

# *In Situ* and *In Vitro* Effects of Bleaching with Carbamide Peroxide on Human Enamel

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## Clinical Relevance

The adverse effects of vital bleaching with carbamide peroxide were not observed when simulating the oral condition. Saliva could have a remineralizing effect over bleached enamel.

## SUMMARY

This study evaluated *in vitro* and *in situ* the potential adverse effects of 10% carbamide peroxide on human enamel using microhardness, calcium loss and surface morphology analysis. Twenty-four enamel slices (4 mm<sup>2</sup>) were obtained from recently extracted premolars. The specimens were polished under water-cooling down to 1,200-grade sandpaper. After initial microhardness readings (100g), the specimens were randomly divided into two groups for *in situ* and *in vitro* conditions. The specimens were covered with 10% carbamide peroxide for eight hours. After removing the bleaching gel, the *in vitro* specimens were stored in deionized water and the *in situ* specimens, included in an intra-oral appliance, were placed in the oral cavity of four volunteers. These cycling sequences took place for 14 days. Upon conclusion of the bleaching

treatment, new microhardness readings were performed on all specimens. Calcium dosage was assessed from the bleaching gel collected after initial exposure on day one, then from gel collected between days two and seven and gel collected between day eight and 14 using an atomic absorption spectrophotometer. Surface morphology was observed from two non-treated control specimens and two specimens of each experimental bleached group under SEM evaluation. Statistical analysis (ANOVA and Tukey tests) disclosed that specimens bleached *in situ* showed similar microhardness to unbleached specimens and had statistically higher ( $p < 0.01$ ) hardness than *in vitro* bleached specimens. The loss of calcium in the *in vitro* situation at 14 days was 2.5 times higher than the *in situ* condition. SEM micrographs demonstrated that surface alterations were more pronounced in the *in vitro* condition. The adverse effects of carbamide peroxide on enamel were evident in specimens bleached *in vitro* but were not seen *in situ*. The presence of saliva could prevent the demineralizing effect of bleaching gel *in situ*.

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## INTRODUCTION

The demands of the population regarding their dental appearance are greater than ever today. Teeth need to be not only aligned properly, they must also be white (Goldstein & Garber, 1995). This, along with a decrease

in dental caries, has directed clinicians to conservative aesthetic dentistry with non-restorative treatments for discolored teeth, an area under rapid development. This has led to the widespread use of bleaching agents to lighten darkened teeth. The technique of bleaching vital teeth is easy and patient acceptance is high.

The nightguard vital bleaching technique, with a build-in reservoir, offers a conservative, cost effective method for bleaching teeth (Haywood & Heymann, 1989). Most commercial products contain 10% carbamide peroxide with carboxypolymethylene polymer (carbopol) as a thickening agent; this polymer improves tissue adherence and allows for longer time release of the bleaching agent (Haywood, 1992; Rodrigues & others, 2001). Despite the favorable results achieved with nightguard vital bleaching, some reports in the literature have related adverse side effects as a consequence of the treatment. Sensitivity following the treatment has been related to the possible removal of mineral content from enamel and dentin (Bitter, 1998). Also, a reduction in enamel bond strength of the adhesive materials has been linked to physical and chemical alterations caused by bleaching materials (Ben-Amar & others, 1995).

There are a number of methods that have been used to assess the effects of bleaching agents on enamel. They involve *in vitro* and *in vivo* exposure to the bleaching agent and the subsequent assessment by visual inspection, SEM, hardness testing and surface loss.

McCraken and Haywood (1995) found an alteration in surface hardness and calcium loss after enamel treatment with 10% carbamide peroxide. A severe reduction in enamel hardness and distinct alterations in the surface morphology of bleached teeth were also reported (Ben-Amar & others, 1995; Josey & others, 1996; Smidt & others, 1998). In a SEM study, Tames, Grando and Tames (1995) found significant surface alterations in enamel topography following enamel bleaching with 10% carbamide peroxide. Different bleaching materials could produce an increase or decrease in enamel microhardness due to bleaching time (Rodrigues & others, 2001). Despite detecting a decrease in the microhardness of dentin bleached with 10% carbamide peroxide, de Freitas and others (2002) have verified that 14 days post-treatment, microhardness values were recovered to baseline levels. Performing a histochemical analysis of dental hard tissues following bleaching, Rotstein and others (1996) detected a significant reduction in Ca/P ratio. These authors advised that bleaching materials could adversely affect dental hard tissues and should be used with caution. An *in vitro* study by Flaitz and Hicks (1996) showed that different concentrations of carbamide peroxide can remove mineral structures from enamel, causing morphological alterations with different forms and intensity and can reach to the subsurface. Crews and others (1997) verified that different

bleaching agents have been known to lower the Ca and P levels in human enamel. Cimilli and Pameijer (2001) showed that some bleaching formulations could lower enamel hardness and cause the dissolution of calcium. In contrast, Murchison, Charlton and Moore (1992) noticed no significant changes in hardness of human enamel *in vitro* after exposure to 10% carbamide peroxide. Lee and others (1995) have also detected no influence on microhardness in bleached enamel using 10% carbamide peroxide *in vitro* condition but found an evident alteration to the enamel surface under SEM examination.

Despite the differences found *in vitro* studies, the clinical significance of these alterations and whether or not they have relevance in daily practice remains unknown (McCraken & Haywood, 1996; Cimilli & Pameijer, 2001). Nevertheless, it has been proposed that the loss of mineral content and increased porosity could explain transitory dental sensitivity during bleaching treatment (Basting, Rodrigues & Serra, 2001).

The *in vivo* dynamic interaction of saliva/enamel is a factor that has generally not been incorporated into *in vitro* experiments (Cimilli & Pameijer, 2001). Saliva has a cleaning action, a buffering capacity and a remineralization ability (Cury, 1989; Thylstrup & Fejerskov, 1998) that could prevent the adverse demineralizing effect of the bleaching agent. In an *in vivo* study, Shannon and others (1993) found lower hardness values for bleached enamel but without statistical significance. In a recent *in situ* study, Basting and others (2001) verified that 10% carbamide peroxide agent altered the microhardness of sound, demineralized enamel but not the microhardness of sound, demineralized dentin. Using an *in vivo* model, Bitter (1998) bleached teeth indicated for extraction and evaluated them for surface morphology using SEM. Non-bleached teeth were used as the control. This author verified surface modifications even after 90 days, suggesting that such alterations could be responsible for sensitivity.

Since little information exists in the literature regarding the clinical response to bleaching treatment, there is a need for studies that simulate clinical conditions in order to evaluate the real effects of such treatment. The hypothesis to be tested is that in a clinical oral simulate condition (*in situ*), the effects of bleaching agents are less evident than when seen in *in vitro* conditions. This study investigated the effect of 10% carbamide peroxide on human enamel using *in situ* and *in vitro* methodologies.

## METHODS AND MATERIALS

### Preparation of the Enamel Slabs

Twelve recently extracted maxillary premolars, removed for orthodontic reasons, were used in this study. The teeth were selected from six individuals between 12 and 14 years of age. All teeth were exam-

ined under magnification (20x) to detect micro-cracks and surface defects. Only premolars without defects were selected for this study. Soon after extraction, the crowns were cut at the CE-junction using a diamond saw under copious water-cooling. The pulp tissue was removed and the teeth were freezer stored. To perform the tests, the crowns were sectioned longitudinally to obtain 4 mm<sup>2</sup> enamel slabs. The enamel slabs were subjected to steam sterilization to avoid bacterial contamination (Amaechi, Higham & Edgar, 1998).

Each slab was included in a PVC matrix using polystyrene matrix, keeping only one side unsealed. Samples were sequentially polished by means 400, 600 and 1200 grade sandpaper. Twenty-four enamel slabs were obtained. Two slabs were used as a control for SEM evaluation and remained unbleached. The enamel slabs were randomly divided into two bleaching groups (n=11), one performed *in situ* and the other *in vitro*.

#### **Exposure of the Slabs to Bleaching Agent (*in situ* condition—experimental condition)**

A commercial bleaching agent Whiteness (FGM, Joinville, SC, Brazil) was used. This gel is a 10% carbamine peroxide agent with carboxypolymethylene polymer (carbopol), having a pH=7.82.

Four undergraduate dental students from the Federal University of Pelotas were used as volunteers for the bleaching treatments. All the procedures were carried out upon approval from the Ethic Committee, and the students provided written consent to partake in the study. Impressions were taken and removable oral appliances were made for each individual. Eleven enamel slabs were removed from individual matrixes using probes and were included in the oral appliances: three volunteers received three enamel slabs and the remaining volunteer received two. The bleaching procedure was performed outside the oral cavity. The enamel slabs were covered with 0.05 ml of 10% carbamine peroxide gel for eight hours at night, with the oral appliance being covered with a PVC film. The bleaching gel was then removed and the oral appliances placed in the mouths of the four volunteers for 16 hours to simulate clinical conditions and the effects of saliva on bleached enamel.

#### **Exposure of the Slabs to Bleaching Agent (*in vitro* condition—control condition)**

Each enamel slab in the individual matrix received an application of 0.05 ml of 10% carbamide peroxide gel for eight hours. After removing the gel, the enamel slabs were placed in individual containers with deionized water for 16 hours. Both bleaching treatments were performed for 14 days.

#### **Microhardness Test**

Microhardness measurements were performed before both bleaching treatments. Vickers microhardness was

measured using a microhardness tester (Buehler, Model 1600, Lake Buff, IL, USA). Three indentations were made on each specimen with 100g for five seconds. The means of each specimen was transformed in Vickers hardness number (VHN) based on a specified calculation formula.

After 14 days of *in situ* or *in vitro* bleaching treatments, the enamel slabs were again tested for microhardness.

#### **Calcium Loss Evaluation**

The bleaching gel from the *in vitro* or *in situ* conditions was collected daily. Three measurements of the calcium content were made for each condition: on the first day, on the seventh day from the gel collected from the second to the seventh day and at 14<sup>th</sup> day using the gel collected between the eighth and 14<sup>th</sup> day. The gel removed after bleaching treatment at each period was added to 0.5 ml of deionized water, resulting in the 10% initial dilution necessary for determining calcium loss. Samples were stored at 8°C.

The calcium readings were performed using an Atomic Absorption Spectrophotometer with a detection limit for Ca of 0.05 µg. The results were expressed in µg Ca/ml gel.

#### **Surface Morphology Analysis**

Six enamel slabs were separated for SEM analysis: two non-bleached specimens, two specimens from the *in situ* condition and two from the *in vitro* condition. The specimens were vacuum desiccated in alcohol and acetone and submitted to gold sputtering (500 Å). Micrographs were taken at different magnifications (600x and 2000x) using a Phillips XL 20 microscope (15 KV). Figure 1 provides a graphic representation of the methods and materials.

## **RESULTS**

#### **Microhardness Tests**

Since data from the microhardness evaluation were normal, parametric tests were employed. Two factors were investigated: the condition (*in situ* x *in vitro*) and the time (before and after bleaching treatment).

The ANOVA test disclosed differences between *in situ* and *in vitro* conditions and between pre- and post-bleaching specimens. Also, interaction between the condition and time was significant, demonstrating that specimens showed a different performance for the same condition.

An additional Tukey post-hoc test was used to clarify differences among the groups. In Table 1, means for the different conditions and times are expressed and the Tukey interval is presented.

In Table 1, it can be noted that the initial specimens from the *in situ* and *in vitro* conditions exhibited similar



hardness values. The hardness of the *in situ* specimens after bleaching treatment was similar to that found in the initial condition. However, bleached specimens from the *in vitro* condition had the lowest hardness values, which was statistically different from all others groups ( $p < 0.05$ ).

### Calcium Loss Evaluation

Table 2 shows the results of the amount of calcium found in gel removed at different times. Analysis of the gel demonstrated that greatest removal occurred during the first day under both conditions. Calcium removal decreased drastically during the second period (second to seventh day) and showed a further small reduction in the third period evaluated (days eight to 14). These findings suggest that the bleaching agent first attacks the superficial layer of the crystallites. The loss of calcium was always significantly higher *in vitro* than *in situ*. The total amount of calcium lost during the entire experiment was 2.5 times higher *in vitro* than *in situ*.

### Surface Morphology Analysis

SEM analysis of the surface showed different patterns in the specimens evaluated. Figure 1 shows the appearance of the enamel surface from unbleached specimens (2000x). The uniform enamel pattern observed, including tiny depressions and elevations, is due to polishing procedures. The surface observed in specimens bleached *in situ* shows patterns similar to the unbleached condition; however, the depressions observed *in situ* are more evident than those in the control slabs (Figure 2). More evident depressions are observed *in vitro* (Figure 3), which indicates a higher mineral loss due to dissolution of the enamel rods. In some regions, bleached enamel resembles phosphoric acid etched enamel.

### DISCUSSION

In this study, microhardness, calcium removal and surface analysis were investigated after enamel treatment with 10% carbamide peroxide with carbopol using *in situ* and *in vitro* methodologies.

A significant effect of bleaching was noted in the enamel specimens in the *in vitro* situation. This was combined with a significant decrease in microhardness, higher calcium loss and deep surface alterations. Such findings confirm the previous results that were reported in the literature that relates to the detrimental demineraliz-

ing *in vitro* effect of bleaching agents on enamel (Ben-Amar & others, 1995; McCracken & Haywood, 1995; Tames, Grando & Tames, 1995; Josey & others, 1996; Flaitz & Hicks, 1996; Rotstein & others, 1996; Crews & others, 1997; Smidt & others, 1998; Cimilli & Pameijer, 2001; Rodrigues & others, 2001).

In contrast, when the results of the *in situ* condition were observed, no significant differences were seen. In relation to surface hardness, the values were similar to unbleached enamel. Morphology analysis disclosed a surface similar to normal enamel, with few alterations from the control group. Also, the amount of calcium loss was significantly lower in *in situ* specimens than for *in vitro* specimens. Using similar methodology to evaluate the microhardness after 10% carbamide peroxide treatment on enamel, Shannon and others (1993) found similar results. However, the findings from this study are not in accordance with those found *in situ* by Basting and others (2001), who found enamel and dentin fragments, sound or demineralized, were bonded in the buc-

Table 1: Means (SD) of Microhardness (VHK) for Different Conditions

	<i>In Situ</i>	<i>In Vitro</i>
Initial	333.88 ( $\pm$ 19.59) <sup>a</sup>	341.68 ( $\pm$ 14.95) <sup>a</sup>
Post bleaching	321.66 ( $\pm$ 30.78) <sup>a</sup>	206.00 ( $\pm$ 40.79) <sup>b</sup>
Tukey interval (5%)=27.99		
<sup>a</sup> Mean values with the same subscript letters were not statistically different ( $p > 0.05$ ).		

Table 2: Calcium Content (mg/ml) Present in Gel Removed at the Different Periods Evaluated

	<i>In Situ</i>	<i>In Vitro</i>
1 <sup>st</sup> day	586.63	1,210.36
2 <sup>nd</sup> to 7 <sup>th</sup> day	18.00	250.85
8 <sup>th</sup> to 14 <sup>th</sup> day	7.82	55.14
Total amount	611.45	1,516.35

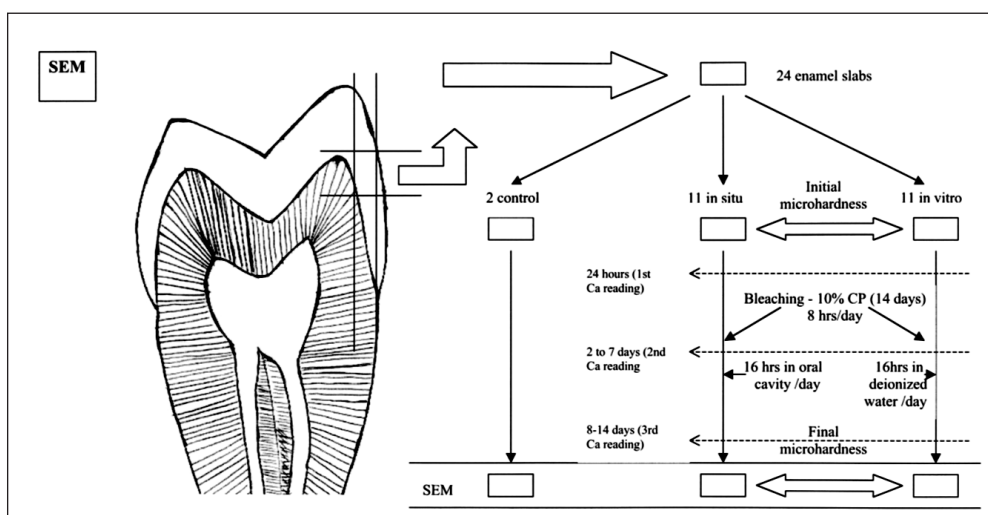


Figure 1. Illustration of the methodology used in the study.

cal surface of upper first molars or second premolars. Their volunteers were submitted to one double blind study using 10% carbamide peroxide or a placebo agent. They also detected a significant decrease in microhardness in both sound or mineralized enamel despite the presence of saliva, fluorides and plaque control. Nevertheless, no significant decrease in dentin microhardness was found.

Differences in the methodologies employed could influence the results found. In the current study, enamel fragments were placed in oral appliances situated in the palate region and the bleaching treatment was performed outside the oral cavity. In a study by Basting and others (2001), the enamel slabs were bonded to the upper molars and premolars, with all the procedures performed in the oral cavity. Also, despite both studies using 10% carbamide peroxide, the materials present with differences in composition such as pH or the amount of carbopol. The pH of the Opalescence material is 6.68 (Basting & others, 2001), while the bleaching agent used in the current study (Whiteness) has a pH=7.82. The most pronounced changes were found in the enamel slabs exposed to lower-pH solutions (Shannon & others, 1993). Bleaching treatments for the two studies were also different, three and two weeks, respectively. More severe changes have been observed in prolonged bleaching treatments (Shannon & others, 1993) and different bleaching times can produce an increase or decrease in enamel hardness (Rodrigues & others, 2001).

In this study, differences in the *in situ* and *in vitro* condition could be attributed to the important role of human saliva in the remineralization process. While enamel slabs from the *in vitro* methodology were stored in deionized water, slabs from the *in situ* group were submitted to a clinical condition in oral appliances used by four volunteers. The oral environment provides conditions for enamel remineralization and demineralized enamel is more susceptible to remineralization (Ten Cate, 1990). When the bleaching agent causes demineralization in enamel, ionic changes are induced, increasing mineral uptake, which replaces the mineral lost during treatment. For example, deciduous enamel eroded by lemon juice was found to be more reactive than non-eroded enamel, and a higher fluoride deposition was observed in eroded enamel after applying 2% neutral sodium fluoride (Rath, 1995).

Saliva has a cleaning function, but it also has a buffering action due to the bicarbonate and phosphate systems. Some inorganic electrolytes contained in saliva (calcium, phosphor and fluorides) are important participants in the remineralization process. When pH is under the physiologic limit, part of the calcium and phosphor complexes are released and added to the ionic

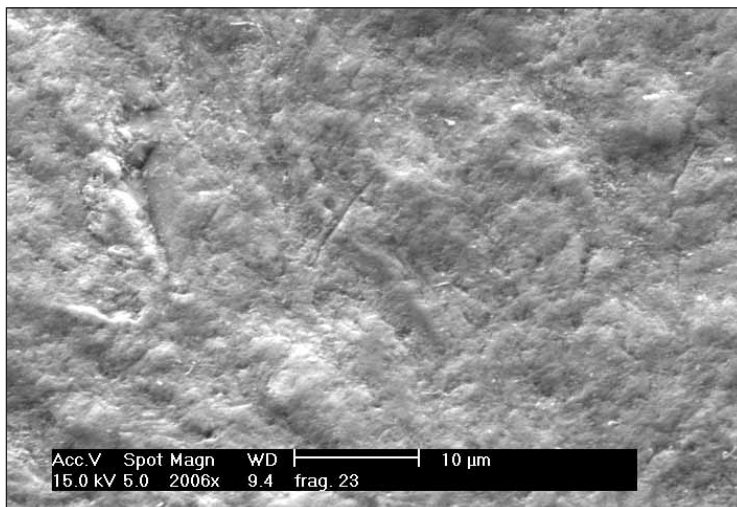


Figure 2. Enamel slab from control group that was not submitted to any bleaching treatment. The tiny depressions and elevations are related to the polishing procedures (2006x).

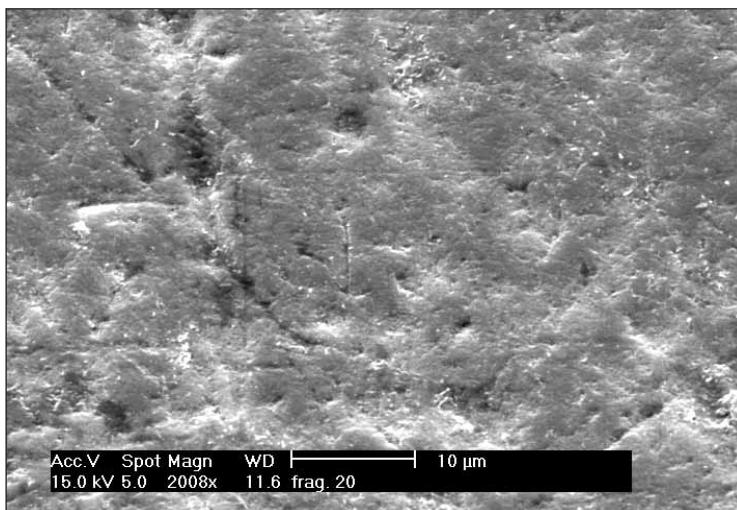


Figure 3. Enamel slab bleached *in situ* disclosing similar pattern to the unbleached specimens, but the depressions observed are more evident (2008x).

calcium and phosphor reservoirs. Consequently, apatite from the enamel surface is protected against dissolution (Thylstrup & Fejerskov, 1998).

There is a correlation between the values of microhardness and calcium loss evaluations allowed by the dynamics of the oral cavity. In a pH of less than 5.5, the amount of Ca and P in saliva is lower than the solubility rate of hydroxyapatite, with enamel having a tendency to lose Ca and P to the oral environment (Cury, 1989). In the enamel slabs cycled *in situ*, saliva interfered in this process, allowing the reposition of mineral and the reestablishment of hardness values similar to non-bleached specimens. However, in enamel slabs cycled *in vitro*, there was no remineralization effect because of the absence of saliva.



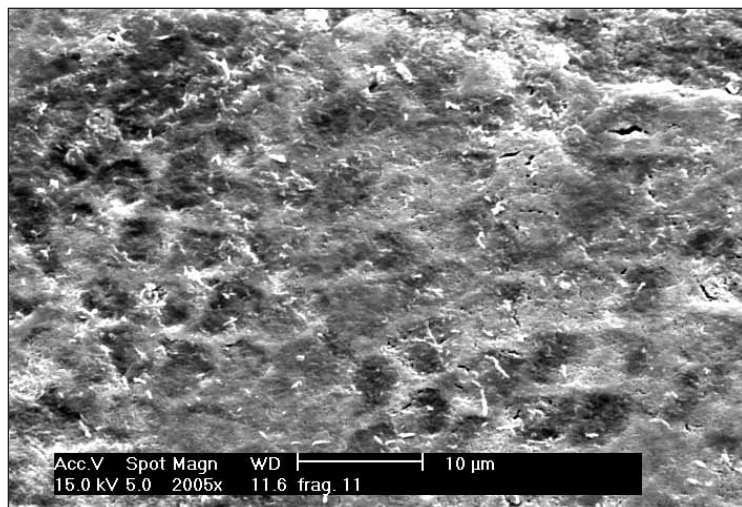


Figure 4. Pronounced depressions are observed in the enamel slab bleached *in vitro*. The higher mineral loss probably is due to the dissolution of enamel rods. A surface that resembles acid etched enamel is observed in some regions of the *in vitro* bleached slab (2005x).

The pH and viscosity of bleaching agents could influence the degree of demineralization on bleached enamel (Smidt & others, 1998). The acidic properties of bleaching agents, prolonged contact time between the bleaching agent and dental structure and the presence of a high percentage of carbopol have been indicated as potential factors responsible for surface alterations (Basting & others, 2001). However, a moderately low-pH bleaching agent solution *in vivo* reduces the pH of saliva in the mouth during the first five minutes. Over the next 15 minutes of treatment, the pH increased above baseline due to the chemical reactions of carbamide peroxide and the neutralizing effect of saliva (Leonard, Bentley & Haywood, 1994). Since the gel in this study presents a non-acidic pH (7.82), changes observed in the enamel structure could be due to the prolonged contact time with the bleaching agent or carbopol concentration.

Although enamel microhardness decreased and the surface morphology suffered a significant change after treatment with carbamide peroxide in a laboratory study, Smidt and others (1998) stated that the buffering capacity and the remineralization potential of saliva might overcome detrimental bleaching effects *in vivo*. Even when artificial saliva is used to simulate the natural saliva function, the detrimental effects of bleaching were less evident (Rodrigues & others, 2001; de Freitas & others, 2002).

Cimilli and Pameijer (2001) pointed out that it is difficult to determine the clinical significance of the results from *in vitro* studies since the calcium and phosphates available in saliva can potentially replenish the lost substance. Some authors have also highlighted the limitations of *in vitro* studies to determine the effects of bleaching on enamel (Leonard, Bentley & Haywood,

1994; Shannon & others, 1998; Smidt & others, 1998; Rodrigues & others, 2001). These limitations reinforce the relevance of this study. The similarity of clinical conditions provided by the *in situ* methodology can offer new perspectives in evaluation of the effects of different dental treatments.

Data from *in situ* and *in vitro* methodologies confirm the correlation between the factors evaluated in this study. When there is significant mineral loss, microhardness values tend to decrease and surface alterations are evident. However, when mineral removal is lower and is replaced with mineral from saliva, the hardness tends to be similar to the control group and surface alterations are less evident.

The results of this study confirmed the hypothesis tested and showed that in simulated clinical conditions bleaching does not produce the detrimental effects observed on enamel *in vitro*. This supports the reported clinical experience that 10% carbamide peroxide vital bleaching is a safe procedure that can be performed in daily clinical practice (Ritter & others, 2002).

## CONCLUSIONS

The limitations of this study show that the remineralization effect of saliva could prevent the demineralization effect of bleaching treatment in human enamel *in situ*.

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## References

- Amaechi BT, Higham SM & Edgar WM (1998) Efficacy of sterilization methods and their effect on enamel demineralization *Caries Research* **32**(6) 441-446.
- Basting RT, Rodrigues AL Jr & Serra MC (2001) The effect of 10% carbamide peroxide bleaching material on microhardness of sound and demineralized enamel and dentin *Operative Dentistry* **26**(6) 531-539.
- Ben-Amar A, Liberman R, Gorfil C & Bernstein Y (1995) Effect of mouthguard bleaching on enamel surface *American Journal of Dentistry* **8**(1) 29-32.
- Bitter NC (1998) A scanning electron microscope study of the long-term effect of bleaching agents on the enamel surface *in vivo* *General Dentistry* **46**(1) 84-88.
- Cimilli H & Pameijer CH (2001) Effect of carbamide peroxide bleaching agents on the physical properties and chemical composition of enamel *American Journal of Dentistry* **14**(2) 63-66.

- Crews KM, Duncan D, Lentz D, Gordy FM & Tolbert B (1997) Effect of bleaching agents on chemical composition of enamel *Mississippi Dental Association Journal* **53(1)** 20-21.
- Cury JA (1989) [Uso de flúor (Fluoride usage)] in: Baratieri LN, Monteiro S Jr, Caldeira MA & others [Dentística—Procedimentos preventivos e restauradores] *Operative Dentistry—Preventive and Restorative Procedures* Rio de Janeiro Santos 43-68.
- de Freitas PM, Basting RT, Rodrigues JA & Serra MC (2002) Effects of two 10% peroxide carbamide bleaching agents on dentin microhardness at different time intervals *Quintessence International* **33(5)** 370-375.
- Flaitz CM & Hicks MJ (1996) Effects of carbamide peroxide whitening agents on enamel surfaces and caries-like lesion formation: A SEM and polarized light microscopic *in vitro* study *ASDC Journal of Dentistry for Children* **63(4)** 249-256.
- Goldstein RE & Garber DA (1995) *Complete Dental Bleaching* Chicago Quintessence p 165.
- Haywood VB & Heymann HO (1989) Nightguard vital bleaching *Quintessence International* **20(3)** 173-176.
- Haywood VB (1992) History, safety and effectiveness of current bleaching techniques and applications of the Nightguard vital bleaching technique *Quintessence International* **23(7)** 471-488.
- Josey AL, Meyers IA, Romaniuk K & Symons AL (1996) The effect of a vital bleaching technique on enamel surface morphology and the bonding of composite resin to enamel *Journal of Oral Rehabilitation* **23(4)** 244-250.
- Lee CQ, Cobb CM, Zargartalebi F & Hu N (1995) Effect of bleaching on microhardness, morphology and color of enamel *General Dentistry* **43(2)** 158-162.
- Leonard RH Jr, Bentley CD & Haywood VB (1994) Salivary pH changes during 10% carbamide peroxide bleaching *Quintessence International* **25(8)** 547-550.
- McCracken MS & Haywood VB (1995) Effects of 10% carbamide peroxide on the subsurface hardness of enamel *Quintessence International* **26(1)** 21-24.
- McCracken MS & Haywood VB (1996) Demineralization effects of 10% carbamide peroxide *Journal of Dentistry* **24(6)** 395-398.
- Murchison DF, Charlton DG & Moore BK (1992) Carbamide peroxide bleaching: Effects on enamel surface hardness and bonding *Operative Dentistry* **17(5)** 181-185.
- Rath IBS (1995) [Análise morfológica e bioquímica da deposição de fluoretos no esmalte de dente decíduo humano erosionado pelo suco de limão. Estudo *in vitro*] Morphologic and biochemistry analysis of the deciduous human enamel eroded by lemon juice. *In vitro* study (Master of Sciences Thesis) Federal University of Santa Catarina p 135.
- Ritter AV, Leonard RH Jr, St. Georges AJ, Caplan DJ & Haywood VB (2002) Safety and stability of nightguard vital bleaching: 9 to 12 years post-treatment *Journal of Esthetic and Restorative Dentistry* **14(5)** 275-285.
- Rodrigues JA, Basting RT, Serra MC & Rodrigues AL Jr (2001) Effects of 10% carbamide peroxide bleaching on enamel microhardness *American Journal of Dentistry* **14(2)** 67-71.
- Rotstein I, Dankner E, Goldman A, Heling I, Stabholz A & Zalkind M (1996) Histochemical analysis of dental hard tissues following bleaching *Journal of Endodontics* **22(1)** 23-25.
- Shannon H, Spencer P, Gross K & Tira D (1993) Characterization of enamel exposed to 10% carbamide peroxide bleaching agents *Quintessence International* **24(1)** 39-44.
- Smidt A, Weller D, Roman I & Gedalia I (1998) Effect of bleaching agents on microhardness and surface morphology of tooth enamel *American Journal of Dentistry* **11(2)** 83-85.
- Tames D, Grando LJ & Tames DR (1995) [Alterações do esmalte dental submetido ao tratamento com peróxido de carbamida a 10%] Enamel alterations following treatment with 10% carbamide peroxide *Revista da Associação Paulista de Cirurgiões Dentistas* **52(1)** 145-149.
- Ten Cate JM (1990) *In vitro* studies on the effects of fluoride on de- and re-mineralization *Journal of Dental Research* **69(Special Issue)** 614-619.
- Thylstrup A & Fejerskov O (1998) [Tratado de cariologia] [Cariology] Rio de Janeiro, *Cultura Médica* p 388.