

INFLUENCE OF ANTIOXIDANTS ON STRESS OF BONDING AGENTS IN RECENTLY WHITENED TEETH

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ABSTRACT

The aim of this study was to evaluate the influence of time of application of antioxidant agents on the bond strength to bovine enamel after bleaching by light emitting diode. Twenty two bovine incisors were used, on which the vestibular surfaces were flattened and divided into four experimental groups ($n = 15$) according to each antioxidant agent, with two groups each, varying the application time of 1 or 10 minutes, all submitted to the same whitening agent (Lase Peroxide) and the control group was only restored. The other groups were treated with antioxidant agents before the restorative procedure using the adhesive system

All Bond 3 and resin Filtek Z-350. To obtain the dental fragments, teeth were placed in a precision cutter (Elquip), obtaining 0.8 mm² with sticks that were submitted to micro tensile testing at a test speed of 0.5 mm / min. The results, statistically analyzed by ANOVA and Tukey test ($p < 0.05$), indicated an increase in the value of bond strength of bleached groups treated with ascorbic acid, and a reduction when combined with sodium thiosulfate. No improvement in bond strength was found in the others.

Keywords: Dental Bleaching, Bond strength, Antioxidants, Adhesiveness.

INFLUÊNCIA DE AGENTES ANTIOXIDANTES SOBRE A RESISTÊNCIA DE UNIÃO EM DENTES RECÉM CLAREADOS

RESUMO

Esse estudo teve como objetivo avaliar a influência do tempo de aplicação de agentes antioxidantes sobre a resistência de união ao esmalte dental bovino após clareamento, através do teste de microtração. Foram utilizados 22 incisivos bovinos, onde as faces vestibulares foram planificadas, sendo divididos em 4 grupos experimentais ($n=15$) de acordo com cada agente antioxidante, com dois subgrupos cada, variando o tempo de aplicação deste em 1 ou 10 minutos, todos submetidos ao mesmo agente clareador (Lase Peroxyde) e o grupo controle foi apenas restaurado. Enquanto os demais grupos receberam tratamento com os respectivos agentes antioxidantes, previamente ao procedimento restaurador, sendo utilizado o sistema

adesivo All Bond 3 e a resina composta Filtek Z-350. Para obtenção dos fragmentos dentais, os dentes foram posicionados numa cortadeira de precisão (Elquip), obtendo-se palitos com 0,8 mm² que foram submetidos ao teste de microtração, a uma velocidade de ensaio de 0,5mm/min. Os resultados, analisados estatisticamente por ANOVA e teste de Tukey ($p < 0,05$), indicaram um aumento no valor da resistência de união dos grupos clareados e tratados com ácido ascórbico, e uma redução quando em associação com tiosulfato de sódio, nos demais não houve melhora na resistência de união.

Palavras chave: Clareamento Dental, Resistência Adesiva, Antioxidantes, Adesividade.

INTRODUCTION

Bleaching treatments are a fast and economical way of restoration of the aesthetics whose indication, prior to restoration procedures, is very frequent. Whitening of teeth has become a routine target in dental practices and is currently a conservative supplementary technique for obtaining satisfactory results when more invasive procedures are selected in aesthetic and cosmetic rehabilitation¹.

Such procedure consists in an oxidation reaction in which the substrate to be bleached supplies electrons to the whitening agent. An example of an oxidizing agent is hydrogen peroxide, an extremely unstable electrolyte, which readily decomposes into water-soluble solvents to form radicals (pehydroxyl), which are highly reactive². Due to its low molecular weight, 30 g/mol, hydrogen peroxide penetrates through the pores of enamel prisms and reaches the

dentine, thus contacting a large amount of pigment molecules and breaking them down into smaller chains of lighter appearance.

As products of such reactions, free radicals and residual oxygen and water are released^{3,4}. The presence of residual oxygen has been considered as the main factor responsible for the decrease in the resistance of the bonding of composites to the whitened dental structure⁵⁻⁸, due to their adverse effect on the polymerization of adhesive systems⁹⁻¹¹.

To enable the total release of peroxide by-products and, thus, to enable definitive restoration, a 14-to-21-day waiting period is recommended^{12,13}. Yet, this interval may be too long for patients who want immediate aesthetic results.

To minimize this inconvenience, the treatment of the whitened dental structure with antioxidants has been recommended, for example, with sodium ascorbate (10%), to enable the completion of aesthetic restorations in shorter periods, as sodium ascorbate removes residual oxygen and promotes higher adhesiveness to the whitened dental substrate^{14,15}.

Antioxidants derived from ascorbic acid are used for decreasing the time interval between dental whitening and definitive restoration, thus enabling for the restoration procedure to be made with the prospect of maintaining longevity and durability of the adhesive¹⁶. However, application time of antioxidants for reverting the adverse effects on adhesion to the enamel and the dentine^{14,15} has not been considered as entirely feasible yet, as it is seen as too lengthy for clinical uses. Bearing in mind that antioxidant agents act as purifiers of free radicals, the purpose of this study was to assess the influence of the use of sodium thiosulfate, BHA, sodium ascorbate and ascorbic acid on the effective removal of residual oxygen and on the adequate adhesive post-whitening bonding in teeth that need immediate restorations and, to enable an ideal clinical timing for their application.

MATERIALS AND METHODS

Twenty-two bovine superior central incisors were used. The specimens were stored in an aqueous medium of thymol (0,9%) (Dilecta Farmácia de Manipulação, Paraíba, Brazil) during 24 hours for disinfection purposes. Each tooth had its buccal surfaces smoothed by means of a polishing device Model SD-10 (Panambra, São Paulo, Brazil) using 320 grit silicon carbide sandpaper to prepare an area appropriate for adhesion. The restorative procedure

in this study was performed using the Lase Peroxide Sensy system (DMC, São Paulo, Brazil), Hydrogen Peroxide (35%), antioxidant agents such as sodium ascorbate, ascorbic acid, sodium thiosulfate and BHA (Butylhydroxyanisole) (10% all of them), the All Bond 3[®] adhesive system (Bisco, Schaumburg, IL, USA) and nanoparticle composite Filtek Z-350[®], color A3 (3M/ESPE, St Paul, Minn, USA).

Specimens were divided into eight groups of fifteen units each, according to the type of antioxidant to be used and to the corresponding application time (1 or 10 minutes), as shown in Table 1, and into a control group that was restored only, with no whitening treatment or use of antioxidants.

Teeth were secured in condensation silicone (Perfil Denso - Vigodent, Rio de Janeiro, Brazil), then, prophylaxis was performed with pumice and water before the whitening agent was applied, as per manufacturer's instructions. Altogether, three applications were performed on each tooth, with three-minute polymerization (LED 470nm, Whitening lase II, DMC, São Paulo, Brazil). After these stages, water rinse was made during 30 seconds, for removal of possible detritus left on the enamel surface. Then, antioxidants were applied on the dental elements during the corresponding time lapses (1 or 10 minutes) and removal of such agents was done through rising during 30 seconds.

The restoration procedure was performed according to manufacturer's recommendations, as follows: Conditioning with phosphoric acid (32%) during 30 seconds (Bisco, Schaumburg, IL, USA), 30-second rinsing with tap water. Application of two layers of adhesive system with a microbrush (Cavibrush – FGM, Santa Catarina, Brazil), subsequent application of light jet of air during 5 seconds and polymerization for 10 seconds. The resin composite restoration was

Table 1: Groups.

Groups	Whitening	Antioxidant	Time
G1	Hydrogen Peroxide 35%	Sodium Ascorbate	1 minute
G2	Hydrogen Peroxide 35%	Ascorbic Acid	1 minute
G3	Hydrogen Peroxide 35%	Sodium Thiosulfate	1 minute
G4	Hydrogen Peroxide 35%	BHA	1 minute
G5	Hydrogen Peroxide 35%	Sodium Ascorbate	10 minutes
G6	Hydrogen Peroxide 35%	Ascorbic Acid	10 minutes
Control	-	Restored Only	-
G7	Hydrogen Peroxide 35%	Sodium Thiosulfate	10 minutes
G8	Hydrogen Peroxide 35%	BHA	10 minutes

about 5 mm in height and each increment was on average of 2 mm and was cured with a light intensity of 500 mW/cm² (Ld max – Gnatus, São Paulo, Brazil), during 20 seconds. The specimens were stored in distilled water in a stove at 37° C for 24 hours.

The teeth had their roots severed at the cervical region. With the help of a double-sided diamond flexible disc (KG Sorensen, São Paulo, Brazil) coupled to a micro-engine with a straight rod (Dabi-atlante, São Paulo, Brazil), with constant irrigation, the lingual crown teeth were then secured with a low fusion compound (DFL, Rio de Janeiro, Brazil) on an acrylic resin base leaving the vestibular surface fully visible and adjusted on a metal support for it to be fixed on the serial section cutting machine (Elquip, São Paulo, Brazil).

By means of a diamond disc (EXTEC, Enfield, USA) rotating at low speed (200 rpm) with constant irrigation, perpendicular serial sections were cut along the axis of the specimen, following cervico-incisal and mesial-distal directions. Finally, the base of the tooth was sectioned parallel to the its longitudinal axis and each tooth supplied an average of 12 stick-shaped specimens, with a cross-sectional area for tests of approximately 0,8 mm². By means of a digital read-out caliper, the bonding area of each specimen was calculated and expressed in mm² for subsequent calculations of micro tensile bonding stress.

The ends of the sticks were fixed to the micro tensile device with a gel-based cyanoacrylate adhesive (Super Bonder – 3M/ESPE, St Paul, Minn, USA) and adapted into a universal test device (KRATOS K2000 – EQUIP. IND. LTDA. /N. M98D301, São Paulo, Brazil) so as to position the bonding interfaces perpendicularly all along the axis of the tensile stress and

were subject to a 0,5mm/min speed, until fracture of the specimen occurred. Then, the maximum load of rupture of each specimen was recorded and the specimen fragments were carefully removed from the grips. The results were obtained in Kgf, then transformed into MPa and subject to variance analysis– ANOVA and TUKEY test with a 5% significance level (p<0,05).

RESULTS

For data analysis, descriptive and inferential statistics of the micro tensile bonding stress were obtained among all the groups (Table 2).

Table 2 shows the descriptive statistics of bonding stress, in all of the groups. It was observed that the averages of bonding stress values were higher for G5, followed by G6, with no statistical difference among the relevant groups. In groups G3 and G7, averages were statistically equivalent and obtained a lower bonding stress level. Even with longer times of application of antioxidant agent, resistance decreased. Groups G3 and G8 also revealed lower bonding stress levels when compared to groups G1, G2, G5 and G6. Even with the increase in bonding stress, with longer times of application of antioxidant agent, this increase was not significant.

The averages of the micro tensile bonding values ranged from 6,10 Mpa to 23,43 Mpa, the latter being the highest for the control group.

In Table 3, results of the F Test, performed via ANOVA, are shown, in which it was possible to veri-

Table 2: Bonding Stress Values of the Groups.

Groups	Statistics				
	Minimum (MPa)	Maximum (MPa)	Average (MPa)	Standard Deviation (MPa)	Rate of Variation (MPa)
G1	5.60	25.52	17.54 ^a	5.22	27.25
G2	9.89	22.96	16.46 ^a	3.23	10.47
G3	1.51	10.71	6.10 ^b	2.91	8.48
G4	2.82	18.94	8.21 ^b	4.59	21.07
G5	7.77	23.79	17.73 ^a	4.19	17.60
G6	7.57	27.54	17.65 ^a	4.79	23.03
G7	1.05	7.86	4.09 ^b	2.09	4.40
G8	2.62	11.52	7.26 ^b	2.55	6.52
Control	16.27	34.91	23.43 ¹	5.88	34.58

Table 3: Averages of micro tensile bonding stress and results of Tukey Test.

Groups	Time of Application	Antioxidant	Average (MPa)	Results of Comparisons of Tukey
Control	-	-	23.43	A
G1	1 Minute	Ascorbic Acid	17.54	B
G2	1 Minute	Sodium Ascorbate	16.46	B
G3	1 Minute	Sodium Thiosulfate	6.10	C
G4	1 Minute	BHA	8.21	C
G5	10 minutes	Ascorbic Acid	17.73	B
G6	10 minutes	Sodium Ascorbate	17.65	B
G7	10 minutes	Sodium Thiosulfate	4.09	C
G8	10 minutes	BHA	7.26	C

fy that, except for the application of antioxidant agents BHA and Sodium Thiosulfate with any time of application, all the other interactions were significant ($p < 0,05$). It was underscored that the hypotheses of the similarity of variances and the normal nature of the data were accepted with a 5,0% significance level ($p = 0.958$, as per Shapiro-Wilk Test).

Still in Table 3, through ANOVA (*one-way*), a significant difference between the groups was proved ($F = 43,83$ and $p < 01$) and through the tests of matched comparisons and between group pairs: As to the Control Group, there was a significant difference vis à vis all the other groups. Through this variance analysis, the difference between groups subject to tests was observed for each one of the combinations. There were significant statistical differences among them.

DISCUSSION

The use of peroxides in dentistry has yielded excellent clinical results in whitening treatments. Yet, some undesirable effects have been identified regarding the decrease in bonding stress to the dental structure¹³, thus affecting the success of the post-whitening restoration procedure.

Some studies have shown that the presence of residual oxygen is the main cause for the decrease in bonding stress of composites against the whitened dental structure⁵⁻⁸. Thus, professionals should try to eliminate any remnant oxygen and radicals derived from the peroxides added to the dental structure to be restored with resinous materials. To perform the restoration procedure, a time interval has been recommended for enabling the entire release of peroxide by-products and, hence, for a successful and definitive restoration^{13, 17}.

Turkun 2004 and Sundfeld et al., 2005, verified that a minimum seven-day term is considered as satisfactory. For Teixeira et al 2004, there is no difference between seven, fourteen and twenty-one days, but Basting et al., 2004, considered that the fifteen-day interval was appropriate. On the other hand, Cavalli et al., 2001 considered that the twenty-one period was ideal as there is a linear relationship between the waiting time and the increase in resistance of the bonding. From the clinical standpoint, this interval may be too long for patients that require an immediate aesthetic treatment.

To minimize this inconvenience, it has been suggested that treatment of the whitened dental structure be

made with antioxidant agents. These are substances that act by neutralizing free radicals by emitting their own electrons, which halts the reaction of loss of electrons. The best known antioxidants are Vitamins A, C (Ascorbic Acid), E, Betacarotene²⁰, Sodium ascorbate, Sodium Thiosulfate solution²¹. These antioxidants may be used to revert the values of bonding resistance of teeth subject to whitening practices^{14, 22} by removing the residual oxygen from the dental structure, with the aim to promote a good adhesion on the enamel and on the dentine¹⁵. Hence, this could be an alternative to decreasing the time interval between whitening and definitive restoration²³.

Antioxidants used in this study were as follows: Sodium Thiosulfate and BHA, Sodium Ascorbate and Ascorbic Acid. Sodium Thiosulfate removes free radicals from neural cells resulting from the oxidation of dopamine. These free radicals are considered as the origin and evolution of Parkinson disease²³. BHA is a synthetic antioxidant used in the food industry for the suppression of oxidation in animal fats. Its efficacy is limited in unsaturated oils derived from vegetables or seeds. It has low stability levels when submitted to high temperatures. Research has shown that these antioxidants may have toxic effects²⁴.

Ascorbic acid and its related salts are powerful antioxidants capable of reducing a variety of compounds, namely, free radicals^{3, 15-17, 24}, and is widely used in the food industry. Sodium Ascorbate is also quite widely used as an antioxidant in biological systems, being considered a good alternative treatment for its capacity for interacting with residual oxygen in teeth post-whitening, by accelerating removal of residual oxygen, and by reverting the decrease in the bonding stress action after dental whitening^{15, 25, 27}. Both are considered as biocompatible antioxidant agents, safe for oral use^{14, 15, 26}.

According to the results in this research, the Control Group obtained the highest bonding stress value. On the other hand, the lowest values were obtained by the groups in which Sodium Thiosulfate and BHA were used as antioxidant agents. Even with an increase in the application time, bonding stress values were equivalent among them, yet, when compared with the other groups in the research, the bonding stress value was lower, with a statistical difference. These results match the findings of Kaya and Turkun (2003), who verified the decrease in the bonding stress of teeth recently whi-

tened and treated with BHA. When compared with Sodium Ascorbate (10%), BHA could not revert the adverse effects of bonding, while Sodium Ascorbate was effective in this regard.

The groups in which Ascorbic Acid and Sodium Ascorbate were used as antioxidants obtained the highest averages of bonding stress, when compared to the remaining experimental groups. Lai et al (2001), Morris et al (2001), Yiu et al (2002), Vongphan et al (2005), Nagpal et al (2007), and Kimyai et al (2008) agreed on the fact that the reduction in the bonding stress in whitened teeth may be reverted by using Sodium Ascorbate or Ascorbic Acid as antioxidants.

In this research, time of application of antioxidant agents was 1 minute and 10 minutes, and time variation did not significantly interfere with the bonding stress level of groups G1, G2, G5 and G6. These results are not consistent with the study undertaken by Lay et al, in 2002, where an alternative period equal to one third of the time devoted to the whitening treatment was suggested, so as to leave Sodium Ascorbate (10%) in contact with the oxidized dental structure for it to have an effect on the removal of free radicals. Yet, other studies also obtained good results when the solution was applied for ten minutes^{15-17,28}.

When we look at the averages of micro tensile bonding stress and the results of the Tukey test, we can see

that the averages are statistically different and that the application of antioxidant agents such as Ascorbic Acid and Sodium Ascorbate proved to be valid.

According to the results in this study, it was verified that an ideal clinical time can be made effective for the use of these agents in post-whitening dental restoration procedures. Yet, it cannot be categorically stated to what extent the results of this *in vitro* study may be extrapolated to the clinical field, in view of the scarcity in research data and of the lack of standardization among them. In fact, supplementary studies are necessary for reaching the conclusion that the clinical use of such antioxidants is the definitive solution to the problem of the decrease in the bonding stress of resinous materials on recently whitened enamel.

CONCLUSIONS

The use of antioxidants is valid in the removal of residual oxygen, yet, not all agents have such capacity as they have varied performance levels when applied at different time periods. Among antioxidants subject to tests, those that got the best results were Sodium Ascorbate and Ascorbic Acid, irrespective of the application time periods. Future studies must be performed so that new formulations are developed for facilitating their use in the dentist's office.

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