Effect of sodium metabisulfite gel on the bond strength of dentin of bleached teeth

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ABSTRACT

Objective: This study aimed to evaluate the effect of the application of sodium metabisulfite (SMB) on the bond strength of bleached teeth. Materials and Methods: The study was divided into two parts. The first part evaluated the application of various concentrations of SMB for 1 h prior to the completion of bonding procedures. Fifty blocks were divided into five groups (n = 10): control; bleaching with 35% hydrogen peroxide (HP); HP + 5% SMB; HP + 12.5% SMB; and HP + 25% SMB. The second part evaluated the application of 25% gel SMB to either enamel or dentin, including the application time. Sixty blocks were divided into six groups (n = 10): control; bleaching with 35% HP; HP + 25% SMB for 1 h in enamel; HP + 25% SMB for 1 h in dentin; HP + 25% SMB for 10 min in enamel; and HP + 25% SMB for 10 min in dentin. Statistical Analysis: Following the completion of microshear bond testing, data were analyzed using one-way analysis of variance as well as Tukey’s and Dunnett’s tests. Results: In part 1, data analysis revealed statistical differences (P < 0.0001) between HP and HP + 5% SMB. No statistical differences were found between the control and both HP + 12.5% SMB and HP + 25% SMB. Part 2 revealed a statistical difference (P = 0.001359) only between the bleached group and others. Conclusions: The use of 25% SMB gel immediately after bleaching was able to reverse the deleterious effect of bleaching on the bond strength of dental composites to dentin.

Key words: Antioxidant, bond strength, free radicals, microshear

INTRODUCTION

Bleaching of vital teeth is a conservative and effective technique for improving dental esthetics. This treatment can be performed in clinical practice, through a supervised at-home technique, or even through a combination of techniques. The substance responsible for tooth bleaching is hydrogen peroxide (HP, H₂O₂). When applied to the teeth, HP diffuses into the dental structure and breaks down into free radicals. Free radicals are usually reactive species because they contain one or more unpaired electrons in their outer electronic shell. Some radicals react with the double bonds of organic molecules that are responsible for tooth pigmentation, and such chemical modifications change the absorbed light spectrum and increase water solubility, thus leading to bleaching.

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Often after bleaching, continuing the esthetic treatment requires restorative procedures with resin materials. However, it is known that when such procedures are performed immediately following the bleaching treatment, the bond strength of the resin to the dental substrate is impaired, which is undesirable. This phenomenon has been attributed to the long-lasting free radicals derived from the HP present in the dental substrate.

A 2-week period is recommended before any restorative procedure is performed. During this time, saliva can reverse the shear bond strength values by presumably leaching the free radicals present in the dental elements. However, restorative procedures often need to be clinically performed immediately following bleaching. Thus, the search for a clinically practical procedure that reverses the effect of the remaining oxidants is an area of interest. The use of several different antioxidants has been proposed for chemically reducing free radicals to harmless compounds, thus minimizing their deleterious effects on composite resin polymerization.

Numerous reducing strategies have been tested to circumvent such a clinical problem, with satisfactory results reported for sodium ascorbate, catalase, grape seed extract, alpha tocopherol, and green tea. Despite some reducing agents’ effectiveness, none of them can be clinically used, probably due to their short shelf-lives. In addition, the lack of clinical evidence and of the standardization of the results point to the need for more studies to find safer and more effective substances for quickly removing oxidants after teeth bleaching, thus allowing a restorative procedure to be performed shortly thereafter.

The reactivity of sodium metabisulfite (SMB) reducing equivalents with oxidants makes SMB useful in the food industry as an antibacterial agent and for preventing the browning of foods due to oxidation. SMB is also used as a preservative agent in fruits, vegetables, seafood, and beverages, such as refreshments, wine, and beer. Many injectable medications, such as antibiotics, analgesics, corticosteroids, and bronchiodilators, have sulfite salt as excipients. In dental practice, the clinician uses SMB routinely to preserve vasoconstrictor agents for local anesthetics. Despite being a rare condition, sulfites can cause allergic reactions, especially in asthmatic individuals. The hypersensitivity of the causative mechanism is not clear but appears to be dose dependent. An average person consumes 2–3 mg of sulfites per day. The most common cause of allergy involves sulfite-based products, which are applied topically. Thus, the quantity proposed in this study (i.e., 22 mg of SMB per specimen) seems to be proven safe, as it is present in both foods and medicines.

However, the nature of the long-lasting oxidant remaining on the dental substrate is not clear, as it remains uncertain whether this oxidant is a free radical. In particular, the product of HP homolytic breakdown is the hydroxyl radical, which is extremely reactive. It oxidizes and abstracts the hydrogen atom and adds to organic molecules’ double bonds rapidly. Consequently, the hydroxyl radical has a short life (in the microsecond range) and thus cannot remain in the dental structure for weeks. HP itself is a better candidate. In addition, the light used in the resin polymerization step could probably lead to hydroxyl radical production from trapped HP. Considering this hypothesis, it would be relevant to use a well-known chemical agent to react with HP as an antioxidant. The current study evaluated the effects of SMB-loaded gel on the bond strength of the resin and dentin of teeth previously submitted to bleaching treatment. SMB is an antioxidant widely used in the pharmaceutical and food industries as a preservative agent, and it reacts with HP rapidly, thus producing the harmless and water-soluble sulfate anion. The null hypothesis tested was that the use of SMB does not influence the bond strength of the dentin of bleached teeth.

**MATERIALS AND METHODS**

**Specimen preparation**

As part of the specimen preparation process, 110 recently extracted bovine incisors were stored in a 0.1% thymol-buffered solution and distilled water after being collected and disinfected. These teeth were examined under a four-time magnifying glass (Carl Zeiss, Santo Amaro, Brazil) to check for cracks or staining, which could have possibly influenced the research results. If a flaw was found, the tooth was discarded and replaced. Then, the remaining teeth were stored in distilled water under cooling until they were ready to be used.

The crowns were separated from the roots using a double-sided diamond disc (KG Sorensen, Barueri, SP, Brazil), which was mounted on a handpiece coupled with an electric micromotor (LB-2000 Beltec, Araraquara, SP, Brazil) operating under constant irrigation.
Through the use of a metallographic cutter (Isomet 1000, Buehler, IL, USA) equipped with a diamond saw, the crowns were sectioned to obtain dental blocks of 6.5 mm × 6.5 mm. After the blocks were obtained, they were flattened using a rotary polisher (AROTEC, Cotia, SP, Brazil) equipped with 400-grit silicon carbide paper (Norton, São Paulo, Brazil) so that each portion of the substrate (i.e., enamel and dentin) was 1.2 mm thick.

The edges of the specimens were embedded in self-curing acrylic resin (Ortho Class, Classic, SP, Brazil), with the major surfaces of enamel and the opposite dentin exposed. Following their inclusion, the specimens were flattened again using 400-grit SiC paper to remove any resin excess remaining on the substrate surface. Next, the enamel facets were polished with 600- and 1200-grit SiC sandpaper and felt discs (TOP, RAM, and SUPRA, AROTEC, Cotia, SP, Brazil) associated with diamond paste (1.0 μm, ½μm, and ¼μm – AROTEC Cotia, SP, Brazil). Between the use of each sandpaper piece and felt disc, the specimens were washed in an ultrasonic tank (Marconi, Piracicaba, SP, Brazil) for 12 min. To standardize the smear layer on the dentin, the specimens were ground with 600-grit SiC paper for 1 min.

This study was divided into two parts as follows: In the first part, the antioxidant was tested at different concentrations. For this purpose, fifty specimens were divided into five groups (n = 10) according to the manufacturer’s instructions, that is, three applications of bleaching gel for 15 min each in a layer of approximately 1 mm.

Thus, the standardization of the gel volume was applied to each specimen. About 0.045 ml of bleaching gel was enough to create a layer of gel with a thickness of 1 mm, as determined by the manufacturer. This volume was applied with the aid of a 1-ml insulin syringe (Injex Safety - Insulin syringe, Injex, Ourinhos, Brazil).

After the three applications, the specimens were thoroughly washed with water and stored in distilled water at 37°C. The bleaching procedure was repeated after 7 days. The specimens were stored again in distilled water and placed in an oven at a temperature of 37°C ± 2°C for a period of 24 h.

**Antioxidant application**

Various antioxidant concentrations were obtained in gel form using Natrosol (hydroxyethylcellulose) thickener before application as described in the division of groups.

Part 1: After 24 h of storage, the groups submitted to bleaching with SMB (HP + 5% SMB, HP + 12.5% SMB, and HP + 25% SMB) were immersed in a plastic cup containing 1.44 ml of antioxidant gel. After 1 h of immersion, the specimens were thoroughly washed in water for 1 min.

Part 2: After 24 h of storage, applications of SMB gel only were carried out on the surface of the enamel (HP + 25% SMB for 1 h in enamel, HP + 25% SMB for 10 min in enamel) or dentin (HP + 25% SMB for 1 h in dentin, HP + 25% SMB for 10 min in dentin). These substrates were delimited with light-cured resin (Top Dam, FGM, Joinville, Brazil). This delimitation was made so that the gel did not rise out of the substrate during the application period and during the standardization of the application volume (0.088 ml) with the aid of an 1-ml syringe insulin. After the removal of the delimitation, the SMB was removed and the specimens were thoroughly washed in running water for 1 min. The volume of 0.088 was defined in a previous pilot study. This volume was sufficient to fill the specimen surface (6.5 mm × 6.5 mm). Furthermore, the area of the specimen surface was set to correspond to the area of the clinical crown of a human tooth.

**Adhesive procedures**

Immediately following the antioxidant application treatments, the dentin facets of all specimens and controls received two resin pillars to be shear tested.
An adhesive tape with two holes of 1.1 mm was used to delimit the area where the bonding was completed.[24] The adhesive procedure followed the manufacturers’ recommendations. The conditioning of the dentin was performed with 37% phosphoric acid (Condac, Joinville, Brazil) for 15 s, followed by rinsing with water for 15 s. Moisture was maintained with cotton balls. Two consecutive layers of adhesive (Single Bond 2, 3M™ ESPE™, St. Paul, MN, USA) were actively applied to the substrate for 15 s. The adhesives were gently air-dried for 5 s. Prior to adhesive polymerization, cylindrical matrices made of perforated noodles each with a diameter of 1.1 mm and a height of 1 mm (Furadinho 6, Pastificio Santa Amalia, SP, Brazil) were positioned onto the bounding tape’s adhesive holes.[25] The adhesive was light cured for 10 s at 618 mW/cm² (FLASH lite 1401, Discus Dental, Culver City, CA, USA). The matrices were then filled with flowable resin composite (Filtek Z-350, 3M™ ESPE™, St. Paul, MN, USA), which was then light-cured for 40 s. Following 2-h storage in distilled water, the matrices were removed along with the adhesive tape.

**Microshear test**

After 24-h storage in distilled water at 37°C, the specimens were placed in a microshear device coupled with a universal testing machine (EZ Test – Shimadzu, SP, Brazil) operating at a 5-N load cell and a speed of 0.5 mm/min. The values found in kilograms-force were converted into Megapascals (MPa). For the analysis of the fracture pattern, the specimens were analyzed in stereomicroscopy at 50-times magnification (Leica Microsystems, Wetzlar, Germany). The fractures were classified as follows: adhesive, cohesive in resin, cohesive in dentin, and mixed (i.e., two or more types of fractures).[24]

**Statistical analysis**

Part 1: Following the exploratory analysis, the data were submitted to one-way analysis of variance (ANOVA). The comparison between the control group and the groups with bleaching was performed using the Dunnett’s test. The degrees of freedom of the SMB concentration were deployed in ANOVA, testing the linear, quadratic, and cubic effects.

Part 2: After the exploratory analysis, the data were submitted to ANOVA according to the 2 × 2 + 2 (substrate × time) + controls. Comparisons with the control groups were performed using the Dunnett’s test.

All analyses were performed in the R* program considering a level of significance of 5%. *R Core Team (2017). A: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

**RESULTS**

In part 1, only the linear effect was significant (P<0.0001). One can see that a statistically significant linear increase in the bond strength occurred with an increasing concentration of SMB, $R^2 = 0.9317$. The application of 5% SMB after bleaching did not differ statistically from the group of bleached specimens only. Applications of 12.5% and 25% SMB did not differ statistically from the untreated group (P > 0.05). These results are shown in Figure 1.

Adhesive fracture was the dominant fracture pattern in all groups as shown in Table 1. The groups bleached with 35% HP and HP + 5% SMB differed statistically from the control group in terms of bond strength, revealing higher rates of adhesive fractures compared with other groups. Only a cohesive fracture in resin was found in the HP + 12.5% SMB group.

In part 2, it can be observed that the groups that received SMB 25%, for both the enamel and the dentin, differed significantly from the control group in terms of whitening (HP), P < 0.05. In addition, no statistical differences were found between all experimental groups and the control group. Furthermore, no difference was found between the application times (10 min or 1 h) and between the substrates (i.e., enamel or dentin) to which SMB gel was applied. The results are shown in Figure 2.
In part 2, adhesive fracture was the most predominant fracture pattern [Table 2], especially in the group with bleached specimens only. Only one cohesive fracture was found in resin in the HP + 25% SMB group for 10 min in dentin.

**DISCUSSION**

The null hypothesis that the SMB gel would not affect the bond strength to bleached dentin was partially accepted because the gel application to 5% for an hour was not able to change the bond strength. All the other concentrations and application times were able to reestablish the bond strength to bleached dentin.

The remaining free radicals used for tooth bleaching have a negative influence on the bond strength of composite to the dental substrate,[6] a finding also confirmed through our study [Figure 1]. Such negative effects have been attributed to HP-derived oxidant species that would oxidize resin monomers and polymers, thus potentially interfering with all steps of polymerization (initiation, propagation, and termination), perhaps even changing the very chemical nature of the resin polymers and their bonds to the dental substrate.[6,8]

The major problem with the strategy employed so far is that it is based on the outdated medical concept of generic antioxidant therapy, which has failed to revert the negative effects of oxidants on human health and aging.[26,27] Reactive oxygen species is a general term used to encompass compounds derived from oxygen by partial reduction (superoxide, HP, hydroxyl radical, etc.), but it often misleads people to treat each of them as a single equally reactive chemical entity. In fact, these species have quite distinct physical and chemical properties. Effective antioxidant therapies or treatments are likely to be more effective when the “reactive species” causing the problem is known, so the reducing agent can be selected considering its specific reactivity.

The dental restorative problems that occur following an HP bleaching procedure can certainly benefit from this new concept. The oxidant responsible for poor dental and resin bond interaction is not known, but it can be inferred from sample histories and from information collected from the current and previous studies. The bond strength problem persists for at least 2 weeks; thus, the HP-derived oxidant should be chemically stable and must be able to strongly associate with the dental structures, being slowly lixiviated by saliva. This rules out superoxide or hydroxyl radical, for example, as they are both short-lived species. Among the possibilities, HP itself is a better candidate, as it gathers such properties. Indeed, HP is stable[22] and can probably substitute structural water in dental substrates given their shared physical properties. In addition, it is likely to be activated by the light used to promote resin polymerization during restorative procedures, generating a hydroxyl radical.

**Table 1: Fracture pattern in part 1**

| Fracture pattern | Control | HP | HP + 5% SMB | HP + 12.5% SMB | HP + 25% SMB |
|------------------|---------|----|-------------|----------------|--------------|
| Adhesive         | 10      | 13 | 14          | 9              | 9            |
| Mixed            | 5       | 6  | 3           | 5              | 7            |
| Cohesive in dentine | 5   | 1  | 2           | 6              | 4            |
| Cohesive in resin |        |    |             | 1              |              |

*HP: 35% hydrogen peroxide application, SMB: Application of sodium metabisulfite according to concentrations*

**Table 2: Fracture pattern in part 2**

| Fracture pattern | Control | HP | HP + SMB 1 h E | HP + SMB 1 h D | HP + SMB 10 min E | HP + SMB 10 min D |
|------------------|---------|----|---------------|----------------|-------------------|-------------------|
| Adhesive         | 10      | 15 | 9             | 9              | 8                 | 9                 |
| Mixed            | 6       | 2  | 4             | 6              | 6                 | 5                 |
| Cohesive in dentine | 4   | 3  | 7             | 4              | 6                 | 6                 |
| Cohesive in resin |        |    |               |                |                   |                   |

*HP: 35% hydrogen peroxide application, SMB: Application of sodium metabisulfite according to concentrations, E: Antioxidant application gel in enamel, D: Antioxidant application gel in dentin*

**Figure 2:** Mean and standard deviation of bond strength as a function of substrates and application times of sodium metabisulfite 25%, in part 2. All groups differed from and hydrogen peroxide group—35% hydrogen peroxide (P < 0.05). No significant difference was found between times or substrates (P > 0.05). The gray ribbon represents the standard deviation.
The relative effectiveness of catalase also points in this direction, as this hemeprotein is a rather specific enzyme for HP removal.

However, the use of an HP-reducing agent could contribute to an improvement in the bond strength between composites and bleached structures. SMB undergoes rapid hydrolysis when dissolved in water, releasing two parts of its reducing active equivalents, which is either sulfur dioxide (SO_2) or the anions of hydrogen sulfite (HSO_3^-) or sulfite (SO_3^{2-}), depending on the pH. In the specific case of reducing experimental gel (pH ≤ 7), sulfite anions are the more relevant species. Nevertheless, all SMB-reducing active equivalents react with HP, yielding water plus the water-soluble sulfate anion.

The decision to perform the specimen immersion in gel rather than completing a gel application, as in the second part of the study, was made to test the experimental gel’s effectiveness at various concentrations. Then, subsequently, time and substrate applications could be evaluated in part 2. Therefore, the immersion of the sample in SMB gel at 5% for 1 h was not effective in reestablishing the bond strength values in dentin. This may be due to the low concentration of SMB in the gel when the remaining HP at the substrate was sufficient to interfere with the polymer chain formation. The highest concentrations of 12.5% and 25% were able to reestablish the bond strength, which corroborates the hypothesis that the concentration of 5% SMB is insufficient for this purpose.

The HP is a molecule with a low molecular weight capable of diffusing the dental structure. According to Eimar et al., the reaction responsible for the color change of the dental substrate occurred in the organic part of the tooth, whose concentration was greater in the dentin. Therefore, a greater concentration of HP was expected to be found in the dentin, which explains the choice to proceed with dentin bonding in both parts of the study. In the substrate, the antioxidant could actually be tested due to higher concentrations of free radicals; thus, the application to enamel was made to evaluate the power of the diffusion of SMB and the application to dentin was performed to simulate the application of the antioxidant gel directly into a cavity. Therefore, a wide range of clinical application possibilities were contemplated in this study. This finding corroborates that of Ismail et al., who observed that the negative effects of bleaching on composite bonding can be neutralized through the application of a reversing agent in dentin, thus increasing the efficiency of clinic chair time. This is clinically relevant for those patients requiring restorative treatment immediately following in-office bleaching.

Although immersion in SMB gel at 12.5% was also effective, a concentration of 25% for the application was chosen for the second part of the study, when its time would be reduced to 10 min. Besides the application in enamel so that bonding procedure was performed on dentin, this could require a higher concentration of SMB, as the first part of the study demonstrated that its concentration is important for bond strength reestablishment. The gel of SMB at 5% was not effective. According to Dishman et al., the waiting period for the substrate to return to a normal bond strength depends on the concentration of HP and on the time of contact with the substrate. This study performed two HP applications at 35%; although in clinical situations, more HP applications may be needed to achieve a satisfactory change in tooth color. Another important consideration is that the amount of dentin in a dental element is larger than the specimen. Dentin, as stated above, has greater organic content and may have more remaining HP. Therefore, the gel at 25% in part 2 was chosen to completely ensure the reversion of bond strength values in cases where greater applications of HP were made.

In the second part of the experiment, the groups that received 25% SMB gel used only 0.088 ml of SMB per specimen. In a clinical situation, the amount applied per dental element would be close to 0.088 ml per tooth. It is also possible that with in-office bleaching, the experimental gel is applied while soft tissues are protected with a light-cured gingival barrier, thus preventing the risk of contact between the gel and soft tissue and making the clinical application safe for patients with hypersensitivity to sulfites. Another possibility for antioxidant gel application prior to a restorative procedure is to use SMB gel in the same tray for tooth bleaching in the case of the at-home bleaching technique. However, the authors believe that the best technique for the application of antioxidant gel would be to apply it directly into a prepared cavity prior to the application of the restorative composite. Thus, the clinician can control the gel application based on the smallest possible amount of gel in direct contact with the area to be restored.

A study evaluated the use of sodium ascorbate in Carbopol®, a thickening polymer, gel, or solution,
and no statistical difference was found between the formulation types. Sodium ascorbate solution is difficult to handle and requires several applications prior to the bonding procedure. Thus, the gel form was proposed in that study. The polymer used as a thickener excipient was Natrosol (hydroxyethylcellulose), which is a cellulose ether derivative. Natrosol is water soluble at room temperature and is presented in gel form, being widely used in the cosmetic and pharmaceutical industries as a stabilizer and emulsifier. This polymer has nonionic characteristics, and its use may be recommended in acidic substances, as it has high pH stability (2.0–12.0). In addition, Natrosol is more convenient for clinical work with an antioxidant in gel form, as it allows the gel application to be localized and controlled.

The substance to be used as a reducing agent prior to bonding procedures must have a low molecular weight to be spread over and permeate through the dental substrate. HP has a molar mass of 34 g/mol, whereas ascorbate, the most-studied reducing agent, has a molecular weight of 175.11 g/mol and perhaps cannot permeate dental structures. In addition, ascorbate has a poor reactivity toward HP. The hydrogen sulfite/sulfite anion released through SMB hydrolysis is water soluble and has a molar mass of 81/80 g/mol. Thus, it can probably spread and access the dental substrate due to its humid environment. In part 2, the HP + 25% SBM group for 1 h and the HP + 25% SBM group for 10 min had the gel applied to the enamel and dentin submitted to mechanical testing, yet the bond strength values were reestablished, thus confirming its ability to penetrate the substrate for a distance of at least up to 2.4 mm.

The groups that were submitted to the bleaching procedure (negative controls of both parts) and the HP + 5% SMB group, in part 1, resulted in bond strength values similar to those of bleached specimens, which have shown more adhesive failures. Turkun and Kaya showed that the bleached teeth had their adhesive interfaces analyzed with scanning electron microscopy, showing a granular and porous appearance similar to bubbles. Such bubbles probably stem from the imprisonment of the bleaching agent in the surface layer of the substrate. In view of this, according to the Griffith’s theory, failures embedded in the specimen (e.g., bubbles) lead to fracture, as the load applied during the mechanical test is concentrated on these defects, as seen in the present study. This explains the high adhesive failure rates in the groups that were bleached, where the antioxidant could not reestablish bond strength values statistically similar to those of the control group.

The dental blocks were stored in distilled water because saliva can reverse the altered bond strength values as can catalase, the natural antioxidant that contributes to removing HP. However, the time for the reestablishment of the bond strength ranges from 24 h to 14 days. Composite resins do not have their color changed through the bleaching procedure as the time for leaching free radicals and for reestablishing the strength bond values becomes an esthetic discomfort to the patient whose restorations must be replaced. In addition, an in vitro study revealed a greater diffusion of peroxide into the dentin of a restored anterior tooth. Thus, it is extremely important to find an agent capable of reestablishing bond strength values in an acceptable clinical time frame, thus allowing for the completion of the esthetic treatment in a shorter time period. Furthermore, restorations whose bond strength is altered immediately following a bleaching treatment allow for greater microleakage. The use of 12.5 and 25% SMB gel proved to be able to neutralize the effects of oxidants after application times of 1 h or 10 min in both the dentin and the enamel. The use of a reducing agent for 10 min prior to the completion of an adhesive restorative procedure is a clinically feasible time period.

**CONCLUSIONS**

The use of 25% SMB gel immediately after bleaching was able to reverse the deleterious effect of bleaching on the bond strength of dental composites to dentin.

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**Conflicts of interest**

There are no conflicts of interest.

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