©Operative Dentistry, 2015, **40-5,** E180-E188

Microshear Bond Strength of Adhesives to Enamel Remineralized Using Casein Phosphopeptide Agents

EH Mobarak • N Ali • LE Daifalla

Clinical Relevance

Remineralization of enamel with casein phosphopeptide—amorphous calcium phosphate with fluoride had no negative effect on its bonding to self-etch adhesives. The newly developed Single Bond Universal adhesive system did not achieve better bonding to enamel than did its predecessors.

SUMMARY

Objective: This study was carried out to evaluate the difference between bonding to demineralized enamel and remineralized enamel using casein phosphopeptide–amorphous calcium phosphate with fluoride (CPP-ACFP) or without fluoride (CPP-ACP) compared to normal enamel. Another aim was to test if the newly introduced Single Bond Universal adhesive system would show better bonding to any enamel condition in comparison to the other tested adhesive systems.

Methods: The lingual enamel surfaces of 40 non carious human third molars were divided into four main groups according to the enamel condition (ground normal enamel [negative control]; demineralized enamel [positive control]; and remineralized enamel with CPP-ACP or with CPP-ACFP, respectively). Within each main group, the lingual enamel surface of each tooth was sectioned into three slabs, resulting in 30 slabs that were distributed into three subgroups according to the adhesive system utilized (Clearfil S^3 Bond Plus, Single Bond Universal, or G-aenial Bond). Two resin composite microcylinder buildups were made on each enamel slab using Filtek Z350 XT. The μSBS was evaluated at a crosshead speed of 0.5 mm/min. Modes of failure were detected using an environmental scanning electron microscope at 300× magnification.

Results: The two-way analysis of variance with repeated measures revealed a significant effect for the enamel condition. However, there was no significant effect for the type of

*Enas Hussein Mobarak, , associate professor, Restorative Department, Cairo University, Cairo, Egypt

Nesma Ali, Master's student, Restorative Department, Cairo University, Cairo, Egypt

Lamia E Daifalla, lecturer, Restorative Department, Cairo University, Cairo, Egypt

*Corresponding author: 14 ElAnsar str, Cairo, 12311, Egypt; e-mail: enasmobarak@hotmail.com

DOI: 10.2341/13-220-L

adhesive system. The interaction between the enamel condition and the type of adhesive system was also not significant. Modes of failure were mainly adhesive except for the demineralized enamel. It showed a mixed type of failure, in which cohesive failure in enamel was recorded.

Conclusions: All single-step self-etch adhesives revealed comparable μSBS values to ground enamel and enamel remineralized with CPP-ACP or CPP-ACFP. Bonding to demineralized enamel was ineffective. With any enamel condition, no tested single-step self-etch adhesive was superior in its bonding.

INTRODUCTION

Scientific advances in restorative materials and techniques as well as in understanding the pathogenesis of caries and methods of its prevention have led to the evolution of minimal intervention dentistry. In this concept, healing of early subsurface lesions by remineralization is preferred to surgical intervention.

For enamel subsurface lesion remineralization, multiple approaches have evolved, including different fluoride regimens and calcium phosphate systems.[1] Previous investigations[2-8] have demonstrated the ability of casein phosphopeptide–amorphous calcium phosphate (CPP-ACP) to control demineralization and enhance remineralization. Casein phosphopeptides are thought to stabilize calcium and inorganic ions. Thus, they provide a reservoir of small CPP-ACP clusters with respect to the enamel surface controlling demineralization and enhancing remineralization The inclusion of fluoride into CPP-ACP resulted in a novel CPP-ACFP material that was suggested to reveal better remineralizing potential than CPP-ACP.[9] The availability of fluoride ions in conjunction with calcium and phosphate ions at the enamel surface was expected to enhance fluorapatite formation. In addition, CPP-ACFP was found to be superior in reducing caries risk, as compared to the products that contain only fluoride "fluoride *per se* products,"[10] in which the enamel has been shown to be more acid resistant.[11] Nevertheless, this acid-resistant enamel substrate might become an obstacle for bonding. Therefore, to clarify this issue, some studies[12-14] have tested the effect of remineralization with CPP-ACP on enamel bonding. However, at present there are no data available on the effect of remineralization with CPP-ACFP on bonding to enamel.

At the same time, the self-etch approach provides dentists with a generation of user-friendly and less technique sensitive adhesives that have paved the way for increasing its usage by general practitioners. The development of self-etch adhesives has continued in recent years and resulted in what is called "universal adhesive." The manufacturer claims that this adhesive system can be used with both direct and indirect restorations. It was also assumed that this adhesive system bonds effectively to all tooth substrates, including enamel and dentin, as well as to glass ceramic, zirconia, noble and nonprecious alloys, and composites without the need for an additional primer. However, there is a lack of information about the bond of this adhesive to enamel compared with other single-step self-etch adhesives. Therefore, a study to determine the microshear bond strength of this new self-etch adhesive system to different enamel conditions (ground enamel with no treatment, demineralized enamel, and enamel remineralized with CPP-ACP or with CPP-ACFP) could be of value. The null hypotheses were the following: 1) The μSBS values of CPP-ACP and CPP-ACFP remineralized enamel specimens are not different from each other or from those of the sound enamel group; and 2) There is no difference in bonding of the newly introduced single-step self-etch adhesive system and that of other adhesives, regardless of the enamel condition.

METHODS AND MATERIALS

Selection of Teeth

A total of 40 normal human third molars, free from previous restoration or decay and with sufficient enamel width lingually, were selected for this study. Teeth were collected from patients within the 20 to 25-year-old age group. Immediately after extraction, the teeth were thoroughly washed, scrubbed, and scaled to remove blood, mucous, and shreds of periodontal ligament. The study was accomplished in accordance with local human subjects' oversight committee guidelines. All teeth were examined using a (6×) magnifying lens (Bausch and Lomb, Opt. Co, Rochester, NY, USA) to ensure that they were free from visible hypoplastic defects or fractures. Teeth were stored refrigerated at 4°C in a phosphate buffer saline solution containing 0.2% sodium azide (pH=7.4) for a period not longer than one month.[15]

Grouping for the Study

The selected 40 teeth were sectioned at the level of the cemento-enamel junction to separate the crown portion and were then divided into four main groups.

Table 1: *Materials Used in the Study*

Material Brand Name	Composition	Manufacturer	Batch No.
Tooth Mousse	Pure water, glycerol, CPP-ACP, D-sorbitol, CMC-Na, propylene glycol, silicon dioxide, titanium dioxide, xylitol, phosphoric acid, flavoring, zinc oxide, sodium saccharin, ethyl *p*-hydroxybenzoate, magnesium oxide, guar gum, propyl *p*-hydroxybenzoate, butyl *p*-hydroxybenzoate	GC Corporation, Itabashi-Ku, Tokyo, Japan	300788TP
MI Paste Plus	Pure water, glycerol, CPP-ACP, D-sorbitol, CMC-Na, propylene glycol, silicon dioxide, titanium dioxide, xylitol, phosphoric acid, sodium fluoride, flavoring, sodium saccharin, ethyl *p*-hydroxybenzoate, propyl *p*-hydroxybenzoate, butyl *p*-hydroxybenzoate	GC Corporation, Itabashi-Ku, Tokyo, Japan	300783TP
Clearfil S^3 Bond Plus	MDP, Bis-GMA, 2 HEMA, hydrophilic aliphatic dimethacrylate, hydrophobic aliphatic methacrylate, colloidal silica, sodium fluoride, DL camphorquinone, accelerators, initiator, ethanol, water, pH = 2.7	Kuraray Medical Inc, Sakazu, Okayama, Japan	00018A
G-aenial Bond	Acetone, distilled water, dimethacrylate, 4-META, anhydride, phosphoric acid ester monomer, silicon dioxide, photo initiator, pH = 1.5	GC Corporation, Itabashi-Ku, Tokyo, Japan	1103231
Single Bond Universal	MDP phosphate monomer, dimethacrylate resins, HEMA, Vitrebond™ copolymer, filler, ethanol, water, initiators, silane, pH = 2	3M ESPE Dental Products, St Paul, MN, USA	468355
Filtek Z350 XT	Bis-GMA, TEGDMA, PEGDMA, Bis-EMA, UDMA (zirconia-silica) 63.3% by volume and 78.5% by weight, filler particle size range (4-20 nm), cluster particle size (0.6-20 μm)	3M ESPE Dental Products, St Paul, MN, USA	N286859

Abbreviations: Bis-EMA, ethoxylated bisphenol A dimethacrylate; Bis-GMA, bisphenol A diglycidyl ether dimethacrylate; CMC-Na, sodium carboxymethyl cellulose; CPP-ACP, casein phosphopeptide–amorphous calcium phosphate; 4-META, 4-methacryloxyethyl trimellitic acid; HEMA, 2-hydroxyethyl methacrylate; PEGDMA, polyethylene glycol dimethacrylate; TEGDMA, triethylene glycol dimethacrylate; 10-MDP, 10-methacryloyloxydecyl dihydrogen phosphate; UDMA, urethane dimethacrylate.

In the first group, bonding was done to ground enamel with no pretreatment; this group served as the control. In the second group, bonding was done to demineralized enamel only. In the third and fourth groups, bonding was done to remineralized enamel with CPP-ACP or with CPP-ACFP, respectively. Then the lingual enamel surface of each coronal portion was vertically sectioned into three slabs, resulting in a total of 30 enamel slabs within each main group. The 30 slabs from each group were distributed equally into three subgroups (10 enamel slabs each) according to the adhesive system utilized. For the first subgroup, Clearfil S^3 Bond Plus was used. For the second and third subgroups, Single Bond Universal and G-aenial Bond adhesive systems were applied, respectively. The brand name, composition, manufacturer, and batch number of the materials used in the present study are listed in Table 1.

Preparation of Enamel Specimens

In order to standardize the position of the slabs during the embedding, a circle of 21 mm in diameter and two perpendicular intersecting lines were drawn on a square-shaped glass piece (50 mm × 50 mm) (Figure 1A). Each enamel slab was fixed from its lingual surface to the center of the circle using glue (Rocket Light, Dental Ventures of America, Corona, CA, USA). Polyvinyl chloride (PVC) tubes of 15-mm internal diameter and 21-mm external diameter were cut into two heights: 1-mm ring and 20-mm cylinder. The first PVC ring of 1-mm height (Figure 1B) was coated with a separating medium and placed on the glass piece coinciding with the drawn circle in order to raise the specimen above the embedding material by 1 mm. The second cylindrical PVC tube of 20-mm height was sealed from one end with an adhesive tape to serve as a mold (Figure 1C). The glass piece with the fixed enamel slab and the encircling 1-mm ring was then placed onto the PVC mold with the drawn circle coinciding with the mold outer margin to centralize the slab within the mold. The polyester embedding material (polyester #2121, ETERNAL CHEMICAL CO, LTD, Hsien, Taiwan) was mixed and poured to fill the mold. After complete setting of the embedding material, the glass slab and the 1-mm ring were removed, at which point the enamel slab was centrally embedded within the mold, while the specimen was protruding by 1 mm above the polyester embedding material. Each embedded enamel specimen was coded according to the enamel condition and the adhesive system

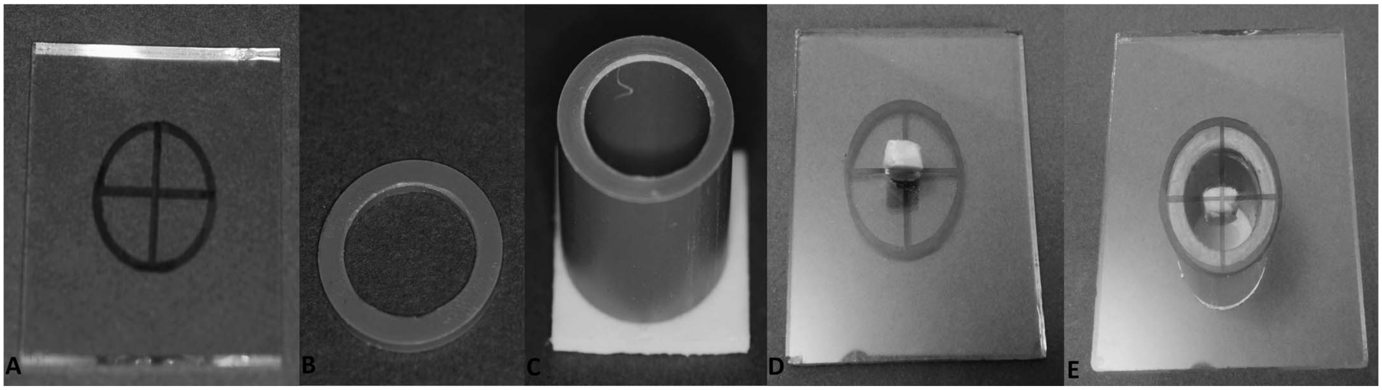

Figure 1. *Steps for embedding of the specimens: two perpendicular intersecting lines drawn on a square-shaped glass piece to standardize the position of the slabs during the embedding (A), 1-mm height PVC ring used to protrude the specimen above the embedding material (B), 20-mm height PVC cylindrical tube sealed from one end to serve as a mold (C). The enamel slab fixed on the glass piece (D). The glass piece with the fixed enamel slab and the encircling 1-mm ring placed onto the PVC mold (E).*

utilized. The enamel surface of each embedded specimen was then ground flat while ensuring that only 0.4 mm was removed from the total height of the specimen using a digital caliper (Proficraft, Mebschibes, Germany). The enamel surface was then finished manually with wet 600-grit silicon carbide waterproof abrasive paper (Grinco Miami, FL, USA) to ensure a uniform smear layer formation.[16]

Preparation for Different Enamel Conditions

Artificial caries-like lesions in the enamel were created by immersing each embedded specimen in 40 mL of demineralizing solution. The solution contained 0.1 mol/L lactic acid, 500 mg/L hydroxyapatite, and 20 g/L Carbopol C907 at pH 4.8.[17] Specially constructed plastic containers were made to hold the embedded enamel specimens during the demineralization and remineralization periods. The embedded specimens were held upside down in the demineralizing solution. The demineralization was done over the course of four days at 37°C, and the solution was changed every two days. After demineralization, specimens were removed and washed with distilled water for 30 seconds, then dried for 10 seconds.

For remineralization, each specimen was subjected to 2 mL of any of the proposed remineralizing solutions (2% CPP-ACP or CPP-ACFP) at 37°C, with daily change of the solution[18] over the course of 30 days. The remineralizing solution was prepared by dissolving 1 g of the remineralizing paste in 4 mL of water, resulting in 160 mL of remineralizing solution from each tube. A specially cut PVC tube (15 mm in length) was glued to the mold to enclose the remineralizing solution. The specimens were held upright during remineralization in the specially constructed plastic containers. Wet cotton was laid at the bottom of the container to maintain the humidity during the remineralization regimen. After the remineralization period, each specimen was removed and washed with 10 mL distilled water for 60 seconds.[18]

Restorative Procedures

The tested adhesive systems were applied to the enamel surfaces using disposable microbrushes (Tokuyama Dental America, Encinitas, CA, USA) according to the manufacturer's instructions, as described in Table 2. A transparent polyethylene tube obtained from a scalp vein infusion set (23G,

Table 2: *Steps of Application for the Adhesive Systems Utilized in the Study*

Adhesive System	Mode of Application
Clearfil S^3 Bond Plus	• Apply bond with a disposable microbrush • Leave in place for 10 s • Dry with mild pressure air flow for five s at 10-mm distance • Light-cure for 10 s
G-aenial Bond	• Apply with a disposable microbrush • Wait 10 s • Dry with oil-free air under maximum air pressure for five s at 10-mm distance • Light-cure for 10 s
Single Bond Universal	• Apply using disposable applicator and rub it for 20 s • Direct gentle steam of air for about five s at 10-mm distance until the film no longer moves • Light-cure for 10 s

JMS Singapore PTE, LTD, Singapore) was cut into small irises of 0.7 mm in length using a sharp lancet. The transparent polyethylene iris was used to assist in packing of the resin composite.[19] The external and internal diameters of the polyethylene iris were measured using a scanning electron microscope (515; Philips, Einhoven, The Netherlands) and were verified to be 2.35 mm for the external diameter and 0.93 mm for the internal diameter. Two resin composite microcylinders were built over each enamel specimen. Each resin composite microcylinder was polymerized for 40 seconds using a Blue Phase C5 light-curing unit (Ivoclar Vivadent, Schaan, Liechtenstein). The light intensity was regularly checked using an LED Radiometer (Kerr Dental Specialties, West Collins Orange, USA). The polyethylene irises were then cautiously removed with the aid of a No. 11 lancet (Wuxi Xinda Medical Device Co, China), leaving the composite microcylinders bonded to enamel surfaces. All resin composite microcylinders were checked using a (6×) magnifying glass lens for any defect or air bubble. Any defective microcylinders were excluded, along with their related slabs.

Microshear Bond Strength Assessment

Each specimen with its two bonded resin composite microcylinders was secured with the four tightening bolts in the lower part of the specially designed attachment jig.[20] The attachment jig was in turn screwed into the lower fixed and the upper movable compartments of the testing machine (Model LRX-plus; Lloyd Instruments Ltd, Ferham, UK). A shear load was applied via the testing machine at a crosshead speed of 0.5 mm/min. Data were recorded using computer software (Nexygen-MT; Lloyd Instruments). The bond strength of the resin composite microcylinders that showed spontaneous interfacial debonding during the handling or the mounting of specimens was recorded as 0 MPa.

Statistical Analysis

Two-way analysis of variance (ANOVA) with repeated measures was used to compare the effect of the enamel condition, the adhesive system, and their interaction. This was followed by the Tukey post hoc test for pairwise comparison. The significance level was set at $\alpha=0.05$. Data were analyzed using the SPSS program for Windows (release 15 for MS Windows, SPSS Inc, Chicago, IL, USA).

Failure Mode Examination

After measuring the bond strength, each fractured specimen was inspected using an environmental scanning electron microscope (ESEM) (Quanta 200, FEI Company, Philips) at 25 KV to determine its mode of failure. The failure modes were allocated to five types, as follows:

1) Type A: Adhesive failure (at the adhesive/enamel interface);
2) Type B: Cohesive failure in the adhesive;
3) Type C: Mixed failure including partial adhesive failure at the adhesive/enamel interface and partial cohesive failure in the adhesive;
4) Type D: Mixed failure including partial cohesive failure in the adhesive system and partial cohesive failure in resin composite; and
5) Type E: Mixed failure including adhesive failure at the adhesive/enamel interface, cohesive failure in enamel, cohesive failure in the adhesive, and cohesive failure in resin composite.

Representative photomicrographs for the most predominant types of failure within each subgroup were captured at 300× magnification.

RESULTS

The μSBS (MPa) results were described in terms of the mean standard deviation (SD). The two-way ANOVA revealed a significant effect for the enamel condition ($p<0.001$), while the type of adhesive system had no significant effect ($p=0.155$). The interaction between the enamel conditions and the type of adhesive system was also not significant ($p=0.700$). The effects of different enamel conditions on the μSBS values of the three tested single-step self-etch adhesive systems are presented in Table 3.

The distribution of failure modes for all tested groups are shown in Figure 2. The ESEMs revealed that the predominant mode of failure for the control enamel groups of the three tested adhesive systems was adhesive failure at the adhesive/enamel interface (type A). For the demineralized enamel groups, all tested adhesive systems showed type E mixed failure that includes adhesive failure at the adhesive/enamel interface, cohesive failure in enamel, cohesive failure in the adhesive, and cohesive failure in resin composite. With regard to bonding to the remineralized groups with CPP-ACP or CPP-ACFP, Clearfil S^3 Bond Plus and Single Bond Universal adhesive systems revealed type A as the predominant failure mode. For G-aenial Bond bonded to the remineralized group with CPP-ACP, type C mixed

Table 3: *The Effect of Different Enamel Conditions on the μSBS (MPa) of the Tested Adhesive Systems, Mean (Standard Deviation)*[a]

Adhesive Systems	Enamel Conditions, MPa				*p*-Value
	Control	Demineralized	Remineralized with CPP-ACP	Remineralized with CPP-ACFP	
Clearfil S3 Bond Plus	11 (2.8) aA (Ptf/tnt=0/20)	4.1 (4.3) aB (Ptf/tnt=7/20)	10.1 (2.9) aA (Ptf/tnt=0/20)	10.9 (4.0) aA (Ptf/tnt=1/20)	<0.001
G-aenial Bond	11.7 (4.0) aA (Ptf/tnt=1/20)	2.1 (2.0) aB (Ptf/tnt=8/20)	10.5 (3.2) aA (Ptf/tnt=1/20)	10.8 (3.3) aA (Ptf/tnt=2/20)	<0.001
Single Bond Universal	13.9 (5.3) aA (Ptf/tnt=0/20)	3.2 (3.3) aB (Ptf/tnt=7/20)	13.0 (4.0) aA (Ptf/tnt=0/20)	11.0 (4.1) aA (Ptf/tnt=0/20)	<0.001

[a] *(ptf/tnt=pretest failure/total number of tested resin composite microcylinders within each group). Within rows, similar capital letters denote no statistically significant difference, while similar lowercase letters within columns reveal no statistically significant difference (Tukey test,* $p \geq 0.05$*).*

failure was found as the predominant failure mode, including partial adhesive failure at the adhesive/enamel interface and partial cohesive failure in adhesive. When it was bonded to the remineralized group with CPP-ACFP, an equal predominance for types A, B, and C failure modes was recorded. Figures 3-6 show representative ESEMs for the predominant failure modes of the tested groups.

DISCUSSION

The results of the current study revealed that the μSBS values of CPP-ACP and CPP-ACFP remineralized enamel specimens were not different from each other or from those of the control group. Thus, the first null hypothesis is accepted. For the remineralized enamel using CPP-ACP, some previous studies[12-14] also had the same findings, despite the fact that these studies used different demineralization and remineralization protocols. Regarding the CPP-ACFP group, the present study is the first of its kind to test the effect of remineralization with CPP-ACFP on bonding of self-etch adhesives to enamel; thus, no literature was available with which to compare our results. Based on the obtained results in the present study, it would be reasonable to infer that like CPP-ACP,[14] CPP-ACFP regained the enamel bonding to a comparable level compared with the sound enamel. Meanwhile, it should be

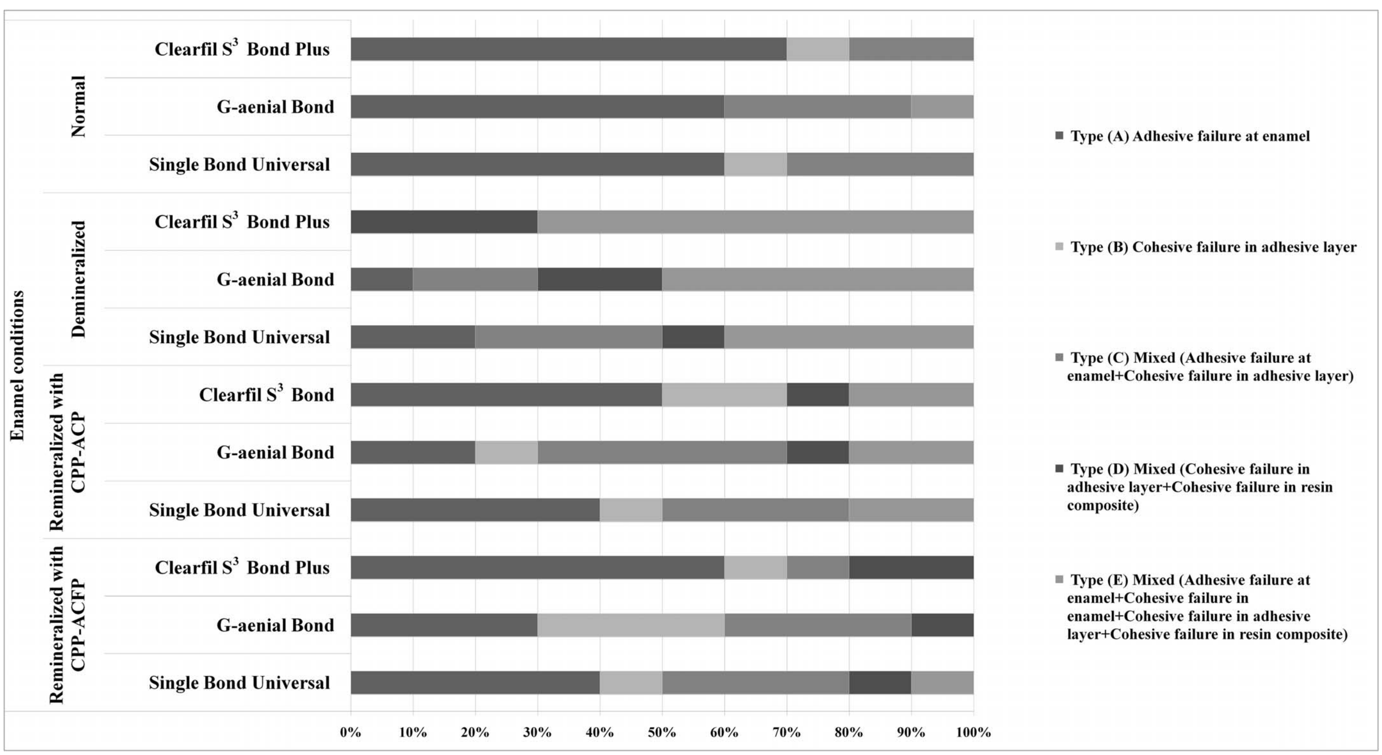

Figure 2. *The distribution of failure modes for all tested groups.*

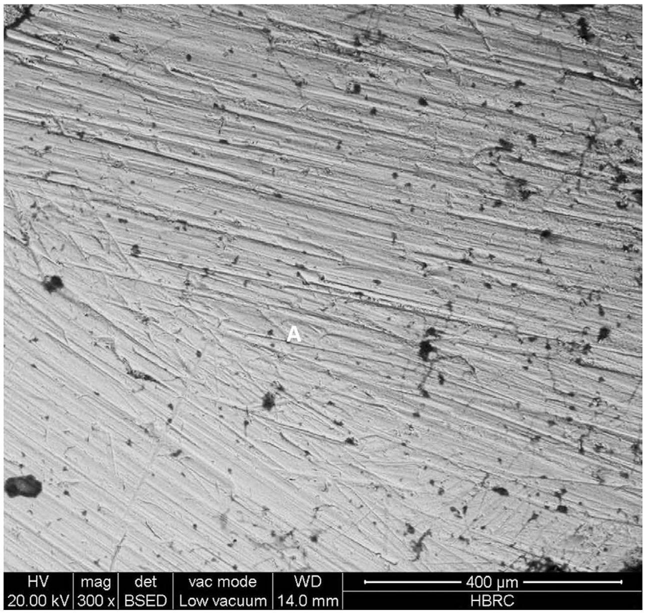

Figure 3. *ESEM photomicrograph showing adhesive failure at enamel/adhesive interface (A) representing failure mode of Clearfil S^3 Bond Plus bonded to control group.*

noted that remineralized enamel does not always have enamel prisms, and it is sometimes composed of a highly dense compaction of calcium phosphate and fluoride.[12] In addition, residual CPP-ACP complexes may remain on the enamel surface and be incorporated into the bonding layer or inhibit the bond between the adhesive system and enamel.[21]

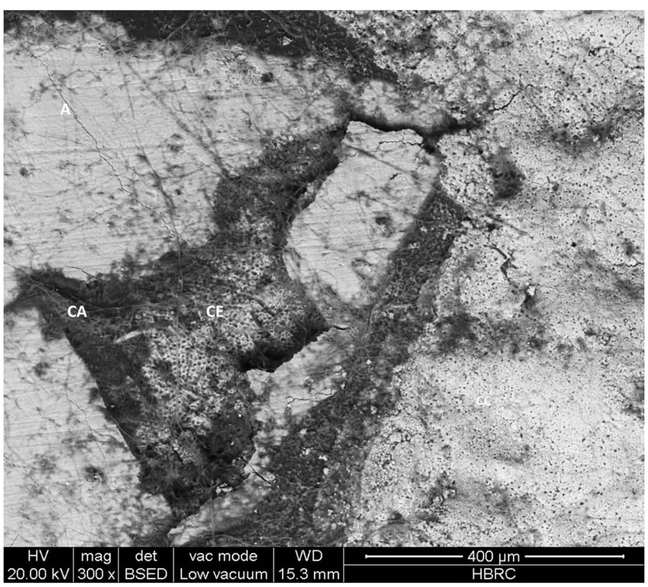

Figure 4. *ESEM photomicrograph showing mixed failure: adhesive failure at enamel/adhesive interface (A), cohesive failure in enamel (CE), and cohesive failure in resin composite (CC) and in adhesive (CA) of Single Bond Universal bonded to demineralized group.*

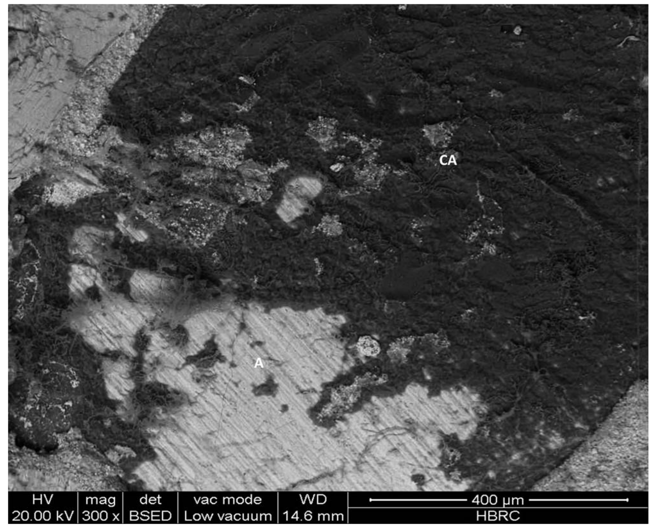

Figure 5. *ESEM photomicrograph showing mixed failure: adhesive failure at enamel/adhesive interface (A) and cohesive failure in the adhesive (CA) of G-aenial Bond bonded to remineralized group with CPP-ACP.*

In the current study, the lowest µSBS values were recorded when the adhesive systems bonded to demineralized enamel. This corroborates with previous studies.[12,14,22] This finding may be attributed to the poor quality of the enamel surface available for bonding, which hinders proper micromechanical interlock. Moreover, mixed failure with cohesive failure in enamel was more often seen in the demineralized group. This finding was in accordance

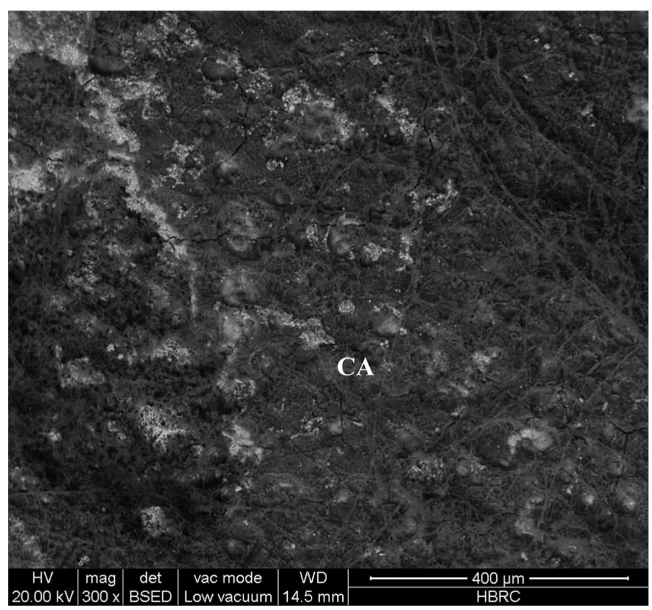

Figure 6. *ESEM photomicrograph showing cohesive failure in the adhesive (CA) of G-aenial Bond bonded to remineralized group with CPP-ACFP.*

with that of the previous study.[23] The reported cohesive failures in enamel may be due to the brittleness of demineralized enamel, which is more likely to fracture under stress. In addition, the self-etching adhesive did not penetrate so deeply as to reinforce the demineralized enamel.[23] This led us to consider demineralized enamel as an improper substrate for bonding, and it should be either remineralized prior to bonding or removed.

Three types of single-step self-etch adhesive systems were selected for this study. No variations in bond strength values among the tested adhesive systems with any of the enamel conditions were recorded. Therefore, the second null hypothesis was not rejected. These similar bond values were recorded despite the differences among them in terms of composition, pH, and type of solvent. Researchers[16,24,25] found that pH alone is not the sole parameter for achieving a good bond. Thus, it appears that other factors, such as the ability of the adhesive to form a chemical bond to enamel and the strength of the adhesive system itself, significantly influence the bond values to enamel. Previous studies[16,26-29] have reported the effective bonding of Clearfil S^3 Bond Plus adhesive system to enamel. The material is stable as a result of the presence of 10–methacryloyloxydecyl dihydrogen phosphate (10-MDP), a molecule with chemical affinity for dental tissues. The two hydroxyl groups in 10-MDP chelate the calcium of the tooth structure. Additionally, Clearfil S^3 Bond Plus adhesive system may resist mechanical fatigue, since it has been reported[27] to be more resistant to mechanical stress. With regard to the G-aenial Bond adhesive system, this 2-hydroxyethyl methacrylate–free, one-step adhesive is known to induce phase separation and therefore requires post-application 'strong' air-thinning. This strong air-thinning is very feasible on flat surfaces, leading to a quite thin and more uniform adhesive layer, which is in contrast to what actually happens in clinical conditions.[30] The Single Bond Universal adhesive system was expected to achieve superior bonding to enamel in comparison to other tested adhesives. This was thought to be due to the existence of 10-MDP and Vitrebond copolymer, with their ability to bond chemically to calcium in enamel hydroxyapatite, providing stable and durable interfaces.[31] In contradiction to our expectation, this adhesive failed to reveal better bonding. At the same time, the recoded failure modes for this adhesive were not different from those recorded with Clearfil S^3 Bond Plus adhesive system.

Nevertheless, attaining very high bond strengths is not necessarily an indicator of clinical success. It would appear that other parameters, such as chemical interaction with the tooth surface and bond stability over the long term, may be important for the clinical success of bonded restorations.[16] This encourages further research that focuses on the long-term bond durability of these adhesives to CPP-ACP and CPP-ACFP remineralized enamel.

CONCLUSIONS

All single-step self-etch adhesives revealed comparable μSBS values to ground enamel and enamel remineralized with CPP-ACP or CPP-ACFP. Bonding to demineralized enamel was ineffective. With any enamel condition, no tested single-step self-etch adhesive was superior in its bonding.

Conflict of Interest

The authors of this manuscript certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

(Accepted 14 November 2013)

REFERENCES

1. Sudjalim TR, Woods MG, & Manton DJ (2006) Prevention of white spot lesions in orthodontic practice: A contemporary review *Australian Dental Journal* **51(4)** 284-289.
2. Cai F, Shen P, Morgan MV, & Reynolds EC (2003) Remineralization of enamel subsurface lesions in situ by sugar-free lozenges containing casein phosphopeptide-amorphous calcium phosphate *Australian Dental Journal* **48(4)** 240-243.
3. Milnar FJ (2007) Considering biomodification and remineralization techniques as adjuncts to vital tooth-bleaching regimens *Compendium of Continuing Education in Dentistry* **28(5)** 234-236, 238-240.
4. Oshiro M, Yamaguchi K, Takamizawa T, Inage H, Watanabe T, Irokawa A, Ando S, & Miyazaki M (2007) Effect of CPP-ACP paste on tooth mineralization: An FE-SEM study *Journal of Oral Science* **49(2)** 115-120.
5. Rahiotis C, & Vougiouklakis G (2007) Effect of a CPP-ACP agent on the demineralization and remineralization of dentine in vitro *Journal of Dentistry* **35(8)** 695-698.
6. Reynolds EC, Cai F, Shen P, & Walker GD (2003) Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum *Journal of Dental Research* **82(3)** 206-211.
7. Shen P, Cai F, Nowicki A, Vincent J, & Reynolds EC (2001) Remineralization of enamel subsurface lesions by sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate *Journal of Dental Research* **80(12)** 2066-2070.

8. Yamaguchi K, Miyazaki M, Takamizawa T, Inage H, & Moore BK (2006) Effect of CPP-ACP paste on mechanical properties of bovine enamel as determined by an ultrasonic device *Journal of Dentistry* **34(3)** 230-236.
9. Pulido MT, Wefel JS, Hernandez MM, Denehy GE, Guzman-Armstrong S, Chalmers JM, & Qian F (2008) The inhibitory effect of MI paste, fluoride and a combination of both on the progression of artificial caries-like lesions in enamel *Operative Dentistry* **33(5)** 550-555.
10. Reynolds EC, Cai F, Cochrane NJ, Shen P, Walker GD, Morgan MV, & Reynolds C (2008) Fluoride and casein phosphopeptide-amorphous calcium phosphate *Journal of Dental Research* **87(4)** 344-348.
11. Iijima Y, Cai F, Shen P, Walker G, Reynolds C, & Reynolds EC (2004) Acid resistance of enamel subsurface lesions remineralized by a sugar-free chewing gum containing casein phosphopeptide-amorphous calcium phosphate *Caries Research* **38(6)** 551-556.
12. Baysal A, & Uysal T (2012) Do enamel microabrasion and casein phosphopeptide-amorphous calcium phosphate affect shear bond strength of orthodontic brackets bonded to a demineralized enamel surface? *Angle Orthodontist* **82(1)** 36-41.
13. Keles K (2010) *Valuation of Shear Bond Strength After Remineralization of Enamel Subsurface Lesion by CPP-ACP: In Vitro Study* Doctoral thesis, University of Cukurova, Adana, Turkey.
14. Uysal T, Baysal A, Uysal B, Aydinbelge M, & Al-Qunaian T (2011) Do fluoride and casein phosphopeptide-amorphous calcium phosphate affect shear bond strength of orthodontic brackets bonded to a demineralized enamel surface? *Angle Orthodontist* **81(3)** 490-495.
15. Nakajima M, Ogata M, Harada N, Tagami J, & Pashley DH (2000) Bond strengths of self-etching primer adhesives to in vitro-demineralized dentin following mineralizing treatment *Journal of Adhesive Dentistry* **2(1)** 29-38.
16. Burrow MF, Kitasako Y, Thomas CD, & Tagami J (2008) Comparison of enamel and dentin microshear bond strengths of a two-step self-etching priming system with five all-in-one systems *Operative Dentistry* **33(4)** 456-460.
17. White DJ (1987) Use of synthetic polymer gels for artificial carious lesion preparation *Caries Research* **21(3)** 228-242.
18. Cochrane NJ, Saranathan S, Cai F, Cross KJ, & Reynolds EC (2008) Enamel subsurface lesion remineralisation with casein phosphopeptide stabilised solutions of calcium, phosphate and fluoride *Caries Research* **42(2)** 88-97.
19. Sattabanasuk V, Shimada Y, & Tagami J (2005) Bonding of resin to artificially carious dentin *Journal of Adhesive Dentistry* **7(3)** 183-192.
20. Mobarak EH (2011) Effect of chlorhexidine pretreatment on bond strength durability of caries-affected dentin over 2-year aging in artificial saliva and under simulated intrapulpal pressure *Operative Dentistry* **36(6)** 649-660.
21. Moule CA, Angelis F, Kim GH, Le S, Malipatil S, Foo MS, Burrow MF, & Thomas D (2007) Resin bonding using an all-etch or self-etch adhesive to enamel after carbamide peroxide and/or CPP-ACP treatment *Australian Dental Journal* **52(2)** 133-137.
22. Attin R, Stawarczyk B, Kecik D, Knosel M, Wiechmann D, & Attin T (2012) Shear bond strength of brackets to demineralize enamel after different pretreatment methods *Angle Orthodontist* **82(1)** 56-61.
23. Jia L, Stawarczyk B, Schmidlin PR, Attin T, & Wiegand A (2012) Effect of caries infiltrant application on shear bond strength of different adhesive systems to sound and demineralized enamel *Journal of Adhesive Dentistry* **14(6)** 569-574.
24. Ostby AW, Bishara SE, Denehy GE, Laffoon JF, & Warren JJ (2008) Effect of self-etchant pH on the shear bond strength of orthodontic brackets *American Journal of Orthodontics and Dentofacial Orthopedics* **134(2)** 203-208.
25. Moura SK, Pelizzaro A, Dal Bianco K, de Goes MF, Loguercio AD, Reis A, & Grande RH (2006) Does the acidity of self-etching primers affect bond strength and surface morphology of enamel? *Journal of Adhesive Dentistry* **8(2)** 75-83.
26. Kimmes NS, Barkmeier WW, Erickson RL, & Latta MA (2010) Adhesive bond strengths to enamel and dentin using recommended and extended treatment times *Operative Dentistry* **35(1)** 112-119.
27. Perdigao J, Dutra-Correa M, Anauate-Netto C, Castilhos N, Carmo AR, Lewgoy HR, Amore R, & Cordeiro HJ (2009) Two-year clinical evaluation of self-etching adhesives in posterior restorations *Journal of Adhesive Dentistry* **11(2)** 149-159.
28. Poggio C, Scribante A, Della Zoppa F, Colombo M, Beltrami R, & Chiesa M (2014) Shear bond strength of one-step self-etch adhesives to enamel: Effect of acid pretreatment *Dental Traumatology* **30(1)** 43-48.
29. Reis A, Leite TM, Matte K, Michels R, Amaral RC, Geraldeli S, & Loguercio AD (2009) Improving clinical retention of one-step self-etching adhesive systems with an additional hydrophobic adhesive layer *Journal of the American Dental Association* **140(7)** 877-885.
30. Van Ende A, De Munck J, Van Landuyt KL, Poitevin A, Peumans M, & Van Meerbeek B (2013) Bulk-filling of high C-factor posterior cavities: Effect on adhesion to cavity-bottom dentin *Dental Materials* **29(3)** 269-277.
31. Mena-Serrano A, Kose C, De Paula EA, Tay LY, Reis A, Loguercio AD, & Perdigao JA New universal simplified adhesive: 6-Month clinical evaluation *Journal of Esthetic and Restorative Dentistry* **25(1)** 55-69.