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ORIGINAL RESEARCH

The Effect of 3% Phosphate Ascorbyl Gel on Bond Strength of Composite Resin to Enamel treated with 35% Hydrogen Peroxide

[1]Milena de Fátima Schalcher de Castro, [2]Alice Carvalho Silva, [3]Marcela Mayana Pereira Franco, [4]Ana Paula Brito Silva [5]Fausto da Silva Bramante, [6]Monica Barros da Silva, [7]Darlon Martins Lima, [8]Adriana de Fátima Vasconcelos Pereira

ABSTRACT

Objective: To evaluate the effect of 3% phosphate ascorbyl gel (PA) in different times onto the microshear bond strength of composite resin (CR) to bovine enamel treated with 35% hydrogen peroxide (HP).

Materials and methods: Thirty enamel blocks of bovine incisors were made and divided into 5 groups (n = 6) with three specimens per group (n = 18), according to treatment: G1= No bleaching + CR; G2 = HP + CR after 15d; G3 = HP + CR after 24 hours; G4 = HP + PA (15 min) + CR after 24 hours; G5 = HP + PA (2 hours) + CR after 24 hours. The resin cylinders were made by Tygon matrices. Microshear bond strength test was performed using universal testing machine with a 50N load at a speed of 0.5 mm/min. Fracture modes were assessed by a stereomicroscope 40 ×. Microshear bond strength values were submitted to the analysis of variance (ANOVA) one-way and Tukey test ($p < 0.05$).

Results: G1 had significant results when compared to G3 and G5 ($p < 0.01$). However, G2, G3, G4 and G5 have showed no significant differences among groups ($p > 0.05$). Failure modes were categorized into adhesive (90%) and mixed (10%).

Conclusion: The use of 3% phosphate ascorbyl gel for 15 minutes was able to improve bond strength of composite resin to bleached bovine enamel, but when 3% phosphate ascorbyl gel was applied during 40 minutes it negatively interfered in the adhesion of the resin to bleached bovine enamel.

Keywords: Antioxidants, Tooth bleaching, Hydrogen peroxide, Composite resins, Shear strength.

[1-4,7,8]Department of Dentistry, Federal University of Maranhão São Luís, Maranhão, Brazil

[5,6]Deparment of Dentistry, CEUMA University, São Luís Maranhão, Brazil

Corresponding Author: Darlon Martins Lima, Adjunctive Professor, Department of Dentistry, Universidade Federal do Maranhão, Av. Portugueses s/n, Campus Universitário Bacanga, 65085-580, São Luís, Maranhão, Brazil, Phone: 55 98 3272-8577, e-mail: darlonmartins@yahoo.com.br

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INTRODUCTION

Tooth bleaching has been introduced in dentistry for over 100 years. Considered the more conservative of cosmetic treatments to improve the smile appearance, tooth bleaching has gained a prominent position being used routinely, mainly with the use of adhesive materials.[1]

Bleaching agents have many compositions, but the most common active ingredient is hydrogen peroxide. These agents may be gel, solution or paste in concentrations ranging 22 to 37% to be used at dentist office.[2,3]

Hydrogen peroxide is a powerful oxidizing agent that generates other reactive forms of oxygen (free radicals), which are responsible for the bleaching process.[4] Due to the presence of residual oxygen percolating through tooth structure after bleaching, at least 7 days are need for the oxygen be removed and does not interfere in the bond strength. Clinically, the interval may be too long for patients seeking immediate cosmetic treatment.[5]

In order to eliminate this inconvenient and enable the restorations at the shortest time interval, the use of antioxidants has been indicated after bleaching.[3,6-10] These products remove residual oxygen from tooth structure and provide good adhesion to ename[l3] and dentin,[7-9,11,12] allowing immediate restorations with longevity, durability and microleakage absence.[13]

The effective use of antioxidants for low concentration of bleaching products are well documented.[10,14-16]

On the other hand, there is a gap in relation to the high concentration of bleaching agents and the efficacy of those antioxidants in remission of oxidation effects, which leads to restricted use by dentists. Sodium ascorbate is an antioxidant widely used,[1,3,6-8,10,17,18] nevertheless some studies have reported that sodium ascorbate is an unstable substance with yellow color that rapidly oxidizes in order to provide staining on teeth freshly bleached.[14,19-21]

To avoid this possible adverse effect, the use of antioxidant gel composed of stabilized sodium ascorbate, white-translucent, chemically known as monophosphate trisodium ascorbate or 3% phosphate ascorbyl has been proposed after bleaching with low concentration of bleaching agents, obtaining favorable results.[19]

The hypothesis to be achieved is that the 3% phosphate ascorbyl is able to improve bond strength of composite resin to enamel bleached with 35% hydrogen peroxide. The aim of this *in vitro* study was to evaluate the effect of 3% phosphate ascorbyl gel in different times in the microshear bond strength of composite resin to bovine enamel treated with 35% hydrogen peroxide.

MATERIALS AND METHODS

Obtaining, Selection and Preparation of Teeth

Healthy and freshly bovine incisors were collected and stored until use in distilled water at a temperature 5°C. The teeth were cleaned with periodontal curettes (SS White, Rio de Janeiro, RJ, Brazil) in order to remove residual periodontal ligaments and soft tissues. After cleaning and polishing with pumice paste (SS White, Rio de Janeiro, RJ, Brazil) and Robinson brush (Dabi Atlante-Ribeirão Preto, SP, Brazil), the teeth were examined in a stereomicroscope with 40× magnification (Meiji 2000, Meiji Techno, Japan). Teeth with cracks or fissures were excluded from the sample, resulting in 30 bovine incisors.

The selected teeth were fixed with godiva thermoplastic (Kerr Corporation, California, USA) in PVC cylinders (Tigre SA, Joinville, SC, Brazil) filled with acrylic resin (JET Classic, São Paulo, SP, Brazil). The PVC cylinders were placed in the cut machine Isomet (South Bay Technology Inc., Buehler, Lake Bluff, USA), and the roots were sectioned with double-sided diamond disk (Extec Day, Wafer blade 5'×. 015 × 1/2, cat. 12240, Extec Corp., Enfield-Connecticut, USA) at low speed under cooling. With a 80-grit sandpaper (3 M ESPE, St. Paul, MN, USA) mounted on a horizontal water-cooled rotary electric polisher (Arotec Cotia, SP, Brazil), the proximal, incisal and lingual enamel surfaces were worn to obtain standardized blocks 70 mm^2 (10 mm high × 7 mm wide) and 2 mm thick, which measurements were confirmed with digital calipers (Vonder, Curitiba, PR, Brazil). The planning of the buccal surface was performed in a polishing machine (Arotec Ind. Com, Cotia, SP, Brazil), using 80-600 grit-growing sandpaper (3M ESPE, St. Paul, MN, USA) under cooling.

Inclusion

The specimens were fixed on a wax plate number 7 (Asfer, Chemical Industry Ltda., São Caetano do Sul, Brazil), with the buccal enamel surface facing down. The PVC cylinder (Tigre SA, Joinville, SC, Brazil) was positioned, such as each specimen was kept in the center of the respective cylinder. Then, each cylinder was filled with acrylic resin (JET Classic, São Paulo, SP, Brazil).

After polymerization of the acrylic resin, the wax number 7 was removed and it was observed if the enamel surfaces were parallel to the surfaces of the acrylic resin cylinder. The wire that pulls the specimen was positioned vertically and, therefore, the possibility strength to be reduced.[9]

The buccal surfaces were polished with 600-grit sandpaper (3M ESPE, St. Paul, MN, USA) under water cooling and covered with cotton soaked in distilled water (37 ± 2)°C until use.

Division of Groups

The specimens were randomly divided into five groups according to the treatment type as shown in Table 1.

Application of Bleaching Agent

To remove any remaining wax or acrylic resin adhered during the inclusion process, the specimens received prophylaxy with pumice paste (SS. White-Rio de Janeiro, RJ, Brazil).

The experimental groups G2, G3, G4 and G5 were exposed to one application of 35% hydrogen peroxide (Whiteness Blue HP, FGM, Joinville, SC, Brazil) for

Table 1: Division of groups according to treatments performed

Groups	*Bleaching agent*	*Antioxidant*	*Time of antioxidant application*	*Waiting period for restoration*
G1	–	–		Over untreated enamel
G2	35% Hydrogen peroxide	–		15 days after bleaching
G3	35% Hydrogen peroxide	–		24 hours after bleaching
G4	35% Hydrogen peroxide	3% Ascorbyl	15 minutes	24 hours after antioxidant
G5	35% Hydrogen peroxide	3% Ascorbyl	120 minutes	24 hours after antioxidant

Table 2: Restorative materials used in this study

Material, manufacturer	*Composition*	*Technique*
Condac 37% FGM Lot:337	Aqueous gel containing 37% phosphoric acid	Application of acid 30 s, washing 30 s, drying air jet 5 s
Scotch Bond- 3M ESPE Primer: Lot: 239896 Adhesive: Lot: N251381	Primer: Aqueous solution of Acrylate-2-hidroxietilmeta (HEMA) and a polialkenolic acid copolymer Adhesive: Solution bisphenol diglycidyl dimethacrylate (BisGMA) 2-hydroxyethyl methacrylate (HEMA) and camphorquinone	Prime: Activate application 10 s, rest 30 s, air jet 5 s, second application, air jet 5 s. Adhesive Application and photoactivation 20 s.
Composite resin Filtek Z350 XT - 3M ESPE Lot: N202748	The resin contains bis-GMA, UDMA, TEGDMA, and bis-EMA resins. The fillers are a combination of non-agglomerated/non-aggregated 20 nm silica filler, non-agglomerated/non-aggregated 4 to 11 nm zirconia filler, and aggregated zirconia/silica cluster filler (comprised of 20 nm silica and 4 to 1 nm zirconia particles)	Application and photoactivation for 40 s

40 minutes. After mixing the thickener and 35% hydrogen peroxide, following the manufacturer's recommendations, approximately 0.04 ml of bleaching agent was applied to the enamel surface in each specimen. After 20 minutes, the whitening agent was stirred using a plastic spatula in order to eliminate oxygen bubbles. During the time of bleaching agent action, the specimens were stored at temperature of 37°C. After bleaching, G2 and G3 were washed with water spray for 10 seconds and stored with cotton soaked in distilled water at 37 °C for 15 days and 24 hours, respectively. The groups G4 and G5 were treated with antioxidants.

Antioxidant Agent

After the bleaching treatment, the specimens of groups G4 and G5 were washed with water spray for 10 seconds and dried with jet-air for 10 seconds. Subsequently, it was applied 0.04 ml of 3% ascorbyl gel (PI0502546-0, Drogal manipulation, Piracicaba, SP, Brazil) in specimens of groups G4 and G5 for 15 and 120 minutes respectively.[14,19] During this time, the specimens were stored at temperature of 37°C.

After treatment with the antioxidant, the specimens were rinsed with water spray for 30 seconds to dissolve and remove the crystals of salts of the antioxidant deposited on enamel surfaces. Then they were stored at 37°C for 24 hours.

Adhesive Technique

The groups G3, G4 and G5 were submitted restorative treatment 24 hours after the whitening session (G3) and antioxidant (G4 and G5). G2 was restored 15 days after bleaching and G1 received no treatment prior to tooth restoration.

The restorative materials used in this study, their classifications, compositions, manufacturers and application modes are described in Table 2.

Initially, dental surfaces were etched with 35% phosphoric acid (30 seconds), washed with water spray (30 seconds), air-dried (30 seconds) and applied the adhesive system Scotch Bond. The methodology developed by McDonough et al[22] and Shimada et al[23] was used to prepare the specimens. Six blocks were used for each group in which three specimens were made, amounting to an 'n' equal to 18 per group for microshear test. The resin cylinders were fabricated using transparent tubes of 0.75 mm diameter and 1 mm in height (Tygon tubing, TYG-030, Saint-Gobain Performance Plastic, Maime Lakes, FL, USA). Before the adhesive system cure, 3 Tygon tubes were placed carefully in each block for subsequent curing of the adhesive for 20 seconds with DEMI-LED (Light-Curing Keer, Switzerland) with intensity 800 mW/cm^2 checked in LED Radiometer (Dementron-Keer, Switzerland). Thus, the tubes were bonded to the enamel surface for subsequent insertion of composite resin.

After fixation of Tygon matrices, the composite resin Filtek Z350 XT color A3 was carefully inserted within each tube with a resin spatula (Duflex, Rio de Janeiro, RJ, Brazil) and an instrument wax dripper No. 2 (Mocar, Surgical and Dental Instruments, São Paulo, SP, Brazil) to adapt the material on the inner surface of the tube and on the enamel surface, avoiding the formation of air bubbles. Then the composite resin was light-cured for 40 seconds.

After the fabrication of composite resin cylinders, the specimens were stored with cotton soaked with distilled water at 37°C for 24 hours before testing adhesion (ISO/TR 11405, 1994). Tygon tubes were removed with the aid

of a fine point explorer (Hu-Friedy Mfg. Co. Inc. Chicago, IL, USA) with extreme care to avoid reduced induction of strength in the adhesive interface.

Microshear Bond Strength Test

The bond strength was evaluated by microshear mechanical test described by Shimada et al.[23] The test was performed on universal testing machine Instron 2519-104 (Instron Corp, Canton, Mass, USA). Each PVC cylinder containing the specimens were mounted in an appropriate device, which was properly fixed to the testing machine so that the composite resin cylinders remain aligned to the load cell and the handle made with round wire stainless steel with a diameter of 0.20 mm (Orthodontics Morelli, Sorocaba, SP, Brazil), noosing the prolonging of load cell and each cylinder composite, maintaining contact with the lower semicircle of the cylinder closest to the adhesive interface to generate the shear stress.[9]

The wire was pulled through 50 N load cell at a strain rate of 0.5 mm/min until the fracture of the composite cylinder. The values of maximum strength applied at fracture were recorded in Newtons (N) by the computer attached to the testing machine. However, according to ISO (TR 11405, 1994) it is desirable to convert these values into Megapascal (MPa) using the following formula: MPa = S/A.

Failure Modes Analysis

After microshear test, the type of fracture of each specimen was evaluated with a stereomicroscope with 40× magnification (u Eye, Germany). Failures were classified as adhesive (A)-fracture located at the interface substrate/restorative material; cohesive in enamel (CE)-when the fracture occurred in the enamel substrate; cohesive composite resin (CR)-when the fracture occurred in restorative material and mixed (M)-combination of adhesive and fracture coesivas.[9]

STATISTICAL ANALYSIS

The normality of the data obtained by the microshear bond strength was confirmed by the Shapiro-Wilk test, with significance level at 5% ($p < 0.05$). To verify the existence of statistically significant differences between experimental groups, an one-way ANOVA was performed followed by Tukey test with a significance level at 5% ($p < 0.05$). The analysis and graphs were performed using the software GraphPad Prism version 5.0.

RESULTS

Table 3 presents data in the microshear bond strength in MPa of different experimental groups and the results of one-way ANOVA test. The findings indicate statistically significant differences ($p = 0.004$). G1 had higher average bond strength (15.52 ± 10.01 MPa) compared to the G3 (5.761 ± 8.024 MPa) and G5 (4.778 ± 4.396 MPa) ($p < 0.01$) However, the experimental groups G2, G3, G4 and G5 showed no significant differences ($p > 0.05$).

Figure 1 presents the mean values of the microshear bond strength groups. The same pattern is observed, as the mean and confidence interval of G1 overlap with G2 and G4. However, the confidence interval for G1 outweigh the confidence intervals predicted from the means of the groups G3 and G5 statistically different from each other ($p < 0.01$).

After microshear bond strength test, the bovine enamel blocks were examined with a stereomicroscope at 40× magnification (uEye, Germany), which allowed to determine the type of fracture occurred. The results (in percentages) can be seen in Table 4.

Most fractures modes were adhesive in the interface enamel/resin (90%) and less frequently (10%) was mixed (adhesive and cohesive in enamel resin). The positive control group G1 had the highest number of mixed fractures and negative control group G3 the most adhesive fractures. The experimental groups G4 and G5 which used the antioxidant showed both 89 and 11% adhesive and mixed fractures, respectively.

DISCUSSION

Bovine enamel was used as a substitute for human enamel in the bond strength test. Although, there are differences in density and porosity between human and bovine enamel,[20] the mechanism of acid-etching is similar.[21] If the presence of peroxide in the prismatic spaces is the explanation for the adverse influence on adhesion, the effect on bovine enamel would not be similar to the

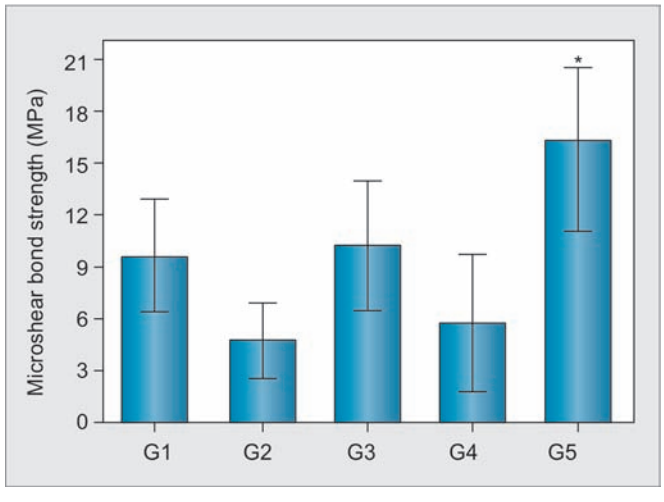

Fig. 1: Mean values of microshear bond strength to (in MPa) of different experimental groups. Legend: Vertical bars (confidence interval of 95% compared to the average, *p < 0.01 compared to G3 and G5)

Table 3: Means of microshear bond strength (MPa), standard deviation, coefficient of variation and sample size of the different groups

Experimental sample	*Number groups*	*Mean ± standard deviation*	*Standard error*	*Coefficient of variation (%)*
G1	18	15.52 ± 10.01^{a}	2.360	64.54
G2	18	10.28 ± 7.426^{ab}	1.750	72.25
G3	18	5.761 ± 8.024^{b}	1.891	139.2
G4	18	9.633 ± 6.517^{ab}	1.536	67.65
G5	18	4.778 ± 4.396^{b}	1.036	92.01

Legend: G1: No whitening + restoration; G2: Whitening + restoration after 15 days; G3: Whitening + restore one day later; G4: Oxidant whitening + 15 minutes + 24 hours after restoration; G5: Oxidant whitening + 2 hours + 24 hours after restoration.
Distinct superscript letters indicate statistically significant differences after one-way ANOVA followed by post hoc Tukey test ($p < 0.001$, $F = 5.803$)

effect on human enamel due to the inherent differences in structure and size of the interprismatic areas.[20] However, while bovine teeth do not lead to results that are identical with those obtained from human teeth, they produce results that are comparable and certainly useful in evaluating the influence of various treatments on enamel bond strengths.[9]

Tooth bleaching and adhesive restorations are commonly associated in esthetic procedures. Both techniques require by the professional scientific knowledge regarding products and the possible adverse interactions.[21]

The active ingredient is mostly hydrogen peroxide, regardless the technique, the type of bleaching agent and the concentration.[3] The bleaching agents applied in office are based only on hydrogen peroxide in gel, solution or cream and show concentrations ranging from 22 to 37%.[2,3] But home bleaching requires lower concentrations of hydrogen peroxide (4% to 7.5%) or carbamide (between 10% and 22%). Hydrogen peroxide (H_2O_2), both at home or in office, dissociates into water (H_2O) and oxygen (O_2), releasing peridroxil free radical (HO_2) for short terms, which is highly reactive due to absence of an electron in the last layer.[9]

The adhesion in both enamel and dentin is based on a process that removes mineral content and permits resin monomers to penetrate the remaining spaces.[24] The effectiveness of adhesive procedures depends mainly on the infiltration capacity of the material in demineralized substrate and the resistance to hydrolytic or enzymatic.[25] Studies have shown considerable reduction in the values of bond strength on enamel and dentin when adhesive systems are used immediately after bleaching with peroxides.[9,19,21] In this study, the dental bleaching with 35% hydrogen peroxide promoted a statistically significant decrease in bond strength of composite resin to enamel in the procedures performed 24 hours after bleaching (G3).

The most accepted hypothesis to explain this result is that peroxides interfere with the infiltration of the adhesive into the substrate and avoid proper curing of the material due to the presence of by-products, such water and mainly oxygen. These products compete with free radicals generated during polymerization,[11,26] and lead to similar effect to the oxygen in the surface layer inhibiting the polymerization of the adhesive.[21]

Thus, it is necessary to wait for an appropriate interval of time so that the residual oxygen is removed and does not interfere in the bond strength.[4] Most studies suggest 2 weeks[4,7,27] to the adhesive restorations be performed safely. Other studies believe that only a week is required for bonding procedures the dental bleaching.[5,28] In the present study, 15 days after bleaching is need for the restoration be able to improve the bond strength with similar results to the positive control group which was not bleached.

In order to accelerate the process of releasing the residual oxygen and reduce the waiting time to perform bonding procedures after bleaching, several techniques have been suggested, but few satisfactory results have been obtained. Among the techniques, there are the removal of the superficial layer of enamel,[29] treatment of bleached enamel with alcohol or acetone prior to performing the restoration to remove residual oxygen and water from surface[8] and the use of adhesives containing organic solvents, such as acetone or ethanol.[20]

Table 4: Analysis of the types of fractures observed by the groups after microshear bond strength

Groups	*Adhesive (A)* *N (%)*	*Cohesive enamel (CE)* *N (%)*	*Cohesive resin(CR)* *N (%)*	*Mixed (M)* *N (%)*	*Total* *N (%)*
G1	14 (78)	0 (0)	0 (0)	4 (22)	18 (100)
G2	17 (94)	0 (0)	0 (0)	1 (6)	18 (100)
G3	18 (100)	0 (0)	0 (0)	0 (0)	18 (100)
G4	16 (89)	0 (0)	0 (0)	2 (11)	18 (100)
G5	16 (89)	0 (0)	0 (0)	2 (11)	18 (100)
Total	81 (90)	0 (0)	0 (0)	9 (10)	90 (100)

The use of antioxidant solutions seems to be the most promising to reduce the side effects of bleaching agents on enamel/dentin. Some authors have shown that oxidizing agents permit reversal of bond strength,[1,3,6,9,11-15] allowing the restorative procedure be performed successfully which lead to longevity, bond durability, and absence of microleakage.[9]

Although, the application of antioxidants is the most studied and promising technique to solve the deleterious effects of peroxides on the adhesive restorations, there is still a difference regard to the minimum time necessary to achieve these results. Most authors recommend 1/3 of the time corresponding to the application of the bleaching agent.[3,6,7,12,19] Another study[11] has reversed the values of bond strength after prolonged treatment with peroxides (7 days) by daily application of antioxidant solution for 10 minutes after bleaching. According to Freire et al[18] using 35% sodium ascorbate, although a greater time between the antioxidant and the tooth results in a longer reaction between the oxidant and antioxidant, the reaction takes only 1 minutes. After this time, the reaction was substantially reduced. Thus, the number of applications of the antioxidant is more important than the contact time.

In this study, hydrogen peroxide was used in a single session of 40 minutes and the antioxidant was tested for 15 minutes (G4 =1/3 the time of bleaching) and 2 hours (G5), to check if there was an improvement in bond strength when the agent was left for longer. The results demonstrated that the oxidizing agent used for 15 minutes (G4) helped in the elimination of residual oxygen by increasing the bond strength values according to several studies.[3,6,7,12,19] The results for G4 were similar to the unbleached group (G1) ($p > 0.05$). However, when the ascorbyl was used for a longer time (G5-2 hours), there was a loss in bond strength, showing statistically significant results in the unbleached control group (G1). It is worth remembering that, despite the results for G2 and G4 were not statistically significant compared to the unbleached group (G1), there was complete recovery of bond strength. The outcomes for G2 and G4 were not statistically significant in relation to G3 and G5.

Further studies are needed to assess if ascorbyl gel when used after bleaching with 35% hydrogen peroxide for a long time (2 hours) is able to change tooth surface or lose some properties invalidating the bond procedure.

The ascorbyl phosphate is a white powder, easily soluble in water at concentrations up to 50% on basic pH. It is able to block the active center of the molecule of ascorbic acid, providing a great stability. On saliva containing the enzyme alkaline phosphatase, the ascorbyl phosphate is converted to free ascorbic acid by the cleavage of carbon-oxygen at C-2 phosphate.[19] Ascorbic acid has pH 1.9 and therefore the application is not recommended for a long time in order not to change the dental structures.[1,21] In this study, saliva was not used. Probably, the breaking of ascorbyl phosphate in the ascorbic acid was due to the presence of remaining alkaline phosphatase in the dental tissue.

Adhesion tests *in vitro* are relevant for the evaluation of materials and treatments, as they allow variables to be isolated and controlled, which aid in understanding and predicting the behavior of materials, which subsequently can be applied *in vivo*.[9]

In the present study, the bond strength was assessed by microshear test. The method allows multiple specimens are obtained from a sample of enamel, dentin (or another substrate) due to the interfaces are very small (about 0.4 mm^2). The method is versatile and useful to evaluate the bond strength of mineralized tissues and polymeric restorative materials. Furthermore, the standardization in the preparation of the specimens can be made, since it is an important factor on bond strength studies[22,23.]

The evaluation of the type of fracture by 40× microscope after the microshear bond strength was also relevant. Mixed and adhesive the failures were mostly found. For all groups, there was a predominance of adhesive fractures (90%), indicating a reduction in bond strength between the tooth and the restorative material. The number of mixed fractures was higher in unbleached group (G1-22%), followed by groups submitted to the application of antioxidant (both G4-G5-11%). Similar results were found by Kimyai and Valizadeh[9] also using the microshear bond strength test.

In this study, it is noted that to avoid side effects after bleaching with 35% hydrogen peroxide it should be expected a suitable time from 15 days to the removal of free radicals, and then performing the restoration. In cases in which the urgency is needed the association of a bleaching agent with an antioxidant 3% ascorbyl phosphate for 15 minutes should replace the waiting time for the elimination of free radicals.

Thus, it is suggested that the dentist should be cautious in the adhesive procedures on enamel bleached with 35% hydrogen peroxide and further studies *in vitro* are needed to assess the longevity and stability of adhesion after using antioxidants, the variability of concentrations and duration time, as well as interference in the dental structure on changes in the pH of antioxidants.

CONCLUSION

The use of 3% ascorbyl gel for 15 minutes was able to improve bond strength of the composite resin to bovine enamel bleached with 35% hydrogen peroxide, but when 3% ascorbyl gel was applied during 40 minutes it negatively interfered in the adhesion of the resin to bleached bovine enamel.

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