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Influence of Delayed Placement of Composites Over Cured Adhesives on Dentin Bond Strength of Single-application Self-etch Systems

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Clinical Relevance

The delay in placement of composite over single-application self-etching adhesive systems was a crucial factor influencing dentin bond strength compared to a composite placed immediately after the polymerization of adhesives.

UMMARY

This study examined the relationship between delay in composite placement over cured adhesives and the dentin bond strength of several single-application self-etch adhesive systems. The adhesive system/resin composite combinations used were: Adper Prompt L-Pop/Filtek Z250 (AP), AQ Bond Plus/Metafil C (AQ), Fluoro Bond Shake One/Beautifil (FB), G-Bond/Solare (GB), One-Up Bond F Plus/Palfique Estelite (OF), Xeno IICF Bond/Xeno CF (XE). Bovine mandibular incisors were mounted in self-curing resin and wet ground with #600 SiC to expose labial dentin. The adhesives were applied according to each manufacturer's instructions, and resin pastes were condensed into a mold (ø4x2 mm) immediately, and 1, 2, 5 and 10 minutes after light irradiation of the adhesives. Ten samples per test group were stored in 37°C water for 24 hours, then shear tested at a crosshead speed of 1.0 mm/minute. One-way ANOVA followed by Tukey's HSD test (α=0.05) was done. SEM observations of the dentin surface after the tests were also conducted. Dentin bond strength ranged from 9.5 ± 2.2 to 14.0 ± 2.4 MPa for AP, 7.3 ± 2.2 to 12.2 ± 3.1 MPa for AQ, 10.0 ± 3.5 to 16.3 ± 2.4 MPa for FB, 11.4 ± 1.5 to 16.3 ± 1.2 MPa for GB, 14.2 ± 3.4 to 15.1 ± 3.0 MPa for OF and 11.5 ± 2.4 to 15.9 ± 2.2 MPa for XE. Except for OF, no significant differences were found among the 2 to

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10 minute delayed placement groups for the systems used. Significant lower bond strengths were obtained for the immediate placement groups except for OF. From SEM observations, cohesive failure of the dentin surface was more pronounced with the longer delay in placement. The data suggest that delayed composite placement over the cured adhesives are suggested for optimum dentin bond strength of single-application self-etch adhesive systems.

INTRODUCTION

The demand for esthetic restorations has placed a focus on the development of bonding systems that provide adequate bonding ability to tooth structure over an extended period of time (Van Meerbeek & others, 1998). The steps required for these bonding procedures have been reduced to eliminate some of the technique sensitivity and practitioner variability associated with using adhesive systems in clinical situations. Single-application self-etch adhesive systems have been developed to simplify and shorten bonding procedures by combining dentin conditioning, priming and bonding steps. These products are simply applied on the dentin surface for the prescribed period of time followed by light irradiation (Van Meerbeek & others, 2003).

Micromechanical interlocking through the creation of a hybrid layer is currently accepted as one of the major mechanisms of resin bonding to dentin (Nakabayashi, Kojima & Mashuhara, 1982; Van Meerbeek & others, 1992; Inokoshi & others, 1993). It is generally accepted that the smear layer that forms on ground dentin should be removed or altered with acidic conditioners to achieve good adhesion between the demineralized dentin and an applied bonding system (Prati & others, 1998; Pashley, 1991; Nakabayashi & Saimi, 1996). After removal of the smear layer, the conditioned dentin surface should be wetted using hydrophobic resin monomers. Single-application self-etch adhesives dissolve the smear layer and demineralize the superficial dentin to facilitate penetration of resin monomers. The etching effect of the single-step self-etch systems is related to acidic monomers or organic acid solutions that interact with the mineral component of dentin and enhance monomer penetration. Water plays an important role as an ionizing medium for etching the dentin and acting as a facilitator of monomer penetration (Ikemura, Kuoro & Endo, 1996; Ikemura & others, 2003).

After infiltration into dentin, the adhesive must sufficiently polymerize to create a durable bond. Hypothetically stronger bonding resin might lead to higher bond strengths to dentin (Pashley & others, 1995). After infiltration of the resin monomers into partially demineralized dentin, subsequent polymerization of the adhesive is required to create a stable bond. The dentin surface is air dried after application of the adhesive, because these adhesives contain solvents such as water, ethanol and acetone. If water or other solvents like ethanol remain in the adhesives, the mechanical properties should decrease due to the incorporation of bubbles inside the polymerized adhesives.

It has been reported that an adverse acid-base reaction and adhesive permeability may contribute to the incompatibility between some simplified adhesives and resin composites (Tay & others, 2003a,b; Suh & others, 2003). The new, simplified adhesives contain uncured acidic monomers in the oxygen-inhibited layer, which is in direct contact with the resin paste. Adverse interactions between acidic functional monomers in adhesive and tertiary amines in resin paste may occur. Clinical procedures to eliminate this kind of incompatibility should be developed.

This study examined the relationship between a delay in composites placed over the cured adhesive and the dentin bond strengths of several single-application self-etch adhesive systems. The null hypothesis to be tested was that the dentin bond strengths of each single-application self-etch adhesive is independent of the time delay before composite placement over the cured adhesives.

METHODS AND MATERIALS

Single-application self-etch adhesive systems with the combination of composites used were Adper Prompt L-Pop/Filtek Z250 (AP, 3M ESPE, St Paul, MN, USA), AQ Bond Plus/Metafil C (AQ, Sun Medical, Shiga, Japan), Fluoro Bond Shake One/Beautifil (FB, Shofu Inc, Kyoto, Japan), G-Bond/Solare (GB, GC Corp, Tokyo, Japan), One-Up Bond F Plus/Palfique Estelite (OF, Tokuyama Dental, Tokyo, Japan) and Xeno CF Bond/Xeno CF (XE, Dentsply Sankin, Tokyo, Japan) as shown in Table 1. Some adhesive systems are only available in Japan. The adhesive system AP is provided by a unique container known as L-Pop; AQ is provided by a bottle with sponge; FB, OF and XE are provided by two bottles and an equal amount of bottle A and B were mixed prior to application; and GB is a one-bottle self-etch system. All adhesive systems were used in combination with manufacturers' recommended restorative resins. An Optilux 501 (Kerr/Demetron, Danbury, CT, USA) curing unit was used, and light intensity of the curing unit was set at 600 mW/cm^2 as measured with a Dental Radiometer (Model 100, Kerr/Demetron).

Mandibular incisors extracted from two-to-three year old cattle and stored frozen (-20°C) were used as a substitute for human teeth (Nakamichi, Iwaku & Fusayama, 1983; Fowler & others, 1992; Schilke & others, 1999). After removing the roots with an Isomet low-speed saw (Buehler Ltd, Lake Bluff, IL, USA), the pulps were removed and the pulp chamber of each tooth filled

Table 1: *Single-application Self-etch Systems Used*

Code	Adhesive (Manufacturer)	Main Components	Lot #	Restorative	Lot #
AP	Adper Prompt L-Pop (3M ESPE)	Methacrylated phospholic eters, Bis-GMA, CQ, initiator, stabilizer, 2-HEMA, polyalkenoic acid, water	127613	Z250	1KKJ
AQ	AQ Bond Plus (Sun Medical)	Water, acetone, 4-META, UDMA, HEMA, MMA, initiator p-toluenesulfinic acid sodium salt	FW1 FX1	Metafil C	GL1
FB	Fluoro Bond Shake One (Shofu Inc)	PRG, fluoroaluminosilicate glass, 4-AET, 4-AETA, bis-GMA initiator, water, solvent	A: 551F B: 551F	Beautifil	090339
GB	G-BOND (GC Corp)	G-BOND 4-MET, UDMA, acetone, water silanated colloidal silica, initiator	031015	Solare	0310811
OF	One-Up Bond F Plus (Tokuyama Dental)	One-up Bond F2 Water, MAC-10, HEMA, MMA, multifunctionl methacrylic monomer fliuoroaluminosilicate glass, photo initiator (aryl borate catalyst)	A: 551F B: 551F	Palfiqu Estelite	J260
XN	Xeno CF Bond (Dentsply Sankin)	Catalyst, PPTM, PEM-F, UDMA, initiator, amine, stabilizer HEMA, ethanol, stabilizer, filler	210000032	Xeno CF	447-05

Abbreviations: Mac-10; 10-methacryloxydecyl di-hydrogen phosphate, HEMA; 2-hydroxyethyl methacrylate, MMA; methyl methacrylate, 4-AET; 4-acryloyloxyethyl trimellitic acid, 4-AETA; 4-acryloyloxyethyl trimellitate anhydride, UDMA; urethane dimethacrylate, bis-GMA: 2, 2bis[4-(2-hydroxy-3-methacryloyloxypropoxy)]phenyl propane, TEGDMA: triethylene glycol di-methacrylate, CQ: dl-camphorquinone

Table 2: *Application Protocols of Single-application Adhesive System*

Adhesive System	Application Protocol
Adper Prompt L-Pop	Activate blister pack by emptying the liquid out of the red blister into the yellow blister. (Blister-Packed). Apply activated solution to dentin for 15 seconds with moderate finger pressure. Gentle stream of air to dry and apply second coat of adhesive. Gently air dry and light irradiation for 10 seconds.
AQ Bond Plus (Single Bottle with Sponge)	Dispense one drop of liquid into well containing one piece of sponge. Apply the mixed sponge to dentine for 20 seconds. Gently air dry for 5~10 seconds, relatively strong air dry for 5~10 seconds, and light irradiation for 10 seconds.
Fluoro Bond Shake One (Two Bottles)	Mix equal amounts of bond agent A and B. Apply to dentin for 20 seconds. Briefly air dry and light irradiation for 10 seconds.
G-Bond (Single Bottle)	Apply sufficient amount of adhesive for 10 seconds. Strong air dry and light irradiation for 10 seconds.
One-Up Bond F Plus (Two Bottles)	Mix equal amounts of the bond agents A and B for until a pink homogenous liquid mixture is obtained. Apply to dentin for 10 seconds with agitation and light irradiation for 10 seconds.
Xeno CF Bond (Two Bottles)	Mix equal amount of the bond agents. Apply to dentin for 20 seconds, and briefly air dry, light-activate for 10 seconds. Re-apply adhesive, air dry, and light irradiation for 10 seconds.

with cotton to avoid penetration of the embedding media. The labial surfaces of the bovine incisors were ground on wet 240-grit SiC paper to make a flat dentin surface. The tooth was then mounted in cold-curing acrylic resin to expose the flattened area and stored in tap water to minimize the temperature rise from the exothermic polymerization reaction of the acrylic resin. Final finish was accomplished by grinding on wet 600-grit SiC paper to expose an area of dentin approximately 6-8 mm in diameter, which is sufficient for bond strength testing. After ultrasonic cleaning in distilled water for one minute to remove any debris, these surfaces were washed and dried with a three-way syringe. The mounted teeth with dentin exposed were randomly assigned to each restorative material, with a sample size of 10 per experimental group.

Adhesive tape was used to define the area of the tooth for bonding, and a Teflon (Sanplatec Corp, Osaka, Japan) mold 2.0-mm high and 4.0 mm in diameter was used to form and hold the materials to the tooth surface. The adhesives were applied to the dentin surface followed by light irradiation according to each manufacturer's instructions (Table 2). Though they are categorized into a single-application self-etch adhesive system, the adhesive application procedures are different. The restorative was condensed into the mold immedi-

Table 3: *Shear Bond Strength (Mean ± SD) to Bovine Dentin*

	Resin Paste Application Delay Period				
Code	**Immediately**	**1 minute**	**2 minutes**	**5 minutes**	**10 minutes**
AP	9.5 ± 2.2^{a} [9/0/1/0]	9.5 ± 2.0^{a} [9/0/1/0]	12.6 ± 1.8^{b} [9/0/1/0]	13.1 ± 2.3^{b} [7/2/1/0]	14.0 ± 2.4^{b} [5/3/1/1]
AQ	7.3 ± 2.4^{c} [9/0/1/0]	7.5 ± 2.7^{c} [9/0/1/0]	12.2 ± 3.1^{d} [5/1/4/0]	11.8 ± 1.5^{d} [5/1/4/0]	11.9 ± 2.2^{d} [5/1/3/1]
FB	10.0 ± 3.5^{e} [7/0/3/0]	11.0 ± 2.8^{e} [8/0/2/0]	15.7 ± 4.7^{f} [5/1/2/2]	15.7 ± 3.6^{f} [4/2/2/2]	16.3 ± 2.4^{f} [4/2/2/2]
GB	11.4 ± 1.5^{g} [6/0/4/0]	11.8 ± 1.3^{g} [6/0/4/0]	16.3 ± 1.2^{h} [2/1/5/2]	15.1 ± 2.6^{h} [2/0/6/2]	15.1 ± 3.0^{h} [6/0/3/1]
OF	14.3 ± 3.7^{i} [6/0/4/0]	14.1 ± 3.0^{i} [6/1/3/0]	14.2 ± 3.4^{i} [4/1/4/1]	15.1 ± 2.6^{i} [3/1/4/2]	15.1 ± 3.0^{i} [4/1/3/2]
XE	11.5 ± 2.4^{j} [9/1/0/0]	12.6 ± 1.9^{j} [9/1/0/0]	15.6 ± 2.8^{k} [2/2/4/2]	15.8 ± 1.5^{k} [2/3/3/2]	15.9 ± 2.2^{k} [4/2/2/2]

Abbreviations: Mac-10; 10-methacryloxydecyl di-hydrogen phosphate, HEMA; 2-hydroxyethyl methacrylate, MMA; methyl methacrylate, 4-AET; 4-acryloyloxyethyl trimellitic acid, 4-AETA; 4-acryloyloxyethyl trimellitate anhydride, UDMA; urethane dimethacrylate, bis-GMA: 2, 2bis[4-(2-hydroxy-3-methacryloyloxypropoxy)]phenyl propane, TEGDMA: triethylene glycol di-methacrylate, CQ: dl-camphorquinone

ately, and 1, 2, 5 and 10 minutes after light irradiation of the adhesives, then light irradiated for 30 seconds. The mold and adhesive tape were removed from the specimens 10 minutes after light irradiation. Then, the specimens were stored in 37°C distilled water for 24 hours and tested in shear mode using a knife-edge shear testing apparatus in a universal testing machine (Type 4204, Instron Corp, Canton, MA, USA) at a crosshead speed of 1.0 mm/minute. The shear bond strength values in MPa were calculated from the peak load at failure divided by the specimen surface area.

After testing, the specimens were examined using an optical microscope (SZ61, Olympus Corp, Tokyo, Japan) at a magnification of 10x to determine the location of the bond failure. The test area on the tooth was divided into eight segments, and the percentage that was free of adhesive or restorative material was estimated. The types of failures were determined based on the predominant percentage of substrate free material as adhesive failure, cohesive failure in resin composite, cohesive failure in adhesive resin and cohesive failure in dentin (Fowler & others, 1992).

The results were analyzed by calculating the mean shear bond strength (MPa) and standard deviation for each group. The data for each group were tested for homogeneity of variance using Bartelett's test, then subjected to an ANOVA followed by Tukey's HSD test at p=0.05. The statistical analysis was carried out with the Sigma Stat software system (Ver 2.01, SPSS Inc, Chicago, IL, USA).

The fractured dentin surfaces were observed by scanning electron microscopy (SEM). All SEM specimens were dehydrated in ascending concentrations of *tert*-butanol (50% for 20 minutes, 75% for 20 minutes, 95% for 20 minutes and 100% for 2 hours), then transferred to a critical-point dryer for 30 minutes. The fractured surfaces were coated in a vacuum evaporator using a thin film of gold. The specimens were observed in a scanning electron microscope (JSM-5400, JEOL Ltd, Tokyo, Japan).

RESULTS

The mean shear bond strengths to bovine dentin are shown in Table 3. Dentin bond strength ranged from 9.5 ± 2.2 to 14.0 ± 2.4 MPa for AP, 7.3 ± 2.2 to 12.2 ± 3.1 MPa for AQ, 10.0 ± 3.5 to 16.3 ± 2.4 MPa for FB, 11.4 ± 1.5 to 16.3 ± 1.2 MPa for GB, 14.2 ± 3.4 to 15.1 ± 3.0 MPa for OF and 11.5 ± 2.4 to 15.9 ± 2.2 MPa for XE. There was a tendency for the bond strength to increase with longer delays in composite placement to the cured adhesives, but no significant differences were found for the groups above two minute placement periods, except for OF. For the adhesive system OF, no significant differences in bond strength among the different composite placement periods were found.

Table 3 summarizes the fracture modes after bond strength tests. The predominant failure was adhesive failure for groups with an earlier composite placement period. The failure modes tended to change to cohesive failure with prolonged delays in composite placement for all adhesive systems tested.

The representative SEM observations of the composite side surfaces after bond strength measurement are shown in Figure 1. The predominant site of failure occurred along a relatively smooth zone between the adhesives and composites. Some voids were observed on the cured adhesives. From SEM observations, the failure site of the bond strength specimens changed dramatically when the composites were placed two minutes after light irradiation of the adhesives. For the delayed composite placement groups, cohesive failure in adhesives, composites and dentin were frequently observed.

DISCUSSION

The results of this study showed that the bond strengths of the one-step self-etch systems, except for OF, were affected by delay in placement of the composites over the cured adhesives. There is a possibility that the polymerization ability of the composite is affected by the acid-base reaction between tertiary amines and the acidic functional monomers in the adhesive, which results in a decreasing rate of polymerization (Tay & others, 2003a,b; Suh & others, 2003). The adhesives used in this study were cured by a free radical polymerization reaction, and a photosensitizer such as camphorquinone (CQ) was employed (Taira & others, 1988; Jakubiak & & Rabek, 1999). CQ requires a coinitiator for an effective polymerization process to occur, and a tertiary amine photoreductant was employed. The tertiary amine interacted with an activated triplet state CQ to form an intermediate excited complex followed by producing reactive radicals for polymerization (Cook, 1992).

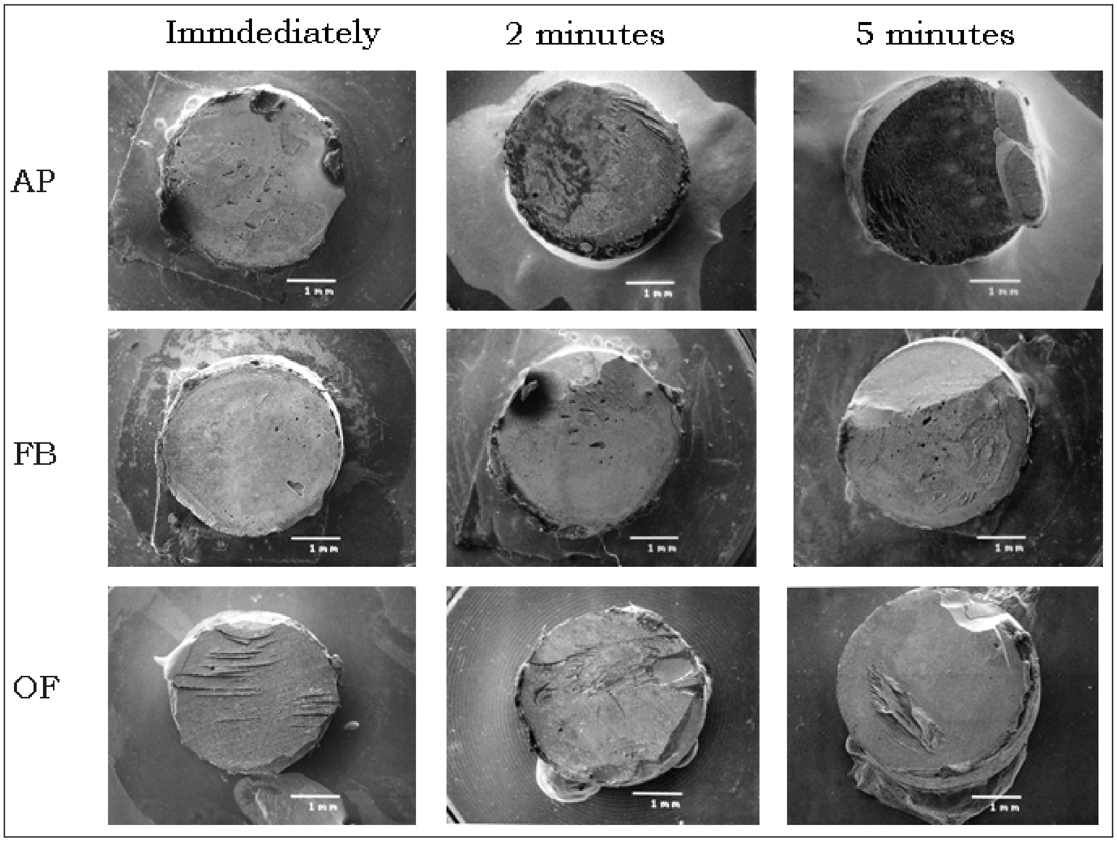

Figure 1. *Representative SEM observations of the composite side surface after bond strength measurement (original magnification 15x).*

It has been reported that adhesive functional monomers affect the polymerization of benzoyl peroxide (BPO)/amine, resulting in poor polymerization and reduced mechanical properties (Bowen, Cobb & Rapson, 1982; Sanares & others, 2001). To improve polymerization in the presence of acidic monomers, accelerators such as aromatic sulfinic acid sodium salts were incorporated together with initiator systems in the adhesives (Nyunt & Imai, 1996). An adverse interaction between acidic monomers and the light polymerized catalyst was thought to not normally occur, since the light polymerization reaction was much faster than the chemical polymerization reaction. However, improper polymerization of the composites occurred at the juncture of the adhesive and the composite when they were placed immediately after light irradiation of the adhesive, which is thought to be caused by the presence of acidic monomers in the oxygen inhibition layers of the one-step self-etch adhesives. There might be an adverse interaction between the nucleophilic tertiary amine in the composites and acidity of the superficial layer of the adhesives (Tay & others, 2001).

From the results of this study, dentin bond strengths significantly increased when the composites were placed two minutes after light irradiation of the adhesives. One possible explanation for this change in bond strengths is the time-dependent acid-base reaction between the remaining acidic functional monomers and the mineral component of the dentin substrate. It has been suggested that the etching effect of the self-etch adhesive is stopped by reaction with the mineral component of the dentin substrate followed by polymerization, which reduces the free acidic monomers. From a report focusing on the effect of self-etching primers on the continuous demineralization of dentin, the etching effect of the acidic functional monomer did not stop with polymerization of the adhesives (Oliveira & others, 2004). Residual acid should be consumed and neutralized by reaction with hydroxyapatite of the dentin substrate (Camps & Pashley, 2000). When the composites were placed on cured adhesives immediately after light irradiation, the remaining acid may have acted as an inhibitor of polymerization of the composites. This phenomenon was seen for most fracture specimens of the bond strength tests for the early placement groups; more adhesive failure between adhesive and composite was observed for these specimens.

For the adhesive system OF, no significant differences were found for dentin bond strengths among the different composite placement periods to cured adhesives. This is presumably the result of the excellent polymerization ability of the dye-sensitized photopolymerization system employed in this adhesive. The initiator system of OF contains dye-sensitizer, co-initiator and borate derivative (Table 1). The energy transfer reaction from dye-sensitizer to co-initiator takes place by light irradiation forming an excited state of co-initiator. Then, the polymerizable radical species are formed by reaction of the borate derivative with the activated co-initiator with hydrogen ions derived from the dye-sensitizer and acidic functional monomers (Kitasako & others, 2000). These unique features of the polymerization reaction of the adhesive might relate to the results of the bond strength test.

The experimental null hypothesis was not confirmed. The dentin bond strengths of several single-application self-etch adhesive systems were affected by the time delay prior to composite placement over the cured adhesives. Since clinical success with these simplified adhesive systems sometimes depends on technique-sensitive and material-related factors, further research based on the clinical situations is still needed to solve these kinds of problems.

CONCLUSIONS

Current developments in adhesive systems have focused on a reduction in the clinical steps with stable bond and hermetic seal of the restoration. Based on the results of this study, the placement time of composite to cured adhesive may be an important factor influencing dentin bond strength of one-step self-etch adhesive systems. Morphological changes of the fracture site also were observed from the SEM analysis of the composite side of adhesive test specimens dependent upon time of placement of the composite. Further research with clinical studies will be required to establish performance of the simplified adhesive systems.

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