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Microleakage and Shear Bond Strength of Composite Restorations Under Cycling Conditions

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Clinical Relevance

The performance of composite restorations can be affected by frequent acid attacks.

SUMMARY

Objectives: The aim of this study was to evaluate microleakage and shear bond strength of composite restorations under different cycling conditions.

Methods and Materials: Class V cavities were prepared in the buccal and lingual surfaces of 30 human molars (n=60). A further 60 molars were used to prepare flat enamel and dentin specimens (n=60 each). Cavities and specimens were divided into six groups and pretreated with an adhesive (self-etch/Clearfil SE Bond or etch-and-rinse/Optibond FL). Composite was inserted in the cavities or adhered to the specimens' surfaces, respectively, and submitted to cycling (control: no cycling; thermal cycling: 10,000 cycles, 5°C to 55°C; thermal/erosive cycling: thermal cycling plus storage in hydrochloric acid pH 2.1, 5 minutes, 6×/day, 8 days). Microleakage was quantified by stereomicroscopy in enamel and dentin margins after immersion in silver nitrate. Specimens were submitted to shear bond strength testing. Statistical analysis was done by two-way analysis of variance and Kruskal-Wallis tests ($p<0.05$).

Results: Microleakage in enamel margins was significantly lower in the control group compared with thermal cycling or thermal/erosive cycling. Erosive conditions increased microleakage compared with thermal cycling (significant only for Clearfil SE Bond). No significant differences were observed in dentin margins. Bond strength of enamel specimens was reduced by thermal cycling and

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thermal/erosive cycling when Clearfil SE Bond was used and only by thermal/erosive cycling when Optibond FL was used. No differences were observed among dentin specimens.

Conclusions: Thermal/erosive cycling can adversely affect microleakage and shear bond strength of composite resin bonded to enamel.

INTRODUCTION

Erosive tooth wear often requires restorative treatment due to hypersensitivity or esthetic or functional limitations when a certain degree of substance loss is reached. As restorative materials and adhesive techniques have been significantly optimized over the past decades, the restoration of even severely eroded teeth using minimally invasive procedures has become possible. While minimal loss of tooth substance can be protected from further progression by sealant application,[1-3] distinct defects can be restored by composite, resin polymer, or ceramic restorations after minimal or even no preparation.[4-8]

Some researchers have purported that adhesion to eroded dental hard tissues might be more difficult to achieve than adhesion to sound enamel and dentin. While only few studies showed that erosion of tooth surfaces does not jeopardize enamel[9] or dentin[10] bonding, most experiments have found a reduced bond strength of adhesives to eroded dental hard tissues.[11-16] However, both adhesion to erosively affected dental hard tissues and the performance of dental restorations under ongoing erosive conditions are of clinical relevance, in cases where a causal therapy of erosive tooth wear cannot be achieved before restoration placement. While several studies have investigated the acid-resistance of different restorative materials per se,[17-22] only a few studies have analyzed the effect of erosive attacks on dental restorations.[23,24] These studies reported some surface erosion of enamel adjacent to cement and composite restorations but did not analyze potential effects on the adhesive interface, for example on microleakage or bond strength of composite restorations.[23,24] Studies on secondary caries development have shown that the application of self-etching adhesives with specific functional monomers, for example 10-methacryloyloxydecyl dihydrogenphosphate (MDP), leads to the formation of a so-called acid-base resistant zone beneath the dentinal hybrid layer[25] or at the enamel-bonding interface.[26] This zone is more resistant to acid and base challenges than the underlying dental hard tissue and might prevent caries development at the tooth-restoration interface. However, the acid challenge of the interface was usually performed with buffer solutions at pH 4.5[25-27] and no information is currently available on the acid resistance of the interface when the demineralizing agent is significantly more acidic, as with erosive solutions.

Therefore, this study aimed to investigate the effect of erosive cycling on the adhesive performance of an etch-and-rinse and a self-etch adhesive by investigating microleakage and shear bond strength of composite restorations. The null hypothesis tested was that erosive challenges do not influence microleakage in enamel and dentin margins of Class V restorations and do not affect shear bond strength of self-etch and etch-and-rinse adhesive systems to enamel and dentin.

METHODS AND MATERIALS

Cavity and Specimen Preparation

Ninety sound human third molars were collected after approval of the local ethics committee (No: 1.190.857), cleaned, and stored in distilled water under refrigeration for less than 3 months.

Thirty teeth were selected for microleakage analysis, and Class V cavities (4 mm in diameter and 1.5 mm in depth) were prepared in the cervical region of the buccal and lingual surfaces using wheel-shaped diamond burs (#909 ISO040, Maxima, Gillingham, United Kingdom) in an air/water cooled high-speed handpiece. Each bur was replaced after five preparations. The cavity margins were located in enamel and dentin as the gingival cavosurface margins were placed 2 mm below the cementoenamal junction. The enamel surface was beveled 0.5 mm using a flame-shaped diamond bur (#832.014 EF, Komet, Gebr. Brasseler, Lemgo, Germany). The cavity size (4 mm $\times$ 1.5 mm) was checked with a periodontal probe. The teeth were randomly assigned into six groups (n=5 teeth, each with two cavities, one for each adhesive used).

For shear bond strength analysis, the roots of the remaining 60 teeth were removed and the crowns cut in a mesial-distal direction. The specimens were embedded in acrylic resin (Paladur, Heraeus Kulzer, Hanau, Germany), and subsequently, the buccal or lingual surfaces were flattened with water-cooled silicon carbide discs (#600 grit, Water Proof Silicon Carbide Paper, Struers, Ballerup, Denmark) until an area with diameter of at least 3 mm in enamel (n=60) or dentin (n=60) was exposed. The specimens were randomly assigned to six test groups (each n=10 enamel and n=10 dentin specimens) according to the adhesive (etch-and-rinse or self-etch adhesive)

Table 1: *Products, Manufacturers, Batch Numbers, Chemical Compositions and Application Instructions for the Materials Tested*

Material	Composition	Application Protocol
Clearfil SE Bond 2 Self-etch (Kuraray, Okayama, Japan)	*Primer (batch: 2B0133)*: 10-MDP, HEMA, hydrophilic dimethacrylate, N,N-diethanol-p-toluidine, di-camphorquinone, water.	Primer applied over tooth substrate actively for 20 s, followed by gentle air-dry for solvent evaporation. Then, application of a thin layer of Bond and light curing for 10 s.
	Bond (batch: 2901214): 10-MDP, BIS-GMA, HEMA, hydrophobic dimethacrylate, N,N-diethanol-p-toluidine, di-camphorquinone, silanated colloidal silica	
Optibond FL Etch & Rinse (Kerr, Orange, CA, USA)	*Primer (batch: 5086326)*: HEMA, PAMM, GPDM, ethanol, water, photoinitiator	Phosphoric acid etching for 30 s in enamel and 15 s in dentin, followed by rinsing with water spray for 30 s. Primer applied over tooth substrate actively for 15 s, followed by gentle air-dry for 5 s to solvent evaporation. Then, application of a thin layer of bond and light-curing for 20 s.
	Bond (batch: 5417219): TEG-DMA, UDMA, GPDM, HEMA, BIS-GMA, filler, photoinitiator	
Filtek Supreme XTE (3M ESPE, St Paul, MN, USA)	*Monomer:* UDMA, BIS-GMA TEG-DMA, BIS-EMA	Application in increments of 2 mm, each followed by light curing for 20 s.
	Filler: 58% volume/volume aggregated zirconia/silica cluster filler and nonagglomerated/nonagregated silica filler. (batch: N669171)	

Abbreviations: MDP, 10-methacryloyloxydecyl dihydrogenphosphate; HEMA, 2-hydroxyethyl methacrylate; BIS-GMA, bisphenol A glycidyl methacrylate; PAMM, phthalic acid monoethyl methacrylate; GPDM, glycerylphosphate dimethacrylate; TEG-DMA, triethylene glycol dimethacrylate. UDMA, urethane dimethacrylate. BIS-EMA, bisphenol A ethoxylate dimethacrylate.

and cycling conditions (no aging/control, thermal cycling or thermal/erosive cycling) used.

Material Application and Aging Conditions

The cavities (for microleakage analysis) or specimens (for shear bond strength analysis), respectively, were treated with an etch-and-rinse adhesive (Optibond FL, Kerr, Orange, CA, USA) or a self-etch adhesive (Clearfil SE Bond 2, Kuraray, Okayama, Japan). For the etch-and-rinse technique, 35% phosphoric acid (Ultra-Etch, Ultradent Inc, South Jordan, UT, USA) was applied onto enamel and then extended to dentin, resulting in 30 seconds of enamel etching and 15 seconds of dentin etching. The adhesives were applied according to the manufacturer's recommendations (Table 1) and light cured (800 mW/cm^2, Optima 10, B.A. International, Northampton, United Kingdom).

Class V cavities were restored with a composite (Filtek Supreme XTE, shade A2, 3M ESPE, St Paul, MN, USA; Table 1) in increments of 2 mm, each light-cured for 20 seconds. After 24 hours, the restorations were finished with a sequence of polishing disks (Sof Lex Pop-On, 3M ESPE) in decreasing roughness.

For shear bond strength analysis, composite was adhered on the enamel or dentin surfaces. After adhesive application, a Teflon split mold (3 mm in diameter, 2 mm in height) was used on the surface, the composite packed against the surface and then light-cured for 20 seconds. After light-curing, the Teflon mold was split and removed.

In the control group, microleakage and shear bond strength analyses were performed after 24 hours of water storage in distilled water. The remaining specimens were submitted to thermal cycling (10,000 cycles, 5/55° C, with a dwell time of 25 seconds and a transfer time of 5 seconds) or thermal/erosive cycling (thermal cycling plus intermittent immersion in hydrochloric acid at room temperature, pH 2.1, six times a day for 5 minutes with an interval of 90 minutes between the acid immersions). The thermal cycling regimen lasted for 8 days, so that the erosive cycling lasted a total of 4 hours.

Microleakage Analysis

The root apices of the teeth were sealed with composite resin (Filtek Supreme XTE) and then coated with two layers of nail polish leaving an area of 1 mm around the margin interface uncoated. The teeth were immersed in 25% volume/volume $AgNO_3$ for 12 hours, followed by immersion in a photo-developing solution (GBX Developer and Replenisher, Carestream Dental, Atlanta, GA, USA) for 8 hours under ultraviolet light.

The teeth were rinsed under running water for 5 minutes and cut in four parallel slices of 1-mm thickness in a buccal-lingual direction, parallel to the tooth long axis. Slices were analyzed under the stereomicroscope with 25× magnification (Carl Zeiss

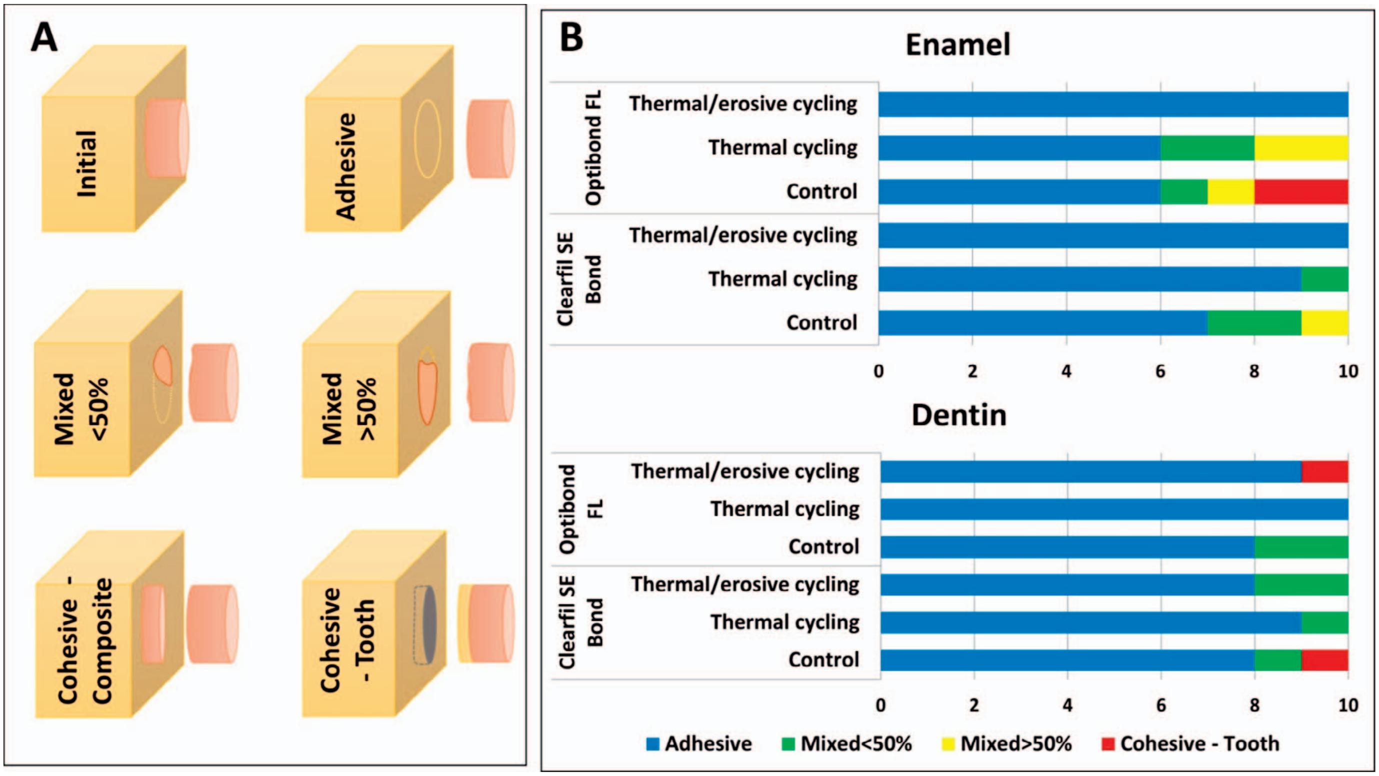

Figure 1. *Failure pattern type and distribution.(A) Schematic drawing of the failure patterns. Adhesive: failure in the adhesive interface; mixed <50%: failure in the composite, with <50% of its total area; mixed >50%: failure in the composite, with >50% of its area; cohesive-tooth: failure affecting the substrate (enamel/dentin) and cohesive-composite: failure affecting only the composite. (B) Failure distribution of enamel and dentin specimens. No cohesive failures were observed in composite.*

Inc, Berlin, Germany), and images were taken by a digital camera (Moticam 2.0, Motic, Hong Kong). The penetration of silver nitrate was measured using the software AxioVision LE 2013 (Carl Zeiss Inc). Data were expressed in percentage of penetration using the formula: $\%p = [P / L] * 100$, where *%p* is the percentage of penetration, *P* is the length of the margin where the silver penetrated and *L* is the total length of enamel/composite or dentin/composite interface.

Additionally, one specimen from each group was randomly chosen and used for scanning electron microscopy (SEM) of the surface margins. Before immersion in the $AgNO_3$ solution, impressions were made using polyvinylsiloxane (President Light Body, Coltene, Altstätten, Switzerland), followed by a preparation of the epoxy replicas (EpoFix, Struers, Ballerup, Denmark). The epoxy resin was placed under vacuum for 20 minutes and cured in a desiccator for 24 hours to avoid the formation of bubbles. Replicas were sputtered with palladium-platinum, and SEM analysis (ULTRA Plus FE-SEM, Carl Zeiss Inc, Oberkochen, Germany) was performed at 200× magnification.

Shear Bond Strength Analysis

Shear bond strength was tested with a universal testing machine (Z010, Zwick GmbH & Co, Ulm, Germany). A shear force was applied to the enamel-composite or dentin-composite interface, respectively, through a chisel-shaped loading device positioned parallel to the enamel or dentin surface at a crosshead speed of 1 mm/min. Shear bond strength (σ) was calculated using the load at failure F (N) and the adhesive area A (mm^2): σ (MPa) = F/A. The debonded area was examined with a stereomicroscope (Carl Zeiss Inc.) at 25× magnification, and the failure modes were classified into one of four categories: adhesive, if it occurred in the adhesive interface; mixed <50%, if occurred in the composite with <50% of the total area adhered to the tooth substrate; mixed >50% if occurred in the composite with >50% of the total area adhered to the tooth substrate; and as cohesive-tooth if the failure affected only the dental substrate (enamel or dentin) or cohesive-composite if the failure affected only the composite. Figure 1A shows a schematic drawing of the failure modes.

Table 2: *Microleakage (Percent of Silver Penetration, Means and Standard Deviations) of Enamel and Dentin Margins* [a]

	Enamel		Dentin	
	Clearfil SE Bond	Optibond FL	Clearfil SE Bond	Optibond FL
Control	10.3 ± 13.4^{Aa}	8.9 ± 11.0^{Aa}	56.0 ± 33.5^{Aa}	61.5 ± 34.4^{Aa}
Thermal cycling	45.6 ± 21.8^{Ab}	31.3 ± 13.3^{Ab}	65.8 ± 30.2^{Aa}	68.0 ± 24.4^{Aa}
Thermal/erosive cycling	71.5 ± 12.6^{Ac}	41.2 ± 19.5^{Bb}	88.6 ± 11.5^{Aa}	77.2 ± 20.5^{Aa}

[a] *Separately for enamel and dentin, different uppercase letters show differences between the adhesives, while lowercase letters show significant differences between the aging conditions.*

Statistical Analysis

Means and standard deviations were determined for each subgroup. Normal distribution was tested by the Kolmogorov-Smirnov test.

Microleakage data were normally distributed for enamel but not for dentin, so two-way analysis of variance (ANOVA) followed by Tukey tests were applied to the enamel data, while the Kruskal-Wallis test was used to analyze the dentin data. As shear bond strength data were normally distributed, two-way ANOVAs followed by Tukey tests were applied separately for enamel and dentin. Considering the kind of tooth substrate and the kind of adhesive used, a χ^2 test was applied to compare the failure patterns in the different aging subgroups. The level of significance was set at 5%.

RESULTS

Microleakage

Microleakage of Class V restorations is presented in Table 2. For enamel, the type of adhesive ($p<0.0001$), the cycling condition ($p<0.0001$), and the interaction between factors ($p=0.004$) were significant with respect to microleakage. Microleakage was significantly lower in the control group than in restorations that were submitted to thermal cycling or thermal/erosive cycling. Thermal/erosive cycling increased microleakage compared with thermal cycling, but this effect was significant only for Clearfil SE Bond. In dentin, thermal cycling and thermal/erosive cycling increased microleakage slightly but not significantly.

Examples of surface margins of each group are shown in Figures 2 through 4. While continuous

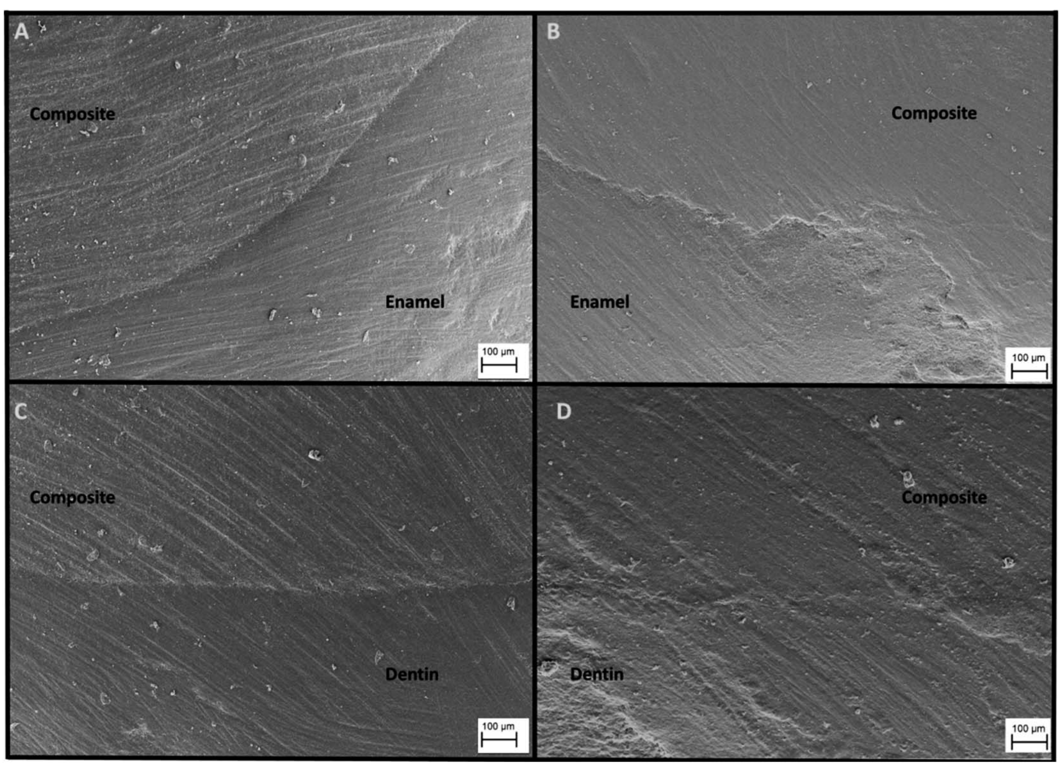

Figure 2. *SEM figures (×200) showing composite-enamel (A, B) and composite-dentin (C, D) interfaces in the control groups when Clearfil SE Bond (A, C) or Optibond FL (B, D) was used. In all restorations, continuous margins were found.*

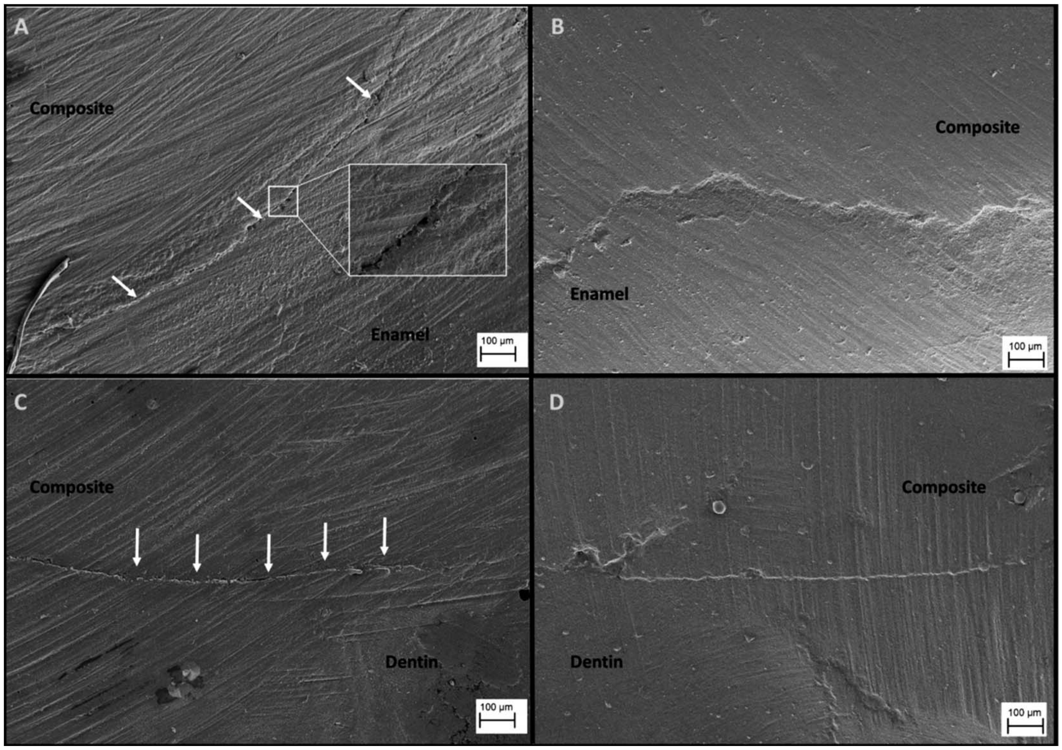

Figure 3. *SEM figures (×200) showing composite-enamel (A, B) and composite-dentin (C, D) interfaces in restorations submitted to thermal cycling when Clearfil SE Bond (A, C) or Optibond FL (B, D) was used. A slight disintegration is visible for Clearfil SE Bond (arrows) compared with the Optibond FL sample. The box in (A) presents the composite-enamel interface at 2800× magnification.*

margins were found in the control groups (Figure 2), thermal cycling resulted in a slight disintegration of the restoration performed with Clearfil SE Bond (Figure 3). Thermal/erosive cycling resulted in a distinct dissolution of enamel and dentin margins (Figure 4).

Shear Bond Strength

Shear bond strength values of differently aged enamel and dentin specimens are presented in Table 3.

For enamel, two-way ANOVA revealed significant effects of cycling treatment ($p<0.0001$) and adhesive type ($p=0.0003$) but not for the interaction between the factors ($p=0.641$). When Clearfil SE Bond was used, thermal cycling and thermal/erosive cycling reduced bond strength significantly compared with the control, but they were not significantly different from each other. When Optibond FL was used, only thermal/erosive cycling reduced shear bond strength significantly compared with the control, while thermal cycling led to a nonsignificant reduction of bond strength.

For dentin, two-way ANOVA revealed no effects of cycling conditions ($p=0.994$) or type of adhesive ($p=0.709$) on shear bond strength, while the interaction of both factors was significant ($p=0.043$).

The failure distribution is presented in Figure 1. Independently of the kind of substrate and the kind of adhesive, χ^2 tests revealed no significantly different failure patterns among the different cycling conditions ($p=0.15$).

DISCUSSION

As thermal/erosive cycling adversely affected microleakage and shear bond strength of enamel but not dentin, the null hypothesis had to be partially rejected.

In the present study, two basic techniques to determine the effect of thermal/erosive cycling on the adhesive interface were used. Microleakage and shear bond strength analyses are surrogate parameters, which are critically discussed in the literature,[28,29] but are still frequently used. As relative effects of thermal/erosive cycling on the composite-tooth interface rather than absolute bond strength or microleakage values were of interest, it seemed acceptable to apply both methods.

Optibond FL as an etch-and-rinse adhesive and Clearfil SE Bond as a self-etching adhesive have

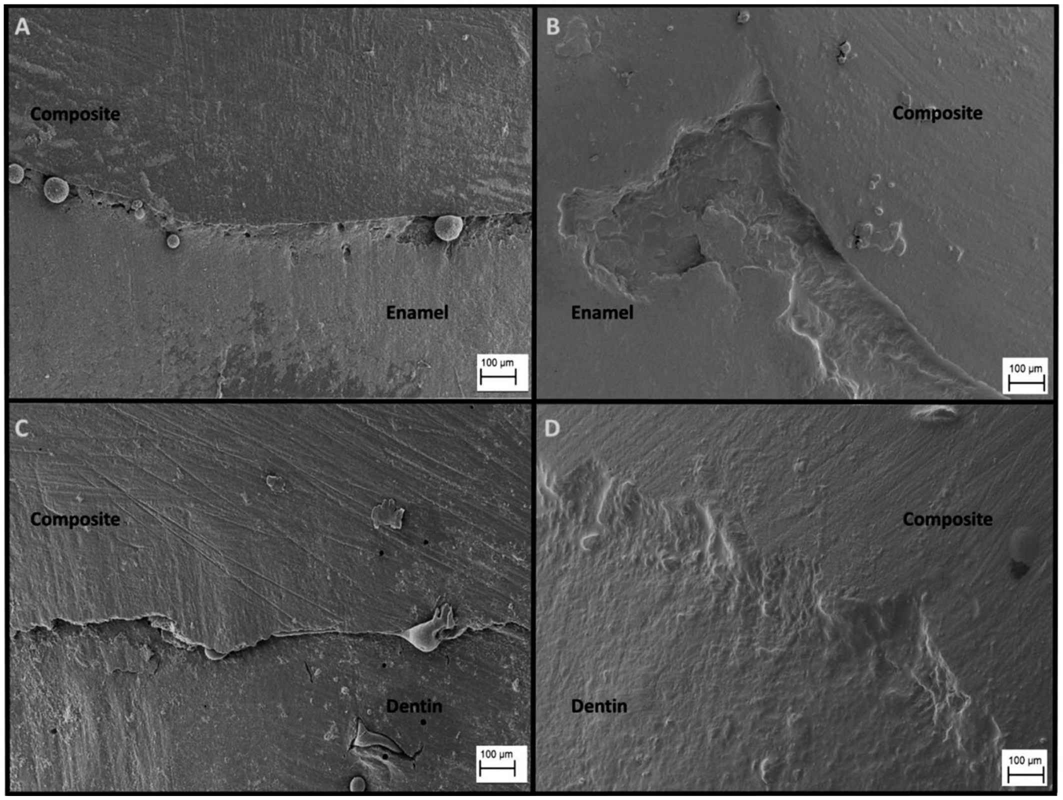

Figure 4. *SEM figures (×200) showing composite-enamel (A, B) and composite-dentin (C, D) interfaces submitted to erosive cycling when Clearfil SE Bond (A, C) or Optibond FL (B, D) was used. Erosive cycling resulted in a distinct surface dissolution of enamel or dentin, respectively, resulting in a step between composite and the tooth surface.*

shown reliable adhesive performance and are considered benchmarks in their respective classes; they are known to be quite resistant to aging.[30] Cycling of enamel specimens resulted in a significant decrease of bond strength and microleakage, while shear bond strength and microleakage of dentin specimens were not significantly changed. Previous studies on the effect of artificial aging found conflicting results. Depending on the kind of aging protocol, some studies showed a significant decrease in bond strength for Optibond FL or Clearfil SE Bond on enamel or dentin surfaces after aging,[31-34] while others did not.[35-37] Microleakage of composite restorations bonded by Optibond FL or Clearfil SE Bond mostly increased by aging.[38,39] Different *in vitro* models to promote the degradation of the adhesive interface have been described in the literature, including aging by storage in water or NaOCl, enzymatic degradation of the organic matrix, thermocycling, pH cycling, or mechanical loading.[40] In the present study, specimens were submitted to 10,000 cycles in water between 5°C and 55°C, which corresponds approximately to 1 year of *in vivo* service.[41] Thermal cycling might accelerate hydrolysis compared with aging by water storage and induce repetitive contraction-expansion stress at the tooth/restoration interface[40]. Specimens submitted to thermal/erosive cycling were intermittently stored in hydrochloric acid at pH 2.1 in addition to thermal cycling. Hydrochloric acid is commonly used to

Table 3: *Shear Bond Strength (MPa, Means and Standard Deviations) of Enamel and Dentin Specimens* [a]

	Enamel		Dentin	
	Clearfil SE Bond	**Optibond FL**	**Clearfil SE Bond**	**Optibond FL**
Control	16.2 ± 5.1 [Aa]	20.0 ± 5.3 [Aa]	14.6 ± 5.5 [Aa]	12.8 ± 5.6 [Aa]
Thermal cycling	8.7 ± 3.5 [Ab]	14.9 ± 4.8 [Bab]	11.0 ± 3.8 [Aa]	16.3 ± 5.4 [Aa]
Thermal/erosive cycling	6.8 ± 2.4 [Ab]	10.6 ± 5.4 [Ab]	14.8 ± 5.2 [Aa]	12.8 ± 5.1 [Aa]

[a] *Separately for enamel and dentin, different uppercase letters show differences between the adhesives, while lowercase letters show significant differences between the aging conditions.*

simulate intrinsic erosion.[42,43] However, the erosive cycling can be classified as relatively mild when considering that the intraoral pH after an acidic attack is reduced for up to several minutes[44] and the total erosion time in the present study lasted for only 4 hours.

As depicted by the SEM pictures, a distinct surface erosion of enamel and dentin developed at the marginal interface. The adverse effect of hydrochloric acid on the marginal interface might increase the flow of fluids through the adhesive interface accounting for microleakage development and decrease of bond strength. However, microleakage and shear bond strength were significantly affected only in enamel but not in dentin.

Bonding to enamel surfaces is mainly achieved by a micromechanical interlocking of resin into microporosities of the acid-etched surface. In contrast, the dentinal hybrid layer is composed of organic matrix, residual hydroxyapatite crystallites, and resin monomers. As erosion primarily affects the inorganic part of the dental hard tissue, the effects on enamel bonding might be more deleterious than on dentin. Nevertheless, it has taken into account that the degradation of the organic matrix was not addressed by the aging protocol of the present study. Considering that intrinsic erosion is caused by gastric juices, not only hydrochloric acid but also proteolytic enzymes of the digestive system (eg, pepsin) come into contact with teeth. Pepsin is capable of degrading the organic matrix of dentin, resulting in a progression of erosive lesions.[45]

In this experiment, only slight differences were found between the adhesive performance of the etch-and-rinse and the self-etching adhesive. Etch-and-rinse adhesives usually lead to higher bond strength values and lower microleakage on enamel than self-etching adhesives, as phosphoric acid etching increases the porosity of enamel compared with the mild etching pattern of the Clearfil SE Bond primer, resulting in an increased micromechanical retention. Thus, Optibond FL revealed higher bond strength values and less adhesive failures after thermal cycling compared with Clearfil SE Bond. On the other hand, the mildly acidic primer of Clearfil SE Bond can easily decalcify the less mineralized dentin. At the same time, MDP can chemically interact with hydroxyapatite resulting in improved dentin bonding performance compared with etch-and-rinse adhesives. In contrast to etch-and-rinse adhesives, self-etching adhesives with specific functional monomers form an acid-base resistant zone, a structural layer on the tooth-bonding interface, which might be responsible for degradation resistance at the interface. Nevertheless, no significant differences between the performance of the etch-and-rinse and the self-etching adhesive under thermal/erosive cycling conditions were seen in the present study. Further studies should analyze possible structural changes of the hybrid layer under highly acidic conditions.

Conclusion

Erosive conditions might adversely affect microleakage development and bond strength of etch-and-rinse and self-etching adhesives on enamel but not on dentin.

Acknowledgements

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Regulatory Statement

This study was conducted in accordance with all the provisions of the local human subjects oversight committee guidelines and policies of the ICT-UNESP. The approval code for this study is 1.190.857.

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.

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