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Effects of Green Tea Application Time on Bond Strength after Enamel Bleaching

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This study evaluated the effect of green tea application time on the bond strength of enamel after enamel bleaching. Enamel samples were obtained from 80 third molars and randomly divided into 7 experimental groups (G1-G7) and 1 group without treatment (G8): G1, bleached with 10% carbamide peroxide (CP); G2, CP + 10% sodium ascorbate gel (SA) for 15 min; G3, CP + SA for 30 min; G4, CP + SA for 60 min; G5, CP + 10% green tea gel (GT) for 15 min; G6, CP + GT for 30 min; G7, CP + GT for 60 min. The CP was applied onto the enamel surface for 8 h for 14 days. The SA was applied in groups 2, 3 and 4, and the GT was applied in groups 5-8 according to the above described application times. Immediately after treatment, the specimens were bonded with Adper Single Bond 2 and Filtek Z350XT. The specimens were prepared to microtensile bond strength analysis. Fracture mode analysis was performed using a stereoscopic loupe. The data were statistically analyzed by two-way analysis of variance, the Tukey's and Dunnett's tests (α=5%). The means (standard deviation) were: G1, 23.3 (3.2); G2, 25.2 (3.9); G3, 26.4 (5.4); G4, 30.2 (4.5); G5, 26.6 (3.4); G6, 22.0 (5.4); G7, 31.4 (3.3); G8, 31.4 (3.2). All groups had a high percentage of adhesive failures. In conclusion, the bond strength values were higher than the value in the bleached group only when the antioxidants were applied for 60 min.

Key Words: green tea, bleaching, bond strength.

Introduction

The mechanism of bleaching agents is based on oxidation-reduction reactions, which release oxygen free radicals. These highly reactive radicals can penetrate the tooth structure through enamel prisms and porosities and reach the dentin by chemical processes that break organic molecules of high molecular weight into inorganic molecules of low molecular weight that are less pigmented (1). Many studies have demonstrated that bleaching agents such as carbamide and hydrogen peroxide may decrease the bonding strength to dental structures when adhesion is performed immediately after bleaching (2). This is caused by the presence of residual oxygen from the bleaching agent, which interferes with and inhibits the polymerization of adhesive systems and thus compromises the bonding strength between dental materials and tooth substrates (3).

In this way, with the aim of avoiding adhesive failures at the restorative material/dental interface, a waiting period ranging from 24 h to 3 weeks has been advised (4,5). These recommendations are based on reports that the reduction in resin bonding strength to enamel or dentin after bleaching treatments is temporary (4). However, some patients need faster treatments, and in some cases, the advised waiting time is not possible. Therefore, antioxidant substances have been studied with the aim of speeding treatment. Some of them are sodium ascorbate (6,7), grape seed extract (3,8), μ-tocopherol (9), catalase, sodium bicarbonate (10), organic solvents like ethanol and acetone (11), and more recently, the green tea (12). Despite the several tested antioxidants, none of them is used clinically. This is probably due to the short shelf life of these products and the storage conditions (temperature, time, light exposure) that may affect them (13). Therefore, a potent antioxidant is needed able to reverse the bond strength values and clinically viable. Additionally, recent studies have shown that the green tea is an antioxidant alternative for use after bleaching (12,14).

The green tea is made from the *Camellia sinensis* plant. It contains flavanols or catechins, such as epicatechin (EC), gallocatechin (GC), epigallocatechin (EGC), epicatechin gallate (ECG), and epigallocatechin gallate (EGCG) (15,16). Green tea catechins have shown to possess potent antioxidant activity that is several times higher than that of vitamins C and E (17). In recent years, the use of green tea has been studied in dentistry. Researches reported that use of green tea decreases dentin erosion (18) and antimicrobial agents (19), and increases enamel bonding strength values after bleaching (12). Additionally, green tea is a natural product, cheap and with an extended shelf life and could be an option for use after bleaching.

A previous study evaluated the effect of green tea applied for 60 min on bleached enamel (12). However, a shorter time application is necessary for clinical practice. Therefore, this study aimed to evaluate different application times (15, 30 and 60 min) of 10% green tea gel (GT) or 10% sodium ascorbate gel (SA) on the microtensile bond strength of human enamel after bleaching with 10% carbamide peroxide (CP). The hypothesis tested in this experiment is that the use of green tea for less than 60

min may reverse the reduction in bonding strength values of teeth after bleaching.

Materials and Methods

Sample Preparation

This study was approved by the Ethics Committee of the University of Parana. Eighty third molars that had been extracted for therapeutic reasons and were free of caries and defects were included in this study. The teeth were maintained in a thymol solution (0.1%; pH 7.0) (9) for no longer than 1 month after extraction, and they were cleaned of gross debris and placed in distilled water for 24 h before starting the experiment.

The root portion of each tooth was removed 2 mm below the cement-enamel junction with a diamond saw (Buelher, Lake Bluff, IL, USA). The coronal portion was sectioned mesiodistally, and the buccal and lingual portions were used. The half-teeth were randomly divided into the following 8 groups (n=10), considering 'half-tooth' as the experimental unit: Group 1: Bleached with 10% CP (negative control group), Group 2: CP + 10% SA for 15 min, Group 3: CP + SA for 30 min, Group 4: CP + SA for 60 min, Group 5: CP + 10% GT for 15 min, Group 6: CP + GT for 30 min, Group 7: CP + GT for 60 min and Group 8: no treatment (positive control group)

The enamel surfaces were flattened using 600- and 1200-grit Al_2O_3 abrasive paper, cooled under running water, and polished with felt discs (Arotec, Cotia, SP, Brazil) impregnated with diamond paste (0.5 µm). Between the paste and disc applications, the teeth were washed ultrasonically in distilled and deionized water for 10 min to eliminate any residues. Then the samples were fixed in an acrylic disc. Two individual trays were made for each specimen using 0.5-mm-thick flexible polymer in a vacuum plasticizer (Plastivac P7; BioArt Equipamentos Odontológicos, São Paulo, SP, Brazil). One tray was used for bleaching, and the other was used for antioxidant application to prevent bleaching gel contamination in the tray during antioxidant application.

Bleaching Procedures

The bleaching procedures were conducted according to previous studies (9,12). The same bleaching agent, 10% CP (Opalescence; Ultradent, South Jordan, UT, USA) was used for all groups. Approximately 0.02 mL of bleaching gel was applied on the enamel surface, and individual trays were placed over the specimens as described before. Then the samples were immersed in a plastic container containing artificial saliva, (composition: 0.0625% potassium chloride, 0.0865% sodium chloride, 0.0055% magnesium chloride, 0.0165% calcium chloride, 0.0006% sodium chloride, 0.08% sodium phosphate, 4.275% sorbitol, 0.1% methylparaben, 0.6% cereal alcohol, q.s.p. 1000) at 37±1 °C for 8 h daily for 14 days, according to the manufacturer's instructions. At the end of each day of treatment, the bleaching gel and individual trays were rinsed with distilled and deionized water using a toothbrush (Colgate, São Paulo, SP, Brazil). During the remaining 16 h, the samples were maintained in artificial saliva at 37±1 °C.

Application of Antioxidants

For the groups 2–7, approximately 0.02 mL of 10% GT (60% catechins and 5% caffeine; Fragon, São Paulo, SP, Brazil) or 10% SA (Fragon) were applied to the samples immediately after the bleaching procedures described above. The individual trays were placed over the samples to prevent dilution of the antioxidant gel in the artificial saliva, as previously described (9), and to simulate the clinical application of this technique. The antioxidant agents were used for 15, 30 or 60 min at 37±1 °C to neutralize the oxidizing effect of CP. Then the individual trays were removed, and the antioxidant agents were washed from the enamel surface with distilled and deionized water.

Bonding Procedures

After the treatments, all the samples, including the group 8 where no treatment was performed, were etched with 35% phosphoric acid (Scotchbond Etchant Gel; 3M ESPE, St. Paul, MN, USA) for 15 s, washed with distilled water for 15 s and dried before application of the adhesive. The bonding adhesive (Adper Single Bond 2; 3M ESPE) was applied according to the manufacturer's instructions. Two consecutive coats of adhesive were applied to the etched enamel. The solvent was then gently air evaporated for 5 s and the material was light cured for 10 s (Radii-cal; SDI, Baywater, Victoria, Australia) with an intensity output excess of 1200 mW/cm^2. Thus, three layers of resin composite (Z350; 3M ESPE), approximately 6.0 mm in height, were applied to the bonded surfaces to build up a cube-like crown. Each resin layer was light cured for 40 s.

Microtensile Bonding Strength

The samples were prepared for microtensile bond strength testing according to Berger et al. (12). Immediately after the bonding procedures, the teeth were stored in distilled and deionized water at 37±1 °C for 24 h. The teeth were then sectioned occlusogingivally into serial slabs, and further sectioned into 0.9 x 0.9 mm composite-enamel beams using a diamond saw in a precision cutting machine (Isomet 1000; Buehler). The number of lost beams (pretest failures) in each group was as follows: Group 1: 2 beams; Group 3: 1; Group 6: 2; in other groups, no pre-failure occurred.

Approximately 10 beams were obtained from each half-

tooth. They were individually fixed in a tensile jig using cyanoacrylate glue (Super Bonder Gel; Loctite, São Paulo, SP, Brazil). The specimens were stressed to failure using a microtensile machine (EMIC; Equipamentos e Sistemas de Ensaios, São José dos Pinhais, PR, Brazil) at a speed of 0.5 mm/min with a 50-kgf load cell, and the kgf values were converted to MPa. The average of each group was obtained calculating the average microtensile bond strength for the beams originating from each half-tooth and these averages were used for statistical analysis.

Fracture

The fracture surface of each specimen was analyzed under a stereoscopic loupe (BelMicroimage Analyser; Bel Photonics, Monza, Italy) at 40× magnification, and the type of failure was determined. The types of fractures were classified as adhesive (lack of adhesion), cohesive (failure of the tooth substrate or resin composite), or mixed (adhesive and cohesive failures).

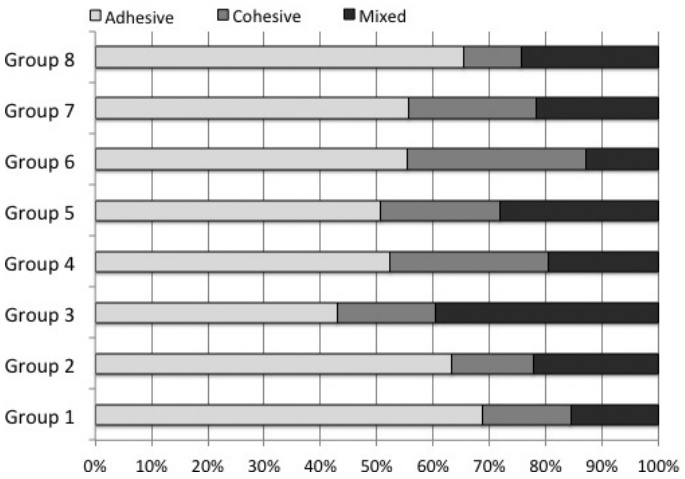

Figure 1. Fracture mode analysis for groups after bond strength test.

Table 1. Mean and standard deviation of microtensile bond strength values (MPa).

Antioxidant	Application time of antioxidant agents		
	15 min	30 min	60 min
10% sodium ascorbate	25.2 (3.9) Ab§	26.4 (5.4) Ab	30.2 (4.5) Aa❖
10% green tea	26.6 (3.4) Ab	22.0 (5.4) Ab§	31.4 (3.3) Aa❖
Bleached enamel (Negative control)		23.3 (3.2) §	
Unbleached enamel (Positive control)		31.4 (3.2) ❖	

Groups with different uppercase letters (column: comparison among antioxidants within the same application time) and lowercase letters (row: comparison among time application within the same antioxidant) are significantly different by Tukey's test ($p<0.05$). ❖ Significant difference from the negative control group by Dunnett's test ($p<0.05$). § Significant difference from the positive control group by Dunnett's test ($p<0.05$)

Statistical Analysis

After the end of the microtensile bonding strength test, the normality of the data was evaluated using the Kolmogorov-Smirnov test. Two-way ANOVA was performed to compare the experimental groups, with Tukey's test for multiple pairwise comparisons and Dunnett's test to compare the experimental groups with the negative and positive control groups (α=5%). The statistical analysis did not include the beams that debonded spontaneously, since the incidence of pretest failures was very low.

Results

Table 1 shows the mean and standard deviations of the microtensile bonding strength test. ANOVA indicated an interaction for the factor time ($p<0.001$). The Tukey's test indicated that when both GT or AS, were used there was no difference in the microtensile bonding strength comparing the 15- and 30-min applications. However, the application times of 15 and 30 min were statistically different from 60 min for GT or AS. The microtensile bonding strength of the negative control group was statistically different from the experimental group on which both antioxidants were applied for 60 min (Dunnett's test). Furthermore, the positive control group was statistically different from negative control group, AS for 15 min and GT for 30 min (Dunnett's test).

In the analysis of the fracture mode, there was a predominance of adhesive failures over cohesive or mixed failures (Fig. 1).

Discussion

The decrease in bond strength and the reduction of the adhesive system interaction on bleached teeth are attributed to the residual oxygen (9). Residual oxygen interferes with resin attachment and inhibits resin polymerization (2,6), which may cause bubble formation at the adhesive interface and lower the adaptation and penetration of enamel tags (7). In addition, other important factors may contribute to the decrease in enamel bond strength, such as calcium loss (20), a decrease in microhardness and alterations in the organic materials (21). Thus, antioxidant agents have been studied with the aim of inactivating free radicals (3,6,7,9,10,12). One of the most investigated antioxidant agents is SA. This salt has low toxicity and it is commonly used in the food industry as an antioxidant agent, indicating that SA is likely to have few or no adverse

biological effects when used for this particular purpose (22). Recently, the use of green tea was studied to increase the bond strength values after bleaching, and a 60-minute application showed satisfactory results in maintaining bond strength (12). However, a 60-minute application time is too long for clinical use.

This study evaluated the capacity of 10% GT and 10% SA when applied for 15, 30 or 60 min after bleaching to reverse the reduction in bond strength of enamel bleached with 10% CP. The results indicated that only the antioxidant application time of 60 min was effective in improving the bond strength values. Furthermore, adhesive failures were prevalent in all the groups. Thereby, the tested hypothesis in the study was rejected, and use of GT for less than 60 min was unable to improve the bond strength values.

Torres et al. (10) evaluated the effects of six antioxidant agents (including 10% AS in aqueous solution) on the bond strength of bleached enamel. The authors recommended waiting for a period of approximately two weeks after the bleaching treatment to proceed with bonding processes because none of the tested antioxidants was able to completely neutralize the deleterious effects of bleaching on bond strength (10). However, in the present study, which evaluated the application of 10% GT, an alternative antioxidant, and 10% SA for 1 h, the bond strength values were statistically higher than in the bleached group. Both antioxidants were used as gels, which may have produced these results. The antioxidant gels, used with an individual tray may have led to greater effectiveness.

Garcia et al. (13) evaluated the percentage of antioxidant activity of 10% SA and other substances. They found that the highest antioxidant activity was for SA, both as a solution or a gel (13). SA is as sodium salt of the ascorbic acid, and it is a potent antioxidant capable of quenching reactive free radicals in biological systems (23). However, SA has a pH of approximately 1.8, which is inappropriate for clinical use. Thus, there is need to study alternative antioxidants that are able to completely reverse the reduction in bond strength of bleached enamel. For this reason, the present study evaluated GT as an alternative to SA and the results showed to be satisfactory, as reported when EGCG, the most active and abundant catechin in GT, was tested (14). EGCG applied for 10 min was able to overcome the compromised bonding strength.

Berger et al. (12) reported that when GT was applied after enamel bleaching for 60 min, the bond strength values were statistically higher than those in the control group (only bleached). This study tested if a shorter application time of GT was able to reverse the reduction in bond strength following bleaching compared to SA. Nevertheless, no significant increase was found in the microtensile values when GT was applied for 15 or 30 min. The green tea catechins, such as EGCG and EGC, have potent antioxidant activities caused by the three adjacent OH groups on the B-ring that scavenge free radicals more effectively than the two adjacent OH groups in ECG and EC (15). Thus, green tea catechins were shown to possess potent antioxidant activity that is several times higher than those of vitamins C and E (17). In this way, it was speculated that EGCG can be the main responsible for the capture of free radicals from the bleaching. As in this study was used GT with 60% catechins, without standardizing the EGCG, it might be necessary an application time of 60 min to obtain satisfactory results.

In contrast to the expected result, when the antioxidant agents were applied for 15 (groups 2 and 5) or 30 min (groups 3 and 6), the bond strength values were not statistically superior to the bleached group (group 1). Turkun and Kaya (7) reported that the application of 10% sodium ascorbate for 10 min was sufficient to reverse the reduction in bond strength, but the sodium ascorbate was used in solution form and the enamel samples were continuously agitated using a sterile brush. Kimyai et al. (24) immersed enamel samples in a 10% sodium ascorbate solution for 3 h according to the protocol published by Lai et al. (6), which advised immersion in antioxidant agents for one third of the time the oxidizing bleaching agent is applied. These divergent results may be due to the different antioxidant forms (e.g., gel or solution) and the mechanical test used for the evaluation (e.g., microshear or microtensile bond strength). Besides, despite the low incidence of premature failures, this could be prevented using microshear test. Enamel is a friable structure and microcracks might be inadvertently produced mainly by the vibrations of the cutting instruments during specimens preparations, necessary for the microtensile bond strength test. This test was chosen because is more relevant clinically (25).

Moreover, the bleaching treatment performed in this study was home bleaching, where the bleaching agent remained in contact with the enamel surface for 8 h daily for 14 days. There are divergences regarding bleaching protocols *vs* antioxidants agents in the literature, for example, Vidhya et al. (3) used 38% hydrogen peroxide (HP) for 10 min; Torres et al. (10), 35% HP: 45 min; Khamverdi et al. (14), 40% HP: 10 min; Sasaki et al. (9), 10% CP: 2 h/14 days; Turkun & Kaya (7), 10% CP: 8 h/7 days; Kimyai & Valizadeh (24) and Berger et al. (12), 10% CP: 8h/14 days. Thus, different results were found for different bleaching protocols and antioxidants, and this should be considered when comparing studies with different methodologies.

Further chemical and analytical studies must be conducted to elucidate the mechanism by which green tea reverses the bond strength of enamel after bleaching. In this study it was speculated that higher concentrations of

green tea may result in a greater reversal of bond strength values in bleached enamel. It is likely that the use of 2,2-2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical method, as used by Garcia et al. (13), can be an effective method to verify this speculation, and future studies applying this method will be conducted. Thus, according the methodology used and the results obtained in the present study, only when 10% green tea gel was applied for 1 h occurred reversal of adverse effect of at-home bleaching on bond strength to enamel, with similar results for 10% sodium ascorbate. Further chemical and analytical studies are required to elucidate how green tea acts on the bleached enamel to improve bond strength.

Resumo

Este estudo avaliou o efeito do tempo de aplicação do chá verde na resistência de união do esmalte após o clareamento. Amostras de esmalte foram obtidas a partir de 70 terceiros molares e aleatoriamente divididas em 7 grupos experimentais (G1-G7) e um grupo sem tratamento (G8). Os 7 grupos experimentais foram tratados como segue: G1, clareado com peróxido de carbamida a 10% (PC); G2, PC + gel de ascorbato de sódio a 10% (AS) por 15 min; G3, PC + AS por 30 min; G4, PC + AS por 60 min; G5, PC + gel de chá verde a 10% (CV) por 15 min; G6, PC + CV por 30 min; G7, PC + CV por 60 min. O PC foi aplicado na superfície do esmalte por 8 h, durante 14 dias. O AS foi aplicado nos grupos 2, 3 e 4 e o CV foi aplicado nos grupos 5, 6 e 7 de acordo com os tempos de aplicação descritos acima. Imediatamente após o tratamento, foi realizado o procedimento adesivo utilizando Adper Single Bond 2 e Filtek Z350XT. Em seguida, as amostras foram preparadas para o teste de microtração. A análise do padrão de fratura foi realizada em lupa estereoscópica. Os dados foram analisados através de ANOVA (2 fatores), testes de Tukey e Dunnett (α=5%). As médias (desvio padrão) foram: G1: 23,29 (3,20); G2: 25,18 (3,95); G3: 26,41 (5,40); G4: 30,17 (4,46); G5: 26,63 (3,43); G6: 22,02 (5,41); G7: 31,40 (3,35); G8: 31,4 (3,2). Todos os grupos apresentaram maior porcentagem de falhas adesivas. Em conclusão, os valores de resistência de união foram maiores que os dos grupos clareados somente quando os antioxidantes foram aplicados por 60 min.

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