EFFECT OF GRAPE SEED EXTRACT ON BOND STRENGTH OF RESTORATIVE MATERIAL TO BLEACHED ENAMEL

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Palavras-chave: Adesão. Clareamento Dental. Extrato de Semente de Uva.

RESUMO

Objetivo: Este trabalho teve por objetivo avaliar *in vitro* o efeito de um antioxidante natural (extrato de semente de uva) em diferentes concentrações, na resistência de união do material restaurador ao esmalte clareado. **Métodos:** Quarenta fragmentos de incisivos bovinos hígidos, foram divididos aleatoriamente em quatro grupos (n=10): Grupo I: sem clareamento; Grupo II: clareado com peróxido de hidrogênio 35% (PH) e sem pós-tratamento; GIII: clareado PH 35% + extrato de semente de uva 5%; e Grupo IV: clareado com PH 35% + extrato de semente de uva 10%. A resistência de união da interface esmalte/material restaurador foi avaliada por meio do teste de cisalhamento (MPa). Os dados foram analisados pela análise de variância (ANOVA) e testes de Tukey (=0,05%). Os tipos de fratura também foram analisados e classificados em: adesiva, coesiva ou mista. **Resultados:** Apenas o Grupo III (clareado + extrato de semente de uva 5%) apresentou aumento estatisticamente significante (p<0,001). dos valores de resistência de união comparado ao Grupo II (clareado e sem pós-tratamento). Todos os grupos mostraram um predomínio do tipo de fratura adesiva. **Conclusão:** O clareamento dental diminui significativamente a força de adesão ao esmalte dental clareado, e o extrato de semente de uva 5% aplicado após o clareamento dental melhora a resistência de união entre o material restaurador e o esmalte clareado.

Keywords: Adhesion. Tooth Whitening. Grape Seed Extract

ABSTRACT

Objective: The aim of this study was to evaluate the effect of different concentrations of a natural antioxidant (grape seed extract) on the bond strength of the restorative material to the bleached enamel. **Methods:** Forty fragments of healthy bovine incisors were randomly divided into four groups (n = 10): Group I: no bleaching; Group II: Bleaching with 35% hydrogen peroxide (HP) and without post-treatment; Group III: Bleaching with 35% HP + 5% grape seed extract; and Group IV: Bleaching with 35% HP + 10% grape seed extract. The bond strength at the adhesive interface was evaluated using the shear test (MPa). The data were analyzed by the analysis of variance (ANOVA) and Tukey test (=0.05%). The fracture types were also analyzed and classified into: adhesive, cohesive or mixed. **Results:** Only Group III (bleached + 5% grape seed extract) had a significant increase (p<0.001) in bond strength values when compared to Group II bleached, without post-treatment). All groups showed a predominance of the adhesive type of fracture. **Conclusion:** It could be concluded that tooth bleaching decreases the bond strength to bleached enamel and 5% grape seed extract applied after dental bleaching improves the bond strength between the restorative material and the bleached enamel.

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INTRODUCTION

Dental esthetics is a concern constantly expressed by patients in dental offices, and there is a great demand for whitening darkened teeth. The main agents used for bleaching vital teeth are carbamide peroxide and hydrogen peroxide. When products derived from peroxides come into contact with the tooth structure, they release oxygen and hydrogen free radicals capable of diffusion through dental tissues, causing degradation of the molecules of pigments into increasingly smaller molecules of a lighter color, promoting bleaching.[1]

Although bleaching agents have been shown to be effective treatment as regards color change, the residual oxygen remained on tooth after treatment has adverse effects on the bond strength.[2,3] Changes caused on the enamel surface may alter microhardness, surface roughness and morphology of the enamel,[4] loss of calcium and phosphate,[5,6] and lead to changes in the bond strength, when teeth are restored in the same session that bleaching is performed.[2,3]

The literature has suggested that the professional should wait from 7[7,8] to 14[9] days after concluding the bleaching procedure to perform restorations. This delay will allow the release of residual oxygen that could compromise the bond strength of restorations.

Taking this into consideration, the ideal would be to use methods for minimizing the effects on the tooth structure caused by residual oxygen, so that the tooth could be restored in the same clinical session. Studies have demonstrated that the use of different antioxidant agents immediately after bleaching could neutralize the oxygen molecules and improve the bond strength of the restorative material to bleached enamel,[10-14] among them are some natural extracts.

After bleaching treatment, the use of plant extract has shown positive results in substitution to chemical agents.[11,13] The grape seed extract is composed of oligomeric proanthocyanidins that present activities of eliminating free radicals, and also has antimicrobial and anti-inflammatory properties and has no mutagenic effect.[15] The application of grape seed extract after bleaching aims to increase the bond strength of the restorative material with the dental structure.[13] Thus, the aim of the present *in vitro* study was to evaluate the effect of applying different concentrations of grape seed extract on the bond strength of the restorative material to bleached enamel.

MATERIALS AND METHODS

Experimental Design

The study was conducted in a randomized manner, and the sample was composed of 40 bovine tooth fragments (n=10). The experimental groups were divided as follows: GI: no bleaching; GII: Bleaching with 35% hydrogen peroxide (HP) and without post-treatment; GIII: Bleaching with 35% HP + 5% grape seed extract; and GIV: Bleaching with 35% HP + 10% grape seed extract. The quantitative response variable was the bond strength at the enamel/restorative material interface, by means of the shear bond strength test (MPa). In addition, qualitative analysis of the fracture patterns (adhesive, cohesive or mixed) was performed.

Sample obtainment

Forty (40) healthy bovine teeth were selected. The teeth had no cracks, fractures or hypoplastic stains. The teeth were cleaned with periodontal curettes, pumice stone and water paste, and Robson brushes. After cleaning, the teeth were section with a diamond coated cutting disc mounted in a cutting machine (Isomet 1000, Model 11-2180), under water cooling, to obtain enamel fragments measuring 5x5x3mm.

The tooth fragments were embedded in transparent acrylic resin (VIPI, Pirassununga-SP, Brazil) and PVC rings, so that the enamel surface remained facing towards the outside medium, to allow flattening with abrasive discs #600 and #1200.

Afterwards, the samples were randomly divided into 4 groups according to the surface treatment.

Surface bleaching

The 35% hydrogen peroxide-based bleaching gel (Whiteness HP; FGM, Joinville, SC, Brazil) had two stages: peroxide (Stage 1) and thickener (Stage 2). The Whiteness HP bleach stages were mixed in the ratio of 3 drops of peroxide to one drop of thickener, and the bleaching agent was applied to the enamel specimen. The bleaching gel application protocol was 3 applications each lasting 15 minutes, per session, in accordance with the manufacturer's recommendations. Between applications, the gel was removed by means of aspiration (endodontic cannula) before re-applying the product. After the bleaching procedure, the specimens were washed with distilled water for 30 seconds and dried with absorbent paper.

The group that did not receive bleaching treatment was stored in relative humidity throughout the procedure.

Post-bleaching surface treatment

Immediately after the bleaching procedure, the specimens of Group GIII and GIV received post-bleaching treatment with grape seed extract. The extracts were used in gel form, in the concentrations of 5% (pH 3.9) and 10% (pH 4.1), which were prepared by a compounding pharmacy (Homeopatia Ouro Preto, Pirassununga, SP, Brazil).

The grape seed extract was applied by a single

operator, for 10 minutes, with constant agitation, by using a disposable applicator.

After applying the grape seed extract, the specimens were washed with distilled water for 30 seconds and dried with absorbent paper. The restorations were performed immediately after the application of the grape seed extract.

Restorative Procedure

After the treatment stage, the specimens of all groups received restorative material. Initially, the surface was etched with 37% phosphoric acid (Condac, FGM, Joinville, SC, Brazil) for 30 seconds on the enamel surface, followed by washing with distilled water for 30 seconds and drying with jets of air. After, the adhesive system (Adper Single Bond; 3M, ESPE St. Paul, MN, USA) was applied on the etched enamel using a disposable applicator, in two consecutive coats, and light polymerized for 10 seconds (SDI-Radical, Ribeirão Preto, SP, Brazil).

Previously flattened enamel surfaces were restored using a split Teflon matrix (3 mm internal diameter, 4 mm height). The center of enamel surfaces were restored with resin composite (Filtek Z250; 3M ESPE, St. Paul, MN, USA), inserted in three increments, and each increment was light polymerized for 20 seconds, at a distance of 10 cm (SDI-Radical, Ribeirão Preto, SP, Brazil). The specimens were stored in relative humidity, at 37°C and after 24h the shear bond strength test was performed.

Bond Strength Test

The specimens were placed on the Universal Test Machine (Model DL 2000, São José dos Pinhais, PR, Brazil, EMIC – Equipamentos e Sistemas de Ensaios LTDA) with a load cell of 50Kg/f, allowing force to be applied at an angle of 45°, without coming into contact with the base of the test specimen. Shear force was applied by means of a rectangular stainless steel tip at a constant speed until the restoration was displaced. The (MPa) values were noted, and later tabulated and sent for statistical analysis.

Fracture Pattern Analysis

After rupture of the specimen, the surfaces were evaluated by means of a clinical microscope (Model ALL 03 - EL) Aliance Comercial de São Carlos Ltda. - ME, São Carlos, SP, Brazil), to identify the type of fracture. The specimens were evaluated at 16X magnification. The failures were classified as:

1.Adhesive (fracture at the substrate/ restorative material interface); 2. Cohesive in enamel (fracture in enamel); 3. Cohesive in resin (fracture in the restorative material); 4. Mixed (Combined adhesive and cohesive fracture).

Statistical analysis

The mean values of bond strength for each group were considered for statistical analysis. Statistical analyses were performed with the SPSS 19.0 program for Windows (SPSS Inc., Chicago, IL, USA). First, the normality of the data was evaluated using the Kolmogorov-Smirnov test, followed by the Analysis of Variance (ANOVA) and the Tukey test (α=0.05).

RESULTS

The Analysis of variance (ANOVA) revealed significant difference among the groups ($p<0.001$). The Tukey test showed that the groups in which the grape extract was used in the concentrations of 5% (Group III) and 10% (Group IV) had higher bond strength values than in those in which bleaching was performed and had no post-treatment (Group II).

However, only Group III had a significant increase in bond strength values, when compared with Group II. Group I, which was not bleached (positive control), showed higher bond strength values than those of the other groups (Table 1).

The fracture patterns were analyzed according to the following classifications: adhesive, cohesive and mixed. In Figure 1, the percentage values of fracture patterns are represented, according to the different experimental groups. Predominance of adhesive failures was observed in all groups, however, in Groups II and IV these types of failures were observed in the total number of their specimens. Group I (control) had the highest number of cohesive failures, followed by Group III. All the cohesive failures in above-mentioned groups were cohesive failures in enamel.

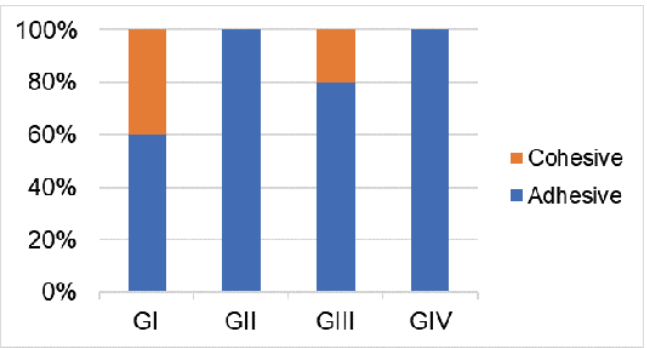

Figure 1: *Percentage of fracture patterns for each studied group*

***Table 1**: Mean (standard deviation) of bond strength values (MPa) observed in the four groups studied (n=10)*

Experimental groups	Mean (±SD)
GI - without bleaching	20.87 (±3.47) a
GII - Bleaching with 35% hydrogen peroxide (HP)	10.72 (±2.71) c
GIII - Bleaching with 35% HP + 5% grape seed extract	16.00 (±2.31) b
GIV - Bleaching with 35% HP + 10% grape seed extract	14.10 (±3.80) bc

Note: Equal letters indicate statistical similarity

DISCUSSION

There has been a growing demand for esthetic procedures in dental offices, among these, tooth whitening is considered a fast, efficient, accessible and conservative treatment.[16] However, when patients need to replace resin composite restorations, the bond strength of the restorative material to the bleached tooth structure may be compromised if the restorative treatment is performed immediately after bleaching. This is due to the presence of residual oxygen in the tooth structure.[7-9,17]

Antioxidants are chemical substances that inhibit the oxidation process,[18] thus the improvement in bond strength occurs due to the fact that the antioxidant agents donate their electrons to the free radicals, thereby interrupting the chain reaction, and thus impeding the formation of these radicals.[19] The grape seed extract is composed of oligomeric proanthocyanidins, in which there are molecules that form part of a specific group of polyphenolic compounds such as the flavonoids. The proanthocyanidins are found in natural sources, such as for example in plants[20] and are considered effective for eliminating free radicals[21] and they have antioxidant action.[22,23] Proanthocyanidins are also considered an atoxic and safe antioxidant,[24,25] that can be well indicated for application during clinical sessions.

This study evaluated the effect of grape seed extract, a natural antioxidant product, on increasing the bond strength. The results obtained showed that the unbleached Group (GI) had the highest bond strength values when compared with the other groups (GII, GIII and GIV). These results were justified by the presence of residual oxygen in the dental structure of the groups that received bleaching treatment, because bleaching agents act mainly by oxidation of organic compounds that release free radicals (particularly nascent oxygen). Due to its low molecular weight, oxygen is capable of penetrating into enamel and dentin, acting on the organic portion, and attaining the dark pigments, thereby promoting bleaching.[26] The oxygen molecule in the tooth structure inhibits polymerization of the adhesive system and resin composite, and therefore, reduced the bonding capacity of the restorative material.[27]

The group that was bleached and treated with 5% grape seed extract (GIII) had the highest bond strength values when compared with the group that was bleached and not treated (GII), in agreement with the results observed in other studies,[10-13] showing a probable reduction in residual oxygen on the enamel surface treated with 5% grape seed extract. On these concern, Manoharam et al. (2016)[12] evaluated the effect of antioxidant agents in the bond strength of resin to bleached enamel (with 15% carbamide peroxide for 8 hours *per* day during 5 days) and the 5% grape seed extract was the most effective.

In the present study, 35% hydrogen peroxide gel was used in 3 applications lasting 15 minutes each, simulating bleaching by the in-office technique using gels with higher concentrations, as were used in previous studies.[10,11,13] The 5% grape seed extract used after bleaching as antioxidant agent was able to reduce the deleterious effects of bleaching, and significantly increased the bond strength of the restorative material to the enamel bleached[10,11,13], which would be clinically beneficial because it allows a decrease in the waiting time after the bleaching sessions to perform the restorative procedure.

The bleaching gel concentration and time of application may influence the changes caused in tooth enamel, such as morphological changes [28] and the bond strength of restorative materials.[29] However, we noted that even when using different times, concentrations and bleaching gels, the 5% grape seed extract increased the bond strength in all previous studies of literature when compared with the untreated groups.[10,11,12,13]

Sharafeddin & Farshad (2015)[30] also used grape seed extract in the concentration of 10% and verified no significant increase in the bond strength values when compared with the control group, corroborating our results. This fact may be associated with the difference in pH of the extracts studied (5% - pH 3.9 and 10% - pH 4.1). At 5%, the gel was more acid than the concentration of 10%. The interaction of acids on the tooth surface may have act in a better manner with the tooth structure.

The fracture test showed predominance of adhesive failures in all groups. However, some cases of cohesive

fracture was observed in the group treated with 5% grape seed extract (GIII), and in the unbleached group (GI), which corroborated the results of the bond strength tests, showed higher bond strength values in these groups when compared with the other groups. Probably there was a higher presence of residual oxygen in the specimens of groups 2 and 4. This fact compromised the bond strength between enamel and restorative material, favoring the high rate of cohesive fractures.

Overall, the results of this study demonstrated that tooth whitening performed with hydrogen peroxide gel at high concentrations negatively affected the bond strength of restorative materials to enamel. The 5% grape seed extract may have promising results in the removal of residual xygen from bleached surface. Further studies are necessary to evaluate whether different application protocols could improve the results achieved for bond strength to bleached enamel.

CONCLUSIONS

Within the limitations of this *in vitro* study, it could be concluded that:

1- Bleaching with 35% hydrogen peroxide significantly reduced the bond strength of restorative material to bleached tooth enamel.

2- The 5% grape seed extract increased the bond strength of restorative material to tooth enamel in restorations performed immediately after bleaching.

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