Demineralization Effects of Hydrogen Peroxide on Bovine Enamel and Relation to Shear Bond Strength of Brackets

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Purpose: To measure the demineralization capacity of 37% phosphoric acid and shear bond strength (SBS) of brackets attached on bovine enamel at different times after bleaching with 30% hydrogen peroxide.

Materials and Methods: Four equally-sized pieces of each crown from 18 bovine incisors were randomly distributed among 7 groups (n = 10). After bleaching with 30% hydrogen peroxide for 1 h, specimens were stored in artificial saliva for 0 h, 24 h, or 1, 2, 3, or 4 weeks before bonding specimens to brackets. An unbleached group of specimens served as controls. Shear bond strength (SBS in MPa) was measured with a universal testing machine. Adhesive Remnant Index (ARI) scores were determined after failure of bracket bonds. To measure demineralized Ca\(^{2+}\), four 4 x 4 mm sections from each of 15 bovine incisors were randomly distributed among 4 groups (n = 15). Specimens were stored in artificial saliva for 0 h, 24 h or 7 days after bleaching and then immersed in 37% phosphoric acid solution. After 15 s, 30 s, and 60 s, 5-ml aliquots of solution were removed for spectrophotometry. Unbleached specimens served as controls.

Results: Larger amounts of Ca\(^{2+}\) were extracted from the enamel by phosphoric acid up to 24 h after application of hydrogen peroxide, when there was also a significant decrease in bracket-enamel SBS. After 1 week, there were no changes in amounts of Ca\(^{2+}\) extracted, and SBS values returned towards unbleached values.

Conclusion: Lower bracket-enamel SBS values at 24 h after bleaching are closely correlated with the larger amounts of Ca\(^{2+}\) extracted from the enamel.

Keywords: 30% hydrogen peroxide, shear bond strength, brackets, decalcifying, phosphoric acid, spectrophotometry.

Tooth bleaching has been considered a safe procedure by some authors, who observed no evidence of deleterious effects on enamel or dentin after the longest applications of the highest concentrations of hydrogen peroxide in normal clinical use for this purpose. Nevertheless, bleaching agents may adversely affect the bond strength of composite to acid-etched enamel. Various studies have reported significant reductions in enamel bond strength of resin composite restorations and resin-bonded brackets when bonding is preceded by tooth bleaching.

Several investigations have attempted to elucidate this decrease in enamel bond strength after bleaching. Due to its low molecular weight, hydrogen peroxide can penetrate enamel and be retained in the bleached enamel. Residual oxygen released from the bleaching agent inhibits polymerization of the resin, resulting in an incomplete curing of the adhesive, and interferes with resin infiltration into the etched enamel. It may therefore be responsible for the time-dependent reduction in the quality of resin-enamel bonds.

There is no general agreement concerning the deleterious effects of bleaching agents on enamel and dentin. Changes to the microstructure of surface enamel have been reported that are proportionally more severe with longer treatment times and higher hydrogen peroxide concentrations. In addition, bleaching agents may alter...
enamel microhardness, with loss of mineral content from the outer tooth structure.2,3,47 The release of calcium and phosphorous ions increases with higher hydrogen peroxide concentrations, leading to a reduction in the Ca:P ratio in bleached samples.2 After prolonged use, the bleaching agent reduces the fracture resistance of dentin,39 and these reductions are greater for longer application times and higher bleach concentrations.40 Carbamide peroxide has an intrinsic demineralizing effect.17,29 Previous bleaching with 30% carbamide peroxide increases the amount of Ca^2+ extracted from enamel after etching with phosphoric acid, with highest losses observed at 24 h after bleaching.28 Many clinical procedures, eg, bonding of orthodontic brackets, porcelain veneers, composite veneers, or composite restorations, require acid etching of the enamel only a short time after completion of bleaching. The effectiveness of these procedures may be affected by this higher demineralizing power of phosphoric acid on bleached enamel.

In the present study, the null hypothesis was that bleaching treatment with 30% hydrogen peroxide does not modify either the decalcifying ability of phosphoric acid in enamel acid etching or the shear bond strength of brackets to enamel. The objectives were: 1. to determine the shear bond strength of metal orthodontic brackets bonded to hydrogen peroxide-bleached enamel after various post-bleaching intervals (immediately, or at 24 h, 1 week, 2 weeks, 3 weeks, and 4 weeks postbleaching); 2. to evaluate by Atomic Absorption Spectrometry the decalcifying effects of 37% phosphoric acid on enamel after clinically relevant application times (15 s, 30 s, and 60 s) following three postbleaching intervals (no interval, 24 h, and 1 week); and 3. to explore the relationship between the decalcifying effects of 37% phosphoric acid and shear bond strength.

MATERIALS AND METHODS

Shear Bond Strength (SBS)

Eighteen bovine incisors with no fractures or defects on the vestibular enamel surface were selected and stored in thymol under refrigeration until use. All roots were cut off at the cemento-enamel junction using an Accutom-50 diamond cutter (Accutom Hard Tissue Microtome, Struers; Ballerup, Denmark). Each crown was then cut (in the incisocervical then mesiodistal direction) into 4 equally-sized sections. Using 20-ml disposable syringes (with conical ends removed) as molds, each specimen was then attached to the central part of the syringe plunger using Coltene utility wax (Whaledent; Mahwah, NJ, USA). The plunger was withdrawn to leave a 1-cm deep container that was then filled with transparent self-curing resin (Ortocryl, Dentaurum; Ispringen, Germany), prepared according to the manufacturer’s instructions. During polymerization, the mold was submerged in water to compensate for the exothermic reaction. The resulting cylinder was expressed from the mold by using the syringe plunger. The exposed enamel surface of specimens was polished with 500-then 1200-grit silicon carbide paper disks in order to remove the surface aprismatic enamel layer and obtain a flat surface.

Specimens were randomly divided into seven treatment groups (n = 10). Group 1 was an unbleached control group. The other six groups were bleached with Illuminé Office bleaching agent (Dentsply Detrey; Konstanz, Germany) according to the manufacturer’s instructions. After mixing the two components, the resulting gel was applied to the polished surface of the enamel for a period of 60 min, during which the specimens were kept in an oven maintained at 37°C and low relative humidity. Although the manufacturer’s information leaflet describes this widely used product as 30% hydrogen peroxide, the mixed gel has a concentration of 15%. After bleaching, specimens were washed with abundant distilled water and dried with absorbent paper. Groups 3 to 7 were then stored in artificial saliva28 in an incubator at 37°C for the required time periods (24 h, 1, 2, 3, or 4 weeks). Group 2 was bonded immediately.

A 37% phosphoric acid gel (3M Dental Products; St Paul, MN, USA) was used to etch specimens for 30 s. They were then rinsed with abundant water and dried with an air syringe until the frosty white appearance of etched enamel was seen. MBT Victoria Series metal brackets for maxillary lateral incisors (3M Uniteck; Puchheim, Germany) were used, with a combined surface area of 12.2 mm^2. They were attached with Transbond XT bracket adhesive (3M Dental Products; St Paul, MN, USA) following the manufacturer’s recommendations.

After surface preparation, Transbond XT liquid primer (3M Dental Products) was applied to the etched surfaces. Sufficient bonding paste was applied to cover the bracket bases. The brackets were then seated firmly on the enamel surface and excess resin was removed with a dental explorer. Light curing was done with an Astralis 10 halogen lamp (Ivoclar Vivadent; Schaan, Liechtenstein) at 700 mW/cm^2 for 20 s. After bonding, the specimens in all groups were stored in artificial saliva at 37°C for 24 h to allow complete resin polymerization. SBS was tested by a method that avoids the generation of leverage forces (Fig 1), using an Electrotest Model 500 universal traction machine (IBERTEST; Madrid, Spain) at a speed of 1 mm/min. The device allows a sharpened blade to be moved in only one direction on the smooth surface of the specimen, always cutting along the bracket/enamel interface in all specimens.

After debonding, all specimens and brackets were examined under 10X magnification and scored on the Adhesive Remnant Index (ARI).5 The ARI scale has a range of 1 to 5. A score of 5 indicated no composite remaining on the enamel; 4: 10% of composite remaining; 3: 10% to 90% remaining; 2: >90% remaining; 1: all of the composite remained on the tooth, bearing the impression of the bracket base.

Spectrometry

Fifteen bovine incisors were stored in distilled water with thymol crystals until use. All roots were cut off at the cemento-enamel junction using an Accutom-50 diamond cutter (Accutom Hard Tissue Microtome, Struers; Ballerup,
Denmark). Crowns were polished with silicon carbide paper disks on a polisher (Exakt-Apparatebau; Norderstedt, Germany) to obtain a flat vestibular surface, and each crown was then cut into 4 equally-sized 4 x 4 mm sections (sample specimens) as described above. After polishing the inner surfaces to remove the dentin, sample specimens were weighed on a Sartorius BL60S precision balance (accuracy of ± 0.0001 g; Sartorius; Mugió, Italy). Maximum weight equality among specimens was achieved by reducing weight when necessary with 600-grit silicon carbide paper disks (WS 18-B, Struers), always reducing the inner surface of the section to avoid modifying its geometry. An Olympus stereomicroscope (Olympus Optical España; Barcelona, Spain) was used to verify that all exposed surfaces were enamel.

One sample specimen from each crown was assigned to one of four groups (n = 15): group I, no bleaching agent (control group); groups II, III, and IV, bleached with 30% hydrogen peroxide for 60 min. Groups III and IV were then stored in artificial saliva for 24 h and 1 week, respectively.

For bleaching, specimens were immersed in 30% hydrogen peroxide (Illuminé Office, Dentsply Detrey) for 60 min, replacing all of the gel at 30 min as per manufacturer's instructions. Specimens were washed with abundant distilled water and dried with absorbent paper. Group III and IV specimens were then kept in artificial saliva at 37°C for the specified time period (24 h or 7 days). Group II specimens were immersed in 37% phosphoric acid solution immediately after bleaching. The 37% phosphoric acid solution was prepared by dissolving 311 ml of 85% phosphoric acid (Scharlan Cemise; Barcelona, Spain) with a concentration of 1.71 g/ml in 689 ml of distilled water to obtain 1000 ml of 37% acid solution with a pH of 0.14, measured with a Micro pH 2000 pH meter (Crisol; Alella, Spain).

A blank 20 ml sample of phosphoric acid solution was first prepared to determine Ca²⁺ levels in the absence of a specimen. All unbleached and bleached specimens underwent the same experimental procedure, ie, immersion in the phosphoric acid solution followed by constant agitation using a magnetic multi-stirrer (SBS A-09 series C, Scientific Basic Solutions; Barcelona, Spain) to uniformly mix the phosphoric acid solution. The 37% phosphoric acid solution had previously been removed. Therefore, the mg of Ca²⁺ corresponding to 5 ml of V1 solution were added to the mg of Ca²⁺ obtained at 30 s. Likewise, since the volume was 10 ml at 60 s, the mg of Ca²⁺ corresponding to 5 ml of V1 solution plus 5 ml of V2 solution were added to those obtained at 60 s.

Statistical Analysis
The effect of storage interval on enamel-bracket SBS was analyzed using one-way ANOVA and the post-hoc Dunnet’s t test, with the unbleached control group as the reference category. A three-way ANOVA was used to explore the relationships among bleaching treatment, acid exposure, and extracted calcium, followed by two-way ANOVA and the post-hoc Dunnet’s t test. Frequencies in each ARI category were analyzed using one-way ANOVA and the post-hoc Dunnet’s t test, with the unbleached control group as the reference category. A three-way ANOVA was used to explore the relationships among bleaching treatment, acid exposure, and extracted calcium, followed by two-way ANOVA and the post-hoc Dunnet’s t test. Frequencies in each ARI category were compared by using a chi-square test. The significance level was set at p < 0.05.

RESULTS

Shear Bond Strength
Table 1 shows the means and standard deviations of SBS measurements. The ANOVA revealed significant effects (F = 9.137; p < 0.001) of the time elapsed before bracket bonding to enamel (bleaching to bonding interval). Dunnet’s t-test showed significant differences in SBS values between the control group and groups with bleaching-to-bonding intervals of 24 h and 1 week. SBS values in the 24-h group were one-third of SBS values in the unbleached control group. After 24 h, SBS values began to recover towards unbleached control values and had doubled after 1 week, although they remained significantly lower than controls. No significant differences in SBS values were ob-

mg Ca²⁺/g = [(ppm Ca²⁺)·10⁻³ l/ml·V]/W

where ppm Ca²⁺ are ppm of Ca²⁺ in each time period, V is volume of solution in ml (at 15 s, V1; at 30 s, V2; and at 60 s, V3), and W is the weight of the sample in mg. For the 15-s period, the volume of solution (V1) was 20 ml, whereas at 30 s, the volume (V2) was 15 ml, since 5 ml of solution had previously been removed. Therefore, the mg of Ca²⁺ corresponding to 5 ml of V1 solution were added to the mg of Ca²⁺ obtained at 30 s. Likewise, since the volume was 10 ml at 60 s, the mg of Ca²⁺ corresponding to 5 ml of V1 solution plus 5 ml of V2 solution were added to those obtained at 60 s.

Fig 1 SBS-testing device that avoids the generation of leverage forces.
served between the unbleached control group and groups with a bleaching-to-bonding interval greater than 1 week. When brackets were attached immediately after bleaching, SBS values appeared slightly lower compared to the unbleached control group, but the difference was not statistically significant.

Analysis of failure type (Table 2) revealed that all failures were adhesive along the resin/enamel interface (ARI score of 5) after a bleaching-to-bonding interval of 24 h, with no resin remaining adhered to the enamel. Fractured specimens from the other postbleaching groups showed various types of failure (around 75% with ARI scores of 4 or 5). All of the composite remained bonded to the enamel surface in 50% of unbleached specimens (score of 1) but in none of the bleached groups. The distribution of fractured specimens across ARI categories significantly differed among the groups ($\chi^2 = 41.194; p = 0.16$).

### Ca$^{2+}$ Demineralization by Phosphoric Acid Etching

Table 3 shows the cumulative amounts of Ca$^{2+}$ extracted from the enamel specimens after subtracting the amount of Ca$^{2+}$ found in the study solution without a specimen. The three-way ANOVA (bleaching, acid exposure time, and sample) showed significant differences between experimental groups ($F = 5.124, p < 0.002$) and acid exposure (etching) times ($F = 115.300, p < 0.001$), with no significant interactions.

The amounts of Ca$^{2+}$ extracted from bleached and unbleached specimens increased with longer etching time, and significantly differed between all etching time periods ($60\ s > 30\ s > 15\ s$). Unbleached and bleached groups showed similar demineralization kinetics.

The total extracted Ca$^{2+}$ was greater for groups with bleaching-to-etching intervals of 0 h and 24 h than for the control group, although this difference was only significant ($p < 0.05$) for the 24-h group ($p = 0.10$ for the 0-h group). Similar amounts of Ca$^{2+}$ were extracted between the group etched at 7 days after bleaching and the unbleached control group. For a given exposure time, groups did not significantly differ in the amounts of Ca$^{2+}$ extracted.

### DISCUSSION

Contemporary bleaching agents are typically either hydrogen peroxide or carbamide peroxide. In-office bleaching generally uses relatively high levels of whitening agents for short time periods. For this study, the in-office bleaching agent Illuminè (Dentsply), usually applied for 60 min in the
clinical setting, was applied on the enamel of bovine teeth, widely recognized as a reliable substitute for human teeth in this type of investigation. It is well documented that bleaching significantly reduces the immediate bond strength of composite to enamel, which has been related to alterations in the attachment surface area, resin/enamel interface, and resin quality. Sundfeld et al found inferior formation of tags, which were shorter, thinner, less frequent, nonuniform, and poorly defined, and a lesser penetration of the adhesive material into the enamel in comparison with unbleached controls. Dishman et al reported a high concentration of residual oxygen in the pores of bleached tooth enamel. This oxygen-rich layer is 5 to 10 μm thick, and may not only inhibit polymerization of the adhesive but also increase the porosity of the resin material by the release of gaseous oxygen. Resin/enamel interfaces on bleached enamel may exhibit more extensive nanoleakage as a result of this interaction.

In this study, SBS values were lower in brackets attached immediately after bleaching than in the unbleached group, but this difference was not significant. However, Unlu et al found significantly lower SBS values for specimens bonded immediately after bleaching than for unbleached specimens. This disparity may be explained by their use of a self-etching adhesive (Clearfil SE Bond), since the susceptibility of adhesives to bleaching may depend on the aggressiveness of the demineralization process. The pH of Illumine is 6.5, much higher than the 1.9 of Clearfil SE Bond adhesive. When the latter

| Table 3 Amounts of Ca²⁺ in mg extracted (as determined by spectrometry) in relation to bleaching-to-etching intervals and acid exposure time |
|-----------------|-----------------|-----------------|-----------------|
| Bleaching-to-etching interval | Acid exposure time | 15s (n=60) | 30s (n=60) | 60s (n=60) | Total (n=180) |
| Group I (control) (n = 45) | 0.614 (0.282) | 0.952 (0.287) | 1.601 (0.456) | 0.758 (0.538) |
| Group II (B- 0 h) (n = 45) | 0.749 (0.388) | 1.089 (0.351) | 1.846 (0.740) | 1.228 (0.726) |
| Group III (B- 24 h) (n = 45) | 0.731 (0.442) | 1.151 (0.628) | 1.880 (0.803) | 1.257 (0.729) |
| Group IV (B- 7 days) (n = 45) | 0.579 (0.167) | 0.875 (0.238) | 1.520 (0.353) | 0.991 (0.473) |
| Total B-E (n = 180) | 0.668 (0.336) | 1.017 (0.454) | 1.714 (0.622) | 1.056 (0.491) |

B-E: Bleaching-to-etching interval. Different capital letters indicate statistically significant differences among experimental groups. In each row, different lower-case letters indicate statistically significant differences among etching times in each bleaching group.
is used immediately after the former, the higher pH of the former could interfere with the effectiveness of the adhesive. When Uysal et al.\textsuperscript{45} used the same adhesive protocol as in the present study (etching with phosphoric and Tans-bond XT), they found that in-office bleaching with 35% hydrogen peroxide did not adversely affect the bond strengths of brackets bonded immediately or at 30 days after bleaching. These results are in agreement with the present findings for these time periods. However, they did not study bonding of brackets to enamel at 24 h after bleaching, when we found the weakest SBS values (and largest Ca\textsuperscript{2+} losses during etching), which were around one-third lower than for unbleached controls. One week later, the SBS had increased to reach the value of the control group, and SBS values then remained the same for all groups up to 1 month. It should be borne in mind that the increase in SBS might have occurred earlier, since it was not evaluated between three and six days after bleaching.

There are concerns that tooth-whitening procedures irreversibly damage tooth structure. Bleaching has been reported to produce microstructural changes to the surface and subsurface layers of enamel,\textsuperscript{23} mainly localized in the outer enamel and diminishing in inner enamel layers,\textsuperscript{16} associated with a loss of Ca\textsuperscript{2+} from the enamel and a significant decline in calcium:phosphate ratios.\textsuperscript{8} A greater loss of Ca\textsuperscript{2+} is produced by 37% phosphoric acid etching of enamel after bleaching,\textsuperscript{24} resulting in loss of prismatic form and a more extensive etching pattern.\textsuperscript{18,22} Although the rougher surface and greater retention obtained might be expected to increase the bond strength, it is in fact reduced because the hydrogen peroxide alters the organic matrix of the enamel,\textsuperscript{17,21} favoring Ca\textsuperscript{2+} loss and weakening the etched enamel.

Josey et al.\textsuperscript{23} using light microscopy, found that the bleaching process resulted in a loss of mineral from the enamel that was evident 24 h after bleaching, similar to the present finding. Although hydrogen peroxide has a high capacity for diffusion,\textsuperscript{46} a time interval is required for the structural alteration of hydroxyapatite that produces Ca\textsuperscript{2+} losses, which are observed after 24 h. In a previous study, Medeiros et al.\textsuperscript{28} found no significant difference in Ca\textsuperscript{2+} loss between a control group and a group etched immediately after bleaching treatment. The pH of the 30% hydrogen peroxide gel used in their study was 6.5, therefore the demineralization observed in the present study cannot be attributed to the low pH of the gel.

We found no statistically significant differences in pair comparisons between groups, which may be explained by the low final concentration of 15% hydrogen peroxide in the bleaching gel used (Illuminé Office, Dentsply Detrey). Significant differences may also have emerged with a longer exposure time, as found in a previous study by our group that used an 11% final concentration of hydrogen peroxide for 90 min, 30 min more than in the present study.\textsuperscript{28} Efegil\textsuperscript{17} found a significant reduction in the mineral content of surface and subsurface enamel specimens after application of high-concentration in-office bleaching for 2 h, the maximum application time recommended by the manufacturer. This demineralization was clearly greater in the outer 150 \(\mu\text{m}\) of enamel. The quantity of ions released increased with higher hydrogen peroxide concentrations.\textsuperscript{2} These greater Ca\textsuperscript{2+} losses might be explained by changes in the chemical composition of dental enamel caused by the bleaching agents.\textsuperscript{15}

Although Josey et al.\textsuperscript{23} suggested that the loss of mineral from enamel was sustained after 12 weeks of storage in artificial saliva, it is also widely reported that microstructural changes in bleached enamel can be reversed by salivary components,\textsuperscript{4,7,14} with consequent restoration of the bond strength to enamel.\textsuperscript{10,43} In the present study, the artificial saliva functioned as a remineralization solution. For this reason, the present findings are similar to those of Unlu\textsuperscript{45} and Machado,\textsuperscript{13} who found that SBS values and levels of extracted Ca\textsuperscript{2+} returned to levels found for unbleached enamel by 1 week after the use of hydrogen peroxide and then remained stable until the end of the study at 4 weeks.

Several methods have been proposed to meet the clinical challenge posed by the effects of bleaching on bond strength. Dishman\textsuperscript{15} suggested that the oxygen-rich layer would be removed by the action of the phosphoric acid applied before application of resin-based adhesive. In contrast, Cadenaro\textsuperscript{14} proposed that etching and rinsing procedures do not eliminate residual oxygen from the surface and that polymerization of the adhesive is reduced. Composite-enamel bond strength was significantly improved by the pre-bonding application of antioxidant agents, eg, 10% sodium ascorbate, catalase or ethanol,\textsuperscript{26} which were claimed to reduce or completely remove residual peroxides and other oxygen radicals. Our results demonstrate that the reduction in SBS is not solely a result of the persistence of oxygen in the enamel. Thus, lowest SBS values were at 24 h, when the largest extraction of Ca\textsuperscript{2+} by phosphoric acid etching was observed. Moreover, the fracture patterns observed indicated poor adhesion. Therefore, the bleached enamel surface did not allow a strong and stable bond to be formed with the composite, and our null hypothesis must be rejected.

We recommend a time interval of at least 7 days between the utilization of peroxide bleaching materials and restorative procedures that require acid-etching and adhesive bonding,\textsuperscript{9} allowing sufficient time for penetration of the adhesive system into the enamel surface, with formation of regular tags.\textsuperscript{13}

**CONCLUSIONS**

At 24 h after application of 30% (final concentration 15%) peroxide, a larger amount of Ca\textsuperscript{2+} was extracted by 37% phosphoric acid from bleached than unbleached enamel, coinciding with a significant decrease in the shear bond strength of brackets to enamel. After one week, the shear bond strength with bleached enamel progressively recovered to the value observed with unbleached enamel, and extracted Ca\textsuperscript{2+} values stabilized.
REFERENCES


Clinical relevance: A minimum interval of 7 days is recommended between the use of peroxide bleaching material and the adhesive bonding of orthodontic brackets which requires acid etching.