

# Bonding stability of adhesive systems to eroded dentin

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**Abstract:** This *in vitro* study evaluated the immediate and 6 months microshear bond strength ( $\mu$ SBS) of different adhesive systems to sound and eroded dentin. Sixty bovine incisors were embedded in acrylic resin and ground to obtain flat buccal dentin surfaces. Specimens were randomly allocated into two groups: sound dentin (immersion in artificial saliva) and eroded dentin (erosive challenge following a pH cycling model comprising 4  $\times$ /day Sprite Light<sup>®</sup> drink for 10 days). Then, specimens were reassigned according to the adhesive system: etch-and-rinse adhesive (Adper Single Bond), two-step self-etch system (Clearfil SE Bond), or one-step self-etch adhesive (Adper Easy One). Polyethylene tubes with an internal diameter of 0.76 mm were placed over pre-treated dentin and filled with resin composite (Z250). Half of the specimens were evaluated by the  $\mu$ SBS test after 24 h, and the other half 6 months later, after water storage at 37°C. Failure mode was evaluated using a stereomicroscope (400 $\times$ ). Data were analyzed by three-way repeated measures analysis of variance and Tukey's *post hoc* tests ( $\alpha = 0.05$ ). After 6 months of water aging, marked reductions in  $\mu$ SBS values were observed, irrespective of the substrate. The  $\mu$ SBS values for eroded dentin were lower than those obtained for sound dentin. No difference in bonding effectiveness was observed among adhesive systems. For all groups, adhesive/mixed failure was observed. In conclusion, eroded dentin compromises the bonding quality of adhesive systems over time.

**Keywords:** Tooth Erosion; Dentin-Bonding Agents; Shear Strength.

## Introduction

An increase in the prevalence of tooth wear in all ages and different populations<sup>1,2</sup> relates to several factors such as higher consumption of acidic foods and gastro esophageal disorders.<sup>3,4</sup>

This has attracted worldwide research interest and studies have shown that the severity of dental erosion is majorly restricted to the enamel.<sup>2,5</sup> However, without controlling etiological factors, the erosive wear can result in dentin exposure over time.<sup>6</sup>

In such cases, restorative treatment is necessary to reduce thermal sensitivity; prevent pulpal involvement; re-establish tooth contour, function, and esthetics; and increase tooth resistance.<sup>7</sup> Currently, adhesive systems associated with resin composite are considered as the most appropriate restorative option because these are more resistant to acidic

conditions than resin-modified and conventional glass ionomer cements.<sup>8</sup>

De and remineralization erosive processes lead to greater wear<sup>9</sup> and softening of the outermost dentin layer<sup>10</sup> compared with sound dentin. In addition, erosion can remove dentinal plugs and organic intertubular dentin, in turn increasing tubule diameter and collagen exposure.<sup>11</sup> Therefore, adhesive materials must adhere to different surfaces than those for which they were originally created and tested, which generates uncertainty regarding their longevity in this structurally altered substrate. Thus, studies on adhesion to eroded dentin are imperative.

The bonding of adhesive systems to eroded dentin has been poorly investigated,<sup>12,13</sup> particularly regarding its long-term effectiveness.<sup>14</sup> Moreover, majority of the results vary. Whereas the bond strength of adhesive systems to eroded dentin has been shown to be lower compared with that of sound dentin,<sup>14</sup> it has been also demonstrated that eroded dentin does not jeopardize the immediate bond strength of the adhesive restorative materials.<sup>13</sup>

Thus, the aim of this *in vitro* study was to evaluate the bond strength stability of adhesive systems to sound and eroded dentin.

## Methodology

### Tooth Selection and Preparation

Sixty bovine incisors stored at 4°C were used in this study. The roots were removed using a low-speed diamond disc in a cutting machine (Labcut 1010, Extec Co., Enfield, USA) and crowns were embedded in self-curing acrylic resin inside polyvinyl chloride rings (JET Clássico®, São Paulo, Brazil). The exposed buccal surfaces were ground under water using a 320-grit SiC paper to obtain flat dentin surfaces and further polished using 600-grit SiC paper for 60 s to create a standardized smear layer.

### Erosive Challenge

Specimens were randomly allocated into two groups: (1) immersion in artificial saliva during the experimental period (control group/sound dentin,  $n = 30$ ) and (2) exposure to erosion challenge according to a pH-cycling model<sup>15</sup> (eroded dentin,  $n = 30$ ).

For 10 days, the erosive cycles were conducted by immersing teeth in Sprite Light® (30 mL/sample; Coca-Cola Co., Spal, Porto Real, Brazil) for 1 min, followed by immersion in artificial saliva (30 mL/sample) for 59 minutes. This procedure was consecutively repeated four times per day at room temperature.<sup>15</sup> Each day, during the remaining 20 h, the teeth were maintained in artificial saliva. The Sprite Light® (pH 2.87) had a buffering capacity of  $0.375 \pm 0.01$ , which was equivalent to 0.375 mL of 0.2 M NaOH/3 mL of the beverage, for an increase of 1 pH unit. The composition of the artificial saliva was 1.5 mM Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O, 0.9 mM NaH<sub>2</sub>PO<sub>4</sub>·2H<sub>2</sub>O, 150 mM KCl, 0.1 M Tris buffer, 0.03 ppm fluoride, and pH 7.0.<sup>16</sup>

### Restorative Procedures

Teeth from each dentin substrate (sound or eroded) were randomly reassigned into three subgroups ( $n = 10$ ), according to the adhesive system used: Adper Single Bond 2 (SB; 3M ESPE, Saint Paul, USA), Clearfil SE Bond (CSEB; Kuraray Medical Inc., Tokyo, Japan), and Adper Easy One (EASY; 3M ESPE, Saint Paul, USA). Table 1 displays the main components and application mode of these adhesive systems.

After adhesive systems' application, according to manufacturers' instructions, polyethylene tubes (Micro-bore® Tygon S-54-HL Medical Tubing, Saint-Gobain Performance Plastics, Akron, USA) with an internal diameter of 0.76 mm and height of 1.0 mm were positioned over the dentin surfaces. Then, the adhesives were light cured using a halogen light-curing unit (Jetlite 4000 Plus, J. Morita USA; Irvine, USA) with 600 mW/cm<sup>2</sup> power density. The device's own radiometer quantified the output power. The tubes were filled with resin composite (Filtek Z250, 3M ESPE, Saint Paul, USA) and light cured. For each tooth, four specimens were built up. A trained operator conducted all bonding and restorative procedures at room temperature.

### Microshear Bond Strength (μSBS) Test

After storage in distilled water at 37°C for 24 h, the polyethylene tubes were removed using a surgical blade, which resulted in cylindrical specimens with a cross-sectional area of approximately 0.45 mm<sup>2</sup>.

**Table 1.** Materials, manufacturers, main components, pH, and application mode.

Adhesive system (Manufacturer)	Main components	pH*	Application mode
<b>SB</b> Adper Single Bond 2 (3M ESPE, St. Paul, USA)	Etchant: 35% phosphoric acid HEMA, water, ethanol, bis-GMA, dimethacrylates, amines, metacrylate functional copolymer of polyacrylic and polyitaconic acids, 10% by weight of 5 nm-diameter spherical silica particles.	0.6 4.7	Etch for 15 s. Rinse with water spray for 15 s, leaving tooth moist. Active application of two consecutive coats of the adhesive with a fully saturated brush tip. Dry gently for 2-5 s. Light cure for 10 s.
<b>CSEB</b> Clearfil SE Bond (Kuraray Medical Inc., Tokyo, Japan)	<i>Primer:</i> MDP, HEMA, hydrophilic dimethacrylate, dl-campherquinone, N,N-diethanol-p-toluidine, water <i>Bonding:</i> MDP, Bis-GMA, HEMA, hydrophobic dimethacrylate, dl-campherquinone, N,N-diethanol-p-toluidine, silanated colloidal silica	2.0	Apply <i>primer</i> on dry surface for 20 s. Slight air drying. Apply <i>bonding</i> . Light cure for 10 s.
<b>EASY</b> Adper Easy One (3M ESPE, St. Paul, USA)	HEMA, water, ethanol, Bis-GMA, dimethacrylates, amines, metacrylate functional copolymer of polyacrylic and polyitaconic acids, 10% by weight of 5 nm-diameter spherical silica particles, phosphate ester of methacrylate.	2.7	Apply one coat of adhesive with a fully saturated brush tip for 20 s. Dry gently for 5 s. Light cure for 10 s.

Abbreviations: MDP: 10-methacryloyloxydecyl-dihydrogen-phosphate; Bis-GMA: bisphenyl-glycidyl methacrylate; HEMA: 2-hydroxyethyl methacrylate). \*pH values were provided by the manufacturers.

Specimens were examined under a stereomicroscope at 20× magnification (Discovery V20, Zeiss, Berlin, Germany), and those with interfacial gaps, bubble inclusion, or other defects were excluded and replaced. Only two specimens were substituted.

Half of the specimens from of each tooth were immediately evaluated by  $\mu$ SBS. The specimens were individually attached to a universal testing machine (Kratos Industrial Equipment, Cotia, Brazil). A thin steel wire (0.20 mm diameter) was looped flush between the load cell projection and resin composite cylinder making contact with the lower half-circumference of the cylinder and touching the dentin surface. A shear load was applied at a crosshead speed of 1.0 mm/min, until failure occurred. The cylinder was maintained in line with the center of the load cell, and the wire loop was parallel to the load cell movement direction and to the bonding interface. The fracture load was recorded and the bond strength was expressed in MPa.

The other half underwent  $\mu$ SBS only after 6 months of storage in distilled water containing 0.4% sodium azide at 37°C. The storage solution was not changed and its pH was monitored each month.

### Failure mode

All debonded specimens were observed in a stereomicroscope (HMV II, Shimadzu, Kyoto, Japan) at 400× magnification to determine failure

mode: adhesive/mixed (failure at the resin–dentin interface or mixed with cohesive failure of the neighboring substrate) or cohesive (failure exclusively within the dentin or resin composite). The specimens that debonded during preparation or water storage period (pretesting or premature failures) were also recorded.

### Statistical analysis

The experimental unit in this study was the hemi-tooth because half of the sample was tested immediately, and the other half after, 6 months. Thus, the mean of  $\mu$ SBS (MPa) values of two specimens of a hemi-tooth was averaged for statistical purposes. The  $\mu$ SBS mean for every testing group was expressed as the average of the 10 hemi-teeth used per group.

Normal distribution of bond strength data and equality of variances were assumed after Kolmogorov-Smirnov and Cochran tests, respectively. The  $\mu$ SBS means were analyzed using three-way repeated measures analysis of variance (substrate vs. adhesive system vs. storage period) and Tukey's post hoc test ( $\alpha = 0.05$ ). The repeated measure was the storage period. Statistical analysis was performed using the GMC software, version 7.7 (FORP USP, Ribeirão Preto, Brazil). Failure mode was only evaluated descriptively.

### Results

$\mu$ SBS (MPa) and standard deviations for all experimental groups are summarized in Table 2.

The factors substrate ( $p = 0.01$ ) and storage period ( $p < 0.01$ ) were statistically significant.

The  $\mu$ SBS values of eroded dentin were lower than those obtained for sound dentin. The bond strength decreased after 6 months of water storage, independent of the substrate. No difference in bonding effectiveness was observed among the adhesive systems.

Figure summarizes the distribution of failure. A predominance of adhesive/mixed failures was observed, whereas no cohesive failures in dentin were verified. Pretesting failures were only observed after 6 months of water aging.

## Discussion

Because of limited scientific evidence on bonding to eroded dentin and of the chemical and microstructural changes in dental substrates arising of erosive challenge, the long-term performance of restorative materials in this type of substrate remains uncertain. Therefore, this investigation evaluated the immediate and 6 months bond strength of different adhesive systems to sound and eroded dentin.

In this study, bovine teeth were used as an appropriate substitute for human dentin because of their relatively close resemblance to their human counterparts.<sup>17</sup> In addition, bovine dentin offers acid resistance similar to that of human dentin.<sup>18</sup>

To simulate dental erosion, the pH-cycling model using a citric acid drink was employed. This beverage has a high erosive potential due to its low pH and low fluoride/calcium concentrations.<sup>15</sup> Although in vitro protocols are fail to reproduce the exact in vivo conditions in clinical settings, the model using cyclic conditions with very short exposure to acids and their removal by saliva appropriately reflects the erosive challenge observed in the oral cavity.<sup>19</sup> Moreover, this model simulates regular

intake of acidic drinks that are considered as risks for dental erosion.<sup>19</sup>

The  $\mu$ SBS values obtained with eroded dentin were lower than those obtained for sound dentin, as also previously reported.<sup>12,14</sup> The erosion process can result in mineral loss, fibril collagen exposure, and opening of dentinal tubules.<sup>14</sup> The higher degree of demineralization in eroded dentin results in deeper demineralized layer, which after adhesive penetration, allows formation of hybrid layers thicker than those in sound dentin. These layers are structurally imperfect and contain porosities,<sup>20</sup> which in turn cause areas of hydrophilic predominance and demineralized zones devoid of resin reinforcement; this may contribute to lower  $\mu$ SBS values for eroded dentin because resin monomers may not penetrate as deeply as acid.<sup>21</sup> Furthermore, the greater collagen exposure creates sites that are more prone to undergo degradation over time.<sup>22</sup> In severe erosive cases, dentin exposure may lead to a sclerotic dentin substrate with a hypermineralized shiny surface layer accomplished by tubular occlusion.<sup>23</sup>

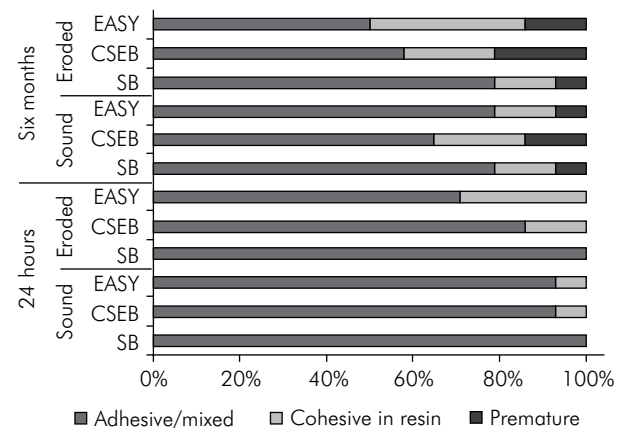


Figure. Failure mode distribution (%).

Table 2.  $\mu$ SBS (MPa) means and standard deviations for all experimental groups (\*).

Storage period	24 h		6 months	
	Sound dentin	Eroded dentin	Sound dentin	Eroded dentin
Adper Single Bond 2	14.11 ± 2.73 <sup>A,a</sup>	11.80 ± 3.55 <sup>A,b</sup>	8.05 ± 1.84 <sup>B,a</sup>	6.49 ± 1.77 <sup>B,b</sup>
Clearfil SE Bond	12.23 ± 4.81 <sup>A,a</sup>	10.89 ± 4.03 <sup>A,b</sup>	9.83 ± 2.94 <sup>B,a</sup>	6.45 ± 2.57 <sup>B,b</sup>
Adper Easy One	11.75 ± 4.23 <sup>A,a</sup>	9.17 ± 2.59 <sup>A,b</sup>	7.79 ± 3.45 <sup>B,a</sup>	4.83 ± 1.85 <sup>B,b</sup>

(\*) Different superscript lower letters indicate a statistically significant difference between substrates. ( $p < 0.05$ ). Different superscript capital letters indicate a statistically significant difference between storage periods ( $p < 0.05$ ).

The *in vitro* model used in this study promotes a substrate with a superficial demineralized layer, simulating initial erosion. The worse performance of adhesive systems in artificially-created eroded dentin compared with sound dentin supports the fact that the protocol is suitable for testing bond strength of eroded dentin. However, it should not replace natural eroded dentin when the use of this substrate is possible. The limitation of this type of simulation in extracted teeth is that the reaction of the dentin-pulp complex to the demineralization process, which is represented by sclerotic dentin, is not present, thus differing from the clinical situation.

Degradation of resin-dentin bonds was observed in this study, represented by a decrease in  $\mu$ SBS values after 6 months of water storage, irrespective of the substrate. A previous study<sup>14</sup> demonstrated that 1 year of storage lowered the bond strength values, although this aging effect was more pronounced for eroded surfaces compared with that for sound dentin. Methodological differences in the storage time and bond strength test (microshear vs. microtensile) may explain these controversial findings.

Metalloproteinases (MMPs) represent a family of zinc- and calcium-dependent endopeptidases present in dentin and saliva and are capable of degrading extracellular matrix components, including collagen, in native and denatured forms.<sup>24</sup> These enzymes are exposed and activated when dentin is solubilized. Dentin erosion is a complex process characterized by mineral dissolution, which exposes the organic matrix to breakdown by bacteria and host-derived enzymes, such as MMPs.<sup>25</sup> Enzymatic removal of the organic matrix by MMPs increases demineralization because the demineralized organic matrix hampers ionic diffusion after an acidic challenge,<sup>25</sup> and subsequently, accelerates the bond degradation to eroded dentin.

The bonding effectiveness to both substrates did not depend on the adhesives tested. Zimmerli *et al.*<sup>14</sup> demonstrated that etch-and-rinse adhesive system showed a superior bonding performance in sound dentin, whereas the self-etch adhesive system (CSEB) showed better results in eroded dentin. CSEB has an acid primer, which promotes simultaneous demineralization and monomer infiltration, preventing denuding of collagen layer, as verified

using phosphoric acid.<sup>26</sup> Moreover, the protective effect of both resin-coated collagen and calcium salts of 10-methacryloxydecyl dihydrogen phosphate<sup>27</sup> may explain the superior bonding produced by CSEB in eroded dentin. Nevertheless, this study did not verify this difference.

The SB and EASY adhesives have similar compositions, except the presence acidic monomer in the one-step self-etching system. We speculated that due to the mild-aggressiveness of the self-etching primer (pH = 2.7), EASY could show better performance in eroded dentin, because of a decrease in the depth of the demineralized layer, which reduces the difference between the depth to which the substrate is demineralized to which monomer can infiltrate. However, this was not observed in this study and further studies are required to test this hypothesis. In another study, a similar performance of self-etch adhesive systems in sound dentin was observed.<sup>28</sup>

We did not perform adhesive area delimitation, although this could have resulted in less questionable outcomes.<sup>29</sup> Shimaoka *et al.*<sup>29</sup> reported significantly higher bond strength values when the bonding area was not confined with perforated tape.<sup>29</sup> Because the adhesive was applied on the dental substrate, it was highly difficult to guarantee that the area being tested was restricted to the circumference of the composite cylinder. However, our immediate results were similar to those of a previous study<sup>29</sup> with delimitation of adhesive area. Moreover, Andrade *et al.*<sup>30</sup> showed that different bonded surface areas did not apparently affect  $\mu$ SBS when the adhesive area was not delimited. Further studies are needed to test this methodological aspect.

It has recently been shown<sup>13</sup> that adhesive systems are preferable to glass ionomer cements for restoring eroded tooth dentin. However, because bond deterioration occurred for all adhesive systems after 6 months of water aging, further studies evaluating factors that increase the longevity of resin-dentin bonds created on eroded substrate such as the use of MMPs' inhibitors, should be conducted.

## Conclusion

Eroded dentin compromises the quality of bonding over time, irrespective of adhesive type.

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