Effect of acid erosion on enamel bond strength of self-etch adhesives and sonic velocity measurement of enamel

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The purpose of this study was to investigate the effect of acid erosion on the bonding performance of universal adhesives. Freshly extracted bovine teeth were cut into enamel slabs and assigned to either the Er or the control group. Specimens in the Er group were immersed in citric acid solution (pH 2.1) twice a day and stored in artificial saliva, while control specimens were simply stored in artificial saliva. Differences in the mean values between the control and Er groups were greater than expected, despite accounting for the effect of differences in the adhesive systems ($p=0.016$). The Er group mainly exhibited cohesive failure in the enamel, while adhesive failure was more frequent in the control group. The sonic velocity was found to decrease over time in the acid attacked specimens. The results indicated that bond strengths of universal adhesive tested increased in eroded enamel.

Keywords: Enamel, Acidic erosion, Bond strength, Ultrasonic velocity

INTRODUCTION

Tooth wear involves destruction of the enamel, often leading to loss of tooth structure and deterioration of oral conditions over time¹. Dental erosion affects the long-term health of dentition². It is also the only type of tooth wear that can be attributed entirely to chemical phenomena not involving any microorganisms³. Moreover, dental erosion combined with the mechanical processes of abrasion and attrition is described as a phenomenon known as erosive tooth wear⁴. Dietary factors and inadequate oral hygiene have resulted in erosion becoming increasingly common among adults of different ages⁵, and physical and chemical factors acting on the tooth surface result in loss of enamel and exposure of dentin⁶. Previous studies have reported that acidic drinks when in direct contact with the tooth surface can damage the enamel, causing a roughened texture and the gradual wear of a tooth⁷. The relative importance of tooth wear has significantly increased even in populations with low prevalence of caries, thus highlighting the need to focus on appropriate preventive procedures and restorative treatments using resin composites.

Bonding restorative procedures are commonly used to restore the tooth's natural appearance and function⁸. Although current bonding systems provide adequate adherence to both enamel and dentin, some concerns have been raised when the enamel is exposed to different chemical and/or mechanical challenges such as bonding of orthodontic brackets and erosive forces^{9,10}. Therefore, the effects of intraoral abrasion or erosion on the susceptibility of enamel and dentin to bonding procedures should also be taken into consideration.

Universal adhesives have been recently introduced as a versatile system that remedies some of the shortcomings of single-step self-etch adhesives^{11,12}. Self-etch adhesives are believed to be user-friendly and less technique-sensitive, resulting in a reliable clinical performance¹³. These adhesives can be used with different types of substrates such as mineralized tooth tissue, silica-based glass ceramics, zirconia ceramics, and metal alloys¹⁴ and are expected to help simplify the bonding of various restorations to a tooth structure. Although universal adhesives provide excellent bonding to the tooth substrate^{15,16}, there is limited information available on bonding with acid eroded enamel substrates. Prior to the present time, most correlations between erosion and enamel alterations were assessed mainly by microhardness and/or wear behavior. However, scarce information is available relating the erosive process itself to altered substrates from a bonding point of view. A few information was available indicating that these processes compromised the bonding to dentin. However, no sufficient information was provided regarding enamel bonding to bonding to acidic eroded enamel substrates.

Ultrasonic imaging is used in many fields as a non-invasive technique with considerable diagnostic potential, and often serves as a valuable research tool¹⁷. Acoustic properties of enamel and dentin have been investigated using these devices, and detection of carious lesions and the thickness of dentin between the tooth surface and the pulp chamber were measured¹⁸. Assuming that the enamel substrate is mainly composed of hydroxyapatite, differences in ultrasonic velocity can reflect changes in the degree of mineralization and the histological structures¹⁹. This is mainly because ultrasonic velocity increases proportionally with the volumetric concentration of minerals²⁰. Moreover, in the case of demineralization of the tooth substrate, both the

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mineral volume concentration and the specific ultrasonic velocity appear to decrease.

The principal objective of the current study was to investigate the influence of acid erosion on the bonding performance of universal adhesives. An ultrasonic device was used to examine changes in the enamel substrate brought about by acid attack. The null hypothesis was that acid attack would not affect enamel bonding performance of the universal adhesives.

The null hypothesis was that there would be no impact of erosion on bonding to enamel using an under an *in vitro* protocol for bond strength evaluation.

MATERIALS AND METHODS

Specimen preparation

Freshly extracted bovine incisors were cleaned and stored in physiological saline for up to 2 weeks. The teeth were sliced longitudinally into 1-mm-thick sections and then cut in the bucco-lingual direction using a diamond disk in a slow-speed saw (IsoMet 1000, Buehler, Lake Bluff, IL, USA). Each slab was carefully shaped into a rectangular form (6×6×1 mm) using a super-fine diamond finishing point (ISO #021; Shofu, Kyoto, Japan). Specimen surfaces were ground using #240-, #600-, and #1200-grid wet silicon carbide (SiC) paper successively to create a flat surface. The thickness and size of the specimens was measured using a dial gage micrometer (CPM15-25DM, Mitutoyo, Tokyo, Japan).

The Er group, comprising ten specimens, was treated with 1.23% citric acid solution (pH 2.1, 6.4×10^{-2} mol/L) five times for one min every 12 h and then was placed in artificial saliva (pH 7.0, 14.4-mM NaCl, 16.1-mM KCl, 0.3-mM $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 2.0-mM K_2HPO_4 , 1.0-mM $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, and 0.10-g-% sodium carboxymethyl cellulose [CMC-Na]). These procedures were repeated throughout the 1-day test period, and the specimens were stored in artificial saliva at 37°C between treatments. These conditions were determined by our preliminary experiment. In the control group, the specimens were simply stored in artificial saliva for the same period of time (Fig. 1).

Enamel bond strength

The three universal adhesives used in this study were Adhese Universal (AU, Ivoclar Vivadent, Schaan, Liechtenstein), All Bond Universal (AB, Bisco, Schaumburg, IL, USA), and Scotchbond Universal Adhesive (SU, 3M ESPE, St. Paul, MN, USA) (Table 1). A resin composite (Clearfil AP-X; Shade A2, Lot No. N416713, Kuraray Noritake Dental, Tokyo, Japan) was used as the restorative material for bonding to enamel. A visible-light curing unit (Optilux 501, Demetron/Kerr, Danbury, CT, USA) was connected to a variable-voltage transformer in order to keep the power density above 600 mW/cm², the value was determined using a dental curing radiometer (Model 100, Demetron/Kerr).

The enamel slabs were adhered to acrylic resin (Tray Resin II, Shofu) blocks using a cyanoacrylate adhesive (Zapit, Dental Ventures of American, Anaheim, CA, USA). A piece of double-sided adhesive tape with a 4-mm-diameter hole was used to define the adhesive area of the enamel for bonding. Each adhesive was

Fig. 1 The Er group was treated with 1.23% citric acid solution (pH 2.1, 6.4×10^{-2} mol/L) five times for one min every 12 h and then was placed in artificial saliva.

These procedures were repeated throughout the 14 days test period, and the specimens were stored in artificial saliva at 37°C between treatments. In the control group, the specimens were simply stored in artificial saliva for the same period of time.

Table 1 Universal adhesives used in this study

Code	Adhesive (Lot No.)	Main components	Manufacturer
AB	All-Bond Universal (1500006939)	MDP phosphate monomer, Bis-GMA, HEMA, ethanol, water, initiators	Bisco, Schaumburg, IL USA
AU	Adhese Universal (U52628)	MDP, Bis-GMA, HEMA, MCAP, D3MA, ethanol, water, initiator stabilizers, silicon dioxide	Ivoclar Vivadent Schaan, Lichtenstein
SU	Scotchbond Universal (566724)	MDP phosphate monomer, HEMA, dimethacrylate resins, Vitrebond copolymer, filler, ethanol, water, initiators, silane	3M ESPE St. Paul, MN USA

MDP: 10-methacryloyloxydecyl dihydrogen phosphate, Bis-GMA: 2,2-bis[4-(2-hydroxy-3-methacryloyloxy-propoxy)-phenyl]propane, HEMA: 2-hydroxyethyl methacrylate, MCAP: methacrylated carboxylic acid polymer, D3MA: decandiol dimethacrylate.

Table 2 Application protocol for universal adhesives

Code	Adhesive application protocol
AB	Apply two separate coats of adhesive to the air-dried enamel surface with scrubbing for 15 s per coat, and then evaporate excess solvent by thoroughly air-drying with an air syringe for 10 s. Light irradiation was done for 10 s.
AU	Adhesive was applied to enamel surface with rubbing motion for 20 s. Medium stream of air applied over the surface for 5 s, then light irradiate was done for 10 s.
SU	Adhesive was applied to air-dried enamel surface with rubbing action for 20 s, and then medium air pressure was applied to the surface for 5 s. Light irradiation was done for 10 s.

applied to the enamel surface in accordance with the manufacturer's instructions (Table 2), and a Teflon mold (2-mm height, 4-mm diameter) was used to form and hold the restorative resin on the enamel surface. The resin composite was condensed into the mold and cured for 40 s. Specimens were prepared in a controlled room with $23\pm 5^{\circ}\text{C}$ temperature and $50\pm 5\%$ relative humidity. Completed specimens were transferred to and stored in distilled water at 37°C for 24 h.

Ten specimens per group for each group were tested in shear mode using a shear knife-edge testing apparatus. The specimens were held at a right angle to the direction of movement of the testing machine (Type 4204, Instron, Canton, MA, USA). A steel blade with a knife edge suspended from the grip of the moving arm of the testing machine engaged the specimen at the adhesive-tooth interface at a cross-head speed of 1.0 mm/min. The shear bond strength values in MPa were calculated by dividing the peak load at failure by the specimen surface area. After testing, the bonding site tooth surface and resin composite cylinders were observed under an optical microscope (SZH-131, Olympus, Tokyo, Japan) at a magnification of $10\times$ to determine failure mode. Based on the percentage of substrate area (adhesive–resin composite–enamel) observed in the de-bonded cylinder and tooth bonding site, the types of bond failure recorded were 1) adhesive failure, 2) cohesive failure in composite, 3) cohesive failure in enamel, or 4) mixed failure—partially adhesive and partially cohesive.

Ultrasonic velocity

The resource equation method was used to calculate the final sample size²¹⁾, and this was then divided into two groups of six specimens each. The ultrasonic velocity was measured using a pulser receiver (Model 5900PR, Panametrics, Waltham, MA, USA), a transducer for longitudinal waves (V112, Panametrics), and an oscilloscope (Wave Runner LT584, LeCroy, Chestnut Ridge, NY, USA). Measurements were recorded before the test and on days 0, 1, 3, 7, and 14. The equipment was initially calibrated using a standard procedure with 304 stainless steel calibration blocks (2211M, Panametrics), and the transducer was oriented perpendicular to the contact surface of each specimen to obtain the echo signal (Fig. 2). The ultrasonic waves were propagated from the transducer to the tooth, transmitted through

Fig. 2 Ultrasonic measuring system used in this study. The ultrasonic velocity was measured using a pulser receiver, a transducer for longitudinal waves, and an oscilloscope.

the tooth, and then detected by the transmitter on the opposite side. Each measurement was made at $23\pm 1^{\circ}\text{C}$ temperature and $50\pm 5\%$ relative humidity.

Statistical analysis

The Kolmogorov-Smirnov test was used to assess the distribution of the data, and the Brown-Forsythe test was used to assess the homogeneity of variance. A two-way analysis of variance (ANOVA) was used to test for differences between the groups, and Tukey's honestly significant difference (HSD) test was used to compare the influence of acid erosion on the enamel bond strengths of the universal adhesives ($\alpha=0.05$). The ultrasonic velocities were analyzed using two-way repeated measure ANOVA followed by Tukey's HSD test ($\alpha=0.05$). All statistical analyses were performed using a statistical software (Sigma Stat version 3.1; SPSS, Chicago, IL, USA).

Laser scanning microscopy (LSM)

Specimens from each group were observed under three-dimensional LSM (LSM, VK-8700, Keyence, Osaka, Japan), with the maximum wavelength of the excitation light being 658 nm. The intensity and amplification of the photomultiplier were kept constant throughout the analysis. The size of the image recorded was $81.5\times 71.5\ \mu\text{m}^2$, and the resolution was $1,024\times 768$ pixels. Images were recorded for four different sites in each specimen.

RESULTS

The results of the effect of acid erosion on the enamel bond strength of universal adhesives have been shown in Table 3. The two-way ANOVA test revealed that the factors of the adhesive system did not influence bond strength values ($p=0.826$). Differences in the mean values between the different surface treatment (control *vs.* Er) were greater than expected, despite accounting for the effect of differences in the adhesive systems ($p=0.016$). The interaction between the factors (adhesive system *vs.* surface treatment) was not statistically significant ($p=0.967$). The mean bond strength in the control group ranged from 15.6 ± 2.6 to 16.5 ± 2.6 MPa, while that in the Er group ranged from 18.1 ± 4.7 to 18.9 ± 4.7 MPa.

The frequencies of different failure modes after completion of the bond strength test have been shown in Table 4. Adhesive failure was most commonly observed in all de-bonded specimens in the control group, while cohesive failure in the enamel was the most common in the Er group, irrespective of the type of adhesive tested.

The average ultrasonic velocities of the enamel specimens have been shown in Table 5. As the differences between storage periods were greater than expected ($p<0.001$), multiple comparisons were performed on the data after taking the effects of storage conditions into

consideration. The average ultrasonic velocity in the intact bovine enamel (control group) ranged from 6,827 to 6,832 m/s and this did not vary significantly with the treatment time ($p=1.00$). The ultrasonic velocities in the Er group decreased over time and were significantly lower than those in the control group after 3 days (6,661–6,827 m/s) ($p=0.033$). However, no significant differences in the ultrasonic velocities were observed between day 1 and day 14 of the study period.

Representative LEM images of enamel specimens (Fig. 3) revealed morphological changes in the different treatment period. The LSM observations showed that demineralization of the enamel surfaces, presented as enamel-etching patterns, and changes in surface morphologies were time dependent. These morphological appearances were corresponding to the changes in ultrasonic velocities.

DISCUSSION

Though the oral condition being the ultimate testing environment for predicting the behavior of restorations due to the complexity and diversity of intra-oral conditions, *in vitro* models are important for providing insight into the fundamental mechanisms of degradation²². The pH cycling used in this study to

Table 3 Influence of acidic erosion on enamel bond strength of universal adhesives

Code	Control group	Er group
AB	16.0 (2.9) ^a	18.1 (4.7) ^b
AU	15.6 (2.6) ^a	18.3 (4.3) ^b
SU	16.5 (2.6) ^a	18.7 (4.7) ^b

Values in parenthesis indicates standard deviation.

Values with the same superscript letters indicate no significant difference.

Table 4 Percentage of each failure mode analysis of debonded specimens

Code	Control group	Er group
AB	[91.1/0/0/8.9]	[0/0/55.7/44.3]
AU	[96.7/0/0/3.3]	[0/0/43.2/56.8]
SU	[93.3/0/0/6.7]	[0/0/56.1/43.9]

Failure mode: [adhesive failure/cohesive failure in resin composite/cohesive failure in enamel/mixed failure]

Table 5 Influence of acidic erosion on average ultrasonic velocities (m/s) of bovine enamel

Group	Treatment time (days)				
	0	1	3	7	14
Control	6,832 (65) ^a	6,829 (66) ^a	6,829 (64) ^a	6,828 (65) ^a	6,827 (65) ^a
Er	6,827 (68) ^a	6,765 (66) ^{a,b}	6,694 (65) ^b	6,671 (66) ^b	6,661 (74) ^b

$n=6$ per group, values with the same superscript letter are not significantly different ($p>0.05$).

Fig. 3 Representative 3D-LSM images of enamel surfaces before adhesive application are shown.

LSM observations (original magnification; $\times 3,000$) revealed differences in morphological features between different treatment conditions. Demineralization of the enamel surfaces was more pronounced in the Er group, whereas enamel surfaces of the control group exhibited homogeneous features. In the LSM images, different colors indicated different depth shown in the vertical burrs. Surface roughness of each group was corresponded to the LSM images of the same groups.

simulate erosive lesion *in vitro* is a common approach used in previous study²³. The changes in surface characteristics induced by the artificial erosion process are in accordance with the results reported previously²⁴.

Although human teeth are thought to be most relevant for *in vitro* studies²⁵, the current study used bovine teeth in accordance with previous reports^{26,27}. Bovine teeth in good condition are easy to obtain in large quantities, have fewer composition variables, and have large flat surfaces²⁸. Moreover, it has been reported that the structural changes and mineral distribution of carious lesions in bovine teeth are similar to that observed in humans²⁹.

The enamel is a mineralized tissue with a complex hierarchical structure and is mainly composed of enamel

rods arranged perpendicular to the tooth surface. Enamel should be modeled as an anisotropic material³⁰, with both the longitudinal section and the buccal surface, so the orientation of the enamel prism may modify the ultrasonic velocity³¹. The anisotropy of enamel appears to be closely related to the prismatic orientation in the longitudinal section³². Because the enamel prisms are oriented at different angles to the direction of the ultrasonic beam based on the plane of the tooth section, enamel specimens in this study were obtained from the labial surfaces of bovine teeth to avoid any effects of the ultrasonic velocity.

Erosion is a superficial demineralization process that softens the surface and causes subsequent wear that extends to the dentin³³. The results of this study demonstrated that ultrasonic velocities of specimens

in the Er group decreased significantly with time but remained constant in the control group. This decreased sonic velocity indicated the release of minerals from the enamel, resulting in dissolution of the prism sheath and prism core. The morphological changes observed in the Er group corresponded to variations in the ultrasonic measurements. However, when comparing ultrasonic velocities between eroded specimens, no significant differences were observed with different storage times.

Upon removal of citric acid from the mouth, the remineralization process commences in which minerals lost during erosion are replaced by subsequent exposure to saliva containing calcium and phosphate ions³⁴. Scanning electron microscopy has shown that the time required for remineralization to occur following an acid challenge *in vitro* ranges between 4 and 24 h, provided there are no further challenges³⁵. Saliva is probably the most important natural defense mechanism against tooth erosion as it provides an acquired pellicle, dilution and buffering, and minerals for remineralization³⁶. A previous *in vitro* study³⁷ reported that enamel eroded by citric acid for 2 h and followed by immersion in artificial saliva exhibited partial re-hardening after 1 to 4 h and was considered completely re-mineralized after 6 to 24 h. The *in vitro* study environment, often but not always, extrapolates clinical findings, giving the worst case scenario as many of the biological factors that afford protective influences are not present. However, artificial saliva does have greater remineralization potential than human saliva as the latter contains protein inhibitors of precipitation³⁸. Blockage of the diffusion channels by proteins in the saliva may limit the remineralization ability of human saliva to a greater extent than artificial saliva. One limitation of assessment of enamel remineralization following an acid attack is that the results are thought to be qualitative and not quantitative due to the *in vitro* study design used.

The LSM images recorded were in accordance with the interpretation of the overall results. In contrast to the control group, specimens of the Er groups presented irregular surface patterns, suggesting erosion. Previous studies^{39,40} have also demonstrated that erosion by soft drinks results in rougher, more irregular enamel surfaces, and this plays an important role in the adhesion mechanism by promoting intense interlocking to the enamel. Conversely, the erosion process leads to the formation of spatial areas with damaged apatite with local structural alterations such as broken and loose atomic linkage. Although increase in the porosity of the acid attacked enamel may contribute to micromechanical interlocking of the adhesive resin, weakening of the enamel substrate may lead to cohesive failure. And thinking about the limitations of this study, erosive enamel lesions were made *in vitro* condition. So, there is little evidence available on the bonding of universal adhesives to enamel previously eroded by acid. While the enamel usually allows the formation of regular and strong adhesive bonds, changes in this substrate may affect the bond strength, failure mode, and resin tag formation. In the presence of acid erosion, this bonding

performance may be compromised further as mineral loss can contribute to weakening of the enamel substrate.

Further investigation into the factors that contribute to the durability of the restorations and their bonding ability to acid eroded enamel is necessary. Future studies should conduct bond strength tests using phosphoric acid etching for comparison with the use of the same technique. And in clinical situations, eroded enamel surface should be checked clearly to get good bonding with resin restorations.

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CONFLICT OF INTEREST

The authors 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.

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