

Efficacy of a Novel At-home Bleaching Technique With Carbamide Peroxides Modified by CPP-ACP and Its Effect on the Microhardness of Bleached Enamel

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Clinical Relevance

The use of a casein phosphopeptide-amorphous calcium phosphate (CPP-ACP)-containing paste with carbamide peroxides could protect enamel against the demineralization caused by these bleaching agents. This improves safety and might even reduce *in vivo* tooth sensitivity during the bleaching process.

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SUMMARY

This study was designed to evaluate *in vitro* the efficacy of a novel at-home bleaching technique using 10% or 16% carbamide peroxide modified by casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) and its influence on the microhardness of bleached enamel. A total of 40 bovine incisors were divided into four groups (n=10) according to the bleaching agent used: 10% carbamide peroxide only; a blend of 10% carbamide peroxide and a CPP-ACP paste; 16% carbamide peroxide only; and a blend of 16% carbamide peroxide and a CPP-ACP paste. During the 14-day bleaching regimen, the samples were stored in artificial saliva. The Vickers microhardness and color of the teeth were assessed at baseline (T0) and immediately after the bleaching regimen (T14) using a microhardness tester and a spectrophotometer, respectively. The degree of color change was determined by the Commission Internationale de l'Eclairage (CIE) L*a*b* system (ΔE , ΔL^* , Δa^* , and Δb^*) and Vita shade guide paramete-

ters. The data were analyzed by analysis of variance and the Tukey test ($p < 0.05$). The teeth that were bleached with a blend of peroxide (10% or 16%) and the CPP-ACP paste presented increased microhardness values at T14 compared with T0, whereas the samples that were bleached with peroxide only did not show any differences in their microhardness values. All of the bleaching agents were effective at whitening the teeth and did not show a statistically significant difference using the CIEL*a*b* system (ΔE , ΔL^* , Δa^* , and Δb^*) or the Vita shade guide parameters. The use of a CPP-ACP paste with carbamide peroxide bleaching agents increased the bleached enamel's microhardness and did not have an influence on whitening efficacy.

INTRODUCTION

The search for a more esthetic smile has grown exponentially during the last few decades, so that tooth color is currently believed to be one of the biggest concerns for patients.¹ With careful diagnosis and appropriate attention to technique, bleaching may represent a more conservative and safer means to lightening discolored teeth.² In particular, at-home bleaching techniques have received worldwide acceptance since first being described in 1989 by Haywood and Heymann³ and have become a widespread method of whitening discolored teeth using low-concentration carbamide peroxides, mainly 10%.^{4,5}

Although at-home bleaching is an effective technique for whitening discolored teeth, whether carbamide peroxide- or peroxide-containing agents can soften dental hard tissues is still debated.⁶ Concerning the effects of whitening products on mineral loss in dental hard tissues, studies that investigate external bleaching therapies often test for microhardness because this is related to the mineral content of the tooth.⁷ The literature has reported a great deal of controversy concerning the effects of this bleaching agent on the microhardness of dental hard tissues, particularly enamel.^{7,8} Some authors have found that low-concentration carbamide peroxides adversely affect postbleaching enamel microhardness,^{9,10} which might be the result of a change in calcium, phosphate, and fluoride mineral content.¹¹ Conversely, other investigators have observed no statistically significant differences between bleached and nonbleached enamel.¹² Moreover, it has also been postulated that although a decrease in the microhardness of bleached enamel

might occur, it can be reversed after a postbleaching period of remineralization through the absorption and precipitation of salivary components, such as calcium and phosphate.¹³ In fact, if peroxides could release remineralizing agents directly onto the tooth surface during the bleaching procedures, a decrease in microhardness would not be observed in postbleached enamel. Besides possibly inhibiting the deleterious effects of bleaching agents on enamel mineral content, the benefit of using remineralizing agents in bleaching peroxides could include a reduction in enamel solubility and reduced sensitivity due to mineral deposition in enamel crystallites.¹⁴

Recently, a new calcium phosphate remineralization technology has been developed based on casein phosphopeptide-amorphous calcium phosphate (CPP-ACP) (Recaldent™ CASRN691364-49-5). The developers of this product claim that the CPP stabilizes high concentrations of calcium and phosphate ions, together with fluoride ions, at the tooth surface by binding to pellicle and plaque.¹⁵ This nanocomplex acts as a calcium and phosphate reservoir that attaches itself to dental plaque and tooth surfaces. On acid challenge, the attached CPP-ACP releases calcium and phosphate ions, thus maintaining a supersaturated mineral environment and thereby reducing demineralization and enhancing remineralization of enamel, as observed in some clinical *in situ* studies.^{16,17} The use of CPP-ACP along with bleaching peroxides might enhance the remineralization of the enamel in contact with the agent by providing calcium and phosphate ions.

The association of a CPP-ACP paste (Tooth Mousse, GC Corporation, Tokyo, Japan) with carbamide peroxide has been studied,¹⁸ and the study's authors suggest that Tooth Mousse can be applied concurrently with the bleach and would not reduce bleaching effectiveness. However, only 16% carbamide peroxide was evaluated and no mechanical test was performed on the enamel to verify the mineral gain. Thus, the present study aims to evaluate a CPP-ACP paste (MI Paste, GC, Tokyo, Japan—an analogue to Tooth Mousse) mixed with 10% or 16% carbamide peroxide concerning its effect on bleaching effectiveness and enamel microhardness. The hypothesis tested is that the combination of a CPP-ACP paste and carbamide peroxide in both concentrations does not adversely affect bleaching efficacy and would increase postbleaching enamel microhardness.

Table 1: *Materials Used in This Study*

Commercial brand and manufacturer	Lot Number	Chemical Composition
Claridex 10% (Biodinâmica, Ibiporã, PR, Brazil)	178/10	10% carbamide peroxide, thickener, sodium fluoride, potassium nitrate, methylparaben, saccharin, and deionized water
Claridex 16% (Biodinâmica, Ibiporã, PR, Brazil)	054/10	10% carbamide peroxide, thickener, sodium fluoride, potassium nitrate, methylparaben, saccharin, and deionized water
MI Paste (GC Corporation, Tokyo, Japan)	090406M	Pure water, CPP-ACP, D-sorbitol, propylene glycol, silicon dioxide, titanium dioxide, xylitol, phosphoric acid, flavoring, zinc oxide, sodium saccharin, ethyl p-hydroxybenzoate, magnesium oxide, guar gum, propyl p-hydroxybenzoate, butyl p-hydroxybenzoate
Artificial saliva (Farmafórmula, Natal, RN, Brazil)	—	K ₂ HPO ₄ , 70% sorbitol, NaF, KCl, NaCl, MgCl ₂ ·6H ₂ O, nipagin, sodium benzoate, hydroxyethylcellulose
Abbreviations: CPP-ACP, casein phosphopeptide-amorphous calcium phosphate paste; K ₂ HPO ₄ , di-potassium hydrogen phosphate; KCl, potassium chloride; MgCl ₂ ·6H ₂ O, magnesium chloride hexahydrate; NaCl, sodium chloride; NaF, sodium fluoride.		

METHODS AND MATERIALS

Experimental Design

The factors under study were 1) bleaching protocols at four levels: 10% Claridex (CP10) (Biodinâmica, Ibiporã, PR, Brazil), CP10/CPP-ACP paste (MI) (MI Paste, GC Corporation, Tokyo, Japan), 16% Claridex (Biodinâmica, Ibiporã, PR, Brazil) (CP16), and CP16/MI; and 2) evaluation periods at two levels: baseline (T0) and immediately after bleaching (T14). Chemical components and lot number of the materials used in this study are listed in Table 1.

The experimental units consisted of 40 crowns of bovine incisors randomly assigned to four groups (n=10) (Table 2). The color of the specimens and Vickers microhardness were evaluated at T0 and T14 in order to assess postbleaching color change and mineral gain or loss.

Specimens' Preparation

A total of 40 recently extracted permanent bovine central incisors were selected and stored under refrigeration in a saturated thymol solution until testing commenced. Teeth with any visible cracks or hypoplastic defects were excluded. The roots were removed 2 mm apically to the cemento-enamel junction using double-faced diamond disks (KG Sorensen, Barueri, Brazil) and were discarded. In this study, the entire crown was used¹⁹ after an artificial staining process, which was performed using the method described by Kielbassa and others.²⁰ A mixture (1:1) of 1 L of red wine (Galiotto,

Flores da Cunha, RS, Brazil) and black tea (Castelari, Guararema, SP, Brazil) was prepared. The tea solution was produced by boiling 2 g of tea in 100 mL of distilled water for five minutes and filtered to remove the tea from the infusion. The teeth were then immersed separately in 10 mL of the staining solution for seven and a half days at 37°C.

After staining, the teeth were positioned in a plastic mold and embedded using a self-curing polystyrene resin (Piraglass, Piracicaba, SP, Brazil). The enamel surfaces of the teeth were ground flat using SiC paper (80-grit) and polished using 600-, 1200-, and 2400-grit aluminum oxide abrasive papers and a 0.4-μm alumina polishing suspension on a polishing machine (APL-4, Arotec, São Paulo, SP, Brazil), exposing enamel in a circular area 10 mm in diameter.

Table 2: *Experimental Groups*

Groups	P	CPP-ACP	Final P:CPP-ACP Proportion
G1	10% carbamide peroxide	No	1:00
G2	10% carbamide peroxide	Yes	1:01
G3	16% carbamide peroxide	No	1:00
G4	16% carbamide peroxide	Yes	1:01
Abbreviations: CPP-ACP, casein phosphopeptide-amorphous calcium phosphate paste – MI Paste (GC Corporation, Tokyo, Japan); P, peroxide.			

Table 3: Representation of Conversion to Numeric Values of the Vita Classical Shades															
B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3.5	B4	C3	A4	C4
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Baseline Color Assessment (T0)

In this study, the color of the specimens was assessed using a spectrophotometer (Easy Shade, Vident, Brea, CA, USA). To standardize the area of the tooth upon which the shade was evaluated, a silicon index covering all the specimens' areas was fabricated with an impression material (Clonage, DFL, Rio de Janeiro, RJ, Brazil). A perforation compatible with the size of the spectrophotometer tip was made in the center of the exposed facial surface (6-mm diameter) with a scalpel blade. The results of the color measurements were evaluated according to the Vita Classical shade guide provided by spectrophotometer and in terms of the three coordinate values (L*, a*, b*) established by the CIE, which locates the color of an object in a three-dimensional color space. The L* axis represents the degree of lightness within a sample and ranges from 0 (black) to 100 (white). The a* plane represents the degree of green/red color (−a* = green and +a* = red), whereas the b* plane represents the degree of blue/yellow color within the sample (−b* = blue and +b* = yellow).

Baseline Microhardness Assessment (T0)

The enamel microhardness determination was performed with a microhardness tester (Pantec HSV 100A, Panambra, São Paulo, SP, Brazil) fitted with a 300-g load, which was used to make indentations on the enamel surface. The loaded diamond was allowed to sink and rest on the enamel surface for 10 seconds and the Vickers hardness number was thus determined. Three indentations were performed on each specimen, with a distance of 100 μm between them, and then they were averaged.

Bleaching Procedures

The bleaching treatment was performed over 14 days, according to the manufacturer's instructions. For each specimen, a tray was fabricated using low-density polyethylene plates (FGM, Joinville, SC, Brazil) in a vacuum plasticizer (Plastivac P7, Bioart, São Carlos, SP, Brazil). For groups 2 and 4, the peroxides were mixed with MI Paste (GC Corporation). The mixtures were freshly prepared by mixing 1 mL of each bleaching gel with 1 mL of MI Paste

until a homogeneous paste was obtained, which was then inserted into a 5-mL syringe (Embramac, Itajaí, SC, Brazil). In addition, the peroxides alone were put into 5-mL syringes. The contents of each syringe were used to bleach the teeth for seven days, and then the mixtures were prepared again. The pH of the mixtures and carbamide peroxides was monitored each of the seven days through a pH Meter (Digimed DM-20, Digicrom Analítica Ltda, São Paulo, SP, Brazil) with an electrode (Digimed DME-CVS) that was calibrated using pH 4.01 and 6.86 solutions before analysis.

Every day, 0.2 mL of the bleaching agents was placed on the enamel. The individual mold was positioned onto each specimen, and each specimen was then positioned for eight hours at 37°C in artificial saliva. After eight hours, the gel was removed from the enamel surface by placing it under running distilled water for 15 seconds. When the specimens were not in contact with the bleaching agents, they were immersed in artificial saliva kept at 37°C, which was changed daily.

Final Color Assessment (T14)

Immediately after bleaching (T14), the color of the specimens was reevaluated. The silicon (DFL, Rio de Janeiro, RJ, Brazil) mold was replaced on a previously demarcated area on the specimens, and the color was reevaluated using the Vita shade guide and CIE L*a*b* parameters. The color assigned at T0 and T14 for each specimen was converted to previously established numeric values (Table 3),²¹ ranging from 1 (B1) to 16 (C4). The smaller the numeric value, the lighter the tooth. Color difference was established by the difference between T14 and T0 numeric values. For CIE L*a*b* data, the color was compared using each of the parameters ΔL*, Δa*, Δb* and the total color difference (ΔE) according to the formula: $\Delta E = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$.

Final Microhardness Assessment

Immediately after bleaching (T14), another Vickers microhardness measurement was taken in the samples following experimental conditions similar to those used at baseline.

Table 4: Means and Standard Deviations of the Vita Shade Data at Different Evaluation Periods

Bleaching agent	Evaluation Periods	
	T0	T14
CP10	11.25 (1.91) Aa ^a	2.75 (0.47) Ba
CP10/MI	11.25 (3.62) Aa	5.37 (1.11) Ba
CP16	11.12 (2.13) Aa	3.50 (0.95) Ba
CP16/MI	11.62 (2.36) Aa	3.25 (0.70) Ba
Abbreviations: CP10, 10% carbamide peroxide; CP16, 16% carbamide peroxide; MI, MI Paste. ^a Different letters (lowercase in the same column, uppercase in the same row) indicate statistically significant differences ($p < 0.05$).		

Statistical Analysis

The color change data for the Vita shade guide and CIE $L^*a^*b^*$ system and the microhardness measurements were analyzed by two-way analysis of variance for repeated measurements and the Tukey test. All tests were performed using the ASSISTAT Beta 7.5 software (Federal University of Campina Grande, Campina Grande, Brazil) at a preset significance level of 5%.

RESULTS

Color Change

Vita Shade Guide Data—The results obtained regarding the Vita shade guide are displayed in Table 4. All techniques were effective for bleaching the teeth and were statistically similar.

CIE $L^*a^*b^*$ System Data—Table 5 displays the mean values and standard deviations of color change

(ΔE^* , ΔL^* , Δa^* , and Δb^* values). There were no differences among the groups concerning all parameters of the CIE $L^*a^*b^*$ system.

Microhardness Data—The mean Vickers Hardness Number (VHN) and standard deviation results for enamel microhardness are presented in Table 6. No differences were found among the groups at T0 (baseline). After bleaching (T14), the groups subjected to bleaching protocols with peroxides mixed into the CPP-ACP paste showed statistically higher microhardness values than at baseline (T0). Moreover, the samples submitted to bleaching agents with the CPP-ACP paste presented statistically higher microhardness values than the samples bleached with peroxides only.

pH of the Bleaching Agents

The four bleaching agents had a comparable and statistically similar pH between 6.49 and 6.65 during the seven days evaluated at room temperature.

DISCUSSION

The hypothesis tested in this investigation was validated because the concurrent application of the CPP-ACP paste MI Paste (GC Corporation) and 10% or 16% carbamide peroxide increased microhardness of the bleached enamel and did not reduce bleaching efficacy. Although the concentration of carbamide peroxides was reduced to half after mixing them with MI Paste, this was not sufficient to affect color change after a 14-day bleaching regimen. This finding corroborates that obtained by Manton and others,¹⁸ who found no reduction in bleaching potential after mixing 16% carbamide peroxide with the CPP-ACP paste Tooth Mousse, an analogue to the MI Paste used in the present investigation.

Table 5: Means and Standard Deviations of the CIE $L^*a^*b^*$ Data

Bleaching agent	ΔE	ΔL^*	Δa^*	Δb^*
CP10	15.13 (6.5) a ^a	13.04 (5.7) a	-5.75 (1.47) a	-5.10 (2.75) a
CP10/MI	11.97 (6.4) a	11.68 (5.54) a	-2.43 (1.48) a	-3.06 (2.86) a
CP16	16.73 (6.72) a	14.39 (5.96) a	-5.50 (2.41) a	-6.54 (1.62) a
CP16/MI	13.38 (4.55) a	12.04 (4.3) a	-3.90 (1.19) a	-4.35 (0.91) a
Abbreviations: CP10, 10% carbamide peroxide; CP16, 16% carbamide peroxide; MI, MI Paste. ^a Different lowercase letters in the same columns indicate statistically significant differences ($p < 0.05$).				

Table 6: Means and Standard Deviations of the Vickers Hardness Number at Different Evaluation Periods		
Bleaching agent	Evaluation Periods	
	T0	T14
CP10	251.00 (38.51) Aa ^a	248.91 (39.04) Ab
CP10/MI	255.33 (37.87) Ba	298.78 (36.42) Aa
CP16	251.33 (38.45) Aa	255.47 (38.98) Ab
CP16/MI	253.36 (39.81) Ba	305.45 (40.23) Aa
Abbreviations: CP10, 10% carbamide peroxide; CP16, 16% carbamide peroxide; MI, MI Paste. ^a Different letters (lowercase in the same column, uppercase in the same row) indicate statistically significant differences (p<0.05).		

A number of methods are available for evaluating the efficacy of bleaching procedures, such as shade guides, photography, colorimeters, spectrophotometers, and computer digitization.⁷ The CIE L*a*b* three-dimensional color space system is the most frequently quoted index used in dental bleaching research and is generated from colorimeters, digital image analysis, or spectrophotometers, such as the Easy Shade (Vident) used in this study. All ΔE values of at least 3.3 are visually perceptible.²² Given that ΔE* values ranging from 11.97 to 16.73 were obtained in the present work, all the bleaching agents were effective to bleach the teeth.

However, color assessment by means of the Vita shade guide parameters might facilitate clinicians' understanding of the level of color change after bleaching because this method is often used to assess the color of teeth in-office. Thus, a complementary color reading of the Vita shade guide was obtained by means of the spectrophotometer Easy Shade (Vident). Because teeth remained at least 5.88 shade-guide units lighter than at baseline, all bleaching agents could also be considered effective to whiten teeth²³ in this *in vitro* study.

When the objective of an *in vitro* study is to evaluate the effectiveness of bleaching agents, it is very difficult to observe differences in the effect of bleaching techniques on samples without staining.²⁴ Thus, the present study used the method proposed by Kielbassa and others²⁰ for artificial staining of bovine teeth. Although tooth fragments are frequently used in bleaching studies,^{2,4,6,8,22} the entire crown was used in the present investigation, as else-

where.¹⁹ Using the entire tooth crown is an easier and lower cost method when compared with enamel blocks. Moreover, this method approximates laboratory conditions in the clinical environment, in that bleaching agents are placed on coronal enamel using trays. However, some procedures that do not mirror clinical conditions were included in this study. Enamel was flattened before subjecting the teeth to bleaching, and no brushing of samples was applied during the bleaching procedures²⁵ in order to be certain that any change on enamel surface was due to the active ingredients of the bleaching gels without external interferences.

It is known that the oxide-reduction reaction of the bleaching agent could lead to the dissolution of the organic and inorganic dental matrix until only carbon dioxide and water remain.¹⁰ Although enamel remineralization is anticipated by the action of saliva during the bleaching, several *in situ*^{9,10,26} studies reported a decrease in microhardness of bleached enamel. A microhardness loss could classically be related to mineral content loss resulting from demineralization²⁷; therefore, the microhardness test is often applied to evaluate the adverse effects of bleaching agents on enamel.⁷

To overcome mineral loss, different carbamide peroxide formulations have been developed, and the addition of fluoride and calcium to agents has been studied by some investigators.^{4,6} It has been shown that carbamide peroxide bleaching gels containing fluoride⁸ and/or calcium⁴ are able to reduce microhardness loss and accelerate microhardness recovery in the posttreatment phase better than nonenhanced gels. This might be due to the fact that fluoride/calcium-containing carbamide peroxide bleaching gels induce fluoride/calcium acquisition of enamel.^{4,6} It is likely that, during bleaching, enamel can become undersaturated compared with the gel and ionic balance occurs,²⁸ leading to enamel ion uptake.⁴ To compensate for this, manufacturers add fluoride or calcium ions to bleaching agents, such as those fluoridated agents used in this investigation.

However, bleaching agents with low concentrations of carbamide peroxide (10%) might result in a change in phosphate, besides the calcium and fluoride content of enamel.¹¹ Thus, a remineralization system should supply stabilized bioavailable calcium, phosphate, and fluoride ions²⁹ because all of these minerals may be lost after bleaching. Some peroxide agents (NightWhite ACP/ACPF, Discus Dental, Culver City, CA, USA) containing an unstabilized ACP system might be commercially available. It has been shown that gels containing

ACP affected remineralization patterns of predemineralized bovine enamel better than fluoridated (sodium fluoride) bleaching agents.³⁰ However, the ACP system stabilized by CPP, otherwise known as CPP-ACP, provides a higher reservoir of bioavailable calcium and phosphate ions in comparison with ACP only, leading to an increased remineralization potential.²⁹ Although a large body of scientific evidence demonstrates that CPP-ACP could promote the remineralization of even enamel subsurface caries lesions,²⁹ this study is the first to analyze the effect of MI Paste in conjunction with carbamide peroxides on bleached enamel microhardness. The increased postbleaching microhardness for samples bleached using carbamide peroxides and MI Paste suggest a mineral deposition on enamel.

Conversely, in the samples bleached with peroxides only, microhardness did not increase after bleaching. The baseline microhardness values were, at most, maintained after bleaching. Given that the pH of the bleaching agents with or without MI Paste were kept statistically similar during the seven-day evaluation period, changes or maintenance in bleached enamel microhardness values are attributed to the composition of each agent. It is likely that the fluoride contained in the gels without MI Paste could provide remineralization during the bleaching procedures associated with the artificial saliva.⁴ Peroxides mixed with MI Paste could provide a higher remineralization process during bleaching, so that the microhardness values attributed to the bleached enamel were statistically superior to those of the samples bleached with peroxides only. From a clinical standpoint, this superior mineral gain also would reduce enamel solubility and tooth sensitivity due to mineral deposition in enamel crystallites,¹⁴ improving safety and comfort for patients. Further studies are needed to evaluate how long this microhardness increase could last. Moreover, other bleaching peroxides should be tested in association with the CPP-ACP paste because the results of this study are not applicable to all types of bleaching systems.

CONCLUSION

The use of CPP-ACP paste with 10% and 16% carbamide peroxides increased postbleaching enamel microhardness and did not adversely affect bleaching efficacy.

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