Analysis of Photoreflectance and Microhardness of the Enamel in Primary Teeth Submitted to Different Bleaching Agents

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Treatment of darkened teeth in children is of great importance from an esthetic-functional point of view and for the psychoemotional development of the child. The objective of the present study was to determine the in vitro efficacy of three bleaching agents for whitening of artificially stained primary teeth. Fifty anterior primary teeth were artificially stained and then divided into three experimental groups (n = 15) submitted to bleaching treatment with 35% hydrogen peroxide gel, 35% carbamide peroxide gel, and 35% carbamide peroxide gel mixed with sodium perborate powder. The control group (n = 5) was not submitted to any bleaching treatment. Color changes were evaluated with a reflectance spectrophotometer and possible alterations in the enamel surface after bleaching were measured by Vickers microhardness testing. The data were assessed using the Student's t test. The results confirmed the bleaching action of the three agents tested. No significant difference in mean microhardness was observed between the three bleaching agents when compared to the control group.

KEYWORDS: *tooth whitening; primary teeth; bleaching agents; enamel microhardness; reflectance.*
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INTRODUCTION

Esthetics in dentistry nowadays represents a source of satisfaction and realization for both the patient and the dentist. In the case of children, esthetics is also a constant concern in daily clinical practice and oral treatment is not only related to preventive and esthetic-functional factors but is also of great importance for the psycho-emotional development of the child in its environment. Stains produced by fluorosis, color changes caused by trauma as well as structural alterations represent a challenge to the pediatric dentist.

Current approaches to the whitening of darkened teeth include tooth bleaching techniques that can be applied by the dentist or patient to both vital and non-vital teeth. Part of the mechanism of bleaching is related to the cleaning of the content that stained the dentinal tubules by the action of the bleaching agent.[6]

Several chemical agents have been employed for tooth whitening. Hydrogen peroxide (35%) is one of the most frequently used agents to remove stains resulting from fluorosis, tetracycline intake and trauma,[16] Hydrogen peroxide acts on the pigments present in enamel and dentin by transforming complex molecules into simpler (less colored) ones.[8] Another bleaching agent used is carbamide peroxide. This product can or cannot contain carbopol, a thyxotropic agent that controls the release of oxygen, prolonging the time of action of the product.[7] Carbamide peroxide is highly unstable and when in contact with saliva and/or dental structures, dissociates into hydrogen peroxide and urea. The latter can be further decomposed into ammonia and carbon dioxide. Ammonia increases the pH, favoring the chemical reaction mediated by hydrogen peroxide.[7] Sodium perborate is also used as a bleaching agent. In the presence of water, it is decomposed into sodium metaborate, hydrogen peroxide and oxygen, thus acting as an oxidizing agent. Sodium perborate is generally used in a mixture with hydrogen peroxide and this combination potentiates the bleaching action, in addition to prolonging the release of free oxygen. Some authors recommend this product to prevent risks during the procedure since sodium perborate is not caustic.[1, 13, 20]

The analysis of bleaching agents, their effectiveness, as well as possible alterations caused by different mixtures of these agents in dental structures, is important in dentistry. Despite the large number of studies available in the literature

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regarding the effect of bleaching agents on permanent teeth, little is known about their action on primary teeth. The objective of the present study was to determine the *in vitro* efficacy of three bleaching agents in the whitening of artificially stained primary teeth.

MATERIAL AND METHODS

Fifty intact anterior primary teeth obtained from the Dentistry College Tooth Bank, Taubaté University (UNITAU), were used. The project was approved by the Research Ethics Committee of UNITAU (CEP/UNITAU 304/03).

The teeth were cleaned with periodontal curettes (Gracey 5-6/Trinity – São Paulo / Brazil), polished with a rubber cup and pumice (Ultrathin, Nupro/Dentsply – São Paulo / Brazil) at low speed, washed, stored in distilled water and autoclaved at 121ºC for 15 minutes. The pulp chamber was then opened in 45 teeth with a spherical diamond tip (No. 1012, KG-Sorensen – São Paulo / Brazil). Next, the pulp chamber and root canal tissue was removed with Kerr files and the teeth were instrumented to a No. 30 file size. The thickness (in mm) of the remaining layer of enamel and buccal dentin was measured in each specimen with a pachymeter (DigitCal, Tesa, type 05.30032 / Switzerland). During and after instrumentation the teeth were abundantly rinsed with sterile distilled water and submitted to a last rinsing step with 15% EDTA (Chng *et al.,*[3] 2002). For handling, the teeth were kept in sterile gauze moistened with sterile distilled water. Next, light reflectance of the teeth was measured with a spectrophotometer (model MS257, Oriel Instruments – Newport / USA) using a xenon lamp (5 mW).

The teeth were artificially stained using the technique of Freccia and Peters,[5] with modifications. The teeth were incubated in 1% sodium hypochlorite solution for 24 hours. Next, they were immersed in test tubes containing human blood (Blood Center of the University Hospital/UNITAU) with negative serologies and the tubes were centrifuged at 11,800 *g* for 20 min twice a day for 3 consecutive days. Distilled water was then added to the blood samples without teeth and the samples were centrifuged at 11,800 *g* for 10 min for erythrocyte hemolysis. The hemolyzed layer was separated and transferred to another test tube containing the teeth. This set was again centrifuged twice a day for a further 3 consecutive days. During the 6 days of staining, the teeth were maintained at 37ºC. After this period, the teeth were washed under running water for 2 minutes, dried with an air stream and stored individually in test tubes containing distilled water. After staining, the light reflectance of the teeth was measured again.

The root canal of each tooth was filled with a resin-modified glass ionomer (Vitremer, 3M – São Paulo / Brazil) up to 1 mm below the cervical margin and its pulp chamber was washed with 1% sodium hypochlorite, and dried with cotton wicks moistened with alcohol ether followed by an air stream.

Next, the teeth were divided into three experimental groups of 15 teeth each and submitted to three types of bleaching treatment: group A - 35% hydrogen peroxide gel (Byofórmula – Taubaté / Brazil), group B - 35% carbamide peroxide gel (Byofórmula), and group C - 35% carbamide peroxide gel mixed with sodium perborate powder (Byofórmula) until forming a paste of thin consistency. The control group (n = 5) was not treated with any bleaching agent but was submitted to all procedures using distilled water.

The pulp chambers were filled with the bleaching agents and access was sealed with cement (New Bond, Technew – São Paulo / Brazil). Light reflectance was then measured on days 1, 7 and 14 after application of the bleaching agent and the teeth were kept at 37ºC in individual flasks containing sterile distilled water. After the first and seventh day of bleaching, the teeth were reopened, the cavity was washed with 1% sodium hypochlorite and again filled with the bleaching agent, and the teeth were returned to their flasks with distilled water for an additional 7 days. After a total of 14 days, the bleaching agents were removed from the teeth. The pulp chambers were washed with 1% sodium hypochlorite and dried with cotton wicks. The teeth were restored with cement and stored in distilled water for 14 days. After this period, the light reflectance of the teeth was measured again. The light reflectance data were tabulated and analyzed by paired Student *t* test. P value < 0.05 was considered statistically significant.

After bleaching treatment, 5 teeth of each group and 5 control teeth, for a total of 20 teeth, were sectioned on the buccal side with a double-sided diamond-coated disk (KG Sorensen- São Paulo / Brazil) under water, thus obtaining standard enamel specimens measuring 3 x 3 mm as confirmed with a pachymeter. The samples were stored in distilled water at 37ºC. Next, after embedding in polyester resin (Orto Cristal, T208, Valglass – São Paulo / Brazil), the samples were polished with a Knuth-Rotor polisher (Struers) using aluminum oxide files of decreasing granulation from 600 to 1200 under refrigeration, and with a rotary electrical polisher (DP, Struers) using 6-µm diamond paste at 250 rpm under abundant water. Final polishing was done with a rotary electrical polisher using a colloidal silica suspension (OP-V) with a felt disc at 250 rpm.

Next, hardness was measured with a Vickers microhardness tester (Future Tech, Digital Microhardness Test FM / USA) using a squared pyramidal indenter. Indentations were made on the enamel surface using a static load of 50 g applied for 7 seconds. Three measurements of surface indentation were obtained for each sample and the Vickers hardness value is reported as the mean of these three indentations. The microhardness data were tabulated and analyzed by the Student *t* test for independent observations. P value < 0.05 was statistically significant.

RESULTS

The thickness of the remaining enamel and buccal dentin layers of the specimens measured during coronal opening was analyzed statistically and the results showed no significant difference between the three groups (Table 1).

The light reflectance data are shown in Tables 2, 3, 4 and 5. Comparison of successive sessions in the same group

Table 1. Statistical comparison of remaining enamel in the specimens between the three groups after coronal opening. There was no significant difference between groups ($P < 0.05$).

Groups (n=15)	*t*	*P* (alpha = 0.05)
Group A vs Group B	0.57	7.7%
Group A vs Group C	0.47	6.1%
Group B vs Group C	-0.15	6.1%

Group A: 35% hydrogen peroxide; group B: 35% carbamide peroxide; group C: 35% carbamide peroxide and sodium perborate.

Table 2. Statistical comparison of light reflectance data of the teeth before and after staining and after the different periods of bleaching in the three groups.

Treatment (n=15)	Group A	Group B	Group C
BS vs AS	S	S	S
AS vs B1	S	S	S
B1 vs B2	NS	NS	NS
B2 vs B3	NS	NS	NS
B3 vs PB	NS	NS	NS

Group A: 35% hydrogen peroxide; group B: 35% carbamide peroxide; group C: 35% carbamide peroxide and sodium perborate; BS: before staining; AS: after staining; B1: 24 h of bleaching; B2: 7 days of bleaching; B3: 14 days of bleaching; PB: 14 days after bleaching; S: significant ($P < 0.05$); NS: not significant.

Table 3. Statistical analysis of light reflectance data of the teeth before and after staining and after the different periods of bleaching comparing group A (35% hydrogen peroxide) with group B (35% carbamide peroxide). There was no significant difference ($P < 0.05$).

Treatment (n=15)	*t*	*P* value
Before staining	-0.5439	0.5875
After staining	1.7684	0.0794
24 h of bleaching	-0.5294	0.5975
7 days of bleaching	-1.2862	0.2007
14 days of bleaching	-0.2975	0.2007
14 days after bleaching	0.3964	0.6925

showed significant differences only between before and after staining. A significant difference was observed between group A (bleaching with hydrogen peroxide) and group C (carbamide peroxide plus sodium perborate) at 7 and 14 days of treatment (Table 4). Comparison of bleaching with carbamide peroxide (group B) and carbamide peroxide plus sodium perborate (group C) revealed a significant difference after 14 days of bleaching.

There was no significant difference in mean microhardness between treated samples and controls (Table 6).

DISCUSSION

The staining technique proposed by Freccia and Peters[5] (1982) continues to be used by some authors for the study of the efficacy of bleaching agents. To this respect, Warren *et al*.[22] (1999) and Ho and Goering[9] (1989) modified the time and speed of centrifugation of the specimens immersed in blood. In addition, Ho and Goering[9] (1989) tested the technique on permanent and primary teeth; however, the number

Table 4. Statistical analysis of light reflectance data of the teeth before and after staining and after the different periods of bleaching comparing group A (35% hydrogen peroxide) with group C (35% carbamide peroxide and sodium perborate). * Significantly different ($P < 0.05$).

Treatment (n=15)	*t*	*P* value
Before staining	0.652	0.5159
After staining	-0.951	0.3434
24 h of bleaching	-0.924	0.3575
7 days of bleaching	-2.360	0.0198*
14 days of bleaching	-2.405	0.0176*
14 days after bleaching	-1.104	0.2717

Table 5. Statistical analysis of light reflectance data of the teeth before and after staining and after the different periods of bleaching comparing group B (35% carbamide peroxide) with group C (35% carbamide peroxide and sodium perborate). * Significantly different ($P < 0.05$).

Treatment (n=15)	*t*	*P* value
Before staining	0.781	0.4365
After staining	-2.679	0.0084*
24 h of bleaching	-0.391	0.6963
7 days of bleaching	-1.160	0.2483
14 days of bleaching	-2.191	0.0303*
14 days after bleaching	-1.474	0.1429

Table 6. Comparison of enamel microhardness between the control group, group A (35% hydrogen peroxide), group B (35% carbamide peroxide) and group C (35% carbamide peroxide and sodium perborate). There was no significant difference between groups ($P < 0.05$).

Comparison between groups (n=5)	*t*	*P* (alpha = 0.05)
Control vs Group A	0.43	5.4%
Control vs Group B	0.33	4.0%
Control vs Group C	0.64	9.0%
Group A vs Group B	0.82	4.0%
Group A vs Group C	1.10	19.3%
Group B vs Group C	0.36	4.4%

of primary teeth was very small and the authors failed to obtain results regarding the success of staining and subsequent bleaching. In the present study, the modifications of the staining technique included the use of 1% sodium hypochlorite solution instead of 5.25% for opening of the dentinal tubules and centrifugation of five specimens in each test tube, whereas in the standard method only one permanent tooth was placed in each tube. These modifications were successful, as shown by the significant difference in the light reflectance of the teeth after staining (Table 2). The color changes observed are similar to those observed *in vivo* for primary teeth suffering trauma, hemorrhage after pulp treatment, incorrect endodontic access and coronal pulp necrosis all leading to stainings of the dental crown.

The choice of bleaching agent and its concentration is an important requisite for the success of tooth whitening. Hydrogen peroxide (35%) is one of the most frequently used bleaching agents which removes stains resulting from fluorosis, tetracycline use and trauma.[11, 16]

In the group receiving hydrogen peroxide as a bleaching agent, a significant difference was only observed between before and after staining. When compared to the group receiving carbamide peroxide plus sodium perborate, significant differences were noted after staining and after the various periods of bleaching. Thus, as also reported in the study of Kwon *et al*.,[10] and Cesar *et al*.,[2] differences in the light reflectance of the teeth were related to a change in color, and the greatest color change was observed on the first day of bleaching with 35% hydrogen peroxide.

Hydrogen peroxide is a highly caustic agent whose application requires special care. In addition, its use has also been associated with root resorption, especially in the cervical region[13]. Thus, some authors recommend the replacement of hydrogen peroxide with agents that have been shown to be as or more effective.[3, 4, 13, 14, 19, 20] In the present study, we only analyzed the *in vitro* effects of bleaching with hydrogen peroxide but did not evaluate parameters such as root resorption.

Sodium perborate is generally mixed with 35% hydrogen peroxide and this combination potentiates the bleaching action, in addition to prolonging the release of free oxygen. In the present *in vitro* study, sodium perborate was mixed with carbamide peroxide and this combination yielded the best results. However, these results differed only slightly from those obtained in the other two groups. Only two of six measurements were compared, a fact that did not permit the confirmation that this combination is more effective as a bleaching agent.

Another important aspect is the presence or absence of alterations in the structure and resistance of dental enamel after bleaching treatment. According to some authors, tooth bleaching with 35% hydrogen peroxide reduces the microhardness of enamel and dentin.[13, 18] The changes in enamel resistance and microhardness are increased when the bleaching agent is applied together with heat. Some investigators have reported morphological changes in the enamel surface caused by carbamide peroxide[17, 21]. On the other hand, bleaching agents containing carbamide peroxide do not seem to cause significant alterations in microhardness values when compared to a normal tooth. The combination of peroxides with sodium perborate seems to be the best option for tooth whitening, also interfering less with enamel microhardness.[12, 17]

Lussi *et al*.[15] compared the reduction in enamel microhardness between primary and permanent teeth submitted to erosive substances and observed that primary teeth were not more susceptible to erosion than permanent teeth. In the present study analyzing primary teeth, no significant difference in enamel microhardness was observed between the groups submitted to bleaching with 35% hydrogen peroxide and 35% carbamide peroxide plus sodium perborate when compared to the control group.

CONCLUSION

On the basis of the method used and the results obtained we conclude that the three bleaching agents tested (35% hydrogen peroxide, 35% carbamide peroxide and 35% carbamide peroxide plus sodium perborate) produced a proven bleaching action. No significant differences in mean microhardness values were observed between the samples treated with the three bleaching agents.

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