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Effect of Prior Acid Etching on Bonding Durability of Single-Step Adhesives

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Clinical Relevance

The ability of acid etching to increase enamel bond strengths varies among single-step self-etch adhesive systems.

SUMMARY

This study investigated the effect of prior phosphoric acid etching on the enamel bond strength of five single-step self-etch adhesive systems: Absolute, Clearfil tri-S Bond, Fluoro Bond Shake One, G-Bond and One-Up Bond F Plus. Bovine mandibular incisors were mounted in self-curing resin, and the facial surfaces were wet ground with #600 silicon carbide paper. Adhesives were applied to the enamel surfaces with or without prior phosphoric-acid etching and light irradiated. The resin composites were condensed into a mold and light irradiated. In total, 40 specimens were tested per adhesive system with and without prior acid etching and were further divided into two groups: those stored in water at 37°C for 24 hours without cycling and those stored in water at 37°C for 24 hours followed by thermal cycling between 5°C and 55°C with 10,000 repeats. After storage under each set of conditions, the specimens were tested in shear mode at a crosshead speed of 1.0 mm/minute. Two-way analysis of variance, the Student's *t*-test and the Tukey HSD test were used to analyze the data at a significance level of 0.05. For the specimens without prior acid etching, the mean bond strengths to enamel ranged from 11.0 to 14.6 MPa after 24-hour storage in water, while the corresponding values for specimens with prior acid etching ranged from 15.2 to 19.3 MPa. When these specimens were subjected to thermal cycling, the

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mean bond strengths ranged from 11.3 to 17.0 MPa without prior acid etching and from 12.3 to 23.2 MPa with prior acid etching. The changes in enamel bond strengths differed among the adhesive systems tested. After 24-hour storage in water, the most common failure modes were adhesive failure and mixed failure for specimens with and without prior acid etching, respectively. Thus, through a careful choice of adhesive system, prior acid etching can increase the bond strengths of single-step self-etch adhesive systems.

INTRODUCTION

The demand for esthetic dental materials has led to the development of various systems that aim to achieve adequate bonding to both enamel and dentin. In order to reduce the sensitivity of techniques that affect the bonding ability of adhesive systems, the number of steps required for bonding procedures have been reduced.[1] Recently, single-step self-etch adhesive systems that combine the functions of a self-etching primer and a bonding agent have been developed.[2] The single-step self-etch adhesive is applied to the tooth surface prior to the resin composite placement in order to ensure maximum adhesion and to improve monomer penetration into the tooth substrate as well as wettability of the tooth surface via the resin components. The use of single-step self-etch adhesives could also eliminate the possibility of discrepancies between the depth of etching and resin monomer penetration.[3]

The depth of the enamel removed during the etching procedure depends on the type of acid, acid concentration, duration of etching and the chemical composition of the surface.[4] 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.[5-6] Single-step self-etch adhesives can be classified as mild, moderate or aggressive, depending on their pH.[2] Mild self-etch systems are moderately acidic, with pH values between 1.5 and 2.8. This weak acidity 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 micro-mechanical retention.[7-8]

The use of acid etching to modify the enamel structure with phosphoric acid[9] has become a standard procedure for conditioning enamel prior to bonding-agent application. The infiltration of adhesive resin into the porous zone results in the formation of resin tags, thereby establishing micro-mechanical retention to the etched enamel.[10–12] Single-step self-etch adhesive systems form a continuous layer by demineralization with acidic monomers, followed by resin monomer penetration into the enamel surface. Penetration of the acidic monomers into etched enamel creates resin tags. Although no relationship between the depth of acid etching of the self-etching primer and bond strength has been demonstrated,[8] the application of self-etch adhesives to unprepared enamel has been shown to produce a shallow etching pattern and insufficient bond strengths.[5-6] By contrast, a comparatively higher bond strength has been obtained using self-etching primer adhesive systems with the creation of submicron resin tags.[13] This raises the question of whether the resulting bonds are stable after thermal cycle stressing.[14]

The current study investigated the effect of prior acid etching on the bond strengths of single-step self-etch adhesive systems to bovine enamel by measuring the shear bond strength, assessing the fracture mode and through field emission scanning electron microscopy (FE-SEM) observations of the treated enamel surfaces. The null hypothesis was that prior acid etching did not affect the bond strength to bovine enamel.

METHODS AND MATERIALS

Materials Tested

The single-application self-etch adhesive systems used in combination with resin composites were as follows (Table 1): Absolute/Esthet•X (code AB; Sankin Dentsply, Tokyo, Japan); Clearfil tri-S Bond/Clearfil AP-X (code CT; Kuraray Medical, Tokyo, Japan); Fluoro Bond Shake One/Beautifil (code FB; Shofu Inc, Kyoto, Japan); G-Bond/Gradia Direct (code GB; GC Corp, Tokyo, Japan) and One-Up Bond F Plus/Estelite Σ (code OF; Tokuyama Dental, Tokyo, Japan). All of the adhesive systems were used in combination with the manufacturers' restorative resins. The application protocols provided by each manufacturer are shown in Table 2.

The visible-light activating unit Optilux 501 (sds Kerr, Danbury, CT, USA) was used, and the power density (800 mW/cm^2) of the light was checked with a dental radiometer (Model 100; sds Kerr) before preparing the specimens.

Bond-Strength Test

In total, 200 mandibular incisors, extracted from cattle and stored frozen (–20°C) for up to two weeks, were used as substitutes for human teeth. After removing the roots with a slow-speed saw and a diamond-impregnated disk (Isomet, Buehler Ltd, Lake Bluff, IL, USA), the pulp was removed, and the pulp chamber of each tooth was filled with cotton to avoid penetration of the embedding media. The labial surfaces of the bovine incisors were ground on wet 240-grit silicon carbide

Table 1: *Materials Tested*

Code	Adhesive System (manufacturer)	pH	Main Components	Lot #	Composite (Shade)	Lot #
AB	Absolute (Dentsply Sankin)	0.8	4-MET, PPTM, PEM-F, UDMA, acetone, initiator	393-016	Esthet•X (Y-E)	0501132
CT	Clearfil tri-S Bond (Kuraray Medical)	2.7	MDP, bis-GMA, HEMA, initiator, ethanol, water, stabilizer, filler, hydrophobic dimethacrylate	040219	Clearfil AP-X (A2)	00987A
FB	Fluoro Bond Shake-One (Shofu Inc)	2.2	4-AET, 4-AETA, bis-GMA, water, PRG, fluoroaluminosilicate glass, initiator, solvent	A: MS-13 B: MS-13	Beautifil (A2)	020135
GB	G-Bond (GC Corp)	2.8	4-MET, UDMA, acetone, water, silanated colloidal silica, initiator	031015	Gradia Direct (A2)	0312121
OF	One-Up Bond F Plus (Tokuyama Dental)	1.4	MAC-10, HEMA, MMA multifunctional methacrylic monomer, fluoroaluminosilicate glass, water, photoinitiator (aryl borate catalyst)	A: 551F-2 B: 551F-2	Estelite (A2)	J279

4-AET, 4-acryloyloxyethyl trimellitic acid; 4-AETA, 4-acryloyloxyethyl trimellitic anhydride;
4-MET, 4-methacryloxyethy trimellitate; bis-GMA, 2,2bis[4-(2-hydrogen-3-methacryloyloxypropoxy)phenyl]propane; HEMA, 2-hydroxyethyl methacrylate; MAC-10, 11-methacryloxy-1,1-undecandicarboxylic acid; MDP, 10-methacryloxydecyl di-hydrogen phosphate; MMA, methyl methacrylate PEM-F, fluoromethacryloxy cyclophosphazen; PPTM, pyrophosphate tetramethacrylate; PRG, pre-reacted glass filler; UDMA, urethane dimethacrylate.

Table 2: *Application Protocols for Single-step Self-etch Systems*

Code	Application Protocol
AB (single bottle)	Dispense one drop of liquid into well. Apply to enamel for three seconds twice. Subject to a mild stream of air for three seconds to dry and light irradiate for 10 seconds.
CT (single bottle)	Dispense one drop of liquid into well. Apply to enamel for 20 seconds. Subject to a relatively strong stream of air to dry and light irradiate for 10 seconds.
FB (two bottles)	Mix equal amounts of bond agents A and B. Apply to enamel for 20 seconds. Briefly air dry and light irradiate for 10 seconds.
GB (single bottle)	Dispense one drop of liquid into well. Apply sufficient amount of adhesive for 10 seconds. Subject to a strong stream of air to dry and light irradiate for 10 seconds.
OF (two bottles)	Mix equal amounts of bond agents A and B until a pink homogenous liquid mixture is obtained. Apply to enamel for 10 seconds with agitation and light irradiate for 10 seconds.

(SiC) paper to a flat enamel surface. Each tooth was then mounted in self-curing acrylic resin (Resin Tray II, Shofu Inc) to expose the flattened area; they were then placed in tap water to reduce the temperature rise caused by 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 excess debris, the surfaces were washed and then dried with oil-free compressed air.

A piece of adhesive tape, with a 4-mm-diameter hole, was firmly attached to delineate an area for bonding. One-half of the specimens were etched with phosphoric-acid (Etchant, 3M ESPE, St Paul, MN, USA) for 15 seconds, followed by 10 seconds of rinsing with a three-way syringe and air drying. The adhesive was applied to the enamel surface according to the manufacturers' instructions (Table 2). The adhesive-covered surfaces were then dried with oil-free compressed air and irradiated with a curing unit. A Teflon mold (Sanplatec Corp, Osaka, Japan), 2.0 mm in height and 4.0 mm in diameter, was used to form and hold the restorative resin onto the enamel surface. The resin composite was condensed into the mold and cured for 30 seconds. The Teflon mold and adhesive tape were removed from the specimens 10 minutes after light irradiation.

The bonded specimens from each group were divided into two treatment groups, with 10 specimens in each. In Group 1, the specimens were stored in distilled water at 37°C for 24 hours without thermal cycling. In Group 2, the specimens were stored in distilled water at 37°C for 24 hours, followed by thermal cycling between 5°C and 55°C for 10,000 repeats.

The specimens in each group were tested in shear mode using the knife-edge 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 under 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 follows: adhesive failure; mixed failure (cohesive failure in the composite and adhesive resin with partial adhesive failure); cohesive failure in enamel and cohesive failure in the composite.[15]

Statistical Analysis

Statistical analysis was used to determine how the bond strengths were influenced by thermal cycling. The data for each group were subjected to Student's *t*-test and analysis of variance (ANOVA), followed by the Tukey HSD test at a significance level of 0.05. The statistical analysis was carried out with the Sigma Stat software system (Version 3.1; SPSS Inc, Chicago, IL, USA).

SEM

The treated enamel surfaces were observed by SEM. To prepare the etched tooth surfaces, the enamel was treated with self-etching primers, then rinsed with acetone and water to rinse off the excess. All of 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 2 hours), then transferred to a critical-point dryer for 30 minutes. The surfaces were coated with a thin film of gold (Au) in a Quick Coater Type SC-701 vacuum evaporator (Sanyu Denshi Inc, Tokyo, Japan). The specimens were observed using FE-SEM (ERA 8800; Elionix Ltd).

RESULTS

The mean shear bond strengths to bovine enamel are shown in Tables 3 and 4. For the specimens without prior acid etching, the mean bond strengths to enamel ranged from 11.0 to 14.6 MPa after 24-hour storage in water, while the corresponding values for the specimens with prior acid etching ranged from 15.2 to 19.3 MPa. When the specimens were subjected to thermal cycling, the mean bond strengths for the specimens without prior acid etching ranged from 11.3 to 17.0 MPa, while the specimens with prior acid etching ranged from 12.3 to 23.2 MPa. The changes in enamel bond strengths differed among the adhesive systems tested. Statistical analysis revealed that the effect of prior acid etching on enamel bond strengths did not depend on specimen storage conditions ($p>0.05$). For the materials tested, with the exception of GB and OF, significant increases in enamel bond strengths were caused by prior acid etching regardless of the storage conditions.

Different trends in the failure mode were seen among the groups with and without prior acid etching. After 24-hour storage in water, the predominant mode of failure for specimens without prior acid etching was adhesive failure; whereas, it was mixed failure for specimens with prior acid etching. After thermal cycling, the predominant mode of failure was mixed failure for both groups of specimens.

SEM images of the enamel surface after acidic solution application are shown in Figure 1. The enamel etching pattern on treated surfaces was more notable in specimens that had undergone prior acid etching.

Table 3: *Effect of Prior Acid Etching on Bond Strength to Bovine Enamel After 24-Hour Storage in Distilled Water*

	Bond Strength*		Failure Mode**	
	Without Acid Etching	With Acid Etching	Without Acid Etching	With Acid Etching
AB	11.0 (1.6)[a]	19.3 (2.4)[c]	9/1/0/0	3/5/1/1
CT	14.2 (1.7)[b]	17.5 (1.5)[c,d]	7/2/0/1	5/3/0/2
FS	14.4 (2.0)[b]	19.2 (1.6)[c]	8/2/0/0	1/4/3/1
GB	13.0 (2.0)[a,b]	15.2 (1.2)[d]	8/2/0/0	3/0/5/2
OF	14.6 (1.3)[b]	16.7 (2.9)[c,d]	5/2/0/3	1/4/0/5

*Mean (standard deviation) in MPa. N=10.
**Failure mode: adhesive failure/mixed failure/cohesive failure in enamel/cohesive failure in composite. Values within same column marked with the same superscript letters showed no significant statistical difference (p>0.05).

Table 4: *Effect of Prior Acid Etching on Bond Strength to Bovine Enamel After 10,000 Thermal Cycles*

	Bond Strength*		Failure Mode**	
	Without Acid Etching	With Acid Etching	Without Acid Etching	With Acid Etching
AB	11.3 (3.2)[a]	19.5 (3.3)[c,d]	8/2/0/0	3/4/3/0
CT	17.0 (1.8)[b]	23.2 (3.0)[c]	5/3/0/2	1/5/1/3
FS	16.7 (2.7)[b]	21.2 (3.5)[c]	5/5/0/0	1/5/2/2
GB	15.0 (2.6)[a,b]	15.6 (2.4)[d,e]	4/6/0/0	2/4/2/2
OF	11.8 (2.3)[a]	12.3 (2.4)[e]	2/6/0/2	1/5/0/4

*Mean (standard deviation) in MPa. N=10.
**Failure mode: adhesive failure/mixed failure/cohesive failure in enamel/cohesive failure in composite. Values within the same column marked with the same superscript letters showed no significant statistical difference (p>0.05).

DISCUSSION

Although it is preferable to use extracted human teeth for bonding research,[15-16] it has become increasingly difficult to obtain such samples for laboratory studies in Japan. In order to compare the 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. Bovine teeth have some advantages, as they are easy to obtain in large quantities, are in good condition and they have less composition variables than human enamel.[17] Bovine teeth also have large, flat surfaces and are unlikely to have undergone prior caries challenges that could affect the test results. Mineral distribution within 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.

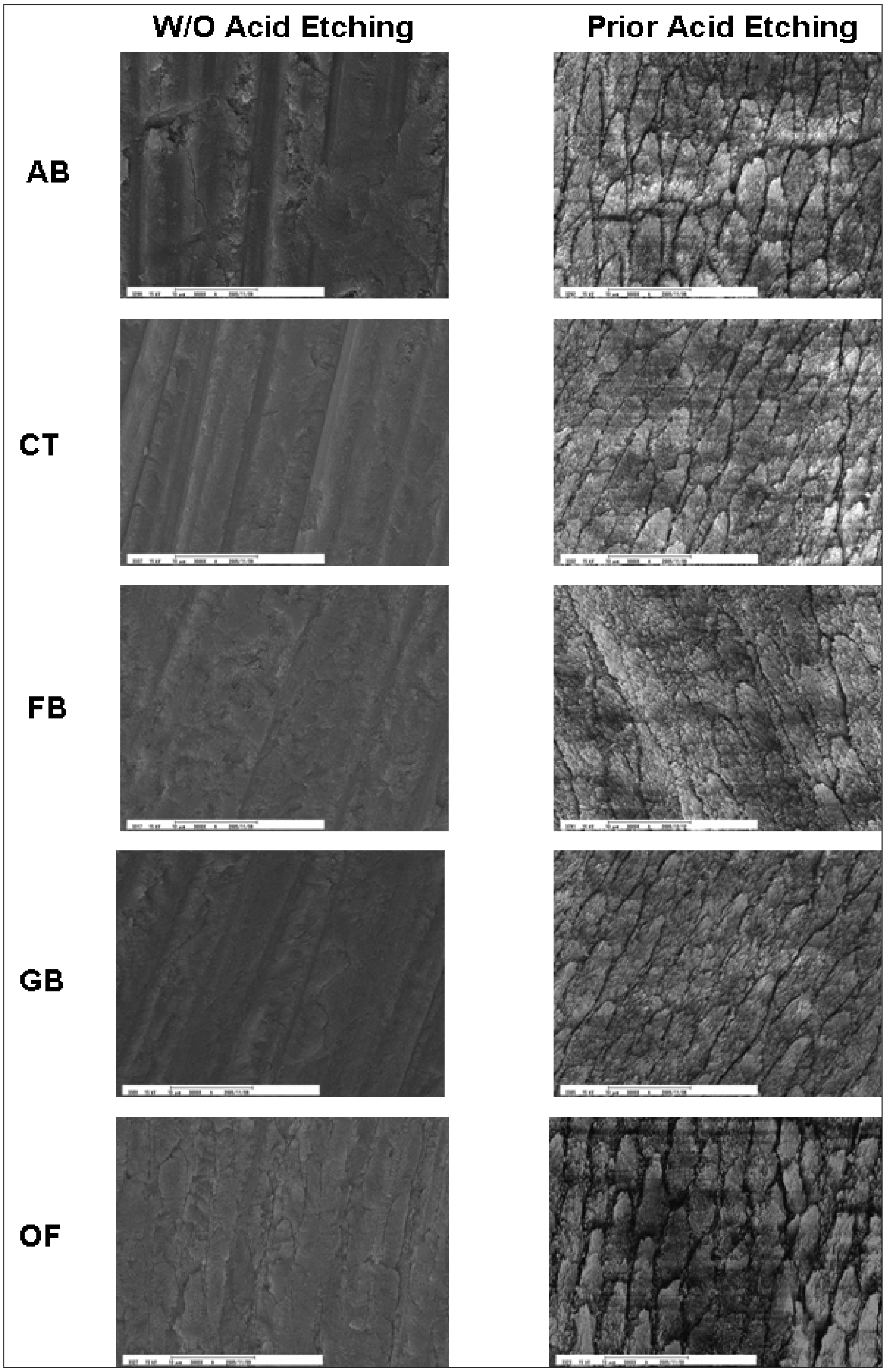

Figure 1. *SEM images of the enamel surface after acidic solution application. The enamel-etching pattern on the treated surfaces was more notable in the specimens that had undergone prior acid etching.*

When the adherent enamel surfaces were treated with phosphoric acid, significant increases in bond strengths were observed using AB, CT and FS. Therefore, the null hypothesis that prior acid etching did not affect the enamel bond strengths was rejected for some of the adhesive systems tested. Higher bond strengths might have been observed with prior acid etching, because a more adherent surface for micro-mechanical retention was produced when enamel was etched with phosphoric acid rather than single-step self-etch adhesives. By contrast, no significant increases in bond strength were found for GB and OB, even when phosphoric acid etching was applied prior to adhesive application. Other factors that might have had an important effect on enamel bond strengths included the cohesive strengths of adhesives and resin composites.

Bonding relies on resin tag formation in the etched enamel surface, which creates micro-mechanical interlocking. The depth of the selective hydroxyapatite removed during the self-etching primer application might depend on the type and concentration of acidic monomers employed, the duration of etching and the chemical composition of the surface enamel.[18] It was previously reported that the etching pattern might not be a determining factor for enamel bond strength;[19] a longer etching time did not lead to increased bond strength, although the respective etching patterns observed by SEM were different. In a clinical situation, no differences were reported when a mild two-step self-etch adhesive system was applied following either a self-etch approach or one that included selective acid-etching of the enamel margins with phosphoric acid.[20] Not only the depth of enamel etching, but also the composition and mechanical properties of the adhesives might play important roles in determining bond strength.

When resin fails to infiltrate the deeper regions of enamel prisms, the resin–enamel bond strength might become weakened by long-term exposure to the oral environment.[21] An ultra-morphological examination of the enamel–resin interface revealed empty spaces in the hybridized area of enamel for an adhesive system.[22] The deficient impregnation of etched enamel by resin could allow water to leach into the bonding resin, resulting in swelling and plasticization.[23] Water might accelerate hydrolysis of the bonding agent and extract poorly polymerized resin oligomers.[24] This deterioration in the mechanical properties of resin might contribute to the decrease in enamel bond strength.

It is important to evaluate bonding durability, as the stability of the bond between the restoration and the tooth substrate is important for the long-term clinical success of tooth-colored restorations.[25] Although the most reliable conclusions about the performance of dental adhesive systems in the oral environment are derived from long-term clinical trials, the long-term storage of a bonded specimen in water or subjecting the specimen to thermal cycling can reveal information about the temperature-dependent degradation of the material.[26] The thermal cycling test exposes specimens to extreme temperatures that simulate intra-oral conditions.[27] Additionally, the cycling induces stress between the tooth substrate and the restorative materials as a result of differences in their coefficients of thermal expansion. The effect of thermal cycling on the bond strength of multi-step bonding systems reportedly depends on the bonding systems used and the number of thermal cycles.[28–30] As single-step self-etch adhesive interfaces are thinner than those created by two-step self-etch systems, thermal stresses are created.

After thermal cycling, significantly greater bond strengths were observed for AB, CT and FS when the adherent enamel surfaces were treated with phosphoric acid. By contrast, for GB and OB, there were no significant differences in bond strength between the groups with and without prior acid etching. The complex thermal cycling process has the potential to trap flaws inside the enamel–resin interface.[31] The thermal cycling test induces stress between the tooth substrate and restorative materials due to differences in the coefficients of thermal expansion. During the thermal cycling test, hot water might accelerate the hydrolysis of resin and extract poorly polymerized resin oligomers.[32-33] Additionally, water and other chemicals leaching from the oral cavity might decrease the mechanical properties of polymers. Deterioration of the mechanical properties of resin composite might contribute to decreased bond strengths of the adhesive systems. These changes in mechanical properties after thermal cycling could facilitate bond failure due to the weakened resin tags that exist between etched enamel and resin.

Achieving harmony between 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. Poor infiltration of the adhesive resin into demineralized dentin leaves nano-spaces in the hybrid layer,[34-35] which might be susceptible to degradation by oral fluids. A similar situation might occur at the resin–enamel interface. It is possible that regions of demineralized dentin that have not been infiltrated by resin monomers might exist within single-step self-etch systems. After application of the adhesive, the enamel surface is air dried, because the adhesive contains solvents that adversely affect polymerization of the resin components. After evaporation, both functional monomers and resin monomers remain on the etched surface. This is followed by polymerization with light irradiation. Therefore, a question remains as to the amount of resin monomers that are required to infiltrate the etched enamel in order to prevent microleakage and maintain bonding durability.

A previous study compared the chemical bonding efficacy of the functional monomers 10-methacryloxydecyl dihydrogen phosphate (MDP), 4-methacryloxyethyl trimellitic acid (4-MET) and 2-methacryloxyethyl phenyl hydrogen phosphate (phenyl-P). MDP was reported to have a high chemical bonding potential to hydroxyapatite within a clinically reasonable application time.[36] Furthermore, the calcium salt of MDP was highly insoluble and, consequently, was able to resist ultrasonic cleaning. According to the adhesion–decalcification concept,[37] 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 leads to higher bond strengths compared with those that rely on micromechanical retention to the enamel substrate alone.

CONCLUSIONS

The results of the current study suggest that the benefits of using phosphoric acid prior to the application of single-step self-etch adhesives, in terms of increased enamel bond strengths, remains even after a number of thermal cycles that simulate long-term exposure to the oral environment. However, general practitioners who use these adhesive systems should be aware that two of the five single-step self-etch adhesive systems tested demonstrated no significant increase in bond strength due to prior acid etching. Further understanding of the factors that contribute to the durability of restorations and their bonding characteristics is needed.

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