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ORIGINAL RESEARCH

Influence of Prolonged Bleaching with 4% Hydrogen Peroxide Containing Calcium and Different Storage Times on the Bond Strength to Enamel

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ABSTRACT

Aims: To assess the influence of different bleaching protocols on the bond strength to enamel.

Materials and methods: In this laboratory experiment were used forty sound bovine incisors were divided into five groups. G1: No bleaching (control). G2: 14 days bleaching with 4% hydrogen peroxide containing calcium (4% HP+Ca^{2+}) (2 hours/day) and 24 hours of artificial saliva (AS) storage. G3: 14 days bleaching with 4% HP+Ca^{2+} (2 hours/day) and 7 days storage in AS. G4: 28 days bleaching with 4% HP+Ca^{2+}(2 hours/day) and 24 hours storage in AS. G5: 28 days bleaching with 4% HP+Ca^{2+} (2 hours/day) and 7 days storage in AS. Following storage times, composite resin cylinders were built upon the enamel surfaces and tested for micros hearing. For statistical analysis, two-way ANOVA and Tukey's test was applied to the data ($p \leq 0.05$), for it was evaluated different times of bleaching and stored in artificial saliva.

Results: The highest mean was observed in G1 (14.61 MPa), and the lowest in G4 (9.22 MPa). Compared to the negative control (G1), no differences were found in 14 days bleaching and the same between G2 and G3 ($p \geq 0.01$). However, in 28 days bleaching, the effects of the storage periods (24 hours and 7 days) were significantly different ($p \leq 0.05$), besides G4 and G5 were statistically different from G1.

Conclusions: Extended bleaching time (28 days) decreased the bond strength, independently of storage time in AS.

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Clinical significance: If adhesive procedures are required after extended at-home bleaching they may need to be delayed for at least for 7 days for the enamel adhesion ability to recover.

Keywords: Dental Enamel, *In vitro*, Hydrogen Peroxide, Shear Strength, Tooth Bleaching.

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INTRODUCTION

In-home bleaching therapies utilize low concentrations of hydrogen peroxide (3.5–10%) within a silicone device. Patients are responsible for filling the silicone tray with bleaching gels and applying the apparatus to the dental surfaces in accordance with the manufacturer's and dentist's instructions, which are important to achieve good results and prevent damage to the teeth and surrounding tissues.[1-3]

To brighten teeth more rapidly, patients sometimes exceed the time recommended by the dentist. Increasing exposure time could lead to adverse dental structure effects. The oxidation reaction must not exceed the saturation point, as the bleaching action may affect inorganic dental structures.[4,5]

The oxygen released during bleaching therapy is believed to increase enamel and dentin permeability. Indeed, the oxygen trapped in the enamel structures may inhibit adhesive and composite polymerization. Peroxides can denature organic matrix proteins (changing their physical and chemical properties) and reduce calcium and phosphate content, which may affect the adhesion

ability of these substrates.[6] For adhesive restorations, an effective bond is required between the material and the dental structure.[7] Therefore, studies have investigated bleaching therapies and the possible effects on adhesion. Some studies have suggested there is an adhesive reduction when restorative procedures are performed following a bleaching procedure.[6,8]

There is a lack of studies focussed on the influence of extended bleaching on the bond strength of restorative materials to enamel. Therefore, the aim of this *in vitro* study was to determine the influence of 4% hydrogen peroxide with calcium on enamel bond strength under different gel exposing times, as well as dissimilar storage periods after bleaching.

MATERIALS AND METHODS

This study was approved by the Research Ethics Committee for Test Animals (#4776201016). Forty bovine incisors (*Bos taurus indicus*; 24 years of age) were selected for the study. All were completely erupted, presenting sound crowns and fully formed roots.

After extraction, the teeth were stored in 0.1% thymol solution (Fórmula, Belém, PA, Brazil)for one week for disinfection. Subsequently, the teeth were completely cleaned to remove all remaining tissue and debris from the surface. As exclusion criteria, a careful inspection was performed at 40× magnification to detect cracks or fractures. In these cases, the defective teeth were discarded. Selected teeth were then stored in distilled water at 4ºC until preparation.[9] A double-sided diamond disc (KG Sorensen, Cotia, São Paulo, Brazil) was used to remove the roots. The crowns were then pumiced and rinsed with distilled water. The crowns were embedded in PVC rings using acrylic resin (JET, Classic, Campo Limpo Paulista, SP, Brasil), and the buccal surfaces of the teeth positioned slightly beyond the limits of the rings to allow grinding. After 24 hours, the embedded teeth were ground wet on a polishing machine (Aropol-E–Arotec, Cotia, SP, Brazil) with #180, 400 and 600 (30 seconds each), sandpaper discs. After flattening, the rings containing the enamel surfaces were randomly assigned (Bioestat software 5.0, Mamirauá, Brazil) to five groups (n = 8 per group) (Table 1).

The bleaching gel was composed of 4% hydrogen peroxide and calcium (White Class, FMG, Joinville, SC, Brazil). Acetate reservoirs were prepared to standardize the amount of gel applied to the enamel surfaces.[10,11] These reservoirs were loaded with 0.5 mL of bleaching gel and 0.1ml of AS (Fórmula, Belém, PA, Brazil) and applied to the enamel surface daily for 2 hours, as directed by the manufacturer (Table 1). Subsequently, an air-water spray positioned 5cm away from the surfaces, was applied for 1 minute, and the rings stored in AS for 24 hours or 7 days at 37ºC, according to the assigned groups.

Table 1: Experimental groups (n = 8)

Groups	*Bleaching (time)*	*Storage time in AS*
G1	No	No
G2	Yes (14 days)	24 hours
G3	Yes (14 days)	7 days
G4	Yes (28 days)	24 hours
G5	Yes (28 days)	7 days

Post-bleaching and AS storage, cylinders of composite resin were built upon the bleached enamel surfaces. To standardize the adhesive area, an 8 mm diameter perforation was made in acid-resistant double-sided tape (Tactape, Manaus, AM, Brazil) that was applied to the surfaces. The demarcated areas were conditioned with 37% phosphoric acid (Condac 37, FGM Joinville, SC, Brazil) for 15 seconds, rinsed and gently air-dried for 30 seconds. Single Bond 2 Adhesive (Adper Single Bond 2, 3M Espe, Sumaré, SP, Brasil) was applied to the conditioned areas for 20 seconds, gently air-dried for 5 seconds and photocured for 10 seconds (Adper Single Bond 2, 3M Espe, Sumaré, SP, Brazil). Next, the external side of the acid-resistant tape was removed, Tygon tubes with 0.5 mm high and 0.8mm internal diameters were positioned on the adhesive area to allow the insertion of the composite resin (Filtek Z350 XT, 3M Espe), which was placed and photocured for 20 seconds.[12] Two cylinders were applied per enamel surface.

For the micro-shear test, specimens were placed on a universal testing machine (Kratos KE, Cotia, SP, Brazil) and a 0.2 mm diameter metallic wire connected to the load cell was tied to the base of the cylinders. The crosshead speed was 0.5 mm/minute, and the micro-shear assay performed until the cylinder was dislodged. The results were recorded in MPa.

The fractured specimens were washed with distilled water and immersed in a solution of 4% methylene blue for 10 minutes to improve visualization with a Leica stereomicroscope (Leica Microsystems GmbH, Wetzlar, Germany) at 35× magnification.[13] The obtained images were examined using software (Leica Microsystems GmbH, Wetzlar, Germany) and the fracture pattern classified as adhesive, cohesive (in enamel or composite) or mixed.

Data from the micro-shear test were evaluated by two-way ANOVA and Tukey's *post hoc* test ($p \leq 0.05$) (Bioestat software 5.0, Mamiraua, Brazil). The fracture pattern was analyzed and calculated as a percentage.

RESULTS

The highest mean was observed for G1 (14.61MPa) and the lowest for G4 (9.22 MPa). The groups with extended

bleaching procedures (G4 and G5) presented significant differences ($p \leq 0.05$) when compared to the control group (G1). However, no significant differences were observed when the groups with bleaching procedures following the manufacturer's recommendations (G2 and G3) were compared to the control group (G1). There were significant differences ineffects based on AS storage times (24 hours and 7 days) for both bleaching protocols (normal/14 days and extended/28 days) (G2 and G4; G3 and G5) ($p \leq 0.0001$). For the recommended bleaching protocol (G2 and G3) there were no significant differences between storage times (24 hours and 7 days; $p \geq 0.05$). However, for the extended protocol (28 days; G4 and G5), the longer storage time (7 days) led to greater bond strength values (Table 2).

A mixed fracture pattern was predominantly observed for all experimental groups, corresponding to 67% of the total specimens analyzed (Fig. 1).

DISCUSSION

This study evaluated the effect of post-treatment storage time after extended bleaching on the bond strength to enamel. Seven days of storage following an extended bleaching treatment (G5) returned bond strength values near to those observed for the control group (G1). This was not observed for 24 hours of storage following the extended bleaching treatment (G4). It was also observed that the specimens treated as recommended by the manufacturer, did not differ compared to the control group (G1) regardless of the post-bleaching storage time. The storage media (AS) used for these experiments may explain some of the results.

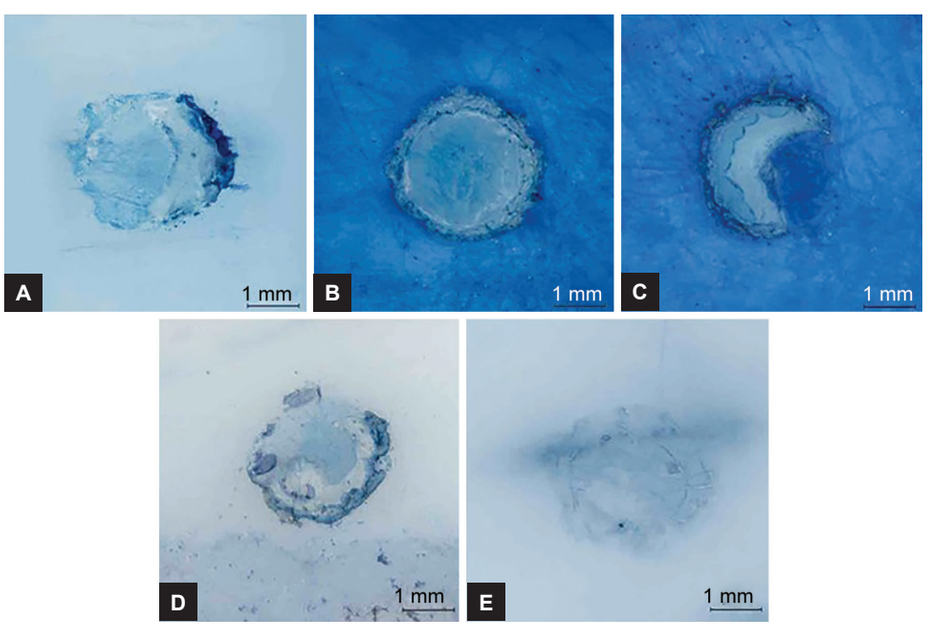
Human saliva is a natural fluid containing calcium, phosphate, and fluoride. Under normal conditions, saliva functions in the partial or total remineralisation process following mineral loss and protects the enamel against demineralisation agents.[14,15] In addition, saliva plays an important role in oxidative stress by presenting anti-oxidative alternatives due to the presence of ascorbic acid and vitamin E.[16] However, *in vitro* studies using artificial saliva, due to the time needed to collect the required volume and the rapid decomposition of human saliva *ex vivo*.[17]

The components present in AS may reproduce the functions of natural saliva.[14,18] This scenario was observed in the present *in vitro* study. When the enamel surfaces were exposed to prolonged bleaching, and subsequently stored in AS for seven days, the bond strength to enamel was recovered to 86.5% of the initial condition. However, this recovery was not statistically significant when compared to the control group.

Table 2: Difference between the mean (and standard deviation) of the microshear test data (MPa). Two-way ANOVA with Tukey's post hoc test at a significance level of $p \leq 0.05$.

	G1	*G2*	*G3*	*G4*	*G5*
Mean	14.61	$14.11^{A,a}$	$13.65^{a,b}$	$9.22^{B,c*}$	$12.22^{c,d*}$
Standard deviation	2.51	2.39	5.76	3.83	5.01

Distinct lowercase letters indicate statistical differences between the same storage times in AS. * indicates significant differences compared to the unbleached group (G1).



Figs 1A to E: Predominantly mixed fracture pattern (A) G1; (B) G2; (C) G3; (D) G4 and (E) G5.

Normal enamel bond strength returned following 14 days of manufacturer-recommended bleaching treatment.[10,11] These results show that even after 24 hours of storage in AS, the bond strength returned to the non-bleached group levels. Thus, it is not necessary to wait seven days to proceed with adhesive restorative procedures when the bleaching gel is used as recommended.[19]

As the main purpose of this *in vitro* study was to determine the influence of gel exposing and storage times intervals on enamel bond strength, after bleaching procedures, only one type of peroxide (hydrogen) was used, and the point that influenced this selection was the presence of calcium, which is not present in Carbamide gels. Moreover, 4% of hydrogen peroxide is equivalent to 10% carbamide peroxide in terms of free radical release; therefore, it was decided to use only the first bleaching gel.

Calcium is present in the bleaching gel composition used herein and could aid the remineralization process.[20,21] The interaction between the bleached enamel and calcium may explain the bond strength values observed in G2 (14.11 MPa) being similar to the control group (14.61 MPa). Nevertheless, a better understanding of the effects of mineralizing agents on enamel is needed, as these calcium ions could be removed within one day post-bleaching.[20]As limitation of this study, different peroxide types/composition, as well their concentrations could influence the results. However, as the main purpose of this *in vitro* study was to verify variations on the gel exposing times and storage periods, only one type of bleaching gel was used.

Another limitation that should be discussed is the absence of brushing and flossing among bleaching treatments. Certainly, this action, present in the mouth, should influence enamel damages, both, due to the abrasion/attrition mechanism and remineralizing agents in the toothpastes.[22,23]

Another issue that should be here addressed is the presence of residual oxygen trapped in the enamel structure. Oxygen is slowly released after bleaching. The presence of free oxygen may interfere with the polymerization process, which could inhibit the hybrid layer formation and compromise the clinical performance of the restoration. Therefore, the adhesive restorative placement should be delayed for up to three weeks. This waiting period may vary, according to the bleaching gel concentration and time established for the bleaching procedure.[7,8,19] This phenomenon may have influenced the reduction in bond strength values in G4 (9.22 MPa) relative to the control group (14.61 MPa). This 36.8% difference was statistically significant and demonstrates that 24 hours of post-bleaching storage in AS is not enough for the oxygen release, especially after 28 days of bleaching.

Morphological alterations on enamel micro-topography may also occur.[1-3] These changes are related to the time that the enamel is exposed to the peroxide.[21] Therefore, extended exposure time could exacerbate these effects. Data from an Energy Dispersive Spectroscopy study[24] demonstrate that calcium reduction is directly related to the time the enamel is in contact with the peroxide. Comparable conditions were found in the enamel ultrastructure after bleaching, when 10, 20 and 35% carbamide peroxide was used.[25] Considering the existing relationship between the morphology, chemical composition, and the bleached enamel bond strength,[21,25] it is possible to explain the lower values observed in the extended bleached groups (28 days), regardless of the AS post-storage time (24 hours or 7 days).

The coloring method used to generate images and identify the fracture patterns herein was recently published.[13] The contrast of the stained areas makes it possible to identify the different interface components (bonding agent, composite and dental structure) present on the fractured area. The observed mixed fractured pattern occurred more frequently within the adhesive interface, and sometimes involved the restorative material (Fig. 1). Adhesive and mixed fracture patterns are normally associated with satisfactory bond strength, which in turn, are related to long-lasting restorations.[26,27]

CONCLUSION

The observed results can be related to the conditions (particularly the bleaching agent/protocol and storage media) in this *in vitro* study, as there was a bond strength reduction proportional to the bleaching protocol (extended). However, it is important to mention the need for clinical studies, which could help in better understanding the damage caused by prolonged exposure to bleaching agents.

CLINICAL SIGNIFICANCE

If adhesive procedures are required after extended at-home bleaching they may need to be delayed for at least for 7 days for the enamel adhesion ability to recover.

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