



Effect antioxidants application on microshear bond strength of universal adhesive to bleached enamel

Efeito da aplicação de antioxidantes na resistência de união do adesivo universal ao esmalte clareado

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ABSTRACT

Purpose: To evaluate, in vitro, the influence of antioxidants (green tea extract - GT and sodium ascorbate - SA) on microshear bond strength (μ SBS) of a universal adhesive system – self-etching mode (UAS) to bleached enamel. **Material and Methods:** After obtaining 50 fragments of human dental enamel (4 mm x 4 mm), forty fragments were submitted to at-home bleaching technique using 10% carbamide peroxide (Opalescence PF, Ultradent) for two h/day, for four weeks. They were randomly divided in four groups (n = 10): GT - 10% aqueous GT solution (60 min); SA - 10% SA solution (10 min); Negative control - no antioxidant agent, immediately restored; PC1 (positive control 1) - no antioxidant agent, restored 14 days the bleaching procedure. Ten enamel fragments were assigned to PC2 group (positive control 2), in which the adhesive procedures were realized in non-bleached enamel. The UAS (Adper Single Bond Universal, 3M ESPE) was applied on enamel surface according to manufacturer's instructions and two cylinders (0.8 mm diameter) of nanoparticulate composite resin (Z350, 3M ESPE) were made on each sample. After 24 h, the cylinders were submitted to μ SBS in a universal test machine (0.5 mm/min). Fracture mode was evaluated in stereomicroscope (30x magnification). SBS data, in MPa, was submitted to one-way ANOVA and fracture mode to Chi-square test ($\alpha = 0.05$). **Results:** There was no statistical difference between the experimental groups ($p = 0.545$) and fracture mode ($p = 0.16424$). There was predominance of adhesive fracture in all groups. **Conclusion:** Neither the bleaching procedure nor the application of antioxidants to bleached enamel interfered in the bond strength of the tested universal adhesive system.

KEYWORDS

Antioxidants; Dental bleaching; Shear strength.

RESUMO

Objetivo: Avaliar, in vitro, a influência de antioxidantes (extrato de chá verde - GT e ascorbato de sódio - SA) na resistência de união ao microcisalhamento (μ SBS) de um sistema adesivo universal - modo de autocondicionamento (UAS) ao esmalte clareado. **Material e Métodos:** Realizou-se a obtenção de 50 fragmentos de esmalte dental humano (4 mm x 4 mm), sendo que desses, quarenta fragmentos foram submetidos à técnica de clareamento caseiro utilizando peróxido de carbamida a 10% (Opalescence PF, Ultradent) por duas horas/dia, durante quatro semanas. Eles foram divididos aleatoriamente em quatro grupos (n= 10): Expostos a GT - solução aquosa de GT a 10% (60 min); expostos a SA - solução 10% SA (10 minutos); Sem exposição ao agente antioxidante e imediatamente restaurado - (controle negativo); Sem exposição ao agente antioxidante e restaurado 14 dias após o clareamento - PC1 (controle positivo 1). Os dez fragmentos de esmalte restantes foram atribuídos ao grupo PC2 (controle positivo 2), no qual os procedimentos adesivos foram realizados em esmalte não clareado. O UAS (Adper Single Bond Universal, 3M ESPE) foi aplicado na superfície do esmalte de acordo com as instruções do fabricante e dois cilindros (0,8 mm de diâmetro) de resina composta nanoparticulada (Z350, 3M ESPE) foram feitos em cada amostra. Após 24 h, os cilindros foram submetidos ao μ SBS em uma máquina de teste universal (0,5 mm/min). O módulo de fratura foi avaliado em estereomicroscópio (aumento de 30x). Os dados do SBS, em MPa, foram submetidos à ANOVA unidirecional e ao modo fratura ao teste do qui-quadrado ($\alpha = 0,05$). **Resultados:** Não houve diferença estatística entre os grupos experimentais ($p = 0,545$) e módulo de fratura ($p = 0,16424$). Houve predomínio de fratura adesiva em todos os grupos. **Conclusão:** Nem o procedimento de clareamento nem a aplicação de antioxidantes no esmalte clareado interferiram na resistência de união do sistema adesivo universal testado.

PALAVRAS-CHAVE

Antioxidantes; Clareamento dental; Força de cisalhamento.

Clinical Relevance: Application of 10% carbamide peroxide or antioxidant agents to enamel did not alter bond strength of a universal adhesive system.

INTRODUCTION

Tooth bleaching can be considered one of the most sought dental treatment for esthetic purposes. Chemical agents containing hydrogen peroxide or carbamide peroxide have been used for this purpose [1].

The bleaching agents are vehicles of oxygen radicals that promote oxidation of pigments embedded in the dental structure. These pigments are fractionated in minor molecular chains, being partially or totally eliminated from the dental structure by diffusion [2]. However, immediately after the dental bleaching, the residual oxygen impairs resin polymerization and, consequently, a decrease in bond strength is expected [3]. Therefore, it is recommended a minimum waiting time of two weeks to make restorative procedures over bleached enamel [3,4].

In order to avoid post-bleaching waiting time, the application of antioxidants immediately after bleaching, has been investigated [5,6,7,8]. Among the most used antioxidants, the sodium ascorbate 10% applied for ten min over the bleached tooth enamel, is one of the most studied agents. Your effectiveness for reverting the decrease in bond strength of bleached enamel has been proven, regardless of whitening gel used [5,6]. Another substance that has been studied is the green tea, a natural antioxidant [7, 8]. The application of 10% green tea extract on bleached enamel was able to recover the bond strength of etch-and-rinse adhesive systems [7,8,9]. Furthermore, other natural antioxidants have also been studied in order to revert the bond strength values, how is the case of grape seed extract, pine bark extract, pomegranate peel extract and white tea [10,11,12].

The evolution of adhesive dentistry has led to the development of new adhesive systems, called universal, that can be applied both in etch-and-rinse and self-etching strategies. Most of these

bond systems contain functional monomers, such as MDP (10-Methacryloyloxydecyl dihydrogen phosphate), that establishes stable ionic bonds with calcium [13]. Even considering this advantage of the universal adhesive system, it has been reported that immediate bond strength of universal adhesive to enamel bleached with carbamide peroxide 35% is decreased but recovered when treating the bleached enamel with sodium ascorbate [14].

Considering the scarcity of studies evaluating effect of other bleaching protocols, such as the at-home whitening technique, as well as the influence of antioxidants agents, like the green tea extract on bond strength of universal adhesive to bleached enamel, the purpose of this research was to evaluate, *in vitro*, the influence of antioxidants applied to bleached enamel on the bond strength and fracture mode of universal adhesive systems, applied on self-etching strategy, when compared to 14-days standard waiting protocol for adhesives procedures.

The null hypothesis tested was that there is no difference in the microshear bond strength of enamel and fracture mode using the universal bond system, applied in the self-conditioning mode, to the bleached enamel that received or not the application of green tea extract or sodium ascorbate, both at 10%.

MATERIAL AND METHODS

Ethical aspects

This research was approved by the Research Ethics Committee of the São Leopoldo Mandic Research Institute, Campinas – SP, Brazil (CAAE n° 97365918.7.0000.5374).

Preparation of specimens

Twenty-five recently extracted third human molars were selected, stored in aqueous thymol solution (0.1, pH 7.0) after the extraction, inspected under a stereomicroscope (EK3S3, São Paulo, SP, Brazil) at 30 times magnification, being discarded those with cracks and stains in the dental enamel.

The teeth were cleaned with scalpel blades and periodontal currettes and sectioned with a flexible double-faced diamond disc (KG Sorensen, Barueri, São Paulo, Brazil) mounted on a low speed handpiece (Dabi Atlante, Ribeirão Preto, São Paulo, Brazil), separating the crown from the root. Fifty quadrangular specimens were obtained from proximal surfaces, measuring 4 mm x 4 mm.

The fragments were embedded in polystyrene resin (Piraglass Com Ind de Art Ornamentais, Piracicaba, SP, Brazil) in polyvinyl chloride (PVC) molds of 2.0 diameter, leaving the outer surface of the enamel exposed. After 24 h, the specimens were removed from the molds and the enamel plates were polish in a polishing machine (Politriz Aropol 2V, Arotec, São Paulo, São Paulo, Brazil) with the decreased granulation (#400, #600, #1200) of sandpaper under irrigation.

Whitening procedures

In forty fragments, the carbamide peroxide 10% whitening gel (Opalescence PF, Ultradent do Brazil, Indaiatuba, São Paulo, Brazil) was applied to the dental enamel, simulating the at-home bleaching technique. The amount of 0.02 mL of whitening gel was applied over each specimen using a 1-mL insulin syringe, for two h a day [15]. Subsequently, specimen was washed with distilled water and stored in artificial saliva at 37 °C. This procedure was repeated for four weeks (28 days). In the period between whitening gel applications, the specimens were kept in artificial saliva, that was renewed once a week.

Application of antioxidant agents to the bleached enamel

The forty bleached specimens were randomly divided into the following groups (n = 10):

- Green Tea (GT) - An aqueous solution containing green tea extract 10% was obtained. Twenty microliters (20µL) of solution was applied to dental enamel with a micropipette, for 60 min [7], and then rinsed with distilled water. Enamel was completely dried with absorbent papers.

- Sodium ascorbate (SA) - An aqueous solution containing sodium ascorbate 10% was manipulated was obtained. Twenty microliters (20µL) of solution was applied to dental enamel with a micropipette, for 10 min [5], and then rinsed with distilled water. Enamel was completely dried with absorbent papers.

- Negative control group (NC) - No antioxidant was applied to enamel. After bleaching, enamel was completely dried with absorbent papers and restored immediately.

- Positive control group 1 (PC1) - Any antioxidant was applied to enamel after bleaching procedure. The specimens were kept in artificial saliva at 37 °C, for 14 days before restoration [3,4].

- Positive control group 2 (PC2) – Neither the bleaching agent nor antioxidant solution were applied to enamel. Adhesive procedures were performed on intact enamel.

Adhesive procedures and preparation for microshear test

The universal adhesive system (Adper Single Bond Universal, 3M ESPE, St. Paul, MN EUA) was applied according to manufacturer's instructions (Table I), with the aid of a disposable brush (Microbrush, KG Sorensen, Cotia, São Paulo, Brazil). Light-curing was performed with a LED device with a power density of 1000 mW/cm² (VALO, Ultradent, Indaiatuba, SP, Brazil), for 10 seconds. After the adhesive application, two tygon tubes (2 mm height) were adapted on each the surface, and inside them, it was inserted the nanoparticulate composite resin, shade A2 (Z350, 3M ESPE, St. Paul, MN, EUA), in a single increment. Light curing was performed for 20 seconds, using a LED device (VALO, Ultradent, Indaiatuba, SP, Brazil), operating with a power density of 1000 mW/cm². Table I describes main materials used in the present study.

Table I - Pulp chamber and root canal volumes measured by ITK-Snap software according to gender

Material (Manufacturer) Batch number (#)	Composition	Application mode
Bleaching gel Opalescence 10% PF (Ultradent Products Inc, South Jordan, UT, USA) # D055I, D04FD, D04KH	Carbamide Peroxide 10%, Potassium Nitrate, 0.1% fluoride.	Applied on enamel for 2 h/day, during 28 days. Light cured for 10 seconds with a LED device (1000 mW/cm ²)
Adhesive system Adper Single Bond Universal (3M ESPE, St. Paul, MN, EUA) # 3296401	Methacrylated 2-hydroxyethyl; Bisphenol A diglycidyl ether dimethacrylate; Decamethylene dimethacrylate; Ethanol; Silane treated Silica; water; 1,10-Decanediol phosphate methacrylate (MDP); Acrylic Copolymer and itaconic acid; N,N-Dimethyl. Ibenzocaine camphorquinone.	A layer was actively applied on enamel for 20 seconds, followed by gently air drying For 5 seconds. Light curing was performed for 10 seconds.
Green Tea extract 10% aqueous solution (Farmácia Santana, Itararé, SP, Brasil) # 0256	Cammelia sinensis (10%); distilled water (90%)	Passively applied over the enamel surface for 60 min.
Sodium ascorbate 10% aqueous solution (Farmácia Santana, Itararé, SP, Brasil) # DY0261520320	Sodium ascorbate (10%), distilled water (90%)	Passively applied over the enamel surface for 10 min.

The restored samples kept in 100% relative humidity in a bacteriological stove at 37 °C, for 24 h. Subsequently they were submitted to microshear test with universal test machine (EZ Test, Shimadzu, Japan) with a crosshead speed of 0.5 mm/min. The values obtained were noted in Kgf and later transformed and expressed in values of load/area (MPa). The enamel fragment was considered as experimental unit, and, therefore, of the two values obtained with the microshear test, arithmetic mean was obtained to assign a single value to the experimental unit.

Fracture mode analysis

The surfaces of the fractured specimens were examined visually using stereomicroscope (EK3S3, São Paulo, SP, Brazil) at 30 times magnification to classify the type of fracture that occurred. The fracture mode was classified as: adhesive (adhesive failure), cohesive in enamel (when there was enamel fracture, keeping adhesive interface intact), cohesive in resin (when there was fracture of the resin cylinder,

keeping the adhesive interface intact) and mixed (two types of failure occurs simultaneously). Despite this classification, only the “adhesive” and “mixed” fractures were submitted to statistical analysis.

Statistical analysis

The results of the microshear test, in MPa, were analyzed regarding its distribution. Having been found the normality of the data and homogeneity of variance between groups, the data were submitted to ANOVA one criteria. The frequencies of “adhesive” and “mixed” fractures were submitted to Chi-square test. The level of significance adopted was 0.05.

RESULTS

Regarding the bond strength data (Table II), one-way ANOVA demonstrated that there was no statistical difference between experimental groups ($p = 0.545$).

Table II - Microshear bond strength data, in MPa

Experimental group	Mean (standard deviation)
GT- Green tea	9.64 (4.13)*
SA – Sodium ascorbate	11.53 (2.50)*
NC - Negative control (immediately restored)	10.13 (4.35)*
PC1 - Positive control 1 (restored after 14days)	9.19 (4.46)*
PC2 - Positive control 2 (not bleached)	8.69 (3.39)*

Means followed by * are statistically similar ($p > 0.05$)

The results of fracture mode (Figure 1) showed no statistical differences among groups ($p = 0.16424$). There was a predominance of adhesive failures in all groups. The GT and the SA groups also presented 20% and 6% of mixed fractures, respectively.

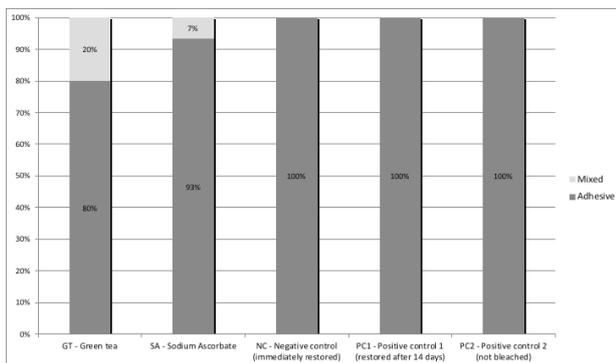


Figure 1 - Distribution of adhesive and mixed fractures among experimental groups.

DISCUSSION

The results of the present study demonstrated that there was no statistical difference between experimental groups. Therefore, the null hypothesis was accepted.

The sodium ascorbate was used as a comparison group because it is one of the most studied antioxidants, in order to revert the effects of dental bleaching on the bond strength of resin to enamel [14,16]. The sodium ascorbate has antioxidants properties, presents low toxicity and neutral pH, being adequate to use in dental structures without producing undesirable damage [14]. The results are in agreement with

some previous studies, that also showed no benefits from the application of sodium ascorbate to increase the bond strength of resin to enamel [17,18]. However, other studies claim that the application of sodium ascorbate can revert the oxidative effects promoted by the whitening gel on the dental structure [10,12,14].

The difference in the results may be due to the methodology employed in these works, taking into consideration mainly the concentration of SA and GT and time of application of whitening gel and antioxidants. In the present study were used human teeth while other studies [14, 19] used bovine teeth. This difference in methodology may have influenced the results.

The green tea has antioxidants properties proven in the literature [13] and therefore was tested as an agent of treatment of the dental bleached structure. However, it was evident there was no difference in bond strength when applying this substance, a result that is corroborated in previous works [18,21,22].

Despite the lack of effect when applying green tea to the enamel, it is important to notice that in the negative control group, where the samples were restored immediately after the bleaching, the bond strength did not decrease significantly as expected. It is speculated that the whitening gel has been applied for less time (2 h) than in studies (6 h) that found decrease of bond strength in this condition. Cavalli et al. [23] for example, applied the bleaching gel for 6 h, during 14 days. In other study from Cavalli et al. [3], the gel was applied for 6 h, during 10 days. Indeed, Barbosa et al. [24] concluded that in enamel that receive application of carbamide peroxide 10% for 14 days, 2 h daily, the restoration with composite resin could be realized immediately after the bleached procedure. Besides that, in other studies that verified decrease in bond strength in bleached enamel, the concentration of carbamide peroxide applied to the enamel was higher (16-

21%) [21] or yet, hydrogen peroxide 38% was used in the in-office technique [12].

Still, in the present study a universal adhesive was used, which contains functional monomer in its composition, the 10-MDP (10-Metacrilóixidecil dihydrogen phosphate), who is capable to establish ionic bonds with hydroxyapatite resulting in effective and stable bond strength [25]. Thus, it is speculated that the effectiveness of the adhesive on the dental enamel has overcome the deleterious effects of the whitening gel, applied for 2 h in the dental structure.

When analyzing the fracture mode, it was observed that the majority was adhesive, including the positive control group 2 (no bleached enamel). This result may be explained by the self-etching strategy chosen for application of the universal adhesive system, which can have led to weaker bond strength than if the previous phosphoric acid was used. In fact, the literature has reported that the phosphoric acid etching improves bonding of universal adhesives and the surface roughness of enamel [26].

Lastly, given the results of the present study, it is not yet possible to recommend the green tea aqueous solution and sodium ascorbate with the aim of revert the effects of 10% carbamide peroxide whitening gel in the dental enamel, since there was no damage in the bond strength to the enamel immediately bleached and neither benefits in the application of these agents. More studies must confirm if the application of 10% carbamide peroxide gel for shorter times than recommended by the manufacturer (8 h) causes significant reduction in bond strength to dental enamel, especially when using adhesives with functional monomer, such as the 10-MDP present in most universal adhesives.

CONCLUSION

In view of the results presented, it was concluded that neither the bleaching procedure nor the application of antioxidants to bleached enamel interfered in the bond strength of the tested universal adhesive system.

Acknowledgements

To CNPq (The Brazilian National Council for Scientific and Technological Development) for the financial support (grant received by the first author Ana Luiza Machado # 143639/2018-8).

Regulatory Statement

This research was approved by the Research Ethics Committee of the São Leopoldo Mandic Research Institute, Campinas – SP, Brazil (CAAE nº 97365918.7.0000.5374).

Conflict of interest

The authors have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

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Date submitted: 2020 May 25

Accept submission: 2020 Jul 14