

Universidade Estadual de Campinas

Faculdade de Odontologia de Piracicaba

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# Efeito de um gel antioxidante experimental na resistência de união à dentina de dentes clareados

EFFECT OF AN EXPERIMENTAL ANTIOXIDANT GEL ON BOND STRENGTH OF DENTIN OF BLEACHED TEETH

> Piracicaba 2016

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Tese apresentada à Faculdade de Odontologia de Piracicaba, da Universidade Estadual de Campinas como parte dos requisitos exigidos para obtenção do Título de Doutor em Clínica Odontológica, na Área de Dentística.

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**Orientadora: Profa. Dra. Debora Alves Nunes Leite Lima** Este exemplar corresponde à versão final da Tese de doutorado defendida pelo aluno Henrique Heringer Vieira e orientada pela Profa. Dra. Debora Alves Nunes Leite Lima

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A Ata da defesa com as respectivas assinaturas dos membros encontra-se no processo de vida acadêmica do aluno. Dedicado à Graziela Meneghetti

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"A grandeza não está na linha de chegada. Está no caminho até ela."

## Resumo

Neste trabalho foi avaliado o efeito da aplicação de um gel antioxidante experimental a base de metabissulfito de sódio (MBS) na resistência de união de resina composta à dentina de dentes bovinos clareados. O trabalho foi dividido em duas partes. A primeira parte avaliou a concentração do gel antioxidante de metabissulfito de sódio, em seguida avaliou-se o tempo e o substrato dental em que o antioxidante foi aplicado. Para as duas partes foram utilizados blocos dentais. O clareamento foi realizado em esmalte e a confecção dos pilares de resina para ensaio de microcisalhamento em dentina. Para a confecção dos pilares de resina composta utilizou-se adesivo Single Bond (3M ESPE) e resina "flow" Filtek Z 350 (3M ESPE). A primeira parte avaliou diferentes concentrações de gel antioxidante (5; 12,5 e 25%) por meio da imersão dos blocos no gel por uma hora, imediatamente previamente ao procedimento restaurador. Para isto, 24 horas após o clareamento cinquenta blocos dentais foram divididos em cinco grupos (n=10): G1A: Sem tratamento; G2A: Clareamento com Peróxido de Hidrogênio 35% (PH); G3A: PH + MBS 5% ; G4A: PH + MBS 12,5%; G5A: PH + MBS 25%. Na segunda parte avaliou-se o substrato dental em que o gel a 25% foi aplicado, esmalte ou dentina, e tempo de aplicação. Sessenta blocos dentais foram divididos em 6 Grupos (n=10): G1B: Sem tratamento; G2B: Clareamento com PH 35%; G3B: PH + MBS 25% por 1 hora em esmalte; G4B:PH + MBS 25% por 1 hora em dentina; G5B:PH + MBS 25% por 10 minutos em esmalte; G6B: PH + MBS 25% por 10 minutos em dentina. Todos os grupos de ambas partes do experimento foram submetidos ao teste de microcisalhamento e à análise do padrão de fratura em lupa estereoscópica (Leica Microsystems). As fraturas foram divididas em adesivas, coesivas em dentina, coesivas em resina e mistas. Os dados de ambas fases foram submetidos à ANOVA um fator e testes de Tukey e Dunnett ( $\alpha$ =0,05%). A análise dos dados da parte um encontrou diferença estatística (p < 0.0001) entre os grupos G2A e G3A e os grupos G4A, G5A e G1A. Assim, o clareamento afetou negativamente a resistência de união, e a aplicação de MBS 12,5 e 25% por uma hora é capaz de reverter estes valores alterados, sem diferença estatística entre estas concentrações. Os resultados da parte dois demonstraram diferença estatística (p= 0,001359) apenas entre o G2B e os demais. Não foi encontrada diferença entre os tempos de aplicação (10 minutos ou 1 hora), bem como entre os substratos em que o gel de MBS foi aplicado (esmalte ou dentina); todos os grupos

testados não diferiram estatisticamente do grupo controle. Ambos os experimentos apresentaram altas taxas de fraturas adesivas. Conclui-se que a aplicação de MBS a 25% por 10 minutos no substrato clareado a ser restaurado é capaz de reverter a redução da resistência de união à dentina causada pelo clareamento dental.

Palavras-Chave: Clareamento Dental. Radicais Livres. Antioxidantes. Dentina.

#### ABSTRACT

In this study was evaluated the effect of the application of an experimental antioxidant gel based on sodium metabisulfite on the bond strength of composite resin to the dentin of bonvine bleached teeth. The study was divided into two parts. The first part evaluates the concentration of the antioxidant gel, and then it was evaluated the time and the application substrate. Dental blocks were used for both parts. Bleaching was performed in enamel and resin pillars used in dentin for microshear testing. For building of the resin composite pillars was used Single Bond (3M ESPE) and resin flow Filtek Z 350 (3M ESPE). The first part evaluated different concentrations of antioxidant gel (5; 12,5 and 25%) by immersing the blocks in gel for an hour immediately prior to the bonding procedures. For this, 24 hours after bleaching fifty blocks were divided into five groups (n = 10): G1A: no treatment; G2A: bleaching with 35% hydrogen peroxide (HP); G3A: HP + 5% SMB; G4A: HP + 12.5% SMB; and G5A: HP + 25% SMB. The second part evaluated the application of 25% gel SMB to either enamel or dentin, including application time. Sixty blocks were divided into six groups (n = 10): G1B: no treatment; G2B: bleaching with 35% HP; G3B: HP + 25% SMB for 1 hour in enamel; G4B: HP + 25% SMB for 1 hour in dentin; G5B: HP + 25% SMB for 10 minutes in enamel; and G6B: HP + 25% SMB for 10 minutes in dentin. All groups of both parts of the experiment were submitted to microshear test and were subjected to standard analysis of fracture in a stereomicroscope (Leica Microsystems). Fractures were divided into adhesive, cohesive in dentin, cohesive resin and mixed. Both parts of experiment were analysed by using one-way ANOVA, and Tukey's and Dunnett's tests ( $\alpha$ =0,05%). Data analysis showed statistical differences (P < 0.0001) between G2A and G3A as well as between G1A and both G4A and G5A. Thus, bleaching affected negatively the shear bond strength, and the application of 12.5% and 25% SMB for one hour can reverse these values, without statistical difference between these concentrations. The results of part two showed statistical difference (P = 0.001359) only between bleached group and others. There was no difference between application times (10 minutes or 1 hour) as well as in none of the groups using SMB gel (enamel or dentin), all groups tested did not differ statistically from the control group. Both experiments have high rates of adhesive fractures. The application of 25% SMB for 10 minutes in the bleached substrate to be bonded is able to reverse the decrease in the bond strength caused by tooth bleaching.

Keywords: Tooth Bleaching. Antioxidants. Free Radicals. Dentin.

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#### 1 INTRODUÇÃO

O clareamento dental afeta negativamente a resistência de união de compósitos restauradores ao substrato (1). O peróxido de hidrogênio, substância responsável por este clareamento, quando em contato com o dente se difunde por estes tecidos e se quebra em radicais livres (2). Radicais livres são agentes oxidantes, ou seja, possuem a tendência de se ligar a outras moléculas para conseguir estabilidade. Os compósitos resinosos possuem em sua composição monômeros com os quais estes radicais livres tendem a se ligar diminuindo a resistência de união das restaurações (1,3).

Duas propostas tem sido estudadas a fim de reverter este efeito deletério do clareamento dental. A primeira é a espera de algum tempo para que os radicais livres sejam eliminados do substrato (4,5), contudo esta proposta não corresponde aos anseios dos pacientes que necessitam de substituição de restaurações com cores destoantes da cor do elemento dental clareado. A outra abordagem é a aplicação de agentes antioxidantes, que são substâncias com a capacidade de se ligar aos radicais livres tornando-os inativos. Alguns antioxidantes já foram testados na literatura (6–8) como ascorbato de sódio (9–11), catalase(6), extrato de sementes de uva (7), alpha tocoferol (10) e chá verde (8). Entretanto ainda não foi encontrado um consenso quanto à utilização destes para reverter a resistência de união prejudicada pelo procedimento clareador.

Apesar da variedade de substâncias, os antioxidantes possuem vida de prateleira curta, o que pode dificultar sua aplicabilidade clínica (12). A diversidade das substâncias testados (6), a falta de padronização da literatura e a ausência de evidências clínicas apontam à necessidade de mais estudos sobre antioxidantes a fim de encontrar uma maneira segura e efetiva em reestabelecer os valores de resistência de união de materiais resinosos aos substratos clareados que possua uma vida de prateleira coerente com a prática clínica.

O metabissulfito de sódio é um sal de enxofre antioxidante amplamente utilizado na indústria alimentícia e farmacológica (13). Quando em contato com água, o MBS libera o dióxido de enxofre, seu produto ativo. Este é capaz de ligar-se aos radicais de oxigênio tornando o MBS um forte agente redutor. O MBS é usado como agente antibacteriano e para prevenir o escurecimento de alimentos devido à oxidação. Outro uso na indústria alimentícia é como conservante de frutas, legumes, frutos do mar, peixes, vinhos e cerveja. (14). Além disso, o MBS é excipiente de medicamentos injetáveis como antibióticos, analgésicos, corticoides e broncodilatadores. Anestésicos locais de uso odontológico possuem MBS, adicionado à solução anestésica para conservação dos vasoconstritores (15). Neste estudo, o MBS foi formulado em gel, para aplicação em substrato dental a fim de reverter o efeito deletério causado pelo procedimento clareador. O MBS possui vida de prateleira de quatro meses na formulação em gel feita para este estudo, tempo viável para uso clínico. Além do que a manipulação em gel de antioxidantes possibilita facilitação da aplicação da substância pelo clínico (16).

Apesar de raro, alguns pacientes podem apresentar hipersensibilidade aos sais de enxofre, sendo a causa mais comum a aplicação de medicamentos tópicos. O mecanismo causador da hipersensibilidade ainda não está claro, mas parece ser dose dependente (15, 17). Contudo a quantidade proposta neste estudo (22mg de MBS por espécime) parece ser bem segura, já que esta substância está presente na indústria farmacêutica e alimentícia sendo consumida diariamente. Uma pessoa comum consome em média 2 a 3 mg de sais de enxofre por dia(13,14).

Este trabalho teve como objetivo avaliar a ação de um gel a base de MBS no reestabelecimento da resistência de união de dentes clareados. O estudo foi dividido em duas partes. Na primeira parte foram avaliadas diferentes concentrações de aplicação do gel experimental, sendo estas 5%, 12,5% e 25% aplicadas por uma hora por meio da imersão dos espécimes. Na segunda parte foi avaliada a aplicação do gel antioxidante experimental a 25% aplicado por 10 minutos ou 1 hora, variando o substrato de aplicação, esmalte ou dentina.

# Effect of Sodium Metabisulphite Gel on the Bond Strength of Dentin of Bleached Teeths

Artigo submetido ao periódico "Journal of Dentistry" (Anexo 1)

Henrique Heringer Vieira; José Carlos T Junior; Anderson Catelan; Flávio Henrique B Aguiar; José R Lovadino; Débora ANL Lima

#### ABSTRACT

In this study was evaluated the effect of the application of sodium metabisulphite (SMB) gel on the bond strength to bleached teeth. The study was divided into two parts, and dental blocks were used for both parts. Bleaching was performed in enamel, and resin pillars were performed in dentin for microshear testing. The first part evaluated different concentrations of SMB by immersing the blocks in gel for an hour prior to the bonding procedures. Fifty blocks were divided into five groups (n = 10): G1A: no treatment; G2A: bleaching with 35% hydrogen peroxide (HP); G3A: HP + 5% SMB; G4A: HP + 12.5% SMB; and G5A: HP + 25% SMB. The second part evaluated the application of 25% gel SMB to either enamel or dentin, including application time. Sixty blocks were divided into six groups (n = 10): G1B: no treatment; G2B: bleaching with 35% HP; G3B: HP + 25% SMB for 1 hour in enamel; G4B: HP + 25% SMB for 1 hour in dentin; G5B: HP + 25% SMB for 10 minutes in enamel; and G6B: HP + 25% SMB for 10 minutes in dentin. All samples were submitted to microshear bond testing. The both parts of this study were analysed by using one-way ANOVA, and Tukey's and Dunnett's tests ( $\alpha$ =0,05%). In the part 1 data analysis showed statistical differences (P < 0.0001) between G2A and G3A as well as between G1A and both G4A and G5A. Thus, bleaching affected negatively the shear bond strength, and the application of 12.5% and 25% SMB for one hour can reverse these values. The results of part 2 showed statistical difference (P = 0.001359) only between bleached group and others. There was no difference between application times as well as in none of the groups using SMB gel, with no statistically significance differences compared to control group. The application of 25% SMB for 10 minutes in the bleached substrate is able to reverse the decrease in the bond strength caused by tooth bleaching.

Keywords: Bond Strength. Microshear. Free Radicals. Antioxidant.

#### INTRODUCTION

Bleaching of vital teeth is a conservative and effective technique to improve dental aesthetics. This treatment can be done in the clinical practice or by supervised at-home technique, or even a combination of techniques(1). The substance responsible for tooth bleaching is hydrogen peroxide (HP,  $H_2O_2$ ). When applied to 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 double bonds of organic molecules that are responsible for tooth pigmentation and such chemical modifications change the absorbed light spectrum and increase water solubility, leading to bleaching (2–4).

Often after bleaching, continuing the aesthetic treatment requires restorative procedures with resin materials (5). However, it is known that when such procedures are performed immediately after the bleaching treatment, the bond strength of resin to dental substrate is impaired, which is undesirable (6,7). This phenomenon has been attributed to long lasting free radicals derived from the hydrogen peroxide present in the dental substrate (6,8).

It is recommended a two-week period before performing any restorative procedure (9,10). During this time, saliva is able to reverse the shear bond strength values by presumably leaching free radicals present in teeth (10). However, restorative procedures often need to be clinically performed immediately after bleaching (5). Thus, a search for a clinically practical procedure that reverse the effect of the remaining oxidants is an open area of interest. The use of several different antioxidants have been proposed (11). to chemically reduce free radicals to harmless compounds, thus minimizing their deleterious effects on the composite resin polymerization (12).

Numerous reducing strategies have been tested to circumvent such clinical problem (9,11,13,14), with satisfactory results reported for sodium ascorbate (9,15–17), catalase (11), and even grape seed extract(9), alpha tocopherol (13), and green tea(14). Despite the effectiveness of some reducing agents, none of them can be clinically used, probably due to their short shelf lifetime (18). In addition, the lack of clinical evidence and of standardization of the results point to the need for more studies to find safer and more effective substances

to quickly remove oxidants after teeth bleaching, thus allowing restorative procedure to be performed shortly thereafter.

The reactivity of sodium metabisulphite (SMB) reducing equivalents with oxidants makes SMB useful in the food industry as anti-bacterial agent and to prevent the browning of foods due to oxidation. SMB is also used as preservative agent in fruits, vegetables, seafood and beverages such as refreshments, wine and beer. Many injectable medications such as antibiotics, analgesics, corticosteroids and bronchodilators have sulphite salt as excipients (19,20). In dental practice, SMB is used routinely by the clinician as a preservative of vasoconstrictor agent for local anesthetics(21). Despite being a rare condition, sulphites can cause allergic reactions, especially in asthmatic individuals. Hypersensitivity of the causative mechanism is not clear, but appears to be dose dependent(21). An average person consumes 2 to 3 mg of sulphites *per* day (20). The most common cause of allergy involves sulphite-based products which are applied topically (22). Thus, the quantity proposed in this study (i.e. 22 mg of SMB *per* specimen) seems to be proven safe, since it is present in foods and medicines(19).

However, the nature of the long lasting oxidant remaining on the dental substrate is not clear and it is highly unlikely that is a free radical. In particular, the product of HP homolytic break down is the hydroxyl radical, which is very reactive. It oxidizes, abstracts hydrogen atom and adds to organic molecules double bonds very rapidly. Consequently, hydroxyl radical has a very short lifetime (in the microsecond range), thus could not remain in the dental structure for weeks (23). Hydrogen peroxide 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 chemical well-known to react with hydrogen peroxide as an antioxidant. The current study evaluates the effects of sodium metabisulphite loaded gel on the bond strength of resin to teeth previously submitted to bleaching treatment.

Sodium metabisulphite (SMB) is an antioxidant widely used in pharmaceutical and food industry as a preservative agent (19), and reacts with HP rapidly, producing the harmless and water-soluble sulphate anion(24). The hypothesis tested was that the use of SMB does not influence the bond strength to bleached teeth.

#### **MATERIALS & METHODS**

#### **Specimen Preparation**

For the specimen preparation, 110 recently extracted bovine incisors were used. The crowns were separated from the roots by 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.

By using a metallographic cutter (Isomet 1000, Buehler, IL, USA) equipped with diamond saw, the crowns were sectioned to obtain dental blocks of 6.5 x 6.5 mm. After obtaining the blocks, they were flattened by using a rotary polisher (AROTEC, Cotia, SP, Brazil) equipped with 400-grit silicon carbide paper (Norton, São Paulo, SP, Brazil) so that each portion of the substrate (i.e. enamel and dentine) could measure 1.2 mm thick.

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

This study was divided into two parts as follows:

In the first part, the antioxidant was tested at different concentrations. For this purpose, 50 specimens were divided into five groups (n = 10) according to bleaching process and subsequent immersion in different concentrations of SMB: G1A: no treatment (positive control); G2A: bleaching with 35% HP (negative control); G3A: HP + 5% SMB; G4A: HP + 12.5% SMB; and G5A: HP + 25% SMB.

The second part evaluated different times of application of 25% SMB in enamel or dentin. The specimens were divided into six groups (n = 10): G1B: no treatment (positive control); G2B: bleaching with 35% HP (negative control); G3B: HP + 25%SMB for 1 hour in enamel; G4B: HP + 25% SMB for 1 hour in dentine; G5B: HP + 25% SMB for 10 minutes in enamel; and G6B: HP + 25% SMB for 10 minutes in dentine.

#### **Bleaching of Specimens**

The bleaching procedure was the same in both parts of the experiment. The specimens were bleached with 35% hydrogen peroxide (Whiteness HP, FGM, Joinville, SC, Brazil) and had their dentine facets enclosed in moistened cotton to prevent dehydration. Gel was applied to enamel according to the manufacturer's instructions, that is, three applications of bleaching gel for 15 minutes each in a volume of 0.045ml. 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 oven for a period of 24 hours.

#### Antioxidant Application

Different antioxidant concentrations were obtained in gel form by using Natrosol thickener before application as described in the division of groups.

Part 1: After 24 hour storage, the groups submitted to bleaching and SMB aplication (G3A, G4A, G5A) had the specimens completely immersed into a plastic cup containing 1.44 mL of antioxidant gel, thus both dentin and enamel had contact with the SMB. After one hour of immersion the specimens were thoroughly washed in water for one minute.

Part 2: After 24 hours of storage, were carried the applications of SMB gel only in the surface of enamel (G3B, G5B) or dentine (G4B, G6B), these substrates were delimited with light-cured resin (Top Dam, FGM, Joinville, SC, Brazil). This delimitation was made so that the gel did not rise out of the substrate during the application period and standardization of the application volume (0.088 mL). After removal of the delimitation, SMB was removed and the specimens thoroughly washed in running water for one minute.

#### Adhesive Procedures

Immediately after the antioxidant application treatments, the dentine facets of all specimens and controls received two resin pillars to be shear tested. An adhesive tape with two holes of 1.1mm was used for delimiting the area where the bonding was made (25). The adhesive procedure followed the recommendations of the manufacturers. The conditioning of the dentine was performed with 37% phosphoric acid (Condac, Joinville, SC, Brazil) for 15 seconds, followed by rinsing with water for 15 seconds. 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 seconds. The adhesive were gently air-dried for 5 seconds. Before adhesive polymerization, cylindrical matrices made of perforated noodles with diameter of 1.1mm and 1 mm height (Furadinho 6, Pastifício Santa Amália, São Paulo, SP, Brazil) were positioned onto the adhesive holes of the bounding tape (26). Adhesive was light cured for 10 seconds at 618 mW/cm<sup>2</sup> (FLASH lite 1401, Discus Dental, Culver City, CA, USA). The matrices were filled with flowable resin composite (Filtek Z 350, 3M ESPE, St. Paul, MN, USA), which was then light-cured for 40 seconds. After 2 hour storage in distilled water, the matrices were removed along with the adhesive tape.

#### **Microshear Test**

After 24-hour storage in distilled water at 37 °C, the specimens were placed in a microshear device coupled to a universal testing machine (EZ Test – Shimadzu, Kyoto, Japan) operating at 5-N load cell and speed of 0.5 mm/min using an orthodontic wire (Morelli Ortodontia, Sorocaba, Brazil), 0.1 mm radius. The values found in kilograms-force (Kgf) were converted into Megapascals (MPa). For analysis of fracture pattern, the specimens were analysed in stereomicroscopy at 50 times magnification (Leica Microsystems, Wetzlar, Germany).

#### Fracture pattern and statistical analysis

The fracture pattern were classified as: adhesive, cohesive in resin, cohesive in dentine and mixed (i.e. two or more types of fractures)(25). Bond strength data of both parts were analysed by using one-way ANOVA (analysis of variance) and Tukey's test at

significance level of 5%, whereas Dunnett's test was used to compare the positive control group with the other groups.

#### RESULTS

In the part 1, whose specimens were analysed according to different concentrations of SMB gel, one-way ANOVA showed statistical difference (P < 0.0001).

Tukey's and Dunnett's tests showed that the bleaching procedure decreased significantly the bond strength of the specimens when G1A was compared to G2A. Application of 5% SMB after bleaching (G3A) did not differ statistically from the group of negative control (G2A). Applications of 12.5% and 25% SMB (G4A and G5A, respectively) did not differ statistically from the positive control (G1A) and among them. These results can be observed in Table 1.

 Table 1 - Mean (MPa) and standard deviation of bond strength values in part 1.

	<b>c</b> .			
Group	Mean and standard deviation			
G2A - HP	12.23 (6.1) b			
G3A – HP + 5% SMB	15.95 (2.27) b			
G4A – HP + 12.5% SMB	19.93 (5.47) a *			
G5A – HP + 25% SMB	22.78 (5.66) a *			
G1A - Control	23.23 (5.58)*			

Means followed by different letters differ in bond strength. "\*" indicates no statistical difference from control group. HP - 35% hydrogen peroxide application; SMB - application of sodium metabisulphite according to concentrations.

Adhesive fracture was the dominant fracture pattern in all groups, as can be seen in Table 2. There was only a cohesive fracture in resin (G3A).

Fracture Pattern						
	G1A - Control	G2A - HP	G3A – HP + 5% SMB	G4A – HP + 12.5% SMB	G5A – 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			

**Table 2** - Fracture pattern in part 1.

HP - 35% hydrogen peroxide application; SMB - application of sodium metabisulphite according to concentrations.

In the part 2, one-way ANOVA showed statistical significance (P = 0.001359). Dunenett's tests showed difference between positive and negative control groups, with the latter showing higher bond strength values compared to the group with bleached specimens only. No difference was found between application times (10 minutes or 1 hour) and between substrates (i.e. enamel or dentine) to which SMB gel was applied. No statistical differences were found between all experimental groups and controls. The results can be seen in Table 3.

Group	Mean and Standard Deviation
G2B – HP	11.84 (4.78) b
G3B – HP + SMB 1 hour E	20.43 (5.51) a*
G4B – HP + SMB 1 hour D	21.48 (5.81) a*
G5B – HP + SMB 10 min E	21.05 (5.94) a*
G6B – HP + SMB 10 min D	21.37 (6.04) a*
G1B - Control	21.75 (5.72) a*

Table 3 - Mean (MPa) and standard deviation of bond strength values in part 2.

Means followed by different letters differ in bond strength. "\*" indicates no statistical difference from control group. HP - 35% hydrogen peroxide application; SMB - application of sodium metabisulphite according to concentrations.

In the part 2, adhesive fracture was the most predominant fracture pattern (Table 4), especially in the group with bleached specimens only (G2B). Only one cohesive fracture was found in resin (G4B).

 Table 4 - Fracture pattern in part 2.

Fracture Pattern						
	G1B - G2 Control H	G2B -	G3B – HP + SMB 1 hour	G4B – HP + SMB 1 hour	G5B – HP + SMB 10 min	G6B – HP + SMB 10 min
		HP	E	D	E	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				1		

HP - 35% hydrogen peroxide application; SMB - sodium metabisulphite application according to concentrations; E - antioxidant application gel in enamel; D - antioxidant application gel in dentin.

#### DISCUSSION

The hypothesis that the SMB gel would not affect the bond strength to bleached dentin was denied because the gel application to 5% for an hour was not able to change the bond strength. All other concentrations and application times were able to re-establish 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 dental substrate (6,15), a finding also confirmed by our study (G1A compared to G2A and G1B compared to G2B). Such negative effects have been attributed to HP derived oxidant species that would oxidize resin monomer and polymers, thus potentially interfering in all steps of the polymerization (initiation, propagation and termination), perhaps even changing the very chemical nature of the resin polymers and their bonds to dental substrate (6,8).

The major problem with the strategy employed so far is that it is based on an outdated medical concept of generic antioxidant therapy that has failed to revert negative effects of oxidants on human health and aging (27,28) Reactive oxygen species (ROS) is a general term used to encompass compounds derived from oxygen partial reduction (superoxide, hydrogen peroxide, hydroxyl radical, etc), but it often misleads people to treat 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 after 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 history and information collected by the current and previous studies. The bond strength problem persists for at least two 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 since it gathers such properties. Indeed, HP is stable (23) 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 hydroxyl radical. The relative effectiveness of catalase also point into this direction as this hemeprotein is a rather specific enzyme for HP removal. Thus, the purpose of this study was to evaluate the effects of an experimental gel containing SMB as a reducing agent for HP, hypothetically trapped in dental structures. SMB undergoes rapid hydrolysis when dissolved water, releasing two parts of its reducing active equivalents, which is either sulfur dioxide (SO<sub>2</sub>) or the anions hydrogen sulphite (HSO<sub>3</sub><sup>-</sup>) or sulphite (SO<sub>3</sub><sup>2-</sup>), depending on the pH. In the specific case of reducing experimental gel (pH  $\cong$  7), sulphite anions are the more relevant species. Nevertheless, all SMB reducing active equivalents react with HP yielding water plus the water-soluble sulphate anion.

The choice to do the specimen immersion in gel rather than gel application, as in the second part of the study has been made to test the experimental gel effectiveness at different concentrations and then subsequently time and substrate application could be evaluated in part two. Therefore, the immersion of the specimen in gel of SMB at 5% (G2A) for one hour 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 remained PH at the substrate are sufficient to interfere with the polymer chains formation. The highest concentrations of 12.5% and 25% (G4A and G5A) were able to re-establish the bond strength, which corroborates to the hypothesis that the concentration of 5% SMB is insufficient for this purpose.

Hydrogen peroxide is a molecule with low molecular weight capable of diffusing the dental structure (29). According to Eimar et al, 2012 (3), the reaction responsible for the color change of dental substrate occurs in organic part of the tooth, which is in greater concentration in the dentine. Therefore, it is expected to be found more concentration of PH in the dentine, which explains the choice to proceed with the dentin bonding in both parts of the study. This being a substrate wherein the antioxidant could actually be tested due to higher concentrations of free radicals, the application of enamel (G3B and G5B) was made to evaluate the power of diffusion of SMB and the application in dentine to be simulated in a situation of application directly in the cavity. Therefore, there is a wide range of clinical application possibilities were contemplated in this study.

Although the immersion in SMB gel at 12.5% was also effective, the concentration at 25% for aplication was chosen for the second part of the study once its time would be reduced to 10 minutes (G5B and G6B). Besides the application in enamel so that bonding procedure was performed on dentin (G3B and G5B) this could require a higher concentration of SMB as the first part of the study demonstrated that its concentration is important for the bond strength reestablishment, as the gel of SMB at 5% was not effective. According to Dishman et al, 1994 (30), the waiting period for the substrate to return to the normal bond strength depends on the concentration of PH and time of contact with the substrate. This study performed two PH applications at 35%, although in clinical situations it may be needed more PH applications to achieve a satisfactory change in tooth color (5). Another important data is that the amount of dentine in a dental element is larger than the specimen. Dentin, as stated above, has greater organic content and may have more remained PH. Therefore, it was opted to use the gel at 25% in part two to ensure completely reversion of bond strength values in cases where greater applications of PH were made.

In the second part of the experiment, the groups that received 25% SMB gel (G3B, G4B, G5B, G6B) used only 0.088 ml of SMB per specimen. In a clinical situation, the amount applied per dental element would be very close to 0.088 ml per tooth. It is also possible that 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 gel and soft tissue and making clinical application safe for patients with hypersensitivity to sulfites. Another possibility of application prior to the restorative procedure is to use the SMB gel in the same tray for tooth bleaching in case of at-home bleaching technique (31). However, the authors believe that the best technique for application of antioxidant gel would be that directly applied into the prepared cavity prior to application of the restorative composite. So, the gel application can be controlled by the clinician based on the smallest possible amount of gel in direct contact with the area to be restored.

A study (32) 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 needs several applications before the bonding procedure, thus a gel form was proposed (32). Therefore, 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 cosmetic and pharmaceutical industry as stabilizer and emulsifier (33).

The substance to be used as a reducing agent prior to bonding procedures must have low molecular weight to be spread over and permeate through the dental substrate(34). HP has a molar mass of 34 g/mol, whereas ascorbate, the most studied reducing agent, has molecular weight of 175.11 g/mol and perhaps is unable to permeate through dental structures. In addition, ascorbate has a poor reactivity toward hydrogen peroxide The hydrogen sulphite /sulphite anion realeased by SMB hydroslyis are water soluble and have molar mass of 81/80 g/mol, thus can probably spread and access dental substrate due to its humid environment (34). In part 2, G3B and G5B had the gel applied to enamel and dentin submitted to mechanical test and yet the bond strength values were re-established, which confirms its ability to penetrate the substrate for a distance of at least up to 2.4 mm.

The dental blocks were stored in distilled water because saliva is able to reverse the altered bond strength values (10) as has catalase, the natural antioxidant that contribute to remove hydrogen peroxide (35). However, the time for re-establishment of the bond strength ranges from 24 hours (10) to 14 days (9). Composite resins do not have their color changed by the bleaching procedure (36) as the time for leaching free radicals and re-establishing the strength bond values becomes an aesthetic discomfort to the patient whose restorations must be replaced (37). In addition, an "in vitro" study showed that there is a greater diffusion of peroxide into the dentin of restored anterior tooth (29). So, it is extremely important to find an agent capable of re-establishing bond strength values in an acceptable clinical time, allowing the completion of the aesthetic treatment in shorter time. Furthermore, restorations with bond strength altered immediately after a bleaching treatment allows greater microleakage (37). The use of 12,5 and 25% SMB gel proved to be able to neutralize the effects of oxidants after application times of 1 hour or 10 minutes in both dentin and enamel. The use of an reducing agent for 10 minutes prior to adhesive restorative procedure is a clinically feasible time (38).

#### CONCLUSION

The experimental use of 25% SMB gel was able to reverse the deleterious effect of bleaching on the bond strength of dental composites to dentin when applied for 1 hour or

10 minutes after the bleaching procedure. After 1 hour of application, no difference was observed at the different concentrations of SMB tested, that is, 12.5% and 25%.

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### **3 CONCLUSÃO**

Com base nos achados deste estudo foi possível concluir que o gel antioxidante experimental é efetivo em restaurar a resistência de união afetada pelo clareamento dental quando usado na concentração de 25% e aplicado por uma hora ou 10 minutos.

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<sup>\*</sup> De acordo com a normativa da FOP/Unicamp baseada na norma International Committee of Medical Journal Editors – Grupo de Vancouver. Abreviatura dos periódicos em conformidade com o Medline.

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# APÊNDICE 1 - Metodologia Ilustrada

## Confecção das Amostras



Figura 1 - Mandíbulas bovinas e incisivos bovinos após extração.



Figura 2 – Fragmentos de esmalte e dentina após corte em cortadeira metalográfica.



Figura 3 – Planificação dos fragmentos em lixa de carbeto de silício #400.



Figura 4 – Procedimento de inclusão dos fragmentos dentais em resina acrílica quimicamente ativada.



Figura 5 – Remoção dos excessos de resina acrílica em politriz metalográfica. Após isto procedeu-se o polimento das amostras.

Fase um do experimento



Figura 6 – Manipulação do peróxido de hidrogênio conforme orientação dos fabricantes.



Figura 7 – Procedimento clareador. Aplicação do gel em esmalte, com face dentinária voltada à algodão umedecido.



Figura 8 – Imersão das amostras em gel antioxidante de variadas concentrações.



Figura 9 – Aplicação de fita delimitadora da área adesiva sobre a face de dentina.



Figura 10 – Condicionamento ácido.



Figura 11 – Lavagem com água.



Figura 12 – Manutenção da umidade da dentina com bolinha de algodão.



Figura 13 – Aplicação do adesivo.



Figura 14 – Posicionamento da matriz previamente à fotoativação.



Figura 15 – Fotoativação do adesivo



Figura 16 – Espécime após inserção da resina composta "flow" na matriz.



Figura 17 – Imersão do conjunto em água para facilitar a remoção da matirz.



Figura 18 – Remoção da matriz com sonda exploradora.



Figura 19 – Corte da fita delimitadora para posterior remoção.



Figura 20 – Espécime posicionado no dispositivo de microcisalhamento.

### Fase um do experimento

O procedimento de confecção das amostras e dos pilares de resina a serem cisalhados foi semelhante para ambas fases do experimento.



Figura 21 – Delimitação da área de aplicação do gel antioxidante.



Figura 22 – Aplicação do gel antioxidante.



Figura 23 – Pilares de resina.

### ANEXO 1 – Documento de submissão do artigo



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