

# Effect of Sodium Ascorbate on Dentin Bonding After Two Bleaching Techniques

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## Clinical Relevance

Bleaching with either 10% carbamide peroxide or 35% hydrogen peroxide impairs the formation of the hybrid layer, resin tags, and bond strength. The use of sodium ascorbate following bleaching diminishes this adverse effect in the case of 10% carbamide peroxide but not so when 35% hydrogen peroxide is used as the bleaching agent.

## SUMMARY

**The purpose of this study was to analyze the influence of 10% sodium ascorbate (SA) on the hybrid layer, resin tag length, and bond strength to dentin after bleaching. Six groups were tested: G C, control; G SA, sodium ascor-**

**bate (SA) + restoration; G CP, bleaching with carbamide peroxide (CP) + restoration; G CP+SA, bleaching with CP + SA+ restoration; G HP, bleaching with 35% hydrogen peroxide (HP) + restoration; and G HP+SA, HP + SA + restoration. After dental bleaching, the dentin was exposed and the antioxidant solution was applied to groups G SA, G CP+SA, and G HP+SA, before bonding procedures. The teeth were sectioned in the mesiodistal direction. One section was decalcified, and the specimens were embedded in paraffin and sectioned in the longitudinal direction with a thickness of 6 µm. Fifteen slices of each specimen were selected according to a systematic sample of slices with an interval proportional to the total number of slices obtained for each tooth. The specimens were stained using the Brown & Brenn method, and an optic microscope was used to analyze the hybrid layer thickness and resin tag length. The remaining tooth segment was sectioned into stick-shaped specimens and used for microtensile bond strength testing (0.5 mm/min). Statistical analysis was performed using two-way analysis of variance and Fisher test. The results for hybrid layer +**

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**tag formation (in micrometers) were G C, 13.27 Aa; G SA, 11.85 Ba; G CP, 6.84 Bb; G CP+SA, 9.02 Ab; G HP, 7.28 Bb; and G HP+SA, 9.22 Ab; bond strength results (in MPa) were G C, 49.5 Aa; G SA, 51.7 Aa; G CP, 37.16 Bb; G CP+SA, 47.69 Aa; G HP, 32.39 Ab; and G HP+SA, 39.67 Ab. Tooth bleaching with CP or HP impairs the formation of the hybrid layer and resin tags and reduces the microtensile bond strength. Statistically, the use of SA significantly increases the hybrid layer thickness and resin tag length. The microtensile bond strength values for carbamide peroxide increased, but the microtensile bond strength for hydrogen peroxide was not affected.**

## INTRODUCTION

In general, the techniques for tooth bleaching are simple and generate positive esthetic results when the manufacturer's directions are followed. In addition to the traditional at-home bleaching technique with carbamide peroxide products in bleaching trays, hydrogen peroxide at high concentration has produced faster results for those patients who cannot or do not want to wear the trays.<sup>1,2</sup>

It is suggested that esthetic restorations should be replaced after tooth bleaching, since color alteration affects tooth structure but not restorative materials.<sup>3,4</sup> A 7-day waiting period following bleaching is recommended before replacing adhesive restorations because bleaching products adversely affect the bonding of resin materials to the tooth and overcome low bond strength values to bleached dental surfaces, which may negatively influence the clinical performance of restorations.<sup>2,4-7</sup> Moreover, there are other reasons for delaying restorative procedures, such as reducing postoperative sensitivity and to allow time for color rebound, which may alter esthetic results.<sup>8-10</sup>

It is worth noting that most of the bonding studies have been conducted with resins placed primarily in enamel,<sup>1,7,11,12</sup> while some recent studies evaluated the effect of tooth bleaching on bonding to dentin.<sup>2,13-17</sup> Most restorative procedures use enamel and dentin as substrates for adhesion.

In the traditional adhesive technique with prior acid etching, the smear layer and smear plug are removed and superficial demineralization of dentin occurs, exposing the collagen fibers. The penetration of the adhesive system into this demineralized structure and in the tubules allows formation of a hybridized region (resin tags + hybrid layer), which

restores the structural resistance of the etched region<sup>18</sup> and prevents the occurrence of microleakage.<sup>19</sup>

Although some authors have indicated that a thin hybrid layer can have a performance similar to a thicker one,<sup>20</sup> other studies<sup>21,22</sup> have reported that a thin hybrid layer and a reduced penetration of the adhesive systems in dentin could result in lower bond strength values.

Many studies consider a 10% sodium ascorbate (SA) solution to be an effective choice to improve dentin bonding and defend the use of SA as the most efficient agent for neutralizing the oxidizing effects of bleaching agents. Its use may also increase the strength of the dentin bond.<sup>11,13,14,23-25</sup> The antioxidant type, concentration, form, and duration of application have been considered important factors for improving bonding after bleaching treatment.<sup>15-17,24,25</sup>

The reducing capacity or ability to neutralize and reverse the oxidizing effects of hydrogen peroxide in biological systems has already been reported.<sup>26,27</sup> Some authors have suggested that the use of SA after bleaching can reverse the reduced adhesion between adhesive materials and dental tissue.<sup>11,13,17,24,25</sup>

However, since there are no significant reports emphasizing the effect of antioxidant agents on the formation of the hybrid layer and resin tags, laboratory studies are necessary to assess the effect of bleaching agents and antioxidants on the micro-mechanical interaction occurring in dentin. The purpose of this current study was to evaluate the influence of 10% SA solution following two bleaching techniques on the bond strength in dentin. The microtensile test was used for evaluation.

The null hypothesis tested was that the use of SA does not influence the hybridization depth or the microtensile bond strength in dentin after bleaching.

## MATERIALS AND METHODS

This study investigated the influence of 10% SA on the hybrid layer, resin tag length, and bond strength to dentin after dental bleaching with two different materials (Table 1).

Thirty-six human premolars without any cracks and that were extracted for other purposes were divided into six experimental groups (n=6) according to the bleaching procedures and the use of antioxidant. The teeth were cleaned with manual periodontal curettes (Duflex Ltda, Rio de Janeiro, Rio de

	Material	Composition	Manufacturer	Lot	Mode of Application
Composite resin	Z-250	UDMA: urethane dimethacrylate; Bis-EMA: bisphenol A polyethylene glycol diether dimethacrylate; TEGDMA: tri-ethylene glycol dimethacrylate; inorganic particles	3M/ESPE, St Paul, MN, USA	6CR	Two 2.0-mm-thick flat composite resin increments
Etching agent	Scotch Etchant	35% Phosphoric acid gel	3M/ESPE, St Paul, MN, USA	5EX	15 s
Adhesive system	Adper Scotchbond Multi Purpose	Primer: HEMA, polyalkenoic acid copolymer; adhesive: Bis-GMA, HEMA, photoinitiators	3M/ESPE, St Paul, MN, USA	Primer: 6BC, adhesive: 6PL	According to manufacturer's instructions
Bleaching products	10% Whiteness standard	Carbamide peroxide, carboxypol, potassium ions, glycol, high content of water, sodium fluoride	FGM – Dental Products, Joinville, Brazil	061008	Four hours per day for 14 days
	Whiteness HP Maxx	35% Hydrogen peroxide, thickening stain's mixture, glycol, and deionized water	FGM – Dental Products, Joinville, Brazil	270206	Four sessions, one session per week, three 15-minute applications per session
Antioxidant	10% Sodium ascorbate	10% Sodium ascorbate and osmolality water	Farmácia Aphoticário, Araçatuba, Brazil	No batch number	10-minute application before bonding procedures

Abbreviations: Bis-GMA, bisphenol-A-glycidyl dimethacrylate; HEMA, 2-hydroxyethyl methacrylate.

Janeiro, Brazil) using distilled water to remove periodontal tissues, washed using pumice and water in a rubber cup, coupled to a slow-speed hand piece (Dabi Atlante, Ribeirão Preto, SP, Brazil), and rinsed and stored in 0.1% neutral thymol solution at room temperature until the beginning of the research.

The current study design was analyzed and approved by the Institutional Review Board for Investigations (2009-2384). Throughout the study, specimens from all study groups were stored in artificial saliva at 37°C.

In groups carbamide peroxide (CP) and CP+SA, a commercial 10% CP at-home bleaching gel (Whiteness Standard, FGM Produtos Odontológicos Ltda, Joinville, SC, Brazil) was applied to the buccal and occlusal surfaces (0.06 mL per tooth) using a graduated 1-mL syringe for four hours per day, according to the manufacturer's instructions. The specimens were thoroughly rinsed with air-water spray and stored in artificial saliva (pH=6.7).

The specimens from groups hydrogen peroxide (HP) and HP+SA were bleached using 35% hydrogen peroxide (Whiteness HP Maxx, FGM Produtos Odontológicos Ltda) applied to the buccal and occlusal surface in a layer of about 1.0 mm in thickness (0.06 mL). After one minute, the product

was exposed to a quartz-tungsten-halogen light (Ultralux, Dabi Atlante) with a light output of 450 mW/cm<sup>2</sup> for 20 seconds, according to the manufacturer's instruction. The product remained on the dental surface for 15 minutes with no light source. Four bleaching sessions were performed, and three applications of the bleaching product were made during each session. After each bleaching sessions, the specimens were stored in artificial saliva until the next session (seven days later).

After performing the bleaching procedures, the occlusal surfaces of all specimens were abraded with 600-grit abrasive paper disks (Carbimet Paper Disks, Buehler, Lake Bluff, IL, USA) in the polisher Aropol E (Arotec Industria e Comércio Ltda) at a constant speed and under irrigation until dentin exposure. The specimens from groups CP+SA and HP+SA, bleached with 10% CP and 35% HP, respectively, had the SA antioxidant solution (Farmácia Aphoticário, Araçatuba, SP, Brazil) applied on the dentin surface for 10 minutes at 1 mL/min. The specimens were then rinsed with distilled water for 30 seconds, and bonding procedures were performed immediately after SA application. For that purpose, the exposed dentin substrate was rinsed, dried, and etched with 35% phosphoric acid gel (Scotchbond Etchant, 3M ESPE, St Paul, MN, USA) for 15

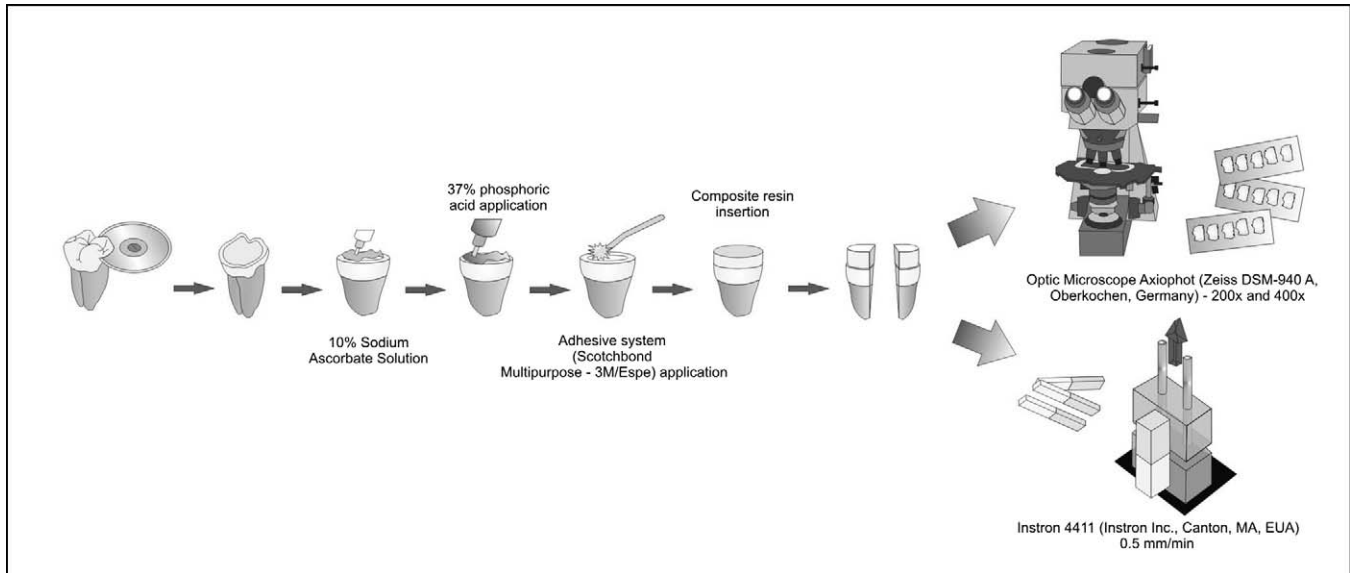


Figure 1. Methodology scheme of the teeth sectioning into two equal parts used for microtensile test and hybrid layer and tag formation analysis.

seconds. Then, the substrate was rinsed again for 10 seconds and dried with compressed air spray with the dentin protected by a small cotton ball to maintain the humidity of the tissue. A layer of the two-step adhesive system primer (Adper Scotchbond Multi-Purpose, 3M/ESPE) was applied on the dentin followed by the application of a layer of the hydrophobic component. Light polymerization was carried out with a halogen light-curing unit for 10 seconds at a light output of 450 mW/cm<sup>2</sup>. The composite resin (Filtek Z250, 3M ESPE), shade A2, was inserted using a Thompson spatula and light polymerized for 40 seconds.

In group SA, 10% SA antioxidant solution (Table 1) was applied as described for groups CP+SA and HP+SA, after dentin exposure. Afterward, bonding procedures were performed.

Group C was the control, and bonding procedures were performed after dentin exposure with no previous treatments.

After the restorative procedures, all of the specimens were sectioned into two equal parts, in the mesiodistal direction, using the metallographic cutter ISOMET 2000 (Buehler) under water cooling and at a speed of 800 rpm and static load of 160 g (Figure 1). One section was decalcified over three months in a solution containing 50% formic acid and 20% sodium citrate. After demineralization, the specimens were immersed in alcohol solutions at different concentrations for dehydration. Immediately after this procedure, the specimens were immersed in xylol for one hour. The restorations were carefully

removed, and the specimens were embedded in paraffin and sectioned in the longitudinal direction with a slice thickness of 6  $\mu$ m. Fifteen slices of each specimen were selected according to a systematic sample of slices with an interval proportional to the total number of slices obtained for each tooth.

The selected slides were stained using the Brown & Brenn method and analyzed with an optical light microscope (Axiophot, Carl Zeiss Company, Oberkochen, Germany). The hybrid layer thickness and resin tag length were analyzed with the micrometric ocular piece 40/075.

Each slice generated three images at 400 $\times$  of the mesial, middle, and distal thirds. In each visual field, the hybrid layer + resin tags were measured at five different areas using the Axiovision Software Rel. 4.6 (Carl Zeiss Company), as demonstrated in Figure 2.

The other tooth section was used for microtensile bond strength testing. A metallographic cutter was used under water cooling at a speed of 800 rpm and static load of 160 g to obtain stick-shaped specimens from the central region of each tooth, totaling 30 specimens per group.

These specimens were fixed to the Universal Test Machine Instron Model 4411 (Instron Inc, Canton, MA, USA), using cyanoacrylate adhesive (Super Bonder, Henkel Ltda, Itapevi, São Paulo, Brazil) and tested with a 50N load cell at a speed of 0.5 mm/min until failure. The bond strength values of the sticks from the same tooth were averaged, and only the bond strength values of the six teeth from each

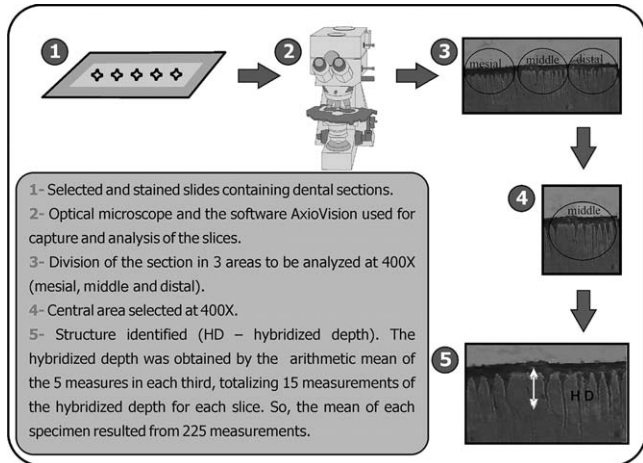


Figure 2. Methodological sequence used to obtain the hybrid layer thickness and resin tags length.

experimental condition were analyzed statistically. Premature failures were not considered in the data analysis.

The results obtained for hybrid layer + resin tags and microtensile bond strength were submitted to two-way analysis of variance (ANOVA) and Fisher PLSD test, at a level of significance of 5%, to verify the effect of the treatments on dentin bonding.

The fracture pattern was analyzed in the stereoscopic Stemi SV 11 (DSM-940 A, Carl Zeiss Company) at 30× magnification. To reveal the fracture area of each specimen more clearly, the revealing solution proposed by Ohkubo and others in 1982<sup>28</sup> was applied on the fractured region, which stained the resin-free dental structure. Images were analyzed using Image J Software (Image Processing and Analysis in Java, NIH, Bethesda, MD, USA), and the fracture patterns were determined.

**RESULTS**

Means of hybrid layer + resin tags (µm) and microtensile bond strength are summarized in Tables 2 and 3. ANOVA showed that significant differences were observed among the groups for both analyzed factors ( $p < 0.05$ ; Tables 4 and 5).

Specimens from G C showed greater resin tag length and hybrid layer thickness, suggesting a continuous and frequent micromechanical interaction (Figure 3). When SA was used before the restoration procedures and without bleaching treatment (group SA), the hybrid layer + resin tags were thinner than the control group ( $p = 0.0038$ ). The bleached specimens from groups CP and HP showed the lowest adhesive penetration means, presenting unusual, fewer, and shorter resin tags and a thinner hybrid layer; no significant difference was found between these groups ( $p = 0.335$ ). It was also verified that SA resulted in an increase in resin tag length and hybrid layer thickness for both CP+SA (Figure 4) and HP+SA (Figure 5).

For the microtensile bond strengths, group C and group SA showed statistically similar values ( $p = 0.59$ ). In the CP group, the SA application increased the values of microtensile bond strength, but the SA application was not effective for the 35% HP bleached teeth (Tables 3 and 5).

Fracture pattern analysis showed a prevalence of adhesive failures. Groups CP and HP+SA presented a high incidence of cohesive failures in dentin, while cohesive failures in resin were frequent in groups C and SA (Figure 6).

**DISCUSSION**

At-home bleaching techniques have been widely used to obtain improved esthetic results.<sup>3</sup> Many studies have been conducted to clarify the interaction of bleaching, its effect on hard tissues, and its influence on bonding adhesive restorative materials to the tooth structure.<sup>12</sup>

In recent studies, microleakage, a decrease in bonding strength, and low micromechanical interaction were associated with bleaching procedures.<sup>6,29,30</sup> These conditions were augmented when the in-office bleaching technique, which uses bleach at a higher concentration, was performed.<sup>31,32</sup> As a result, a 24-hour to one-month waiting period after bleaching has been recommended before performing restorative procedures.<sup>2,6,29</sup>

	Group C	Group CP	Group HP
No SA	13.27 ± 0.94 A a	6.84 ± 0.41 B b	7.28 ± 0.36 B b
+SA	11.85 ± 0.76 B a	9.02 ± 0.84 A b	9.22 ± 1.10 A b

Abbreviations: C, control; CP, carbamide peroxide; HP, hydrogen peroxide; SA, sodium ascorbate.  
<sup>a</sup> Means followed by different letters (capital letter in vertical; lower case in horizontal) represent a significant difference according to analysis of variance and Fisher test,  $p < 0.05$ .

Table 3: Values of Microtensile Bond Strength (MPa) for All of the Studied Groups (Means ± Standard Deviation)<sup>a</sup>

	Group C	Group CP	Group HP
No SA	49.54 ± 8.09 A a	37.16 ± 3.10 B b	32.39 ± 11.73 A b
+SA	51.73 ± 5.52 A a	47.69 ± 6.47 A a	39.67 ± 7.27 A b

Abbreviations: C, control; CP, carbamide peroxide; HP, hydrogen peroxide; SA, sodium ascorbate.  
<sup>a</sup> Means followed by different letters (capital letter in vertical; lower case in horizontal) represent significant difference according to analysis of variance and Fisher test, p ≤ 0.05.

Table 4 Analysis of Variance Table for Hybrid Layer + Resin Tags

	df	Sum of Squares	Mean Square	F-Value	p-Value	Lambda	Power
Bleaching agent	2	160.661	80.330	131.124	<0.0001	262.248	1.000
Antioxidant agent	1	7.326	7.326	11.958	0.0017	11.958	0.934
Bleaching agent/antioxidant agent	2	24.255	12.128	19.796	<0.0001	39.592	1.000
Residual	30	18.379	.613				

The decrease in bond strength and reduction of the adhesive system interaction in bleached teeth are attributed to the presence of residual oxygen.<sup>13,17,33,34</sup> This highly reactive chemical element eliminates pigmentation but also reacts with the free radicals of the resin materials, which inhibits polymerization and generates polymers with reduced mechanical properties.<sup>25,33,35,36</sup> Some studies in dental enamel suggest that the amount of residual oxygen is proportional to the reduction in bonding and the reduced length and frequency of resin tags in the bleached substrate.<sup>6,29</sup> Data found in the current study also showed a reduced hybrid layer and resin tag formation. This result may be related to mechanical modifications in peritubular and intertubular dentin produced by bleaching agents that could lead to biomechanical alterations, but there is no evidence to substantiate this.<sup>30,37</sup>

Histochemical studies have shown reduced levels of sulfur in samples bleached with HP or CP. Sulfur is a component of proteoglycans (chondroitin sulfate and keratan sulfate), and changes in their levels indicate damage to the organic dentin matrix.<sup>38-40</sup>

These changes in proteoglycans might interfere in the maintenance of interfibrillar spaces,<sup>41</sup> which may have compromised diffusion of the adhesive into the collagen network, potentially reducing the bond strength in this current study.<sup>42,43</sup>

The specimens from group C (Figure 3) presented a thick hybrid layer + resin tags and also high microtensile bond strength values. The bleached groups (G CP and G HP), which presented similar results to those from previous studies, demonstrated the deleterious effects of the oxygen released by the bleaching agents.<sup>5,13,29,35</sup>

For those groups that received the solution of 10% SA, the group SA exhibited a reduced hybrid layer thickness + resin tag length (11.85 μm) and was considered as a control group. Although many studies report the advantages of using this antioxidant substance with bleached teeth,<sup>11,13,24</sup> few studies considered the use of ascorbate with unbleached teeth.<sup>13,44</sup> In the current study, the use of SA alone led to limited resin tags and hybrid layer when compared with the control group, although their formations remained continuous and homogeneous across the adhesive interface. Although the hybrid layer + resin tag thickness decreased in the SA group when compared with the control group, the microtensile bond strength values were similar. This difference might not be clinically significant, since the bond strength was satisfactory for both groups.

The use of SA immediately after bleaching (groups CP+SA and HP+SA) increased the hybrid layer and resin tag length when compared with groups CP and HP (Figures 4 and 5), but the effect was not as great

Table 5 Analysis of Variance Table for Bond Strength

	df	Sum of Squares	Mean Square	F-Value	p-Value	Lambda	Power
Bleaching agent	2	1287.886	643.943	11.423	0.0002	22.846	0.993
Antioxidant agent	1	400.067	400.067	7.097	0.0123	7.097	0.738
Bleaching agent/antioxidant agent	2	105.963	52.982	.940	0.4019	1.880	0.191
Residual	30	1691.203	56.373				



Figure 3. Photomicrograph (group C) showing a continuous hybrid layer and frequent resin tags formation analyzed by common optical microscopy (400x magnification).

as that from group C. Nevertheless, the hybrid layer and resin tag formation were homogeneous and continuous, especially for group CP+SA.<sup>13,25,30</sup> Moreover, although some authors<sup>20</sup> reported that hybrid layer depth is not usually related to bond strength, higher bond strength values were also verified in this experimental group.

However, in the hydrogen peroxide group (HP+SA), the bond strength values were not improved when the bleaching agent was followed by an application of SA. This may be explained by the use of highly concentrated products<sup>45</sup> and suggests that the use of SA was not sufficient to improve the resin tag and hybrid layer formation. It has been demonstrated that, as the duration of application for the antioxidant increases, the bond strength of the composite to dentin tissue also increases.<sup>24</sup>

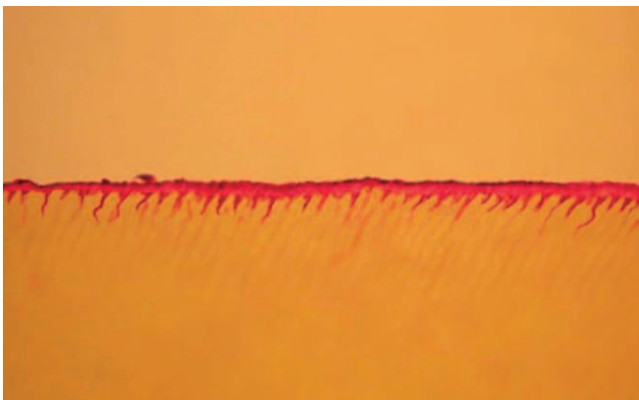


Figure 4. Photomicrograph (group CP+SA) analyzed by common optical microscopy (400x magnification), showing a thick continuous hybrid layer and frequent but short resin tags formation.

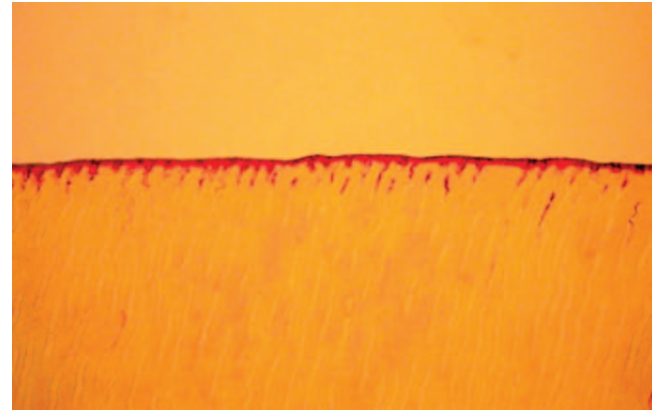


Figure 5. Photomicrograph (group HP+SA) analyzed by common optical microscopy (400x magnification), showing a thin and continuous hybrid layer but sparse and short resin tags formation.

According to the results of the present study, the use of SA improved adhesion in bleached teeth, suggesting that the antioxidant action may reverse (partially or fully) the damaging effects of the bleaching products.<sup>11,13,17,24,25</sup> It is possible that by restoring the reducing potential (redox) of the oxidized substrate, SA limits premature polymerization of the free radicals of the adhesive and therefore cancels (reverses) the effects of the impaired bonding on the bleached dentin.<sup>13,34</sup>

Additional studies are required to further explain the mechanism of this reversal process, its role on bonding, and the possible beneficial effects of its systemic application. It is important to suggest additional studies comparing the effect of different antioxidant solutions considering the instability of these compounds,<sup>46</sup> which limit their use to only a few hours after manipulation. The clinical implica-

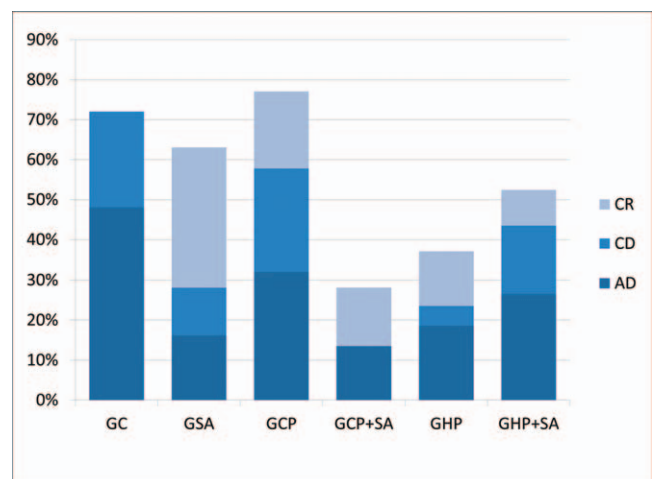


Figure 6. Fracture pattern for the study groups. Cohesive in resin (CR); cohesive in dentin (CD); adhesive (AD).

tion of this present study is that the application of an antioxidant can shorten the time period spent in the clinic by both dentists and patients to allow restorative procedures to be performed after bleaching treatments.<sup>16</sup>

Therefore, the current results do not support full acceptance of the null hypothesis, that the use of SA does not influence the hybrid layer and resin tag formation and the microtensile bond strength in dentin after bleaching.

### CONCLUSIONS

Based on the present results, dental bleaching using 10% CP or 35% HP is damaging to resin tag and hybrid layer formation. When SA was applied following bleaching with CP, a better hybrid layer thickness and resin tag length developed. In this case, there was an improvement in the microtensile bond strength values. This was not the case when SA was applied following bleaching with 35% HP.

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### Conflict of Interest

The authors 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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