirmed that the bond strength value of RB significantly increased after 1 year when compared with that after 1 week. The bond strength values of CPB remained stable.

As fluoride-containing adhesives are in direct contact with the cavity wall, it has been shown that fluoride ions released from these adhesives easily penetrated and diffused into dentin at the cavity wall. Subsequently, fluoride ions which penetrated dentin enhanced mineralization and reduced dentin demineralization[29]. Itota *et al.* also showed that fluoride-containing adhesives were capable of remineralizing non-resin-infiltrated, demineralized dentin[16]. Theoretically, dentin which is incompletely infiltrated with resin can be circumvented by using self-etching adhesives. Carvalho *et al.* confirmed the existence of this non-infiltrated region —it consisted of dissolved calcium and phosphate ions which were formed during the self-etching bonding procedure[12].

It was therefore speculated that when a fluoride-containing adhesive is used, fluoride can be released into the spaces and a probable reaction between fluoride and other products prevents future demineralization of the hard dental tissues[15].

Similar to our *in vitro* results, Dönmez *et al.* demonstrated that bonds created by fluoride-releasing self-etch adhesive Clearfil Protect Bond remained stable after 1 year of *in vivo* and *in vitro* evaluations, while bonds created by a fluoride-free, self-etch adhesive system (Clearfil SE Bond) were significantly degraded[27]. They explained that the slow fluoride release from Clearfil Protect Bond reduced the solubility of calcium phosphates within the hybridized smear layer, and the resultant hybrid layer provided more stable bonding to dentin over time[26]. Bonding stability of CPB was also attributed to an antibacterial monomer, 12-methacryl oyloxydodecylpyridinium bromide (MDPB). In MDPB, a quaternary ammonium salt possesses anti-matrix metalloproteinases (anti-MMP) activities[14]. MMPs are a family of host-derived proteolytic enzymes trapped within the mineralized dentin matrix that can hydrolyze the organic matrix of demineralized dentin[30-32]. Most MMPs are synthesized and released from odontoblasts in the form of proenzymes that require activation to degrade extracellular matrix components[33]. Unfortunately, they can also be activated by modern self-etch and etch-and-rinse adhesives[34,35]. After bonding with resin to dentin, exposed collagen fibrils at the bottom of the hybrid layer that resulted from imperfect resin impregnation of demineralized dentin matrix might be affected by dentin MMPs, thereby leading to reduced bond strength[36].

In the present study, a functional monomer (10-methacryloyloxydecyl dihydrogen phosphate (10-MDP)) in the composition of CPB chemically interacted with hydroxyapatite and contributed to superior and stable bonding[37,38]. The chemical bonding potential of 10-MDP with hydroxyapatite was significantly high and hydrolytically stable because of the production of calcium salt, which is barely soluble[38].

In a study by Shinohara *et al.*, CPB specimens demonstrated significant increase in bond strength after 3 months of water storage[15]. This high bond strength was attributed to the wet bonding technique used in the study (35% phosphoric acid+Scotchbond Multi-Purpose Primer+CPB adhesive). It was probable that more non-infiltrated demineralized dentin was present and more dissolved calcium and phosphate ions reacted with fluoride to increase bond strength. Further, the storage of specimens in water differed from that employed in our study. The teeth were stored in water only after restoration. In the present study, test sticks were exposed to water immediately after they were obtained from each tooth.

In the current study, as in several other studies, the bond strength of RB significantly increased after 1 year of storage in water[39-41]. RB can be classified as a glass ionomer-based adhesive, as it contains basic ion-leachable fluoroaluminosilicate glass (FASG), fully pre-reacted glass polyalkenoate fillers (F-PRG), and adhesion-promoting monomer 4-AET (4-acryloethyltrimellitic acid) in the adhesive formulation[42]. F-PRG enhanced the sustained fluoride releasing and recharging potential of the adhesive material[43]. 4-AET was also shown to chemically bond to both dentin apatite and collagen[44]. This provided the potential of chemical bonding *via* an ion exchange process between the glass particles and the partially demineralized tooth substrate[45]. In an aqueous medium, the two ionized carboxylic groups of 4-AET molecule could also react with the calcium component of dentin apatite to form insoluble calcium carboxylate salts[46].

Geiger and Weiner[45] demonstrated the formation of an intermediate layer between a glass ionomer restoration and dentin. They claimed that the presence of soluble minerals in this layer probably presented high resistance to secondary caries. It was assumed that this layer might have also occurred between dentin and the glass ionomer phase of RB. Additionally, Hashimoto *et al.* found that crystal growth had developed within the resin-dentin bonds during long-term water storage of RB[41]. They speculated that fluoride released from the restorative material might be responsible for this crystal formation. The ability to grow crystals between fluoridated restorative compounds and hard dental tissues protected tooth surfaces at interfacial gaps, thus contributing to long-term stability.

In the present study, bond strength of RB to dentin considerably increased after 1 year of accelerated aging in water. Our results agreed with those of Shirai *et al.*[39], which showed that the bond strength durability of RB improved after 1 year of water storage. RB with a pH of 3.2 is also categorized as a mild self-etch adhesive, and the demineralization depth produced by these mild adhesive systems in coronal dentin was reported to be less than 1 μm[47]. Shirai *et al.* demonstrated that RB exhibited nearly no mechanical interactions with dentin[39]. Therefore, it was speculated that the bonding mechanism of RB to dentin might be mainly based on ion exchange mechanism and chemical interaction with dentin. This chemical bonding mechanism *via* an ion exchange process between glass particles and dentin was a water-dependent reaction which might take a few weeks to establish[48].

Failure analysis in the current study revealed that adhesive failure was the main failure mode after 1 week of water storage for both adhesive materials. Occurrence of this failure mode decreased after 1 year. These results agreed with the findings of De Munck *et al.*, who showed that low bond strengths correlated with high percentages of adhesive failure[8]. The decrease in adhesive failure mode might be an indication of improved bonding efficacy over time.

The findings of our study presented promising expectations for the long-term clinical durability of fluoride-containing adhesive systems. Since these systems were already found to be responsible for the remineralization of decalcified dentin by creating an acid inhibition zone[15,41,45], glass ionomer type of

fluoride-containing adhesives may be a solution to increasing the long-term durability of resin-dentin bonding in the oral environment.

CONCLUSIONS

Within the limitations of this *in vitro* study, it was concluded that the bonding performance of two fluoride-containing adhesive systems used in this study did not decrease after 1 year of accelerated aging in water. However, the glass ionomer composition of RB had a greater impact on the increased bonding performance of the adhesive material after 1 year.

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