

Influence of Adhesive Application Time on Enamel Bond Strength of Single-step Self-etch Adhesive Systems

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Clinical Relevance

Proper application duration of single-step self-etch adhesive plays an important role in obtaining optimal enamel bond strength.

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SUMMARY

This study examined the relationship between the adhesive application time and enamel bond strength of several single-step self-etch adhesive systems. The adhesives used were: Bond Force (BF, Tokuyama Dental Corporation, Tokyo, Japan), Clearfil tri-S Bond (CT, Kuraray Medical Inc, Tokyo, Japan), iBond Self-Etch (IB, Heraeus Kulzer, Armonk, NY) and OptiBond All-in-One (OB, sds Kerr). Bovine mandibular incisors were mounted in self-curing resin and wet ground with #600-grit silicon carbide paper to expose labial enamel. Adhesives were applied for 10, 20 or 40 seconds and the resin composites were condensed into a mold (ø4 x 2 mm) on the enamel and light irradiated. 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 and the Tukey HSD test ($\alpha=0.05$) were performed within each adhe-

sive system. The enamel surfaces after adhesive application were also observed in a Field Emission Electron Probe Surface Roughness Analyzer and the Ra value was calculated. The enamel bond strength ranged from 13.6 to 17.8 MPa for BF, 13.6 to 15.9 MPa for CT, 14.3 to 16.2 MPa for IB and 13.4 to 19.8 MPa for OB. Significant increases in bond strength were found for the 40-second application groups, except for IB. No changes in Ra value were found for BF and IB, while significant decreases in Ra value were recorded with 40-second application for CT and OB. The data suggested that the duration of the single-step self-etch adhesive application was a crucial factor for determining the enamel bond strengths of some of the single-step self-etch adhesives employed in the current study.

INTRODUCTION

The acid-etch technique to modify enamel structure with phosphoric acid has become a standard procedure for the surface conditioning of enamel prior to adhesive resin application.^{1,2} The retentive ability of resin composite to etched enamel is described as being a function of the increase in bonding area and wettability of the adherend surface.³ The infiltration of adhesive resin into the porous zone resulted in the formation of resin tags, establishing micromechanical retention to etched enamel. Recently, a new approach to bonding to tooth substrate without phosphoric acid etching through the use of self-etch systems has been introduced.⁴ These simplified systems are suggested to reduce technique sensitivity and shorten clinical procedures.⁵

The depth of the enamel removed during the etching procedure depends on the type of acid, the acid concentration, the duration of etching and the chemical composition of the enamel surface.⁵ A morphological study demonstrated that the application of a self-etching primer did not create a deep enamel etching pattern, unlike the application of phosphoric acid.⁶ Single-step self-etch adhesives can be classified as mild (pH ≥ 2), intermediate (pH ≈ 1.5) and strong (pH ≤ 1), depending on their pH.⁷⁻⁸ The weaker acidity of mild self-etch systems raises the question of whether the adhesives are able to penetrate the enamel surface and yield durable bonding with the restored teeth. Concern about accelerated degradation of the enamel-resin bonds of single-step self-etch adhesive systems also exists, as the weaker acidity might create a more shallow etching pattern on enamel and, consequently, weaker micromechanical retention.⁹⁻¹⁰

A precursor of the single-step self-etch adhesive is a self-etching primer that contains acidic functional monomers, such as 10-methacryloyloxydecyl dihydro-

gen phosphate (MDP), 4-methacryloxyethyl trimellitic acid (4-MET) and 2-methacryloxyethyl phenyl hydrogen phosphate (phenyl-P).¹¹ The self-etch adhesive is a hydrophilic solution that is extremely effective in wetting the tooth surface. The etching effect of these systems is related to the acidic monomers or organic acid solutions that may interact with the mineral component of enamel and enhance monomer penetration.¹²⁻¹³ An increase in roughness results in a greater available area for mechanical interaction and it facilitates wetting of the enamel surface. On the other hand, narrow grooves could lead to the inclusion of air between the enamel surface and adhesives. Poor infiltration of the adhesive resin into demineralized enamel leaves nano-spaces in the bonding interface,¹⁴ which might increase susceptibility to degradation by oral fluids. Water may accelerate hydrolysis of the bonding agent and extract poorly polymerized resin oligomers.¹⁵⁻¹⁶ A decrease in mechanical properties of the resin may contribute to a decrease in enamel bond strengths.¹⁷⁻¹⁸ Achieving harmony between the depth of demineralization and the extent of resin monomer penetration might be the key to creating a high-quality bonding interface between the resin and enamel.

Each self-etching adhesive system has its own recommendation for duration of adhesive application. Although the application time for altering the enamel surface is recommended by the manufacturer, there is a risk that variations in application duration exist in clinics. Since self-etching adhesives contain acidic functional monomers and the pH of these solutions is low, the thickness of the demineralized layer might be affected by the application time. This leads one to question whether changes in adhesive application time might affect the enamel bond strength obtained.

The current study was carried out to examine the relationship between the adhesive application time and enamel bond strength of several single-step self-etch adhesive systems. The null hypothesis to be tested was that longer duration times of self-etch adhesive application would not improve the enamel bond strength of commercially available systems.

METHODS AND MATERIALS

Materials Tested

The single-step self-etch adhesives that were used are listed in Table 1. All the adhesive systems were used in combination with the manufacturers' recommended restorative resin composite. A visible-light activating unit, Optilux 501 (sds Kerr, Orange, CA, USA), was used and the power density (800 mW/cm²) of the light was checked with a dental radiometer (Model 100, Demetron Kerr, Danbury, CT, USA) before making the specimens.

Table 1: Single-Step Self-etch Adhesives Used

Code	Adhesive (Manufacturer)	Main component	Lot #	Restorative	Lot #
BF	Bond Force (Tokuyama Dental)	Phosphoric acid monomer, bis-GMA, HEMA, TEGDMA, water, ethanol, initiator	011M	Estelite Σ Quick	018058
CT	Clearfil tri-S Bond (Kuraray Medical)	MDP, bis-GMA, HEMA, water, ethanol, initiator, microfiller	00080A	Clearfil AP-X	00878A
IB	iBond Self Etch (Heraeus Kulzer)	4-META, methacrylate, glutaraldehyde, HEMA, acetone, water, initiator, filler	010067	Venus	010129
OB	OptiBond All-in-One (3M Unitek)	GPDM, methacrylate monomer, water, ethanol, acetone, initiator, filler, sodium hexafluorosilicate	453080	Premise	446158

bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloyloxypropoxy)phenyl]propane, HEMA: 2-hydroxyethyl methacrylate, TEGDMA: triethyleneglycol-dimethacrylate, MDP: 10-methacryloyloxydecyl dihydrogen phosphate, MAC-10: 11-methacryloyloxy-1,1-undecandicarboxylic acid, 4-META: 4-methacryloyloxyethyl trimellitate anhydride, GPDM: glycerol phosphate dimethacrylate.

Bond Strength Test

Mandibular incisors extracted from two-to-three year-old cattle and stored frozen (-20°C) for up to two weeks were used as a substitute for human teeth.¹⁹⁻²⁰ After removing the roots using a slow-speed saw with a diamond-impregnated disk (Isomet, Buehler Ltd, Lake Bluff, IL, USA), the pulps were removed and the pulp chamber of each tooth was filled with cotton to avoid penetration of the embedding media. The labial surfaces of the crowns were ground on wet 240-grit silicon carbide (SiC) paper to a flat enamel surface. Each tooth was then mounted in self-curing acrylic resin (Resin Tray II, Shofu Inc, Kyoto, Japan) to expose the flattened area and placed in tap water to reduce the temperature rise from the exothermic polymerization reaction of the acrylic resin. The final finish was accomplished by grinding on wet 600-grit SiC paper. After ultrasonic cleaning with distilled water for one minute to remove debris, these surfaces were washed and dried with oil-free compressed air.

A piece of double-sided adhesive tape with a 4-mm diameter hole was firmly attached to define the bonding area. The adhesives were applied to the enamel surface as described by the manufacturer, except that the time of application was for 10, 20 or 40 seconds. Oil-free compressed air with an air pressure of 0.2 MPa was used to evaporate the solvents of the adhesive for five seconds from 5 cm above the enamel surface using a three-way syringe, then irradiated with the curing unit. A Teflon (Sanplatec Corp, Osaka, Japan) mold 2.0-mm high and 4.0-mm in diameter was used to form and hold the shade A2 restorative resin onto the enamel surface. The resin composite was packed into the mold and irradiated for 30 seconds. The Teflon mold and adhesive tape were removed from the specimens 10 minutes after light irradiation. The specimens were then stored in 37°C distilled water for 24 hours.

Ten specimens for each application time—10, 20 and 40 seconds—were tested in shear mode using a knife-edge testing apparatus in a universal testing machine (Type 5500R, 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 in an optical microscope (SZH-131, Olympus Ltd, Tokyo, Japan) at 10x magnification to define the location of the bond failure. The type of failure was determined based on the percentage of substrate-free material as adhesive failure, cohesive failure in composite and adhesive resin, and cohesive failure in enamel.

Scanning Electron Microscopy (SEM)

For the treated enamel surface observation, the enamel surfaces were treated with adhesive, then rinsed with acetone and water. All the 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 two hours), then transferred to a critical-point dryer for 30 minutes. The surfaces were coated in a vacuum evaporator, Quick Coater Type SC-701 (Sanyu Denshi Inc, Tokyo, Japan), with a thin film of Au. The specimens were observed in a Field Emission Electron Probe Surface Roughness Analyzer (3D-SEM, ERA-8800FE, Elionix Ltd, Tokyo, Japan) at an accelerating voltage of 15 kV. The arithmetic means of the absolute values of distances from the mean line to the profile were calculated (R_a ; μm). The number of specimens was five for each measurement.

Statistical Analysis

The results were analyzed by calculating the mean and standard deviation for each group. A statistical analysis was done to show how the data were influenced by adhesive application duration. The data for

each group were tested for homogeneity of variance using Bartlett's test, then subjected to one-way ANOVA followed by the Tukey HSD test at $\alpha=0.05$ within each adhesive system. The statistical analysis was carried out with the Sigma Stat software system (Ver 3.1, SPSS Inc, Chicago, IL, USA).

RESULTS

The enamel bond strength ranged from 13.6 to 17.8 MPa for BF, 13.6 to 15.9 MPa for CT, 14.3 to 16.2 MPa for IB and 13.4 to 19.8 MPa for OB as shown in Table 2. With longer application times, increases in bond strength were found for BF, CT and OB. For BF and OB, significant increases in bond strength were found when the adhesive was applied for 20 seconds or longer, compared to that obtained with the 10-second application. For CT, a significant increase in bond strength was found when the adhesive was applied for 40 seconds. No significant difference was observed in bond strength for IB. The predominant failure was adhesive failure for the 10- and 20- second application duration groups. When the adhesives were applied for 40 seconds, cohesive failure in resin and enamel increased except for IB.

Representative pictures with 3-D images of SEM observations of enamel specimens are shown in Figure 1 a-d. From the SEM observations, each single-step self-etch adhesive presented different levels of surface changes. For BF and IB, the Ra value ranged from 0.12 to 0.13 μm , and no morphological alteration was observed, regardless of the different adhesive application times. Significant decreases in Ra values were observed for CT and OB with the 40-second application.

DISCUSSION

Although it is preferable to use extracted human teeth for bonding research,¹⁹⁻²⁰ it has become increasingly difficult to obtain such samples for laboratory studies in Japan. In order to compare data from the current study with that reported in previous bovine enamel bond strength tests, bovine teeth were used as a substitute for human teeth in the current study. Bovine teeth have some advantages, as they are easy to obtain in large quantities, are in good condition and have less composition variables than human enamel.²¹ Bovine teeth also have large, flat surfaces and are unlikely to have undergone prior caries challenges that could affect the test results. The mineral distribution within the carious lesions in bovine teeth is reportedly similar to that found in human teeth, and the structural changes that occur in human and bovine teeth are also similar.

Table 2: Influence of Adhesive Application Time on Enamel Bond Strength

Code	Adhesive Application Time (Seconds)		
	10	20	40
BF	13.6 (3.3) ^a [7/2/1]	16.7 (2.5) ^b [6/2/2]	17.8 (3.4) ^b [5/2/3]
CT	13.5 (2.3) ^c [6/3/1]	13.9 (1.7) ^{c,d} [7/2/1]	16.1 (1.8) ^d [4/0/6]
IB	14.3 (2.8) ^e [5/5/0]	15.7 (1.8) ^e [4/4/2]	16.2 (2.4) ^e [4/5/1]
OB	13.4 (2.8) ^f [9/1/0]	17.5 (2.0) ^a [8/2/0]	19.8 (3.3) ^a [2/5/3]

Unit: MPa, values in parenthesis indicate standard deviation.
Values with the same superscript letters are not significantly different ($p>0.05$).
Failure mode: [adhesive failure/cohesive failure in resin/cohesive failure in enamel]

The use of single-step self-etch adhesives can prevent discrepancies occurring between the depth of etching and the resin monomer penetration.²² These simplified systems are expected to reduce technique sensitivity by reducing the number of clinical steps involved.²³⁻²⁴ These single-step self-etch adhesives may form a continuum between the tooth surface and the adhesive by the simultaneous demineralization and resin penetration followed by polymerization. Penetration of the acidic monomers into the enamel surface creates resin tags. Based on morphological observations made using SEM in the current study, the single-step self-etch systems produced a shallow demineralized surface. Application of only the single-step self-etch adhesive resulted in a shallow etching pattern that might be the result of limited penetration of the adhesive into the enamel micro-porosities or a result of calcium precipitation on the enamel surface masking the etch pattern, then interfering with resin penetration.²⁵⁻²⁶

For BF, CT and OB, prolonged application duration of the adhesive led to higher enamel bond strength. However, the specific demineralization of enamel may not be a critical factor in determining enamel bond strength for IB. From the results of the current study, the influence of adhesive application duration was different among the adhesive systems employed. Single-step self-etch adhesives are formulated with hydrophobic and hydrophilic resin monomers dissolved in solvents, such as ethanol, acetone and water.¹¹ It has been reported that the extent of solvents and water remaining in the polymer network is related to the polarity of the resin monomer.²⁷ After application of the adhesive, the enamel surface is air dried, because the adhesive contains solvents that adversely affect polymerization of the resin components. This is followed by polymerization with light irradiation. Ethanol and acetone play an important role in evaporating the water present in single-step self-etch adhesives.²⁸ When considering the ability to remove water from an adhesive, ethanol-based adhesives have been considered to be more difficult than acetone-based adhesives. Since acetone has a relatively high vapor pressure, evaporation occurs

Figure 1

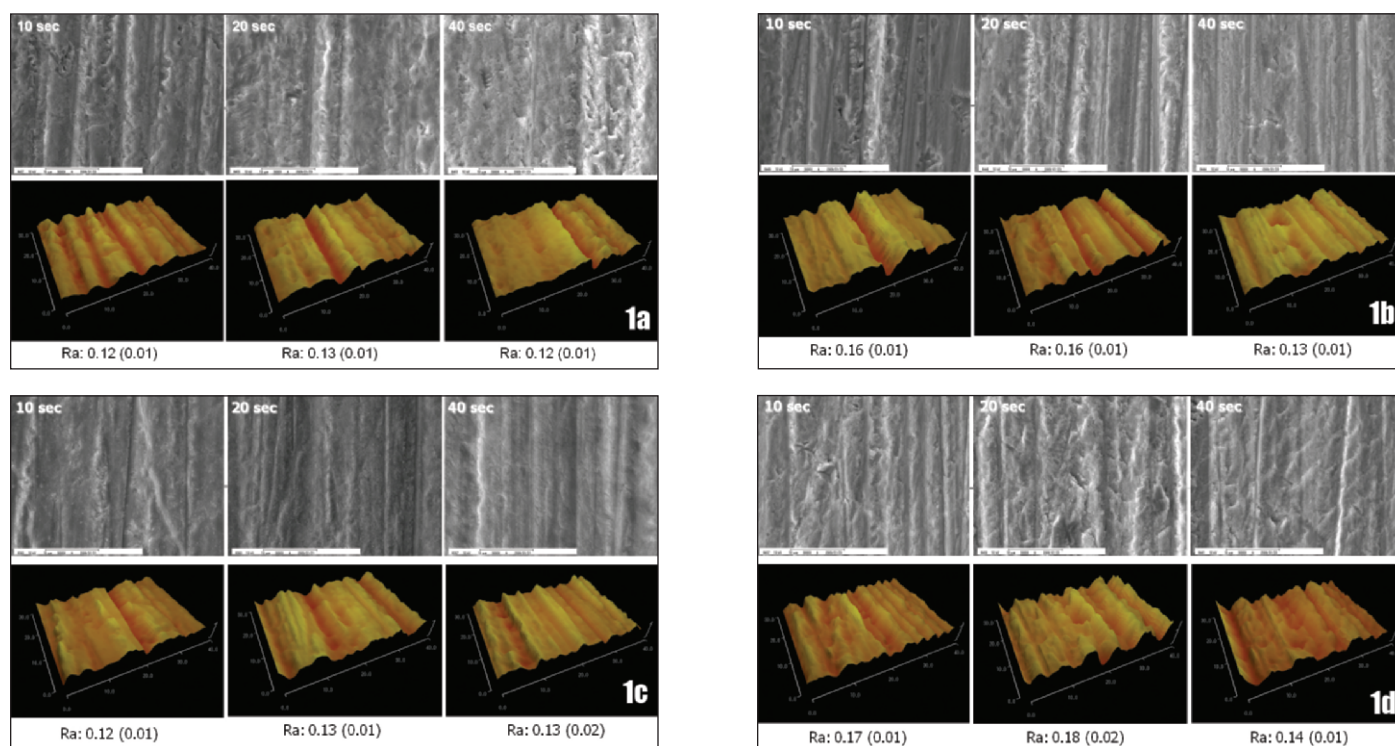


Figure 1a-d: SEM observation and corresponding 3-D image of the adhesive treated enamel surface with different application durations: Figure 1a) Bond Force, Figure 1b) Clearfil tri-S Bond, Figure 1c) iBond Self Etch, Figure 1d) OptiBond All-in One. After longer application time of the adhesives, slight surface alteration was observed for Clearfil tri-S Bond and OptiBond All-in One. No apparent morphological changes were found for Bond Force and iBond Self Etch. Unit: μm , values in parenthesis indicate standard deviation.

much faster than ethanol, resulting in less impact on bond strength. Because IB is an acetone-based adhesive, no significant difference in enamel bond strength was observed, regardless of adhesive application time.

With single-step self-etch systems, the possibility of monomer not infiltrating demineralized enamel is reduced. Another factor for the observed increase in enamel bond strength is the quality of the formulated polymer. Since the etching effect on an enamel surface is an ionic process, water is indispensable for ionization of the acidic functional monomers. The amount of water to be included in the single-step self-etch adhesive is critical. It should be large enough for ionization of the acidic functional monomers, yet sufficiently small to preserve a high amount of resin monomer concentration.²⁹ The remaining water content has an adverse effect on the mechanical properties of the cured adhesive layer. Even if complete penetration into the demineralized enamel can be achieved, the degree of conversion of resin monomers might be impacted by the presence of the remaining water in the adhesive. It is known that the cohesive strength of an adhesive is correlated to the rate of solvent evaporation and bond strength.³⁰ When self-etch adhesives were applied for a prolonged length of time, a higher amount of water could have evaporated, leading to higher mechanical

properties of the adhesive resin and higher enamel bond strengths.

For CT and OB, longer adhesive application duration (40 seconds) created a smoother enamel surface. Surface irregularity caused by grinding with SiC paper was altered by longer duration of the adhesive application. Under the conditions of the current study, there was no correlation between surface roughness caused by adhesive application and bond strengths. This indicates that the wettability and quality of the cured adhesive layer might be of greater importance for optimal bond strength. Therefore, a question remains as to the amount of resin monomer that is required to infiltrate the etched enamel in order to prevent microleakage and maintain bonding durability. Several factors, such as the type of acid, concentration, application duration and chemical reactivity of acidic monomers, may influence the bonding potential to enamel substrate. A previous study compared the chemical bonding efficacy of the functional monomers MDP, 4-MET and phenyl-P.³¹ According to the adhesion-decalcification concept,³¹ the less soluble the calcium salt of an acidic molecule, the more intense and stable the molecular adhesion to a hydroxyapatite-based substrate. A chemical interaction between hydroxyapatite and functional monomers in an adhesive may lead to higher bond strengths com-

pared with those that rely on micromechanical retention to the enamel substrate alone.³²

Current developments in adhesive systems have focused on simplifying the application methods by decreasing the steps required for placement. A reduction in the clinical steps for adhesive materials with a stable bond and hermetic seal has been desired by dentists. However, the results of the current study indicate that some of the single-step self-etch adhesives show enamel bond strengths that are less than maximum if the application time of the adhesive on an enamel surface is too short. Although longer application time of an adhesive leads to higher enamel bond strengths, there might be a greater chance for it to be contaminated by moisture conditions in the oral environment. Further research with clinical studies will be required to establish the performance of the single-step self-etch adhesive systems.

CONCLUSIONS

Within the limitations of the current study, the following conclusions were drawn:

1. The increases in adhesive application time significantly influence the enamel bond strengths of three out of the four single-step self-etch adhesives.
2. Predominant failure was adhesive failure for the 10- and 20-second application duration groups. When the adhesives were applied for 40 seconds, cohesive failure in resin and enamel increased.
3. From the SEM observations, each single-step self-etch adhesive presented different levels of surface changes.

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