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In vitro evaluation of the effect of antioxidants on the bond strength of bleached teeth

Avaliação *in vitro* do efeito de antioxidantes sobre a resistência de união de dentes clareados

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ABSTRACT

Objective: To evaluate the *in vitro* effect of antioxidants on the bond strength between composite resin and enamel subjected to bleaching agents. **Methods:** Nineteen sound human molars had their roots sectioned, while their surfaces were flattened and filled with composite resin to produce the specimens, which were then divided into seven groups: G1 – unbleached and without antioxidant (control); G2 – 36% carbamide peroxide; G3 – 36% carbamide peroxide and 10% ascorbic acid solution; G4 – 36% carbamide peroxide and 10% ascorbic acid gel; G5 – 16% carbamide peroxide; G6 – 16% carbamide peroxide and 10% ascorbic acid solution; and G7 – 16% carbamide peroxide and 10% ascorbic acid gel. The results of microtensile strength were submitted to an analysis of variance (ANOVA) and Tukey's test, with a significance level of 5%. **Results:** The bond strength between composite resin and enamel was not affected after the use of bleaching agents. In addition, ascorbic acid did not appear to neutralize the oxidative effects of these agents, given that group 3 showed the lowest mean of bond strength (13.85 MPa). In other groups, the mean of bond strength ranged from 24.06 MPa to 32.02 MPa, with no significant differences when compared to the control. **Conclusion:** The presence of ascorbic acid immediately after bleaching did not increase the bond strength between the resin and the enamel surface.
Uniterms: Tooth bleaching. Antioxidants. Ascorbic acid. Dental enamel. Composite resins.

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INTRODUCTION

Bleaching of vital teeth usually aims at esthetic improvement of darkened and pigmented teeth[1]. However, despite the fact that teeth bleaching is becoming increasingly common as a procedure included in a restorative treatment plan, it may lead to side effects, such as decreased bond strength between composite resin and the bleached tooth structure[2,3].The presence of residual oxygen free radicals has been reported as the main reason for the occurrence of these phenomena, particularly because of their effects on the polymerization of resins[4,5]. Remineralization of bleached areas and an increase in bond strength occur only days after exposure of bleached teeth to saliva, reasons for which it is not recommended to perform immediate restoration after tooth bleaching. This procedure requires a minimum of one week between the procedure and restorative treatment so that the enamel bond strength can be reverted. Nevertheless, this timeframe may be considered too long, depending on the esthetic needs of the patient[5,6].

In this sense, antioxidants, such as ascorbic acid derivatives can act as free-radical stabilizers, which may represent a viable alternative in these cases, as described in many studies[6-12]. These agents act by neutralizing the etiological factor responsible for the incomplete polymerization of the resin monomers, thus resulting in a successful and long-lasting restorative procedure, with adhesive durability and absence of microleakage[13].

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Given that antioxidant agents can reduce the amount of free radicals in the bleached tooth structure, favoring a complete polymerization in immediate composite resin restorations, and taking into account the patients' desire to have their esthetic treatments completed in a timely manner, this study aimed to evaluate the effect of antioxidants on the bond strength of resins in bleached teeth.

MATERIAL AND METHODS

The present study received approval from the Research Ethics Committee of the State University of Paraíba (Brazil), logged under protocol number 0422.0.133.000-10, in compliance with Resolution 196/96 of the Brazilian National Health Council.

Nineteen sound human molars with indication for extraction were collected in this study. These teeth were stored in 0.2% thymol aqueous solution for 24 hours until disinfection, and were then washed in running water and cleaned with curettes and Robson brushes (KG Sorensen, Cotia, SP, Brazil) with pumice/water paste, coupled to a micromotor (Dabi Atlante, Ribeirão Preto, SP, Brazil), ensuring the removal of soft tissues. The teeth were stored in distilled water at 4°C and replaced weekly for a maximum period of six months to prevent bacterial growth.

To prepare the enamel surface, the roots were sectioned with the aid of a double-sided diamond disc (SSWHITE, Rio de Janeiro, RJ, Brazil), and the crowns were hemi-sectioned in the mesiodistal direction. The coronal portions were immobilized using an adhesive gel of cyanoacrylate (Henkel, Itapevi, SP, Brazil) to self-curing acrylic resin plates (Classic, Sao Paulo, SP, Brazil), embedded in PVC tubes (Tiger, Escada, PE, Brazil) with 25 mm in diameter and 10 mm in height. The buccal and lingual faces were exposed 1 mm beneath the tube.

The buccal and lingual surfaces were flattened by a polishing machine (Panambra, São Bernardo do Campo, SP, Brazil) and silicon carbide-based sandpaper (3M, Sumaré, SP, Brazil) with granulation #320 in order to flatten a suitable area for adhesion. Next, another prophylaxis, using Robson brushes and pumice/water paste coupled to a micromotor with handpiece, was performed in order to remove possible debris remaining on the enamel surface.

In all steps of this study, i.e., preparation of specimens, bleaching with carbamide peroxide (Ultradent, Indaiatuba, SP, Brazil) and exposure to ascorbic acid, specimens were randomly allocated into groups, with or without ascorbic acid (Pharmaface, Campina Grande, PB, Brazil), as shown in Table 1.

Table 1 - Distribution of specimens by group and corresponding treatment

Group	Treatment
G1	No treatment (control)
G2	36% carbamide peroxide
G3	36% carbamide peroxide + 10% ascorbic acid solution
G4	36% carbamide peroxide + 10% ascorbic acid gel
G5	16% carbamide peroxide
G6	16% carbamide peroxide + 10% ascorbic acid solution
G7	16% carbamide peroxide + 10% ascorbic acid gel

All groups consisted of 15 specimens, with some exceptions: group 3 had only five specimens; there was and a loss of two and one specimens from groups 2 and 5, respectively, when they were being immobilized in Geraldeli's device. The control group received no bleaching or antioxidant treatment; specimens were restored according to the manufacturer's recommendations.

After preparation of whitening trays through a vacuum laminator (Bioart, São Carlos, SP, Brazil), the specimens were bleached. Groups to be subjected to in-office bleaching (groups 2, 3, and 4) first underwent prophylaxis with pumice/water paste, followed by color selection using the VITA scale and the application of the whitening agent on the enamel surface, which was removed afterwards. The application and removal of the bleaching agent was repeated two more times, followed by the prophylaxis procedure.

As for the other groups that underwent at-home bleaching (groups 5, 6, and 7), after prophylaxis and the selection of color, the bleaching agent was applied to the tray, which was kept in contact with the

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specimen for four hours. Then the agent was removed and prophylaxis was performed. Ascorbic acid was applied onto the dry bleached surface for 10 minutes using a microbrush (FGM, Joinvile, SC, Brazil), followed by cleaning in running water for 30 seconds.

According to manufacturers' specifications, 37% phosphoric acid (Biodinâmica, Ibiporã, PR, Brazil) and two layers of conventional adhesive (3M/ESPE, Seefeld, BA, Germany) were applied to the surface. One-milliliter increments of nanoparticle composite resin (3M/ESPE, Sumaré, SP, Brazil) were homogeneously applied for a total of 5 mm. Each layer was light-cured using LED light with an intensity of 500 mW/cm^2 (Gnatus, Ribeirão Preto, SP, Brazil).

The specimens were obtained using a serial sectioning machine (Equilp, São Carlos, SP, Brazil) to perform serial sections perpendicular to the long axis by means of a diamond disc (Extec, Enfield, CT, United States) turning at low speed (200 rpm) under constant irrigation. The sections had 1 mm thickness in the mesiodistal and cervical-incisal directions. The base of the tooth was then sectioned parallel to the long axis. Each tooth provided 15 'stick-like' specimens, with transversal areas of approximately 1 mm^2.

The specimens were fixed at their ends to a microtensile device (Geraldeli's device) and adapted in a universal testing machine (Instron, Barueri, Brazil) at 0.5 mm/min. The results were converted into Mpa and submitted to ANOVA and Tukey's test, with a significance level of 5% ($p < 0.05$).

For data analysis, we used descriptive statistics (mean, median, and standard deviation) for numerical variables, as well as the one-way ANOVA (F test) with Tukey's comparisons. The hypothesis of equality of variances was verified by Levene's F test, and the hypothesis of data normality was performed using the Shapiro-Wilk test applied to the standardized residuals.

The margin of error used in the decision from the statistical tests was 5%. Statistical Package for Social Sciences version 17.0 (SPSS Inc., Chicago, IL, USA) was used for data entries and statistical calculations.

RESULTS

Table 2 shows the comparative results between groups (at-home bleaching with 16% carbamide peroxide and in-office bleaching with 36% carbamide peroxide) with no antioxidant or with a 10% ascorbic acid solution/gel. The mean of bond strength were found to be higher in the groups using 16% carbamide peroxide than in those using 36% carbamide peroxide; the lowest mean (13.85 Mpa) was found in group 3 (36% carbamide peroxide plus 10% ascorbic acid solution), while the highest mean (32.02 Mpa) was found in group 5 (at-home bleaching with 16% carbamide peroxide without antioxidant). Either with or without bleaching, the only significant difference was seen in the groups treated with a 10% acid solution.

The only difference regarding the use of antioxidant (solution/gel) was detected in the group bleached with 36% carbamide peroxide. Moreover, pairwise multiple comparisons (Tukey's test) confirmed significant differences in the bond strength values between a 10% acid solution and no antioxidant. The variability expressed by the coefficient of variation was not found to be high, as it was lower than 50% in each group.

Table 2 - Bond strength values by group according to the antioxidant agent used

		Group			
Antioxidant	**Statistical measures**	**No treatment**	**16% Carbamide**	**36% Carbamide**	**p-value**
		G1	G5	G2	
No antioxidant	Number of stick-like specimens	15	14	13	
	Mean	26.91	32.02	29.92 $^{(A)}$	**$p^{(1)} = 0.429$**
	Median	26.30	33.08	30.17	**$p^{(2)} = 0.639$**
	Standard-deviation	9.60	12.10	10.71	
	Coefficient of variation	35.67	37.79	35.80	
	Minimum	14.37	7.81	8.70	
	Maximum	43.70	49.68	45.30	
		-	G6	G3	
10% ascorbic acid solution	Number of stick-like specimens		15	5	
	Mean		25.93	13.85 $^{(B)}$	**$p^{(3)} = 0.002$***
	Median		30.15	13.39	
	Standard-deviation		11.99	2.13	
	Coefficient of variation		46.24	15.38	
	Minimum		2.34	11.72	
	Maximum		43.23	17.41	

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Antioxidant	Statistical measures	Group			
		No treatment	16% Carbamide	36% Carbamide	p-value
		-	G7	G4	
10% ascorbic acid gel	Number of stick-like specimens		15	15	
	Mean		26.81	24.06 (AB)	**p(2) = 0.483**
	Median		27.30	22.00	
	Standard-deviation		10.00	11.17	
	Coefficient of variation		37.30	46.43	
	Minimum		9.48	5.52	
	Maximum		39.50	48.24	
	p-value		**p(4) = 0.312**	**p(4) = 0.019***	

(*) Significant difference at a 5% level.

(1) F test (ANOVA) for comparison between three groups: No treatment, 36 % Carbamide and 16% Carbamide.

(2) Student's t test with equal variances for comparison between groups: 16% Carbamide and 36% Carbamide, without antioxidant.

(3) Student's t test with unequal variances.

(4) F test (ANOVA) for comparison between the dosage forms of the antioxidants.

Note: Distinct letters in parentheses indicate significant difference between the respective groups by Tukey's pairwise comparisons.

The two-factor F test (ANOVA) was used to verify significant differences between the bleaching agents (p = 0.040) and the antioxidants (p = 0.008), regardless of the control group. The interaction was not significant (p = 0.318). Multiple comparisons showed no difference between the levels 'no antioxidant' and '10% acid solution'.

DISCUSSION

It has been pointed out that bleaching agents can cause possible structural and morphological changes in enamel and dentin, which could interfere with the bond strength of the adhesive system to these tooth substrates[4,7]. Studies have shown that the bond strength of usual adhesive systems to enamel and dentin is significantly reduced upon immediate use after bleaching[11,14,15].

These changes in the ability of composite resins to adhere to the bleached tooth structure were observed by other authors performing experiments on shear bond strength[6], tensile[4] and microtensile bond strength[16], scanning electron microscopy[8], and microleakage[17].

The main cause of loss of adhesion at the tooth/filling interface is reported to be the presence of oxygen free radicals remaining on the bleached tooth structure and complete inhibition of polymerization of the adhesives[5]. The influence of oxygen in the polymerization is ruled by many factors, such as temperature, light intensity, and chiefly O_2 concentration. Therefore, the greater the amount of oxygen free radicals, the greater the impact on the bond strength of resin materials to the tooth structure[4].

In our study, these same findings were not observed, however. There was no significant difference in the resin bond strength to enamel when restoration was carried out immediately after bleaching with carbamide peroxide. This can be seen in the results of group 2 (bleaching with 36% carbamide peroxide) and group 5 (bleaching with 16% carbamide peroxide), which showed the highest mean of bond strength (29.92 MPa and 32.02 MPa, respectively) when compared to the other groups, including the control group (group 1), which was not subjected to bleaching.

These findings corroborate those found by Marson, Sensi & Arruda[18], who evaluated the bond strength between resin and bleached enamel in fifty molars randomly divided into five groups: G1 – unbleached, G2 – bleached with 10% carbamide peroxide and restored after 24 h, G3 – bleached with 10% carbamide peroxide and restored after seven days, G4 – bleached with 35% hydrogen peroxide and restored after 24 h, and G5 – bleached with 35% hydrogen peroxide and restored after seven days. The authors observed a decrease in the bond strength between the composite resin and the bleached enamel in G4.

Given the influence of post-bleaching oxygen radicals in the decreased bond strength of fillings to the tooth surface, several studies have investigated and confirmed the effectiveness of different antioxidant agents in preventing this phenomenon from occurring[6-12]. Antioxidant agents, such as ascorbic acid derivatives, can act as free radical stabilizers and are therefore capable of neutralizing the etiological factor responsible for the incomplete polymerization of the resin monomers, resulting in a successful long-lasting restorative procedure, with adhesive durability and absence of microleakage[7,13].

This possible reversing effect of antioxidants after bleaching was reported in the study by Kunt et al.,[6] who used 10% ascorbic acid. Likewise, Kimyai & Valizadeh[19] observed that the bond strength of the composite resin increased after treatment of bleached teeth with 10% ascorbic acid for 10 minutes.

In the present study, we also evaluated the role of 10% ascorbic acid in the form of gel and solution

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applied for a period of 10 minutes onto the bleached tooth surface. However, based on this protocol, our findings differed from those previously reported.

Ascorbic acid used at the same concentration (10%) for the same period of time, as described by other authors, was unable to neutralize the oxidizing effects of bleaching agents. This can be seen in groups 3 and 4, which showed the lowest means of bond strength (13.85 MPa and 24.06 MPa, respectively), with a significant difference only for group 3 when compared to the other groups, considering a 5% margin of error.

Some studies recommend a waiting period of one to two weeks after tooth bleaching before proceeding with adhesive restorations[4,6,20]. Nevertheless, the results found in this study indicate that such a time interval before the completion of the esthetic treatment is unnecessary and impractical, given the patient's needs. Thus, the adhesive restoration can be carried out soon after bleaching.

Taking into account the results obtained in this study and those described elsewhere, what becomes evident is the importance of the consensus on the use of antioxidants on the bleached tooth surface as a way to reduce in-office time and rehabilitate the patient's esthetics quickly, but without further damage. We could not verify significant difference in bond strength between groups, showing that the use of 10% ascorbic acid (either gel or solution) shortly after the bleaching procedure was not able to neutralize the oxidative effects of the bleaching agent.

CONCLUSION

There was no association between the use of 10% ascorbic acid, either solution or gel, and an increase in bond strength values between the composite resin and the enamel surface after bleaching.

RESUMO

Objetivo: Avaliar *in vitro* o efeito de antioxidantes sobre a resistência de união entre resina composta e o esmalte sujeito a agentes clareadores. **Métodos:** Dezenove molares humanos hígidos tiveram suas raízes seccionadas e suas superfícies planificadas e restauradas para obtenção dos corpos-de-prova, os quais foram divididos em sete grupos: G1 - não clareado e sem agente antioxidante (controle); G2 - Peróxido de carbamida a 36%; G3 - Peróxido de carbamida a 36% e Ácido ascórbico 10% solução; G4 - Peróxido de carbamida a 36% e Ácido ascórbico 10% gel; G5 - Peróxido de carbamida a 16%; G6 - Peróxido de carbamida a 16% e Ácido ascórbico 10% solução; G7 - Peróxido de carbamida a 16% e Ácido ascórbico 10% gel. Os resultados da resistência a microtração foram submetidos à análise de variância (ANOVA) e ao teste de Tukey em nível de significância 5%. **Resultados:** A resistência adesiva entre resina e o esmalte não foi afetada após a utilização dos agentes clareadores, além disto, o ácido ascórbico não se apresentou capaz de neutralizar os efeitos oxidantes destes agentes, tendo em vista que o grupo 3 mostrou menor média quanto a resistência de união (13,85 Mpa). Nos demais grupos, as médias variaram de 24,06 Mpa a 32,02 Mpa, não havendo diferença significante entre essas médias e o controle. **Conclusão:** A presença do ácido ascórbico logo após o clareamento dentário não aumento a resistência de união entre a resina e a superfície do esmalte.
Descritores: Clareamento dental. Antioxidantes. Ácido ascórbico. Esmalte dentário. Resinas compostas.

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