Tooth Bleaching by Different Concentrations of Carbamide Peroxide and Hydrogen Peroxide Whitening Strips: An In Vitro Study

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ABSTRACT

Objective: To investigate the tooth whitening effects of various concentrations of carbamide peroxide (CP) gels and 6% hydrogen peroxide (HP) whitening strips used on an intrinsic, in vitro stain model in a simulated home-applied bleaching protocol.

Method: Extracted third molars were sectioned and stained to Vita shade C4 using a standardized tea solution. Stained specimens were then bleached with 10, 15, 20, 22, and 30% CP gels applied in custom-made trays for 8-hour sessions for 14 days. A 6% HP whitening strip product was also tested in a regimen of twice-daily 30-minute treatments for 14 days. Shades were assessed at baseline and at 2, 5, 7, 10, and 14 days of treatment using a shade guide (SG) and a shade vision system (SVS), recorded as shade guide unit (SGU) changes from baseline, and CIE L*a*b* recordings using a chromometer.

Results: By day 14, all CP treatments resulted in at least 12 SGU improvements by SG and SVS methods: the HP treatment mean was just less than 12 SGU. With the chromometer, the CP improvements ranged from approximately 19 to 28 units and 16 units for the HP whitening strips. Observationally, by SG and SVS, CP treatments achieved the maximum improvement (12–13 SGU) at different time points: day 5 for 30% CP, day 10 for 22% CP, and day 14 for the other three treatments. SG and SVS data were virtually binary, switching from 0 to scores of 9 or above as bleaching progressed. The differences between the six treatments in the mean day to achieve a positive SG or SVS score (9 or more units) approached significance. For each of the SG, SVS, and L*a*b* scores, the dose-response correlation with CP concentration was significant at one or more assessment times. SG and SVS showed extremely strong agreement in detecting change and substantial agreement with L*a*b*.

Conclusion: This in vitro study supports the limited data available from the very few available randomized controlled clinical trials indicating that CP and HP home-use bleaching systems can achieve considerable tooth whitening outcomes, albeit at different rates, which appear to be concentration dependent.

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CLINICAL SIGNIFICANCE

There is a clear significant relationship for both concentration and duration of exposure for CP bleaching agents. The final shade change is independent of the concentration of bleaching agent, with time as the dominant variable. Higher concentrations of CP that have not been investigated previously may be a treatment option for esthetic improvement of shade where time is at a premium, but caution must be exercised in view of the possible increased incidence of sensitivity.

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Tooth whitening techniques **L** have seen renewed interest from the dental profession as a result of the public's desire for whiter, brighter teeth. The technique of bleaching or whitening teeth was first described in 1877¹ but gained general acceptance by the profession in 1989 with the introduction of the so-called nightguard vital bleaching (NGVB) technique, which involved the application of a 10% carbamide peroxide (CP)-based gel in a bleaching tray worn for up to 8 hours at night.² Since the introduction of the NGVB technique there have been many different modifications involving the bleaching agents used, the type of tray provided, and the treatment regimen employed. Bleaching agents have included different concentrations of CP and hydrogen peroxide (HP) with different flavors, thickeners such as carbopol, and desensitizing agents, such as potassium nitrate. As the technique has developed, the tray material has tended to become softer and thinner and the tray design has alternated between the use of reservoirs and scalloping or a straight-cut tray without reservoirs.

Other treatment regimens developed include day use of various concentrations of CP and HP for between 30 minutes and 4 to 8 hours per day. Clinical studies have shown that a 10% CP gel used in a bleaching tray at night produces predictable results,^{3–11} as do 5 to 6% HP whitening strips.¹² A laboratory study found that a 16% CP solution bleached more quickly than the same 5 or 10% solution. However, all concentrations produced a bleaching effect if used long enough.¹³ Similarly, clinical studies have compared 10% CP with 15% CP14 and 20% CP with 7.5% HP.15 Both studies found no difference in the eventual final shade even though there were initial quicker results with the higher concentration of CP. There have been no reported controlled randomized trials or in vitro studies comparing a range of different concentrations of CP that are available to the dental professional, namely, 10, 15, 20, 22, and 30% solutions.

A recently developed in vitro method that quantitatively assesses the effects of tooth whitening products¹⁶ was used to investigate the effect of varying HP concentrations, from 5 to 35%, on the tooth whitening process.¹⁷ The relationship between the number of bleach applications and the bleaching gel concentration was exponential rather than linear. The aim of the present study was to investigate the tooth whitening effects of a wide range of professionally available concentrations of CP gels (10, 15, 20, 22, and 30%) and 6% HP whitening strips used in simulated home-applied bleaching using the same model in vitro.¹⁶

MATERIALS AND METHODS

The in vitro model used in this study involves the preparation of internally stained tooth specimens and three tooth shade assessment methods, as described previously.¹⁶ In brief, 15 third molar teeth underwent root removal and vertical bisection beginning at the occlusal fissure level. The exposed dentin surface was polished using a 1,000-grit silicon carbide paper in a lapping and polishing unit with water irrigation. It was then etched with 35% phosphoric acid to remove the smear layer and expose the tubule network to promote

stain uptake into the tooth. Teeth were placed in a standard tea solution for stain development for 24 hours. The tea solution was produced by boiling 2 g of tea (Marks & Spencer's Extra Strong Tea, Marks & Spencer, London, UK) in 100 mL of water for 5 minutes and filtered through gauze to remove the tea from the infusion.

Three methods were used to assess staining:

- 1. A standard Vita shade guide (SG) (Vita Zahnfabrik, Bad Säckingen, Germany)
- 2. A commercially available clinical colorimeter with imagegrabbing technology, Shade Vision System (SVS; X-rite, Grandville, MI, USA)
- An electronic colorimeter (Chromometer, Minolta CR 221, Minolta UK, Milton Keynes, UK)

SG and SVS results were recorded as shade guide unit (SGU) values, whereas the chromometric method yielded CIE L*a*b* values.¹⁸

Tooth specimens were prepared, and baseline shades were assessed. One investigator (M.S.) performed all the shade comparisons and was blind to the allocation of specimens. Shade guide assessments were made with specimens lying on a black background under the same fluorescent lighting conditions. A black background was chosen following initial work to determine shade consistency using the SVS.¹⁶ The SVS was used to give a mean Vita shade for each of the specimens. As with the SG, SVS recordings were made with specimens lying on a black background. The chromometer was applied to the outer surface of the enamel not at a single site but across the whole width of the specimen and therefore without the use of a jig. The mean L*a*b* values for each specimen were then calculated.

Only specimens with shade C4 as assessed by the SG and SVS were accepted for the study. Thirty specimens were allocated randomly to six treatment groups, each comprising five specimens. The shade tabs were arranged in a permuted sequence suggested by the manufacturer, and each was assigned a numeric value ranging from 1 to 16 (B1, A1, B2, D2, A2, C1, C2, D4, A3, D3, B3, A3.5, B4, C3, A4, C4). L*a*b* values for all specimens were assessed using the chromometer. Five concentrations of commercially available CP home bleaching agents, 10%^A, 15%^A, 20%^A, 22%^B, and 30%^C, applied in custom-made trays (10%^A, 15%^A, 20%^A Opalescence PF: Ultradent Products Inc., South Jordan, UT, USA; Polar 22%^B: SDI, Bayswater, Victoria, Australia; 30%^C Quick white: DMDS UK, Canterbury, UK), and one over-the-counter brand of 6% HP (equivalent to approximately 18% CP) polyethylene whitening strip (Crest

Whitestrips, Procter & Gamble, Cincinnati, OH, USA) were used.

All exposed dentin surfaces were sealed using clear nail polish and then fixed onto a microscope slide using double-sided adhesive tape so that only the enamel surface was exposed for the application of the bleaching agents. The five specimens for each treatment group were adhered to the same microscope slide in line and separated by approximately 5 mm. The CP bleaching agent was applied to groups 1 to 5 using custom-made bleaching trays. Thus, plastic impression trays were cut and modified with brown stick compound, and then an alginate impression was taken of the specimens on each slide and a stone model was cast within 30 minutes. Finally, a whitening tray was fabricated with a reservoir depth of 0.5 mm to allow the bleaching agent to be applied to the enamel surface of the specimens. Groups 1 to 5 were treated with the appropriate concentration of CP bleaching agent placed in the trays for 8 hours. Group 6 specimens were treated with the HP whitening strips in a manner simulating the manufacturer's instructions for use. Thus, each strip was molded by hand to envelop the five specimens on the slide and left in place for 30 minutes. This procedure was performed twice per day. The treatments were carried out in an airtight container with a moist atmosphere created by

placing wet paper towels in the container base, and all treatments were terminated after 14 days of active treatment. Following each treatment cycle, both the teeth and the whitening tray were cleaned with a toothbrush and rinsed with water for 2 minutes. The teeth were then placed in water for 16 hours to allow them to rehydrate between treatments (Figure 1).

After rehydration following 2 days of treatment, all samples were assessed for shade using all three methods. The bleaching treatment/rehydration cycles were then applied again, and the shade assessments were repeated at 5, 7, 10, and 14 days. The shade assessments were made in the order of SG, SVS, and chromometer with groups of specimens numerically coded to achieve examiner blindness. The SVS and SG data were expressed as SGU change. The equivalent chromometer data were expressed as overall color difference ΔE^* calculated using the following expression: $\Delta E^* = \sqrt{[(\Delta L^*)^2 +]}$ $(\Delta a^*)^2 + (\Delta b^*)^2].$

The outcome measures for analysis were SGU change as recorded by SG, SVS, and ΔE^* change measured by the chromometer.

Summary statistics were calculated for the three outcomes by treatment and duration of exposure. SG and SVS scores behaved in an essentially binary manner, starting at a shade change of zero (baseline, C4) for each specimen and switching to a positive shade change of 9 (C2) to 13 (B2) SGUs from baseline at some subsequent assessment. Thus, for each specimen, the day at which it first reached a positive value of nine SGU changes or greater was determined. One-way analysis of variance was then used to assess whether this differed between the



Figure 1. Bleaching treatment cycle. CP = *carbamide peroxide; HP* = *hydrogen peroxide.*

six treatments. To determine whether there was a significant dose response for the CP treatments (ie, excluding the HP product), correlation coefficients at each measurement day (days 2, 5, 7, 10, and 14) were calculated for L*a*b*, SG, and SVS. A cross-tabulation was constructed to assess the similarity of SG and SVS, expressed as binary variables. Finally, the degree of agreement of binary SG and SVS with L*a*b* was assessed using the statistic U/mn, which is the Mann-Whitney *U* statistic divided by the product of the two sample sizes. A value of 1 (or 0) corresponds to perfect separation between L*a*b* values corresponding to positive and negative SG or SVS and 0.5 corresponds to no difference. This statistic is equivalent to the area under the receiver operating curve. Finally, 95% confidence intervals (CIs) for U/mn were calculated. All statistical analysis was performed by the medical statistician author (R.G.N.).

RESULTS

Tables 1 and 2 show summary statistics for the bleaching effect of the various gels measured using the SVS and SG expressed as SGU change. The equivalent data, determined using the chromometer, are shown in Table 3.

After 14 treatment days, all CP treatments resulted in a mean of at least 12 SGU improvements by SG and SVS methods: the HP treatment mean was just under 12 SGU. Assessed by the chromometer, the CP improvements in shade change expressed as ΔE^* ranged from approximately 19 to 28 (ΔE^*) units: the HP treatment achieved around 16 (ΔE^*) units of improvement. As an observation both by SG and SVS, CP treatments reached the final shade change (12–13 SGU) at day 5 for 30% CP, day 10 for 22% CP, and day 14 for the other

TABLE 1. MEAN (SD) CHANGE IN SHADE VISION SYSTEM SCORES ON MEASUREMENT DAYS WITH CARBAMIDE PEROXIDE AND HYDROGEN PEROXIDE TREATMENTS.					
Bleaching Agent	Day 2	Day 5	Day 7	Day 10	Day 14
10% CP	0.0 (0.0)	4.4 (6.07)	4.8 (6.57)	4.8 (6.57)	12.0 (0.0)
15% CP	4.8 (6.57)	7.2 (6.57)	7.2 (6.57)	9.6 (5.37)	12.0 (0.0)
20% CP	7.2 (6.57)	9.6 (5.37)	9.6 (5.37)	9.6 (5.37)	12.0 (0.0)
22% CP	4.8 (6.57)	9.6 (5.37)	9.6 (5.37)	12.0 (0.0)	12.0 (0.0)
30% CP	7.2 (6.57)	12.0 (0.0)	12.0 (0.0)	12.0 (0.0)	12.0 (0.0)
6% HP	0.0 (0.0)	4.8 (6.57)	4.8 (6.57)	7.2 (6.57)	11.4 (1.34)
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CP = carbamide peroxide; HP = hydrogen peroxide

TABLE 2. MEAN (SD) CHANGE IN SHADE GUIDE SCORES ON MEASUREMENT DAYS WITH CARBAMIDE PEROXIDE AND HYDROGEN PEROXIDE TREATMENTS.					
Bleaching Agent	Day 2	Day 5	Day 7	Day 10	Day 14
10% CP	0.0 (0.0)	4.0 (5.52)	4.4 (6.03)	4.8 (6.57)	12.0 (0.0)
15% CP	4.8 (6.61)	7.2 (6.57)	7.4 (6.77)	9.8 (5.50)	12.2 (0.45)
20% CP	7.0 (6.44)	9.8 (5.50)	9.8 (5.50)	9.8 (5.50)	12.2 (0.45)
22% CP	4.8 (6.57)	9.4 (5.27)	11.8 (0.44)	12.0 (0.0)	12.0 (0.0)
30% CP	7.2 (6.57)	12.0 (0.0)	12.0 (0.0)	12.0 (0.0)	12.0 (0.0)
6% HP	0.0 (0.0)	4.6 (6.31)	4.6 (6.31)	7.0 (6.40)	12.0 (0.0)

CP = carbamide peroxide; HP = hydrogen peroxide.

TABLE 3. MEAN (SD) CHANGE IN CHROMOMETER READINGS (Δ E*) ON MEASUREMENT DAYS WITH CARBAMIDE PEROXIDE					
AND HYDROGEN PER	OXIDE TREATMENTS				
Bleaching Agent	Day 2	Day 5	Day 7	Day 10	Day 14
109/ CD	0.95(4.46)	12 25 (6 14)	1(25/(04)	17(2(7.05))	10.42(9.7)
10 % CP	9.83 (4.46)	13.23 (6.14)	16.23 (6.94)	17.63 (7.93)	19.42 (8.67)
15% CP	8.71 (4.36)	13.75 (5.47)	14.75 (4.73)	16.15 (5.83)	18.55 (5.80)
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20% CP	10.17 (2.01)	14.24 (2.29)	15.71 (2.61)	16.39 (2.89)	20.02 (3.16)
220/ CD	15 56 (2 21)	19.04(5.12)	22.01(5.94)	24.54(6.21)	29.62(7.11)
22 /0 CF	13.36 (3.21)	19.04 (3.13)	23.01 (3.94)	24.34 (0.31)	20.03 (7.11)
30% CP	13.26 (3.15)	17.68 (7.10)	20.32 (10.32)	22.02 (10.06)	25.27 (11.33)
(0/ LID	0 (1 (5 0 2)	12 21 (2 7()	12 22 (4 00)	14.27 (4.00)	1(10/11)
6% HP	9.64 (5.03)	13.21 (3.76)	12.23 (4.98)	14.37 (4.98)	16.49 (4.46)
CP = carbamide peroxide; HP = hydrogen peroxide.					
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three treatments. For the HP treatment, not all specimens reached the 12 to 13 units of improvement by day 14. Chromometer values generally showed steadily increasing values with increasing duration of exposure to bleaching agents.

Table 4 shows summary statistics for the day on which specimens in each treatment group reached a positive score (9 SGU or higher). For CP treatments the shortest time was for 30% CP, after which time increased successively as the CP concentration decreased; HP and 10% CP were essentially similar. Analysis of variance over the six treatments approached significance (SG, p = .064; SVS, p = .091).

Table 5 shows dose-response correlation coefficients for CP treatment outcome at each measurement day for SG, SVS, and L*a*b*. All correlations were positive, and each measurement system gave a significant correlation on at least one occasion. For both SG and SVS scores, the time to switch to positivity was, correspondingly, significantly inversely correlated with CP concentration. The order of sensitivity to detect a significant positive dose-response relationship appears to be SG > SVS > $L^*a^*b^*$. Crosstabulation of SG by SVS as binary variables shows that their patterns of switching from negative to positive are very similar, with only one disagreement for the 150 assessments. The degree of agreement of

TABLE 4. MEAN DAY TO ACHIEVE A POSITIVE SHADE CHANGE SCORE (9 UNITS OR GREATER) BY SHADE GUIDE AND SHADE VISION SYSTEM WITH CARBAMIDE PEROXIDE AND HYDROGEN PEROXIDE TREATMENTS. Measurement SD Treatment Mean 10% CP SG or SVS 10.4 4.9 SG or SVS 15% CP 6.6 5.3 SG or SVS 5.0 5.2 20% CP 22% CP SVS 3.3 4.8 22% CP SG 4.2 2.2 30% CP SG or SVS 3.2 1.6 6% HP SG or SVS 4.5 9.6

 $\rm CP$ = carbamide peroxide; $\rm HP$ = hydrogen peroxide; $\rm SG$ = shade guide; $\rm SVS$ = shade vision system. Based on five specimens per group.

SG and SVS with L*a*b* using U/mn was 0.764 (95% CI 0.671–0.835) for SG and 0.760 (95% CI 0.667–0.831) for SVS, indicating substantial but not perfect separation.

DISCUSSION

The aim of this study was to assess the effect of various concentrations of commercially available homeapplied bleaching agents on the response of internally stained teeth to bleaching. In common with previous studies in vitro,^{16,17} it seems that teeth bleach to an approximate end point (Vita shade D2 in this study).^{16,17} The present in vitro study showed that the HP gel and each concentration of CP could

TABLE 5. PEARSON CORRELATION COEFFICIENTS (WITH *P* VALUES) BETWEEN CARBAMIDE PEROXIDE CONCENTRATION AND SHADE CHANGE ON EACH MEASUREMENT DAY RECORDED BY L*A*B*, SHADE GUIDE, AND SHADE VISION

STOTEM METHODS.			
Assessment Time	Chromometer	SG Score	SVS Score
Day 2	0.411 (.042)	0.365 (.073)	0.363 (.074)
Day 5	0.331 (.106)	0.491 (.013)	0.471 (.018)
Day 7	0.297 (.150)	0.520 (.008)	0.447 (.025)
Day 10	0.293 (.155)	0.465 (.019)	0.475 (.016)
Day 14	0.337 (.099)	0.124 (.554)	_
Time to reach positive	_	-0.521 (.008)	-0.504 (.010)
value (9 or higher)			

SG = shade guide; SVS = shade vision system. N = 25 throughout.

improve the shade of internally stained extracted teeth by 12 SGU in 14 days of use. Using the day to reach a positive change (9 SGU or greater units), the mean SG and SVS scores showed a clear pattern, with 22% and 30% CP achieving these effects earlier than other concentrations. The results obtained were in agreement with those of previous researchers who compared different concentrations of CP.13 The magnitude of the shade change in the present study was larger than the previous study in which the teeth used were less heavily stained: the acceptance criterion for the Leonard and colleagues study being shade A3,¹³ which is much lower than shade C4 used in the present study. Leonard and colleagues found the average shade change to be 4.9 and 6 for incisors and canines, respectively.¹³ Similarly, the higher concentration of 16% CP produced faster shade changes than 5 and 10% CP, but, by the end of treatment, all teeth reached approximately the same end point whitening for all concentrations.

The present study employed the original intrinsic stain model devised to study bleaching in vitro.¹⁶ The same model was used to investigate the effect of different concentrations of HP on the outcome of tooth whitening.¹⁶ In both studies the total magnitude of bleaching was similar to that achieved in the present study.^{16,17} There was also a clear, significant

dose-response relationship for the duration of exposure in the HP concentration study,¹⁷ as also shown here for CP. For all measurement methods the correlation coefficients were significant on at least one recording day, with the greatest sensitivity shown with SG and the least with the chromometer. Nevertheless, the agreement between dichotomized SG and SVS was extremely high, with substantial agreement between these systems and L*a*b*. The magnitude of shade changes in the present study was broadly similar to, albeit larger than, that of previous clinical studies.^{19–21} Heymann and colleagues reported a mean change of 7 units on the Vita shade guide after bleaching with a 10% CP gel over 7 days,²⁰ whereas Gerlach and Zhou reported a mean change of 5.5 units using a whitening strip (6% HP); a quarter of their sample had a shade change in excess of 8 units.¹⁹ In the present study, the mean change in color (ΔE^*) by chromometer was used, which is mainly dependent on the change in lightness/darkness (ΔL^*). Observationally, changes in a* and b* were as expected, representing a reduction in redness and yellowness, an effect reported by others.^{16,17,19,22}

The chromometer was applied to the outer surface of the enamel not at a single site but across the whole width of the specimen and therefore without the use of a jig. The shade of each specimen was recorded

from three separate locations taken across the full width of the specimen, working from right to left: the instrument automatically taking three readings from each site and providing the average on the printout. Although a jig is used to ensure repeat chromometer measurement from the same point on a tooth clinically, the nature of the specimens and their setup on the slides in this study meant that the use of a jig would not be possible. Hence, readings across the whole width of the specimen from right to left provided a mean change in shade across the specimen.

The results of the present study, in common with previous uses of this model in vitro, must be interpreted with caution. With in vivo conditions in vital teeth, there is a continuous outward movement of fluid through dentinal tubules and porous enamel, which would tend to impede the penetration of an applied bleaching agent. The use of extracted teeth devoid of dentinal fluid probably allowed the bleaching agent to permeate the tooth more quickly than would occur in vivo. Indeed, it would be of interest to determine in vivo whether nonvital teeth bleach more easily than vital teeth. In spite of this reservation, the results of the present study suggest that the final color change is independent of the concentration of bleaching agent used and that time is the dominant variable. Practitioners, however, may wish to use

a higher concentration of bleaching agent to achieve a quicker result for those patients for whom time is at a premium. This approach may run a greater risk of side effects, such as tooth sensitivity.

In conclusion, this in vitro study supports the limited data from the few available randomized controlled clinical trials involving different concentrations indicating that CP and HP home-use bleaching systems can achieve considerable tooth whitening outcomes, albeit at different rates, which appear to be concentration dependent. In addition, the study showed that even using the higher concentration of 30% CP gave the same end shade result as the lowest concentration of 10% CP, but the whitening result was achieved much more quickly.

DISCLOSURE

The authors do not have any financial interest in the companies whose materials are discussed in this article.

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