Effectiveness of different carbamide peroxide concentrations used for tooth bleaching: an *in vitro* study

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ABSTRACT

Objectives: This *in vitro* study evaluated the effectiveness of three carbamide peroxide concentrations used for tooth bleaching treatments. Material and Methods: Sixty bovine dental slabs (6x6x3 mm) were obtained, sequentially polished, submitted to artificial staining (baseline) and randomized into four groups (n=15), according to the bleaching agent concentration: distilled water (control), 10% (CP10), 16% (CP16) or 37% (CP37) carbamide peroxide. CP10 and CP16 were covered with 0.2 mL of the respective bleaching gels, which were applied on enamel surface for 4 h/day during two weeks. Samples of CP37 were covered with 0.2 mL of the bleaching gel for 20 min. The gel was light activated by two 40-s applications spaced by 10-min intervals. The gel was renewed and applied 3 times per clinical session. This cycle was repeated at 3 sessions with 5 days of interval between them. Tooth shade evaluations were done with a digital spectrophotometer at T0 (baseline), T1 (after 1-week of treatment) and T2 (1-week post-bleaching). Tooth shade means were statistically analyzed by Kruskal-Wallis and Friedman's tests and color parameters were analyzed by two-way ANOVA and Tukey's test (p<0.05). Results: At T1 and T2 evaluations, tooth shade was significantly lighter than at baseline for all treatment groups, considering the color parameters ΔL^*, Δa^*, Δb^*, ΔE^* (p<0.001) or tooth shade means (p<0.001). CP37 group showed lower shade mean change than CP10 and CP16 at T1 (p<0.01), but this difference was not statistically significant at T2 (p>0.05). Conclusions: One week after the end of the treatment, all carbamide peroxide concentrations tested produced similar tooth color improvement.

Key words: Tooth bleaching. Peroxides. *In vitro*.

INTRODUCTION

Tooth color is an important issue for esthetic dentistry professionals and for patients that wish to enhance the appearance of their smiles. Nowadays, at-home vital tooth bleaching with custom trays is well accepted as a safe, conservative, low cost and effective esthetic procedure for treatment of discolored teeth[10]. Considering the active products available for vital tooth bleaching, carbamide and hydrogen peroxide are the most commonly used agents for different bleaching modalities[4,9,10], which vary according to peroxide concentration, mode of application and time of exposure[6,16].

The whitening products available for vital tooth bleaching can be applied on tooth surface at the office by a dentist or at home by the patient, under the dentist's supervision[4]. The only concentration that so far received the seal of acceptance by American Dental Association (ADA) was the 10±1% carbamide peroxide, which assures its safety and efficacy for at-home tooth bleaching with custom

trays[2]. Previous studies have reported the efficacy and safety of this bleaching gel concentration[18,20]. However, randomized clinical trials showed that the increase of the carbamide peroxide concentration for home-use did not increase the efficacy and longevity of the tooth whitening effect[20,21].

Manufacturers have introduced many different bleaching agents' concentrations and methods with the premise of "improving" the whitening effect[10]. As a consequence, there was a significant increase of the variety of in-office tooth bleaching products available in the market, which usually uses high hydrogen (15 to 40%) or carbamide (30 to 37%) peroxide concentrations combined or not with a supplementary light source (i.e. laser, LED, halogen lamp or plasma arc)[7,16]. Despite the method of activation of these agents, the data available in the literature are conflicting. While some studies have reported that the use of light sources can enhance the whitening effect[13,22], others have shown that the use of light-activation with in-office tooth bleaching did not affect the outcome of 35% hydrogen peroxide treatment[1,14,15].

Considering the peroxide concentration and its time of application as the main factors that can affect the effectiveness of tooth whitening, the aim of the present study was to evaluate the effectiveness of three carbamide peroxide concentrations used in tooth bleaching treatments. The null hypothesis for this study was that the high-concentration carbamide peroxide gel evaluated is not more effective than the low-concentration carbamide peroxide bleaching agents.

MATERIAL AND METHODS

Specimen preparation

Sixty recently extracted bovine incisors without cracks and surface defects (40x magnification) were used in this study. The roots were cut with a water-cooled low-speed diamond saw (Isomet 1000, Buehler, Lake Bluff, IL, USA) and, the pulp tissue was removed. Then, teeth were stored in 0.5% chloramine-T solution for one week.

After disinfection, each crown was sectioned in both buccolingual and mesiodistal directions to obtain one enamel-dentin slab (6x6x3 mm), which were measured with a digital caliper (DC 500, Mitutoyo, Suzano, SP, Brazil). Each slab was individually embedded in self-curing acrylic resin in a ring model and then, enamel surfaces were flattened with 600- and 1200-grit silicon-carbide paper under water cooling to create a standardized buccal enamel surface. Samples were washed and stored in distilled water at 37°C for 1 week until starting the experimental procedures.

Staining procedure

In an attempt to standardize the tooth shade, an artificial staining of the specimens was carried out following the method proposed by Sulieman, et al.[24] (2003). Enamel surfaces were etched with 35% phosphoric acid gel for 60 s and rinsed with distilled water for 30 s. Specimens were immersed daily over a 1-week period in a standardized coffee solution at room temperature (25±2°C) in screw capped plastic universal containers. The daily renewed coffee solution was produced by boiling 12 g of coffee (Melitta, Avaré, SP, Brazil) in 200 mL of distilled water for 5 min followed by filtering in a coffee maker (Melitta). After the immersion period, the specimens were washed and stored in distilled water at room temperature.

Tooth shade measurement

Tooth shades of the stained specimens were assessed with a digital spectrophotometer (Vita Easyshade, Vita Zahnfabrik, Bad Sackingen, Germany) after 1, 3 and 7 days of immersion in coffee solution (baseline). Three measurements were done with the active point of the spectrophotometer in the center of each sample. The instrument automatically averaged the three readings for each sample, which were use for overall data analysis. The digital spectrophotometer measures the tooth shade based on two methods: by grouping shades according to the 16 shade tabs in the guide (Vitapan Classical, Vita Zahnfabrik), which were numbered from 1 (highest value – B1) to 16 (lowest value – C4) (Figure 1)[19] and, the CIEL*a*b* color system, which was defined by the International Commission on Illumination. In the CIELAB color system, the L* axis represents the value (lightness or darkness), the a* axis is a measure of redness (positive a*) or greenness (negative a*), and the b* axis is a measure of yellowness (positive b*) or blueness (negative b*). The difference between the color coordinates was calculated as: $\Delta E^* = [(\Delta L^*)^2 + (\Delta a^*)^2 + (\Delta b^*)^2]^{1/2}$ [8].

The same protocol used to determine the tooth shade at baseline (T0) was used in all other periods evaluated: after 1 week of treatment (T1) and 1 week post-bleaching (T2). All teeth shades measurements were performed objectively by an experienced examiner, which was blinded to the agent concentration.

Vitapan shade guide	B1	A1	B2	D2	A2	C1	C2	D4	A3	D3	B3	A3,5	B4	C3	A4	C4
Scores	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16

Figure 1- Color scores by sequence of colors in the shade guide (from lighter to darker colors)

Bleaching procedure

The same examiner responsible for the staining procedure carried out all bleaching treatments. The coffee-stained samples were randomly allocated into four groups (n=15), according to the bleaching agent concentration: distilled water (control group), 10% (CP10), 16% (CP16) or 37% (CP37) carbamide peroxide. The manufacturer and chemical composition of each bleaching agent used in this study are listed in Figure 2.

The control group was stored in distilled water at 37°C, which was daily renewed during 2 weeks. Samples of CP10 and CP16 groups were covered with 0.2 mL of the respective bleaching gels, which were applied on enamel surface for 4 h/ day during 2 weeks. Samples of CP37 group were covered with 0.2 mL of the bleaching gel for 20 min. The gel was light activated by two 40-s applications (LED Radii, SDI, Bayswater, Victoria, Australia) spaced by 10-min intervals. The gel was renewed and applied 3 times *per* clinical session. This cycle was repeated at 3 sessions with 5 days of interval between them. After bleaching treatments, the gels were carefully removed with sterile gauze and tap water and then the samples were stored in distilled water at 37°C until the next application.

Statistical analysis

Tooth shade means were analyzed statistically by Kruskal-Wallis and Friedman's tests and color parameters were analyzed by two-way analysis of variance (ANOVA) and Tukey's test. The factors investigated were treatment group and evaluation period. The level of confidence was established at $\alpha=0.05$.

RESULTS

Tooth shade means and tooth color parameters (ΔL^*, Δa^*, Δb^*, ΔE^*) for the treatment groups at the different evaluation periods are presented in Tables 1 and 2, respectively. At T1 and T2 evaluations, CP10, CP16 and CP37 groups showed a statistically significant decrease in tooth shade means when compared with baseline and control group ($p<0.001$). At T1, the group treated with CP37 presented lower tooth shade means than the other bleaching treatments tested in the same period ($p<0.01$). However, the tooth shade means resulting from the 1-week post-bleaching procedures (T2) were not statistically different between CP10, CP16 and CP37 groups ($p>0.05$).

Considering tooth color parameters at T1 and T2, all treatment groups showed an increasing of the lightness (ΔL^*), a decreasing of the redness (Δa^*) and yellowness (Δb^*) compared with baseline ($p<0.001$). However, the increasing of the lightness (ΔL^*) in the group treated with CP37 was lower than CP10 and CP16 at both evaluations periods ($p<0.001$). Although a* values decreased for all treatment groups, at T2, there was no statistically difference among CP10, CP16, CP37 and control ($p>0.06$). The decrease of b* values in CP37 was significantly lower than in the other treatment

Product	Manufacturer	Chemical composition
Whiteness Perfect 10%	FGM, Joinville,SC, Brazil	10% carbamide peroxide, carbopol, potassium nitrate, sodium fluoride, glycol and deionized water.
Whiteness Perfect 16%	FGM, Joinville, SC, Brazil	16% carbamide peroxide, carbopol, potassium nitrate, sodium fluoride, glycol and deionized water.
Whiteness Super 37%	FGM, Joinville, SC, Brazil	37% carbamide peroxide, carbopol, potassium ions, glycol and deionized water.

Figure 2- Description of the bleaching agents used in this study

Table 1- Tooth shade means and standard deviations (±SD) for the different treatment groups according to the evaluation periods

Treatment groups	Evaluation periods		
	T0 (baseline)	T1 (1-week of treatment)	T2 (1-week after treatment)
Control	[A]12.9 (±1.7)[a]	[A]12.4 (±1.3)[a]	[A] 12.5 (±0.7)[a]
CP10	[A]12.7 (±2.6)[a]	[B]4.4 (±2.1)[b]	[C]2.9 (±0.7)[b]
CP16	[A]12.7 (±1.8)[a]	[B]4.1 (±2.2)[b]	[C]2.3 (±1.3)[b]
CP37	[A]11.8 (±2.5)[a]	[B]7.0 (±3.2)[c]	[C]2.4 (±0.6)[b]

Different capital letters indicate statistically significant differences only among the evaluation periods ($p<0.001$). Different lowercase letters indicate statistically significant differences only among the treatment groups ($p<0.001$).

Table 2- Tooth color parameter means and standard deviations (±SD) for the different treatment groups according to the evaluation periods

Color parameters	Treatment groups	Evaluation periods		
		T0 (baseline)	T1 (1-week of treatment)	T2 (1-week after treatment)
ΔL^*	Control	A 77.2 (±4.2)a	B 1.9 (±2.4)a	B 2.4 (±3.0)a
	CP10	A 76.4 (±4.5)a	B 10.1 (±4.2)b	C 12.1 (±5.6)b
	CP16	A 75.6 (±4.2)a	B 12.6 (±3.7)b	C 15.6 (±6.7)b
	CP37	A 79.3 (±2.8)a	B 5.9 (±2.2)c	B 6.2 (±2.3)c
Δa^*	Control	A 2.5 (±2.3)a	B -1.0 (±0.8)a	C -1.7 (±1.6)a
	CP10	A 2.4 (±2.9)a	B -2.6 (±1.9)b	B -2.7 (±2.1)a
	CP16	A 1.7 (±2.1)a	B - 2.5 (±1.5)b	B - 2.3 (±1.4)a
	CP37	A 2.5 (±2.4)a	B -2.8 (±2.0)b	C -3.4 (±1.9)a
Δb^*	Control	A 36.4 (±5.0)a	B -1.4 (±1.9)a	C -2.7 (±3.4)a
	CP10	A 36.1 (±4.2)a	B - 7.6 (±3.1)b	B -7.6 (±2.2)b
	CP16	A 32.0 (±4.0)a	B - 5.8 (±3.4)b	B -5.0 (±3.3)ab
	CP37	A 36.0 (±5.2)a	B - 7.1 (±4.1)b	C - 11.0 (±4.0)c
ΔE	Control	---	A 3.6 (±1.9)a	B 5.5 (±2.9)a
	CP10	---	A 13.5 (±3.9)b	A 15.1 (±5.0)b
	CP16	---	A 14.4 (±3.9)b	B 16.8 (±4.1)b
	CP37	---	A 10.1 (±3.9)c	B 13.6 (±3.5)b

At baseline, L*, a*, b* means were shown in absolute values. Different capital letters indicate statistical significant differences only between evaluation periods (p<0.05). Different lowercase letters indicate statistical significant differences only between treatment groups (p<0.05).

groups (p<0.001). Despite ΔE^* values, there was no difference among the treatment groups at the end of the treatment (p>0.1).

DISCUSSION

Previous studies have suggested that the two main factors that determine the tooth whitening efficacy are the peroxide concentration and its time of application[11,16]. There is a belief that the increase of the bleaching agent concentration can produce a higher and faster whitening effect compared with whitening products with low-concentration bleaching agents. However, this study showed that after the first week of treatment, 10 and 16% carbamide peroxide concentrations were significantly more effective than the 37% concentration. From this study, it appears that the factor contact time of bleaching agent was more important than the concentration of gels. Although this result might seem surprising, similar effects in tooth shade improvement were achieved with longer treatment times, independently of the peroxide concentration used[16,17]. A review that compared 9 clinical trials and the effectiveness of 25 tooth whitening products showed that tooth whitening is more effective when the bleaching agent is placed in trays[16].

As the present *in vitro* study followed the manufacturers' recommendations in order to evaluate the effectiveness of carbamide peroxide-based gels available for in-office bleaching (37%) or home-use (10 and 16%), the mode and duration of applications were different among the groups. Certainly, these different characteristics of application for each bleaching agent might have influenced the outcome of tooth whitening[9,12,23]. A clinical trial that evaluated the effectiveness of 8 in-office tooth whitening systems verified that the contact time between bleaching gel and tooth surface is an important factor that can improve the outcome of the bleaching treatment[17].

The necessity of light-activation of peroxide-based gels has already been questioned by some authors[7,13,15,22]. Even though the use of light in bleaching procedures has been considered unnecessary[1,14] because of the presence of other agents (*i.e.,* carbopol) that assure the catalyze of peroxides, light-activation of 37% carbamide peroxide was performed in our study to accelerate the whitening process, following the manufacturer's instructions.

Despite the subjectivity of visual tooth color assessment, it has been well documented that the tooth color changes can be successfully

assessed using both shade guides and digital spectrophotometry[3,19]. Nevertheless, when employing an objective method for measuring the tooth color changes, it is also possible to evaluate color parameters (L*, a* and b*) based on the CIELAB system, an three-dimensional color space[8]. According to spectrophotometer data, the whitening improvement occurs mainly by increasing the lightness (higher L*) and reducing the redness (lower a*) and yellowness (lower b*)[3]. At 1 week post-bleaching, all treatment groups showed an increase of the L* and a decrease of the a* and b* color parameters. There are clearly limitations in comparing *in vitro* results with clinical studies once that changes in ΔL*, Δa* and Δb* need to be interpreted with caution. Nevertheless, the color parameters data of this *in vitro* study is in agreement with the results of clinical trials, indicating that lower concentrations of tooth bleaching gels are as effective as higher concentrations[5,17,20,21].

The control group showed an increase of the lightness (L*) and also a decrease of the yellowness (b*) and redness (a*) during water storage. These changes in the parameters are probably due to the removal of tooth surface pigments, as a consequence of the coffee staining procedure.

The overall color change (ΔE*) values are often used in tooth whitening studies in order to indicate the perceptible tooth color changes after treatment[3,11]. At 1 week post-bleaching, all treatment groups showed ΔE* values higher than 13.0. Clinically, such finding could demonstrate that the lower concentration of peroxide-based gels would achieve the same perceptible results as the 37% carbamide peroxide, frequently used for in-office tooth bleaching. Besides, previous studies have stated that color reversal after bleaching treatment occurs quicker for in-office products than home tray-based bleaching agents[16,17,25].

Considering that all carbamide peroxide gels evaluated in this study resulted in a similar tooth shade improvement, it could be suggested the use of low carbamide peroxide concentrations for vital tooth bleaching, in accordance with the American Dental Association guidelines, which only assures the safety and efficacy of bleaching treatment using trays with 10%±1% carbamide peroxide application[2].

The null hypothesis for this study was accepted as the high-concentration carbamide peroxide produced similar tooth color improvement than the bleaching agents containing low carbamide peroxide concentration. Further studies and clinical trials are needed to determine if the same color improvement is obtained with in-office or home-use carbamide peroxide bleaching agents.

CONCLUSIONS

Within the limitations of this study, it may be concluded that at-home (10 or 16% carbamide peroxide) or in-office (37% carbamide peroxide) bleaching agents produced a similar color improvement after the end of the treatment.

Clinical relevance

Tooth bleaching with high or low concentrated carbamide peroxide agents were similarly effective after treatment period. Practitioners must be informed about bleaching techniques in order to provide an evidence-based choice for their patients.

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