

# Effect of Sodium Ascorbate on Resin Bonding to Sodium Perborate-bleached Dentin

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## Clinical Relevance

Resin dentin bonding can be performed immediately after sodium perborate bleaching without the need for sodium ascorbate pretreatment; however, the two-step self-etching adhesive performed better than the all-in-one system. The all-in-one adhesive, Xeno IV, exhibited significantly lower microshear bond strength when bonded to positions associated with larger-diameter dentinal tubules.

## SUMMARY

This was an *in vitro* study to evaluate the effect of sodium ascorbate on the microshear bond strength (MSBS) of resin composite to sodium perborate-bleached dentin. Molar dentin sections were divided into six groups: 1) control, 2) sodium perborate (SP) bleach and immediate bonding, 3) SP and 30 second sodium ascorbate (SA); 4) SP and 1 minute SA; 5) SP and 2 minute SA; and 6) SP and 7 day delay

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before bonding. They were further divided into two-step self-etching (Clearfil SE Bond) or all-in-one self-etching (Xeno IV) adhesive systems. Resin composite microtubes were bonded according to dentin location—center, pulp horn, and peripheral positions—and an MSBS test was carried out. Failure mode was determined using light microscopy and scanning electron microscopy. There were no significant differences between the treatment types/groups. MSBSs were significantly higher for two-step self-etching adhesive compared with all-in-one self-etching adhesive ( $p=0.028$ ). For the all-in-one adhesive, MSBSs at the center and pulp horn positions were significantly lower than the peripheral positions ( $p<0.001$ ). All-in-one groups had significantly more adhesive failures than two-step adhesive groups ( $p=0.015$ ). The odds of adhesive failure were higher at the pulp horn position than the peripheral position ( $p=0.004$ ). Sodium perborate bleaching of dentin had no effect on MSBS or mode of failure for either two-step or all-in-one self-etching adhesives; therefore, the effect of sodium ascorbate was negligible. The two-step

**adhesive groups demonstrated the highest MSBS, and the all-in-one groups, when bonded to center and pulp horn dentin, exhibited the lowest MSBS.**

## INTRODUCTION

In the current era of minimally invasive dentistry, bleaching is readily considered when treatment planning for discolored teeth. Tooth discoloration can occur intrinsically and extrinsically, and the origin and nature of the stain will determine whether external and/or internal bleaching will be most effective.

Bleaching agents composed of hydrogen peroxide, in particular sodium perborate (SP), have been reported to be very effective when bleaching internally in endodontically treated teeth. The success rate ranges from 93% to 100% with 1- to 6-year follow-ups,<sup>1-4</sup> although other studies have reported lower rates of 50% to 79% over 3 to 8 years.<sup>5,6</sup>

Despite the obvious benefits of the ability of hydrogen peroxide-containing agents to eliminate stains by cleaving double bonds of pigmented molecules, negative effects on dentin at the morphological,<sup>7</sup> chemical,<sup>8</sup> and biomechanical<sup>9</sup> levels have been reported. The pH of the bleach formulation may play a critical role in these effects.<sup>10</sup> Reductions in the resin bond strength to dentin have been reported when dentin was exposed to SP<sup>11,12</sup> as well as to a variety of concentrations of hydrogen peroxide<sup>13,14</sup> and carbamide peroxide.<sup>14,15</sup>

Recent developments of resin bonding agents have led to a move toward self-etch adhesion and away from the classic etch-and-rinse approach. Self-etch adhesives have several advantages: the rinsing phase is eliminated and clinical time and risk of errors during application are reduced. Despite the apparent advancement in resin adhesives, self-etch resin bond strengths have been reported to be compromised by prebleaching of the dentin substrate.<sup>11</sup>

The probable mechanism for the reduction in bond quality in bleach-treated dentin is the residual bleach within the collagen matrices and dentinal tubules after bleaching that eventually break down to oxygen and water.<sup>16</sup> The liberation of oxygen could either interfere with resin infiltration into enamel and dentin<sup>17</sup> or directly inhibit the free-radical polymerization of resins,<sup>18</sup> which would lead to a defective resin-dentin interface that cannot sufficiently withstand debonding forces, thus reducing the strength.

The most common method of reversing the compromised resin bond to bleached dentin has been to delay bonding long enough to allow the residual peroxide to leach away. The time required to achieve a return to baseline bond strengths has been reported to be approximately 1week when exposed to SP with or without hydrogen peroxide<sup>11,12</sup> however, others have reported that a delay of 1week was not long enough when the dentin was exposed to hydrogen peroxide and carbamide peroxide as the bleaching agent<sup>14,19</sup> before bonding.

Of promise is pretreating bleached dentin with sodium ascorbate (SA), a neutral biocompatible antioxidant, based on its ability to neutralize and reverse the oxidizing effects of hydrogen peroxide<sup>20,21</sup> by altering the redox potential of the bleached tooth, thereby preventing the premature termination of the free-radical polymerization of the adhesive.<sup>16</sup> The ability of SA to reverse the effects of 5% sodium hypochlorite and 10% hydrogen peroxide on resin-dentin bonding was initially found to have positive results because it restored the microtensile bond strengths when SA was applied for the equivalent duration of peroxide exposure (1-minute or 10-minute applications).<sup>16</sup> Further evidence of its action has been reported with 10%-22% carbamide peroxide-bleached enamel<sup>22,23</sup> and 35% hydrogen peroxide-bleached human dentin.<sup>24</sup> Both solution and hydrogel forms of SA significantly increased the shear bond strength when dentin was exposed to 10% carbamide peroxide; no differences were associated with two forms.<sup>25,26</sup>

However, the limitation of the action of SA noted in the literature has been the time required for complete recovery to nonbleached bond strength levels, as the durations of application required have been reported to range from 10 minutes<sup>23</sup> to 3 hours.<sup>22,27</sup> Previous studies of the action of SA have tested the microtensile bond strength of resin composite bond to bleached dentin or enamel. As yet, no studies have evaluated the effect of SA on microshear bond strength (MSBS) of resin composite to SP-bleached dentin.

This study aimed to investigate the effect of SA on MSBS and mode of failure of a resin composite bonded to SP-bleached dentin, while considering the resin adhesive type and dentin location as possible variables. The null hypothesis tested was that SA has no effect on MSBS or mode of failure of the resin composite bond to SP-bleached dentin with no difference between the types of resin adhesive or dentin location.

## MATERIALS AND METHODS

### Overview

Ethics approval was obtained for the collection of teeth from The University of Melbourne Human Research Ethics Committee (ID 1033324). Forty-eight extracted non-carious human molars were collected. Immediately after extraction, the teeth were stored in 1% chloramine-T solution (pH 9.1) (Dentalife, Melbourne, Australia) for at least 2 weeks, transferred to phosphate buffered saline solution (Dentalife) and stored at 4°C for no longer than 1 month after extraction.

### Tooth Sample Preparations

Horizontal mid-coronal sections were obtained at the level coronal to the pulp chamber using a diamond saw at slow speed under water cooling to produce dentin disc sections approximately 2 mm thick. Each tooth section was mounted in dental stone in a 30-mm diameter polyvinylchloride cylinder and polished using 600-grit silicon carbide paper at 150 rpm under running water (Tegrapol-25, Struers, Ballerup, Denmark) to ensure that a standardized, flat smear-covered surface of dentin was produced. The teeth preparations were subsequently stored in an incubator (Thermoline Scientific, New South Wales, Australia) at 37°C and 95% relative humidity throughout the experiment.

### Resin Composite Bonding

The specimens were divided into six groups of eight teeth as follows:

*Group 1:* No prebonding treatment (control) was applied.

*Group 2:* SP bleaching agent (2 g SP and 1 mL of water) was applied on the specimen. It was stored for 7 days and rinsed off with air-water spray for 10 seconds.

*Group 3:* SP bleaching agent was applied on the dentin surface of the specimen. It was stored for 7 days and rinsed off with air-water spray for 10 seconds. The specimen was exposed to 10% SA (Dentalife) solution for 30 seconds and rinsed off with air-water spray for 10 seconds.

*Group 4:* SP bleaching agent was applied on the specimen. It was stored for 7 days and rinsed off with air-water spray for 10 seconds. The specimen was exposed to 10% SA solution for 1 minute and rinsed with air-water spray for 10 seconds.

*Group 5:* SP bleaching agent was applied on the specimen. It was stored for 7 days and rinsed off with

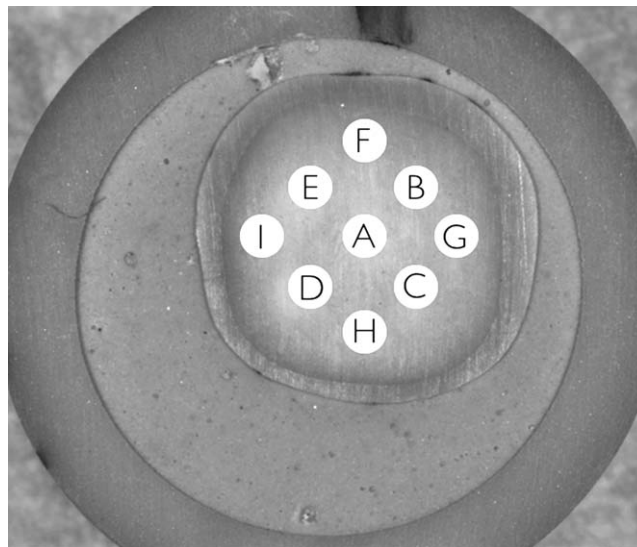


Figure 1. Predetermined microtube positions: Center position (A); pulp horn positions (B, C, D, and E); peripheral positions (F, G, H, and I).

air-water spray for 10 seconds. The specimen was exposed to 10% SA solution for 2 minutes and rinsed with air-water spray for 10 seconds.

*Group 6:* SP bleaching agent was applied on the specimen. It was stored for 7 days and rinsed off with air-water spray for 10 seconds. The specimen was stored in the incubator at 37°C for 7 days without further treatment.

Following the procedures described earlier, the specimens from each group were divided equally into two subgroups: the two-step self-etching adhesive, Clearfil SE Bond (Kuraray-Noritake, Tokyo, Japan), which was designated SE, and the all-in-one adhesive, Xeno IV (Dentsply Caulk, Milford, CT, USA), which was designated X. All specimens were subjected to bonding procedures on the final day of the surface treatments.

The specimens in the SE group were dried but not desiccated, and SE was applied according to the manufacturer's instructions to the dentin surface in a predetermined configuration (Figure 1). Seven to ten translucent polyvinylchloride microtubes (Microtube extensions, New South Wales, Australia), 0.75 mm internal diameter by 1.5 mm high, were placed perpendicular to the adhesive-covered specimens, and the adhesive was cured using a light-emitting diode light unit of 800 mW/cm<sup>2</sup> irradiance (BluePhase C8, Ivoclar Vivadent AG, Schaan, Liechtenstein) for 10 seconds. The microtubes were filled with a nano-filled hybrid resin composite,

Table 1: Compositions of the Adhesives Used in the Study

Adhesive	Code	Contents	Manufacturer	Batch No.
Clearfil SE bond	SE	Primer: 10-MDP; HEMA; hydrophilic DMA; dl-camphorquinone; N,N-diethanol-p-toluidine; water	Kuraray Medical, Tokyo, Japan	51800
		Bond: 10-MDP; Bis-GMA; HEMA; hydrophobic DMA; dl-camphorquinone; N,N-diethanol-p-toluidine; silanated colloidal silica		
Xeno IV	X	UDMA, PENTA, acetone, polymerizable trimethacrylate resin, 2 polymerizable DMA resins, photoinitiator	Dentsply Caulk, Milford, CT	100608

*Abbreviations: Bis-GMA: bisphenol A glycidylmethacrylate; HEMA: 2-hydroxyethyl methacrylate; 10-MDP: 10-methacryloyloxydecyl dihydrogen phosphate; UDMA: urethane dimethacrylate; PENTA: dipentaerythritol pentaacrylate phosphate; DMA: dimethacrylate.*

Esthet.X HF (Dentsply Caulk), and light cured for 30 seconds.

The specimens in the X group were dried in the same way as the SE specimens, and X was applied according to manufacturer's instructions to the dentin surfaces in the predetermined orientation. Microtubes were placed, filled with the nano-filled hybrid resin composite, and cured in the same manner as the SE specimens.

The composition and batch numbers of the adhesives are listed in Table 1.

After the bonding procedures, specimens were checked under a light microscope to detect any defects in the resin packing of the microtubes and discarded if voids or defects were evident. All specimens were stored for a further 24 hours before testing.

### MSBS Testing

Each specimen was placed in the jig of the universal testing machine (Imperial 1000, Mecmesin, Slinfold, UK) so that the shear force could be applied parallel to the specimen surface. MSBS tests were carried out on each of the resin composite cylinders via a 0.35 mm diameter stainless steel wire loop placed around the base of each resin cylinder at the dentin/resin composite interface. The load was applied to the resin cylinders at a crosshead speed of 1.0 mm/min until bond failure occurred. The maximum load applied (N) was recorded using computer software (Emperor version 01, Mecmesin, Slinfold, UK) and converted to MSBS (MPa) by dividing the load by the bonded surface area (mm<sup>2</sup>). Statistical analyses of MSBSs were performed with GenStat for Windows, 14th edition SP1 (VSN International Ltd, Hemel Hempstead, UK), using both three-way and two-way analysis of variance with the three factors being treatment type (groups 1-6), bonding agent (SE Bond or Xeno IV), and bond position (center, pulp horn, or periphery). This was followed by multiple compari-

sons using Tukey's test, with statistical significance set at  $\alpha = 0.05$ .

### Fracture Analysis

Fractured surfaces were examined under a light microscope at  $\times 10$  magnification (Leica S8 APO, Leica Microsystems, Wetzlar, Germany) by a single observer (MY), who was blinded to the experimental groups of the specimens. The specimen fractures were classified as A = adhesive failure when it occurred at the resin-dentin interface over more than approximately 75% of the bonding surface, C = cohesive failure when it occurred within the resin more than approximately 75% of the bonding surface, or M = mixed failure when either modes of failure occurred on approximately 25%-75% of the bonding surface. Data were analyzed using a generalized linear mixed model (GenStat 14, a = 0.05).

Scanning electron microscopy (SEM) (FEI Quanta Scanning Electron Microscope, FEI, Hillsboro, OR, USA) was carried out on randomly selected resin-dentin interfaces to further examine the previously resin-bonded substrate and to identify any effects of the various treatments. Images for analysis were acquired at  $\times 2000$  and  $\times 8000$  magnification by Image Pro Plus 6.0 (MediaCybernetics, Rockville, MD, USA).

## RESULTS

### MSBS

The effects of the various treatments involving SP and SA on the MSBS of two-step and all-in-one adhesives are shown in Table 2. There were no significant differences between the treatment groups; however, the MSBS values for two-step adhesive groups were significantly higher than those of the all-in-one groups ( $p=0.028$ ). No interaction was found between the treatment and bond type factors.

Table 2: *Microshear Bond Strength of Resin Adhesives to Dentine After Various Treatments*

Group	Treatment	Clearfil SE Bond, Bond Strength* (MPa)	Xeno IV, Bond Strength* (MPa)
1	Distilled water (control)	8.98 ± 4.72 (36) <sup>a</sup>	7.39 ± 3.82 (34) <sup>b</sup>
2	7 d SP	10.49 ± 2.45 (36) <sup>a</sup>	6.63 ± 3.90 (33) <sup>b</sup>
3	7 d SP + 30 s 10% SA	9.49 ± 4.08 (36) <sup>a</sup>	8.06 ± 4.92 (33) <sup>b</sup>
4	7 d SP + 1 min 10% SA	9.27 ± 4.09 (33) <sup>a</sup>	9.84 ± 4.78 (34) <sup>b</sup>
5	7 d SP + 2 min 10% SA	9.94 ± 4.50 (35) <sup>a</sup>	8.27 ± 4.48 (32) <sup>b</sup>
6	7 d SP	9.86 ± 3.43 (34) <sup>a</sup>	7.60 ± 3.27 (33) <sup>b</sup>

Abbreviations: SA: sodium ascorbate; SP: sodium perborate.

\*Values are mean ± SD. The number of specimens tested is included in parentheses. Bond strength results were analyzed by two-way analysis of variance and Tukey's multiple-comparison tests. Groups identified by the same superscript letter are not significantly different ( $p > 0.05$ ).

Table 3: *Microshear Bond Strength of Resin Adhesives to Dentine Based on Microtube Position*

Microtube Position	Clearfil SE Bond, Bond Strength* (MPa)	Xeno IV, Bond Strength* (MPa)
Center (A)	9.40 ± 3.65 (23) <sup>a</sup>	6.64 ± 3.95 (21) <sup>b</sup>
Pulp horn (B, C, D, E)	9.97 ± 4.49 (92) <sup>a</sup>	6.73 ± 4.11 (89) <sup>b</sup>
Peripheral (F, G, H, I)	9.45 ± 3.42 (95) <sup>a</sup>	9.55 ± 4.08 (89) <sup>a</sup>

\*Values are mean ± SD. The number of specimens tested is included in parentheses. Bond strength results were analyzed by two-way analysis of variance and Tukey's multiple-comparison tests. Groups identified by the same superscript letter are not significantly different ( $p > 0.05$ ).

The effect of bond site on the MSBS of two-step and all-in-one adhesives is shown in Table 3. The microtube position had no significant effect on the MSBS of the two-step adhesive groups; however, the all-in-one adhesive bond strengths at the center (A) and pulp horn positions (B-E) were significantly lower than those at the peripheral position (F-I) ( $p < 0.001$ ).

### Failure Mode

Under light microscopy analysis, no cohesive failures were detected on any of the bond interfaces; most of the failures were adhesive in nature ( $n=323$ ), and the remainder were mixed failures ( $n=86$ ). There were no significant differences between the treatment types on the proportion of adhesive failures. In contrast, bond type had a significant effect; the all-in-one adhesive groups showed significantly more adhesive failures than the two-step adhesive groups ( $p=0.015$ ). Microtube position had a significant effect on the bond failure mode; the odds of adhesive failure were 0.47 at the peripheral position compared with the pulp horn position ( $p=0.004$ ).

SEM analysis demonstrated areas of dentin with variable smear layer and resin bond coverage (Figures 2 through 4); however, no differences could be determined between treatment groups and bond types. Generally, larger diameter dentinal tubules were evident in pulp horn and center dentin positions compared with the peripheral dentin position.

### DISCUSSION

The fundamental mechanism of resin bonding to dentin is essentially a two-stage exchange process between the synthetic resin and the dental substrate resulting in a micromechanical interlocking between the resin monomers and the components of the prepared dental hard tissues.

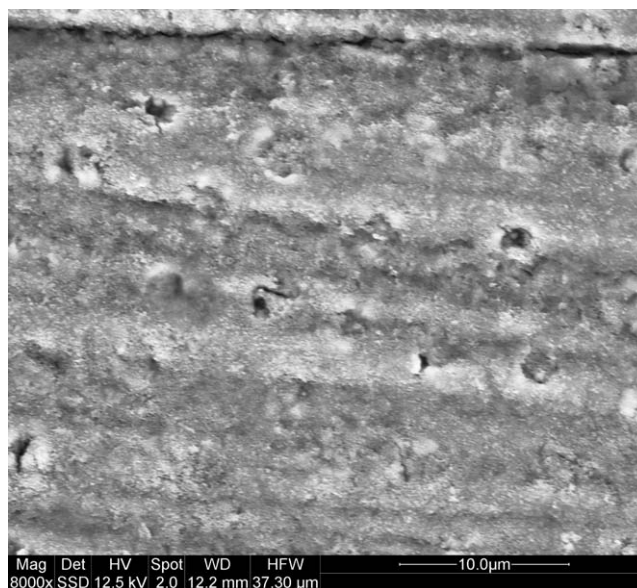


Figure 2. SEM micrograph ( $\times 8000$ ) of microshear failure interface of the all-in-one bonded peripheral dentin (2 min SA treatment after 7 d SP bleach) showing presence of smear layer.

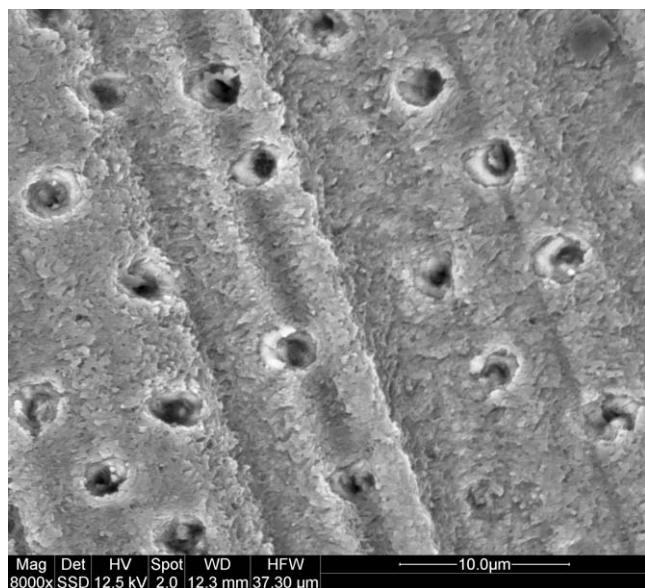


Figure 3. SEM photograph ( $\times 8000$ ) of microshear failure interface of the two-step adhesive bonded pulp horn dentin (1 min SA treatment after 7 d SP bleach) showing a predominantly absent smear layer and patent dentinal tubules.

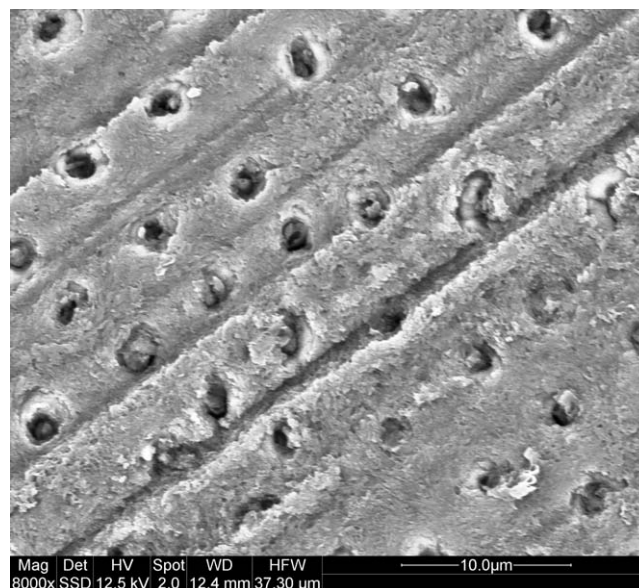


Figure 4. SEM photograph ( $\times 8000$ ) of microshear failure interface of the two-step adhesive bonded peripheral dentin (7 d delay after 7 d SP bleach) showing a predominantly absent smear layer and some filled dentinal tubules.

Both bonding agents tested in this study are self-etch adhesives with the difference being the number of procedural bonding steps required: SE bond is a two-step self-etch resin-bonding agent with a separate mild acidic primer (pH 2.0) and adhesive resin monomer containing 10-MDP; Xeno IV is acetone-based all-in-one self-etch resin bonding agent that combines all the components into a single solution. It is also classified as a mild etching adhesive (pH=2.1).<sup>28</sup> The other significant difference between the two adhesives is the use of 10-MDP as the functional monomer in SE bond, which enables chemical bonding to tooth structure. Using x-ray photo-electron spectroscopy, phosphate groups of 10-MDP have been shown to form an ionic bond with calcium of hydroxyapatite and also seem to form a regularly ordered nano-layered structure at the hydroxyapatite surface.<sup>29</sup>

In this study, SP bleaching of dentin before resin bonding had no significant effect on the MSBS of either the two-step or the all-in-one adhesives. This is in contrast to reports in the literature,<sup>11,12</sup> although this may be attributed to differences in study designs. Elkhatib and others<sup>11</sup> used a sodium perborate-hydrogen peroxide mixture and reported microtensile bond strengths, whereas Shinohara and others<sup>12</sup> investigated shear bond strengths to SP-bleached bovine dentin. The use of human dentin in this study may also explain the variation in the resin

bonding outcome compared with studies utilizing bovine dentin. Furthermore, the minimal effect of SA on subsequent resin bonding is likely to be related to the low concentration of the oxidizing breakdown products compared with those produced from hydrogen peroxide and carbamide peroxide bleaching agents, which, in general, have been reported to negatively affect resin-dentin bond strengths.<sup>13-15,19,30</sup>

The post-bleach application of the antioxidant SA on the bleached dentin surface had no significant effect on the MSBS of the two-step adhesive and all-in-one adhesives. This is not surprising, as there was no reduction in bond strength as a result of bleaching. It also demonstrated that SA did not interfere with the resin-dentin bonding. The potential beneficial effect of SA on restoring resin bond strengths of enamel and dentin has been reported widely in the literature when bond strengths have been compromised by carbamide peroxide<sup>22,23</sup> and hydrogen peroxide<sup>16,24</sup> bleaching agents.

In general, the two-step adhesive groups were found to have significantly higher MSBS values than the all-in-one adhesive groups, a finding that has been supported by other studies comparing the same two-step adhesive with other all-in-one bonding agents.<sup>31,32</sup> It can be postulated that the combined micromechanical and chemical bonding mechanism of mild self-etch adhesives containing 10-MDP, as is

the case with the two-step adhesive in this study, may contribute to the higher MSBS values, although there is a lack of evidence proving that chemical interaction improves immediate bond strength. However, improved bond stability has been proven by clinical data, as they demonstrated that two-step self-etch adhesives, in general, show reliable long-term performance.<sup>33,34</sup>

The reliable bonding ability of the two-step adhesive was further evident in this study after assessing the effect of bond position on the MSBS. The microtubes were positioned in a particular orientation (Figure 1) to determine whether the dentin location, as established by the depth and position of dentin relative to the pulp chamber, affected resin bonding. Dentinal tubules, which are wider and have a greater surface area as they approach the pulp chamber, were simulated by the center (A) and pulp horn (B-E) positions in this study. Superficial dentin, which is characterized by smaller tubule diameters, was simulated by the peripheral positions close to the dentinoenamel junction (F-I). This difference in tubule diameter was evident from the SEM analysis of selected specimens performed (Figures 3 and 4). The microtube position had no significant effect on the MSBS of the two-step adhesive; however, in the all-in-one adhesive groups both center and pulp horn positions had significantly lower MSBS than the values found in the peripheral position. Any conclusions from these statistical findings, especially those involving the center position, must be carefully made because of the difference between specimen numbers according to position: center ( $n=21$  for two-step adhesive  $_{SE}$ ;  $n=23$  for all-in-one adhesive  $_{X}$ ), pulp horn ( $n_{SE}=92$ ,  $n_X=89$ ), and peripheral ( $n_{SE}=95$ ,  $n_X=89$ ) positions.

The increased distance from the periphery means that the center and pulp horn positions represent areas of deeper dentin. Dentin that is closer to the pulp chamber is characterized by wider dentinal tubules, increased permeability, and greater dentin wetness.<sup>35</sup> The reduced dentin surface area and increased moisture content most likely explain the inconsistent behavior of the all-in-one adhesive, Xeno IV, which is believed to rely on primarily micromechanical bonding for adhesion to the tooth surface. All-in-one self-etching resin adhesives, with their greater degree of hydrophilicity, are prone to attract more water from the intrinsically moist dentin substrate, and this property has raised concerns about these bonding agents possibly acting as semipermeable membranes.<sup>36</sup> Besides resulting

in a decrease in resin bond strength,<sup>37</sup> such permeability of the adhesive layer appeared to contribute to the hydrolysis of resin polymers and the resultant degradation of resin bond over time.<sup>38</sup> Furthermore, adhesives containing acetone as the solvent, as present in the all-in-one system used, have been found to be more susceptible to the formation of defects along the bond interface, such as primer globules and blisterlike spaces when used on wet dentin, that is, the overwet phenomenon.<sup>39,40</sup> Although the two-step adhesive may also be affected by wetter dentin through the dilution of the monomer, its intrinsic ability to form a chemical bond may overcome the potentially moisture-compromised micromechanical bond at the center and pulp horn regions.

Although treatment type (SP bleach and SA application of various exposure times) had no effect on the proportion of adhesive failures, the Xeno IV groups had significantly more adhesive failures than the SE bond groups. The overwet phenomenon affecting Xeno IV is a possible explanation for its susceptibility of failure at the dentin-bonding agent interface. This explanation is further supported by the observed higher frequency of adhesive failures at the wetter pulp horn position compared with bonding to peripheral dentin, which is drier and composed of smaller diameter dentinal tubules.

## CONCLUSION

The results of this study support resin bonding with either the two-step adhesive, SE Bond, or the all-in-one adhesive, Xeno IV, immediately after bleaching with SP with no need for the antioxidant SA. Generally, SE Bond performed more consistently than Xeno IV, as higher MSBS values were evident with SE bond groups and lower MSBS values were evident in the Xeno IV groups when bonded to wetter center and pulp horn dentin.

The null hypothesis cannot be rejected completely as SA had no effect on the MSBS or mode of failure of the resin bond to bleached dentin; however, there were differences between the types of resin adhesive and dentin.

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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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