Protocols for sodium ascorbate application on intracoronary dentin bleached with high-concentrated agent

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Abstract

Purpose: Composite resin restorations are normally replaced after the internal bleaching of endodontically treated-teeth because the bleaching agent does not alter the color of the restorative material. This study evaluated the effect of 10% sodium ascorbate (SA) applied at different protocols on bleached dentin.

Materials and Methods: One-hundred slabs of intracoronary bovine dentin were divided into 5 groups: 2 controls-G1 without bleaching (positive), GII bleached with 35% hydrogen peroxide (HP) (negative); and 3 experimentals – GIII. 35% HP + SA at protocol 1 (dripping, washing and drying the solution), GIV. 35% HP + SA at protocol 2 (dripping and aspirating the solution) and GV. 35% HP + SA at protocol 3 (dripping, rubbing and aspirating the solution). Sixty fragments were restored and subjected to shear bond strength test (n = 12). Forty fragments (n = 8) were prepared for chemical analysis (energy dispersive X-ray spectrometry) and surface morphology (scanning electron microscopy). Data were analyzed by ANOVA and Tukey test (P < 0.05).

Results: G1 (3.169 ± 1.510a) had the highest means values, similar to GIV (2.752 ± 0.961a) and GV (2.981 ± 1.185a) (P < 0.05). Inferior values were obtained in GII (1.472 ± 0.342b) and GIII (2.037 ± 0.742ab) had intermediate values (P > 0.05). Oxygen concentration was reduced in groups treated with SA, and the surface exhibited residual granules of the solution.

Conclusion: The 10% SA solution reestablishes the bond strength of restorative material to bleached dentin, especially if active protocols of application and aspiration were used.

Keywords: Adhesion; bleaching; hydrogen peroxide; sodium ascorbate

INTRODUCTION

In-office bleaching of endodontically treated-teeth is a routinely procedure of dental practice and hydrogen peroxide (HP), at concentrations above 30%, is the main chemical agent used. Preexisting composite resin restorations are normally replaced after the internal bleaching because the bleaching agent does not alter the color of the restorative material. The literature recommends 7–14 days of postbleaching period before a new restorative procedure be performed. The residual oxygen resulting from decomposition of the bleaching agent inhibits the resin polymerization and reduces the bond strength of the restorative material to the dental substrate.

In an attempt to reduce or neutralize the effects of oxygen released during tooth bleaching, the application of antioxidant agents has been suggested before the restorative

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MATERIALS AND METHODS

Sample selection and preparation
The teeth were stored in 0.1% thymol solution at 9°C and rinsed in running water for 24 h to complete eliminate the thymol residues. After, they were examined with stereomicroscope (Leica Microsystems, Wetzlar, Germany) under ×20 magnification. Fifty incisors were selected and sectioned transversally in cementoenamel junction to separate crowns from roots, and longitudinally, separating the buccal and lingual surfaces, using precision cutter machine (Isomet 1000; Buehler, Germany). Subsequently, the crown of each tooth was sectioned to obtain two intracoronary dentin fragments, totalizing 100 fragments (5 mm × 5 mm).

The study was conducted in a randomized design, and the sample was composed by 100 intracoronary slabs of bovine dentin, in which 60 fragments were used in the bond strength test (n = 12), and 40 on the EDS and SEM analysis (n = 8).

The specimens intended to the shear bond strength test were embedded in bakelite resin (Arotec, Cotia, SP, Brazil) using an automatic pressure impregnation system (Arotec Pre30; Arotec, Cotia, SP, Brazil) with the intracoronary portion of dentin facing upward. Then, the dentin surfaces were flattened under irrigation, with 400- and 600-grit sandpapers (Norton; Lorena, SP, Brazil) and polished with 1200-grit sandpaper, to standardize the smear layer.

Bleaching protocol
The bleaching agent was 35% HP (Whiteness HP, FGM, Joinville, SC, Brazil) and it was applied on intracoronary dentin surface for 15 min, totaling 3 applications, with an interval of 5 min between each one, as recommend by manufacturer. After the applications, the bleaching agent was completely removed from the surface using high-power suction (endodontic cannula).

The sample was stored at 37°C, covered with gauze soaked in artificial saliva during 7 days. After this period, a new bleaching procedure was performed, as previously described. Specimens were sealed with cotton pellet and eugenol-free temporary filling material (Coltosol F; Coltene, Altstätten, Switzerland), and then stored at 37°C in artificial saliva.

The specimens of GI, which were not bleached remained stored at 37°C, covered with gauze soaked in artificial saliva (DaTerra, Ribeirão Preto, SP, Brazil) during the entire experimental period.

Postbleaching treatment with sodium ascorbate
The specimens were randomly distributed into 5 groups (n = 12) according to postbleaching treatment: GI—not bleached (positive control), GII—bleached with 35% HP without posttreatment (negative control), GIII—bleached and treated with SA using the Protocol 1, GIV—bleached and treated with SA using the Protocol 2, and GV—bleached and treated with SA using the Protocol 3 [Table 1]. The 10% SA solution was prepared using 10 g of powder of SA (Sigma-Aldrich, Louis, MO, USA) and 100 mL of distilled water.

Restorative procedure
For the specimens that were submitted to restorative procedure was applied 35% phosphoric acid (3M ESPE St. Paul, MN, USA) in the surface for 15 s, followed by rinsing with distilled water, and drying with absorbent paper. Then, Adper Single Bond 2 (3M ESPE St. Paul, MN, USA) was applied and photopolymerized according to the manufacturer.

The dentin surfaces were restored with Filtek Z250 XT composite resin (3M ESPE, St. Paul, MN, USA) using

| Table 1: Bond strength means and standard deviations (MPa) of restorative material to dentin in different experimental conditions |
|-------------------------------------------------|
| Experimental groups | Mean±SD |
|---------------------|---------|
| Group I: Without bleaching (positive control) | 3.16±1.51* |
| Group II: Bleached without posttreatment (negative control) | 1.47±0.34* |
| Group III: Bleached and treated with sodium ascorbate - protocol 1 | 2.14±0.74** |
| Group IV: Bleached and treated with sodium ascorbate - protocol 2 | 2.75±0.96* |
| Group V: Bleached and treated with sodium ascorbate - protocol 3 | 2.98±1.18* |

Different letters indicate statistically significant differences (Tukey test - P<0.05).

*Protocol 1: Dripping, washing, and drying, **Protocol 2: Dripping, leave act and aspirating, ***Protocol 3: Dripping, rubbing, and aspirating. SD: Standard deviation
Teflon molds (3 mm internal diameter, 4 mm in height) stabilized with wax, to obtain resin cylinders with the above-mentioned measures. The composite resin was included in three increments, polymerized using light-emitting diodes (LED) light source (Gnatus, Ribeirão Preto, SP, Brazil), with light intensity of 1200 mW/cm (420–480 nm wavelength range), and the intensity of the LED was measured with curing radiometers. Then, the wax was removed with a scalpel, and Teflon mold was opened. The dentin/restorative material set was stored in relative humidity at 4°C for 24 h.

Shear bond strength test
After 24 h, the specimens were positioned in universal test machine (Instron Corporation, Canton-Massachusetts, USA) with a load cell of 2 kN. The force was applied with a rectangular stainless steel tip, at a speed of 0.5 mm/min until the displacement of the restoration.

Failure patterns were analyzed using a ×40 stereomicroscope (Leica Microsystems, Wetzlar, Germany) and were classified as adhesive, when the dentin surface was covered by a thin layer of adhesive material; cohesive of the material, when the area was covered with composite resin; cohesive of the substrate, when failure occurred in dentin; and mixed, in situations in which there was a combination of adhesive and cohesive.

Chemical analysis of the elements by energy dispersive X-ray spectrometry and morphological surface by scanning electron microscopy
The 40 fragments were prepared for EDS, and SEM was sectioned into two halves and polished with 600- and 1.200-grit for 30 s and with alumina 0.3 μM and 0.05 μM for 5 min. After polishing, the sections received phosphoric acid 35% (3M ESPE St. Paul, MN, USA) for 20 s, followed by rinsing at the same time and then were ultrasonically cleaned for 10 min. The specimens were immersed in 2.5% glutaraldehyde buffered with 0.1 M sodium cacodylate at pH 7.4 at 4°C for 12 h, followed by washing in distilled water for 3 min and immersion in distilled water for 1 h, with changes every 20 min. Then, the specimens were dehydrated using increasing concentrations of ethanol of 25°, 50°, 75°, and 95° GL, with ethanol change every 20 min intervals, subsequent immersion for 1 h in ethanol 100° GL, and finally, were immersed in HMDS for 10 min to obtained chemical drying of the fragments, dried, and stored at 37°C for 24 h.

After this period, they were fixed in stubs with carbon tape and submitted to the scanning electron microscope with a detector of EDS (Carl Zeiss, Cambridge, England). The quantitative elemental analysis was carried out by EDS at ×5.000 magnification. After the quantification of chemical elements on the surface, the specimens were visualized by gold sputtering (Bal-Tec SCD 005 Sputter Coater, Balzers, Liechtenstein) and analyzed under a scanning electron microscope (SEM; S3400N, Hitachi, Tokyo, Japan) and the most representative area was photographed.

Data analysis
Data analysis of the shear bond strength test (MPa) was performed using SigmaPlot 11.0 software (San Jose, CA, USA), showing normal and homogeneous distribution. Then, they were analyzed by ANOVA and Tukey test, performed at a preset alpha of 0.05.

The concentration of the chemical elements was expressed as percentage (%), and the photomicrographs were evaluated qualitatively.

RESULTS

Bond strength of the restorative material to dentin
Means and standard deviations of the experimental groups are shown in Table 1. The analysis of variance showed significant difference among groups (P < 0.05).

The Tukey test showed that the specimens without bleaching (GI-positive control) had the highest bond strength, statistically similar (P > 0.05) to specimens that were bleached and treated with SA using the Protocol 2 (GIV-dripping the solution and aspirating) and Protocol 3 (GV-dripping the solution, rubbing, and aspirating).

The lowest bond strength values were obtained in specimens that were only bleached, without posttreatment (GII-negative control) (P < 0.05). The fragments that were bleached and treated with SA using the Protocol 1 (GIII-just dripped on the surface) had intermediate values (P > 0.05).

Considering the failure patterns, it was observed the predominance of the adhesive type in all groups. Cohesive failures of the material were not recorded. Cohesive failures in dentin were verified in specimens treated with SA using active protocols (GIV and GV). The failures percentage of each experimental group is shown in Table 2.

Quantification of chemical elements by energy dispersive X-ray spectrometry
Table 3 represents the data from EDS analysis. It was possible to quantify mainly the concentrations of the elements: carbon (C), oxygen (O), phosphorus (P), and calcium (Ca). The magnesium (Mg) was detected in low amount (<2% by mass-wt%) in all samples.
Table 2: Failure mode distribution (%) in experimental groups

| Failure types       | Group I (without bleaching) | Group II (bleached without posttreatment) | Group III (bleaching + protocol 1) | Group IV (bleaching + protocol 2) | Group V (bleaching + protocol 3) |
|---------------------|-----------------------------|------------------------------------------|-----------------------------------|-----------------------------------|----------------------------------|
| Adhesive (%)        | 11 (91.7)                   | 12 (100)                                 | 10 (83.3)                         | 10 (83.3)                         | 9 (75)                           |
| Cohesive of dentin (%) | 0                           | 0                                       | 0                                 | 1 (8.3)                           | 1 (8.3)                          |
| Mixed (%)           | 1 (8.3)                     | 0                                       | 2 (16.7)                          | 1 (8.3)                           | 2 (16.7)                         |

Table 3: The averages and standard deviations of atomic percentage (wt%) each element analysis in experimental groups

| Chemical elements | Group I (without bleaching) | Group II (bleached without posttreatment) | Group III (bleaching + protocol 1) | Group IV (bleaching + protocol 2) | Group V (bleaching + protocol 3) |
|-------------------|-----------------------------|------------------------------------------|-----------------------------------|-----------------------------------|----------------------------------|
| Carbon            | 25.45±3.7                   | 18.4±2.2                                 | 24.8±2.1                          | 29.01±7.9                         | 25.7±9.5                         |
| Oxygen            | 30.64±4.0                   | 53.15±4.5                                | 44.15±9.5                         | 35.4±4.9                          | 34.8±5.3                         |
| Sodium            | 3.6±0.12                    | 0.45±0.15                                | 4.25±0.12                         | 5.39±0.1                          | 5.54±0.31                        |
| Magnesium         | 1.85±0.32                   | 1.0±0.2                                  | 1.3±0.2                           | 1.90±0.13                         | 2.00±0.3                         |

The largest difference was observed in GI (bleached dentin, without posttreatment). In this group, oxygen concentration reached 53.15%, and there was a reduction of the amount of carbon. The posttreatment with SA by Protocol I (GIIV) showed a reduction in oxygen levels on surface, being in average in 44.15%. In bleached surface treated with SA by Protocols 2 (GIV-35.4%) and 3 (GV-34.8%), the oxygen concentration of the dentin reached values close to the specimens that did not receive the bleaching treatment (GI-30.64%, control).

The concentrations of calcium (Ca) and phosphorus (P) remained stable in all groups, and the concentration of sodium (Na) was above 4% in specimens that received the treatment with SA (GIIV, GIV, and GV).

**Surface morphology by scanning electron microscopy**

The SEM images showed morphological differences in the dentin surface between the unbleached specimens (GI-positive control), specimens bleached with 35% HP (GIIV-negative control), and other specimens that were bleached and treated with 10% SA solution at different protocols (GIIV, GIV, and GV).

It was verified that the control group (GI, unbleached) had homogeneous surface, covered by smear layer and obliterated dentinal tubules [Figure 1a]. In the specimens bleached with 35% HP (GIIV), the surface was covered by smear layer, but less regularly, with cracks and small fissures, probably due to the action of the bleaching gel [Figure 1b]. In those bleached and treated with 10% SA, it was also observed the presence of smear layer, but GIIV had larger fissures in dentin surface and few residual granules from SA solution [Figure 1c]. In GIV, it was observed fewer fissures and larger amount of residual granules [Figure 1d] while GV exhibited fewer fissures and a large amount of residual granules from SA [Figure 1e].

**DISCUSSION**

Esthetic interventions, such as replacement of preexisting restorations after bleaching, are often necessary since the bleaching agent does not alter the color of the restorative material. However, the chemical reactions that occur in the bleaching process can change the dental surface, interfering in the formation of resin tags and negatively affects the adhesiveness of the restorative material on the substrate.

SA is a biocompatible, nontoxic, and neutral antioxidant able to reduce a wide variety of oxidizing compounds, mainly the free radicals, thus can restore the redox potential of oxidized substrate. Recently, Lima et al. found slight reduction in the initial pulp damage when using oral ingestion of ascorbic acid before bleaching.
bleached and treated with SA by Protocol 2 (dripping, leave act, and aspirating) and Protocol 3 (dripping, rubbing, and aspirating). The lowest values were obtained in the dentin that was only bleached (negative control). These findings evidenced that the bleaching agent affected negatively the bond strength of restorative material to dentin. This can be explained by the presence of OH-radical, responsible for bleaching. For being a strong oxidizing agent with high penetration power, this radical acts on intertubular and peritubular dentin through the degradation of organic portion, breaking the polypeptide chain of the dentin matrix. The residual oxygen resulting from the decomposition of bleaching agent inhibits the polymerization of adhesive materials and reduces the bond strength of the resin to the dental structures.

In the groups that received the posttreatment with 10% SA solution, the bond strength was reestablished. The SA is a salt of ascorbic acid (vitamin C) with molecular formula $\text{C}_6\text{H}_8\text{O}_6$. Its main function is the hydroxylation of collagen, in addition, to be a potent antioxidant, being used to transform the oxygen free radicals into inert forms. In this study, the treatment with SA on bleached dentin was able to reduce the residual oxygen from surface, especially when using active protocols (GIV and GV). It is speculated that the friction of the solution on the surface by the total time of 10 min contributes to the penetration of SA in dentin, in sequence, the rinsing of the surface, followed by aspiration with high-power suction, may results in the removal of residues from dentin, mainly the free oxygen.

Some studies evaluated the use of 10% SA solution in dentin immediately after the bleaching procedure. Recently, the application of 35% SA for 2 min reversed the negative effect of 35% HP on resin bond strength to dentin. In our study, the 10% SA enhances the bond strength of the restorative material to dental substrate. The analysis of the failure pattern after the bond strength test showed a higher percentage of adhesive failures, emphasizing the fragility of the bonding interface. Basting et al. observed a higher percentage of adhesive failures in groups bleached with 10% and 22% carbamide peroxide. Previous studies verified the predominance of adhesive failures in the experimental groups that used at high-concentrated bleaching agents.

In the EDS analysis, it was found that the oxygen concentration reached 53.15% in GII (dentin only bleached), and this concentration was reduced in groups treated with SA (GIII-44.15%, GIV-35.4% and GV-34.8%). The EDS (energy dispersive X-ray) is an essential accessory in the microscopic characterization of biological materials. A detector installed in the vacuum chamber of SEM measures the energy associated with the electrons. The use of the EDS together with the SEM is of great importance in the characterization and mapping of the distribution of chemical elements in the surface.

It was observed under SEM that the specimens treated with 10% SA solution had smear layer with less fissures when compared with the other groups and was found residual granules of the solution. In the specimens only bleached with 35% HP (GII), the surface was covered by the smear layer, but with some fissures, probably due to the action of the bleaching gel. Vieira et al. also found these fissures on dentin surface bleached with 35% HP. The results of this study demonstrate that HP at high concentrations may reduce the adhesiveness between dentin and restorative materials, but the active protocols with antioxidant agents such as SA can restore the bond strength, allowing the esthetic restoration be performed in the same session of the dental bleaching.

In the oral cavity, there are several forces acting simultaneously on a restoration and in the long-term may negatively affect the bond strength. Further studies should be conducted to evaluate the potential of other antioxidants on tooth surface, to minimize the undesirable effects of HP.

**CONCLUSION**

The use of 10% SA in the intracoronary dentin bleached with high concentration agent reestablishes the bond strength between dentin and restorative material, especially if active protocols were used during application and aspiration of the solution.

**Clinical significance**

Clinicians should be aware that SA solution applied on bleached dentin of endotype restorations can be an alternative protocol to restore the adhesiveness capacity and therefore, the composite restoration could be performed in the same session of bleaching treatment.

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**Conflicts of interest**

There are no conflicts of interest.

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