©Operative Dentistry, 2013, **38-2,** 177-185

The Relationship of Hydrogen Peroxide Exposure Protocol to Bleaching Efficacy

SR Kwon • PW Wertz • DV Dawson
DS Cobb • G Denehy

Clinical Relevance

The use of a linear low-density polyethylene wrap as advocated in the sealed bleaching technique can minimize hydrogen peroxide penetration into the pulp cavity without compromising bleaching efficacy *in vitro*.

SUMMARY

The purpose of this study was to compare two in-office bleaching methods with respect to tooth color change and level of hydrogen peroxide penetration into the pulp cavity and to evaluate relationships between penetration level and color change. Eighty extracted canines were exposed to two different bleaching regimens (conventional vs sealed bleaching technique). After exposure to 38% hydrogen peroxide gel for one hour, hydrogen peroxide amount was estimated spectrophotometrically. Color change was measured per Commission Internationale de l'Eclairage methodology. Linear regression was used to evaluate factors affecting color change, including bleaching technique. The conventional and sealed bleaching groups showed no difference for any color change parameters (ΔL, Δa, Δb, ΔE); however, there was significantly greater hydrogen peroxide penetration in the conventional bleaching group ($p<0.05$). Linear modeling of the change in lightness (ΔL) showed that the increase in lightness tended to be greater for teeth with lower initial L* values ($r=-0.32$, $p<0.05$). After adjustment for initial L*, there was no evidence that ΔL differed with hydrogen peroxide penetration

*So Ran Kwon, DDS, MS, PhD, MS, Loma Linda University, School of Dentistry, Center for Dental Research, Department of Restorative Dentistry, Loma Linda, CA, USA

Philip W. Wertz, PhD, University of Iowa, College of Dentistry, Dows Institute for Dental Research, Iowa City, IA, USA

Deborah V. Dawson, PhD, ScM, University of Iowa, College of Dentistry, Dows Institute for Dental Research, Iowa City, IA, USA

Deborah S. Cobb, DDS, MS, University of Iowa, College of Dentistry, Operative Dentistry, Iowa City, IA, USA

Gerald Denehy, DDS, MS, University of Iowa, College of Dentistry, Operative Dentistry, Iowa City, IA, USA

*Correspondence: Loma Linda University, School of Dentistry, Center for Dental Research, Department of Restorative Dentistry, 24876 Taylor Street, Loma Linda, CA 92350, USA; e-mail: sorankwon@llu.edu

DOI: 10.2341/11-351-L

levels ($p>0.05$) or bleaching technique (mean group difference in $\Delta L=0.36$; $p>0.05$).

INTRODUCTION

Tooth bleaching is a conservative and highly effective method to whiten discolored teeth. It is a treatment option to enhance the esthetics of the teeth that has been practiced in dentistry for more than 100 years. Thus, the safety and efficacy of this procedure have been well established.[1]

In-office bleaching is generally preferred by both dentists and patients in that the responsibility for the procedure of bleaching teeth is transferred to the dental office.[2] In-office bleaching produces immediate bleaching results and can also be used as a kick start so that patients better comply with home bleaching procedures.

During in-office bleaching, the highly concentrated hydrogen peroxide bleaching gel is usually left on the tooth surface for 5 to 20 minutes and replenished according to the manufacturer's directions. However, irritation to the nasal mucosa caused by evaporation of volatile components in the bleaching gel, inadvertent exposure to the highly concentrated bleaching gel, as well as inconvenience and increased costs associated with multiple replenishment of bleaching gel during one bleaching session have been pointed out as disadvantages of conventional in-office bleaching procedures.[3] To prevent evaporation and desiccation of active agents, placement of a linear low-density polyethylene (LLDPE) wrap onto the bleaching gel had been described as the sealed bleaching technique.[3]

Bowles and Ugwuneri[4] were the first to show that in extracted teeth exposed to hydrogen peroxide, significant levels of hydrogen peroxide could be detected in the pulp cavity. Many studies followed, adopting the newly introduced *in vitro* model to investigate various factors that might influence the amount of hydrogen peroxide penetration into the pulp cavity. Studies have shown that higher concentrations of hydrogen peroxide,[4] heat and prolonged bleaching time,[5] light activation,[6] altered surface due to restorations,[7] and characteristics such as large open dentinal tubules of young teeth[8] facilitate the diffusion and penetration of hydrogen peroxide molecules from the outer tooth surface into the pulp cavity.

However, the effect of different in-office bleaching protocols on the amount of hydrogen peroxide penetration into the pulp cavity has not been investigated. It is also not known whether there is a relationship between hydrogen peroxide penetration levels and the color change of the tooth.

Thus, the purpose of this *in vitro* study was to compare the relationship of the amount of hydrogen peroxide penetration into the pulp cavity between the conventional and sealed bleaching technique and correlate penetration levels with the color change of the tooth. The null hypotheses to be tested were that color change and hydrogen peroxide penetration levels would not differ between the two in-office bleaching methods and that there would be no correlation between hydrogen peroxide penetration levels and tooth color change.

METHODS AND MATERIALS

Sample Selection and Preparation

Eighty extracted human canines were collected three months prior to the study and stored in 0.2% Thymol (Sigma-Aldrich, St Louis, MO, USA) and distilled water at 4°C. All teeth were cleaned and observed for the absence of developmental anomalies, caries, existing restorations, deep crack lines, or severe attrition. The roots were trimmed 3 mm apical to the cementoenamel junction (Figure 1a), and the pulpal tissue was removed with #25 to #40 H-files (Maillefer files, Dentsply Maillefer, Ballaigues, NA, Switzerland). The pulp chamber was slightly enlarged with a round carbide bur (Midwest, Dentsply Professional, Des Plaines, IL, USA) toward the lingual to maintain intact labial tooth structure and still be able to encompass 30 μL of acetate buffer (Figure 1b).

Bleaching Protocol

Fifty maxillary and 30 mandibular canines were randomly assigned to the conventional bleaching group and the sealed bleaching group. Tooth thickness was measured from the outer labial surface to the outer boundary of the pulp cavity at the cross-sectioned root 3 mm below the cementoenamel junction using an electronic digital caliper (Harbor Freight Tools, Pittsburgh, PA, USA; Figure 1c). A jig was fabricated for each tooth by gently placing the lingual surface of the tooth into a polyvinylsiloxane putty impression material (Exaflex, GC America Inc, Alsip, IL, USA) at a 30° angle from the bottom. The baseline color was measured with a spectrophotometer (Spectroshade Micro, MHT, Niederhasli, Switzerland) to provide a topographical color map of the entire tooth in one image (Figure 1d). A resin barrier (OpalDam, Ultradent Products Inc, South Jordan, UT, USA) was placed to cover 0.5 mm of tooth

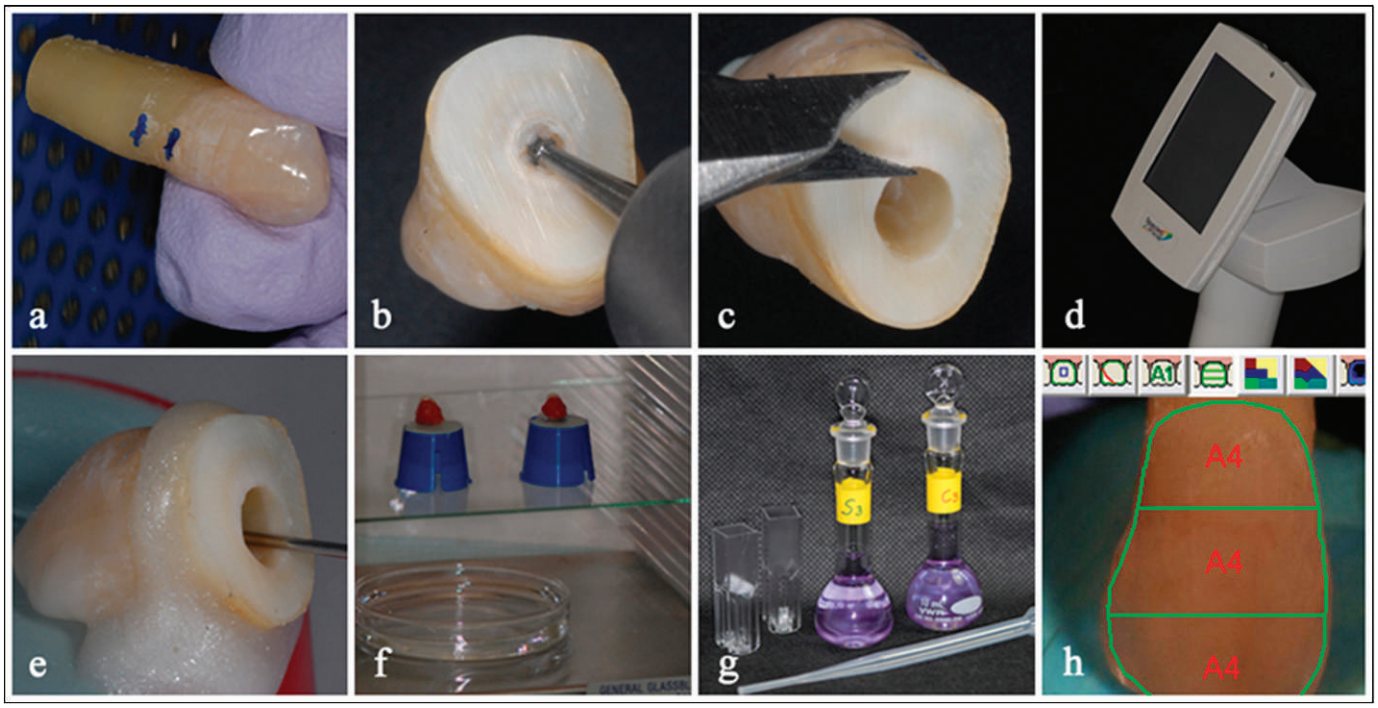

Figure 1. *Step-by-step procedures. (a): The roots of canine teeth were trimmed 3 mm apical to the cementoenamel junction. (b): The pulp chamber was enlarged to encompass 30 μL of acetate buffer. (c): Tooth thickness was measured from the outer labial surface to the outer boundary of the pulp cavity at the cross-sectioned root. (d): Tooth color was measured with a spectrophotometer. (e): Acetate buffer is placed into the pulp cavity. (f): Teeth were placed in a closed humid chamber during the bleaching process. (g): The mixture in the volumetric flask is transferred to cuvettes to be measured in a UV/Visible Spectrophotometer. (h): The color difference is measured with a software analysis program.*

coronal as well as 2 mm of root apical to the cementoenamel junction and light cured for 20 seconds (Elipar S10 LED curing light, 3M ESPE, St Paul, MN, USA).

The pulp cavities were rinsed twice with 30 μL of distilled water and dried with coarse paper points prior to the placement of freshly mixed 30 μL, 2M acetate buffer (pH 4.5; Figure 1e). The acetate buffer acted as a stabilizing agent of hydrogen peroxide that might have diffused into the pulp cavity. Thirty-eight percent hydrogen peroxide gel (Opalescence Boost, Ultradent Products Inc, South Jordan, UT, USA) was then placed onto the labial surface of the canines and subjected to the following groups.

Conventional Bleaching Group—The bleaching gel (80 μL) was applied onto the tooth surface and replenished every 20 minutes for three times according to the manufacturer's directions. A microbrush was used for the removal of the bleaching gel, but no irrigation was performed to avoid any contamination with the acetate buffer in the cavity.

Sealed Bleaching Group—The bleaching gel (80 μL) was applied onto the tooth surface and covered with a linear low-density polyethylene wrap (Professional Plastic Food Wrap Film, Bakers & Chefs Food Equipment Pte Ltd, Singapore) without replenishment of the gel during the bleaching procedure (60 minutes).

All teeth were kept in a closed humid chamber (General Glassblowing Co. Lab Apparatus, Richmond, CA, USA) at room temperature (25°C) with 100% relative humidity during the bleaching procedure (Figure 1f). At the end of the bleaching procedure, the acetate buffer was retrieved and placed in 10 mL volumetric flasks. The pulp cavities were thoroughly rinsed twice with 30 μL of distilled water, and the washes were added to the flasks. After removal of the acetate buffer, the bleaching gel was removed with microbrushes, and the teeth were rinsed with distilled water and stored in individual glass vials for two hours prior to taking postoperative shades with the spectrophotometer.

Measurement of Hydrogen Peroxide Penetration Levels

Hydrogen peroxide penetration levels were estimated according to the method of Mottola and others.[9]

Table 1: *Baseline Data (Mean/Median [SD]) for Conventional and Sealed Bleaching Group*[a]

Baseline Parameter	CBG	SBG	*p* Value*
L_1*	70.2/70.4 (3.43)	69.8/69.6 (3.78)	0.56
a_1*	2.9/2.7 (1.58)	3.2/3.0 (1.65)	0.54
b_1*	23.7/23.7 (2.62)	24.0/24.2 (2.40)	0.51
Tooth thickness (mm)	2.6/2.2 (0.20)	2.64/2.4 (0.19)	0.95

Abbreviations: CBG, conventional bleaching group; SBG, sealed bleaching group.
[a] *n = 40 in each group.*
* *Significance probability associated with Wilcoxon rank sum test.*

One milliliter of leucocrystal violet solution (0.5 mg/mL), 0.5 mL of horseradish peroxidase solution (1 mg/mL) was added to the volumetric flasks containing the acetate buffer retrieved from the cavity. After addition of 4 mL acetate buffer, the total volume was adjusted to 10 mL with distilled water, and the intensity of the color was measured in a UV/Visible Spectrophotometer (Model Lambda 20, Perkin Elmer, Branford, CT, USA) at a wavelength of 596 nm (Figure 1g). The evaluator taking the spectrophotometer reading was blinded regarding the treatment group. A standard calibration curve with known amounts of hydrogen peroxide was used to determine the amount of hydrogen peroxide in microgram equivalents in the samples.

Determination of Color Change

The color difference of the tooth before and after bleaching was measured as ΔE from the Commission Internationale de l'Eclairage. It was calculated from the following equation: $\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$ with the use of a software analysis program (MHT Software Analysis version 2.43; Figure 1h).

Statistical Methods

Measurements of color change included overall color change (ΔE) as well as changes in lightness (ΔL), the red-green dimension (Δa), and the blue-yellow dimension (Δb). Other measures of interest included hydrogen peroxide penetration and tooth thickness. The nonparametric Wilcoxon rank sum (Mann-Whitney) procedure was used to assess whether the two treatment groups differed at baseline with respect to L*, a*, b*, and tooth thickness. This procedure was also used to evaluate group differences in color change and H_2O_2 penetration following treatment. Multiple linear regression was used to evaluate factors affecting color change, which was measured as ΔL. Candidate covariates entertained in the modeling of a given color change outcome included bleaching technique, tooth thickness, H_2O_2 penetration, and the relevant baseline values of the particular color dimension. Standard residual analyses were carried out to assess validity of assumptions associated with the regression modeling, including residual plots and Shapiro-Wilk tests of normality. Throughout, the level of significance was set at $\alpha = 0.05$.

RESULTS

The conventional and sealed bleaching groups were similar at baseline with respect to the L_1*, a_1*, and b_1* color dimensions, as well as tooth thickness (Table 1; $p > 0.05$ in all instances, Wilcoxon rank sum test).

Based on the Wilcoxon rank sum test, there was no evidence that the two groups differed for any color change measurement (Table 2; Figure 2). In con-

Table 2: *Color Change Data and Hydrogen Peroxide Penetration (Mean/Median [SD]) by Bleaching Group*

	ΔL	Δa	Δb	ΔE	HPP (μg)
CBG	2.35/2.00 (1.58)	−0.87/−0.77 (0.60)	−2.06/−2.13 (1.23)	3.60/3.36 (1.37)	0.54/0.50 (0.20)
SBG	2.05/2.11 (1.49)	−0.73/−0.70 (0.44)	−1.83/−1.87 (1.19)	3.11/2.92 (1.48)	0.33/0.31 (0.16)
p value*	0.62	0.66	0.38	0.15	<0.0001

Abbreviations: CBG, conventional bleaching group; HPP, hydrogen peroxide penetration level; SBG, sealed bleaching group.
* *Significance probability associated with Wilcoxon rank sum test.*

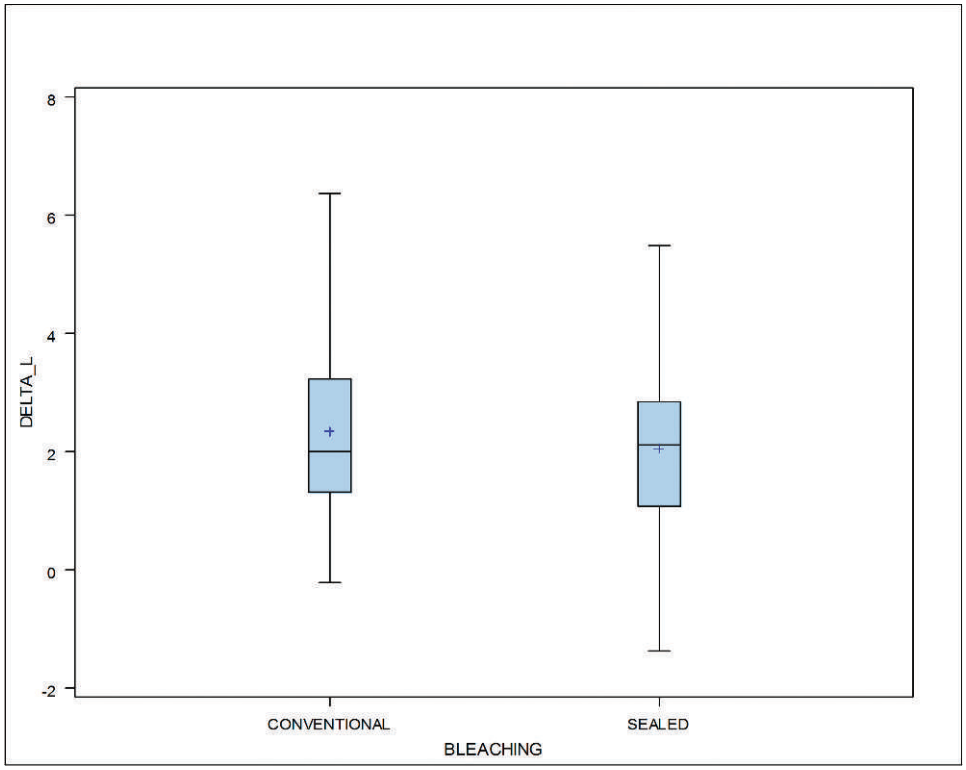

Figure 2. *Box plots for change in lightness (ΔL) relative to baseline by bleaching group.*

trast, the two groups were found to differ significantly in the level of hydrogen peroxide penetration following bleaching ($p<0.05$). In the conventional bleaching group, the mean level of H_2O_2 penetration was significantly higher than in the sealed bleaching group (Table 2). The distribution of hydrogen peroxide penetration levels is illustrated in Figure 3.

There was interest in whether color change was also related to other measures, such as tooth thickness and H_2O_2 penetration. Pearson correlation coefficients were used as measures of bivariate association (Table 3). None of the four color change measures appeared to be correlated with hydrogen peroxide penetration. Noteworthy are the highly significant correlations of ΔL with baseline lightness ($r=-0.32$, $p<0.05$) and the correlation of Δa with baseline values of a* ($r=-0.42$, $p<0.05$). In addition, changes in the red-green color dimension (Δa) were strongly correlated with tooth thickness ($r=0.37$, $p<0.05$).

These bivariate correlations indicate that those teeth that were initially darker tended to show greater increases in lightness after bleaching treatment. In the case of the a* dimension, the changes were primarily negative, that is, toward the green end of the red-green dimensional scale. Those teeth that showed the greatest change tended to be those that had the highest baseline a* levels and the smallest tooth thicknesses.

Change in the blue-yellow color dimension (Δb) was also correlated with tooth thickness ($r=0.25$, $p<0.05$). These changes were also overwhelmingly negative, that is, shifted toward the blue end of the blue-yellow dimensional scale. Those teeth that showed the greatest change tended to be those that had the smallest tooth thicknesses.

The issue of group comparisons was therefore revisited in the context of multiple linear regression, which made it possible to reassess group differences after adjustment for covariates.

Linear modeling of the change in lightness (ΔL) showed that the increase in lightness tended to be greater for teeth with lower initial L* values ($r=-0.32$, $p<0.05$). After adjustment for initial L*, there was no evidence that ΔL differed with bleaching technique ($p>0.05$). The mean difference in ΔL between the two treatment groups was 0.36 lightness units. The adjusted (for baseline L) means for ΔL were 2.38 for the conventional bleaching group and 2.02 for the sealed bleaching group. No

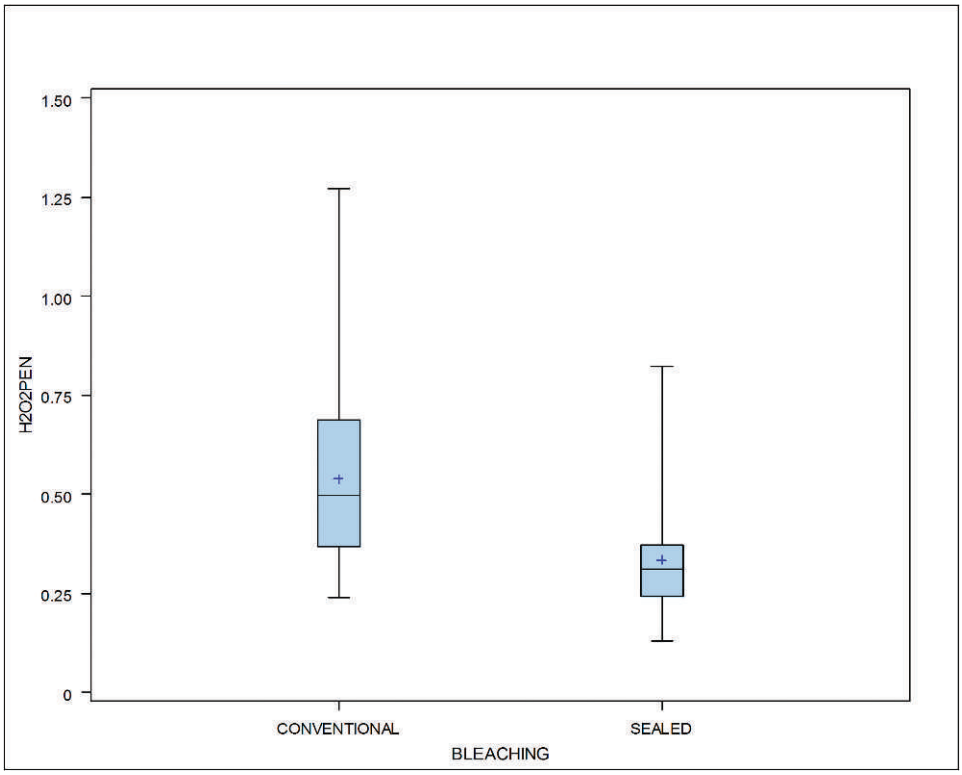

Figure 3. *Box plots for hydrogen peroxide penetration level by bleaching group.*

other covariate was significantly associated with change in lightness (Figure 4).

DISCUSSION

This study compared two in-office bleaching techniques, the conventional and sealed bleaching group, with respect to four color change parameters and hydrogen peroxide penetration levels. Following bleaching treatment, the two groups were similar in terms of color change relative to baseline. The mean overall color changes for the conventional and sealed bleaching groups were 3.60 and 3.11, respectively, which is considered to be discernible to the naked eye[10] and reflects the clinical relevance of this study.

The results indicated that the application of a 38% hydrogen peroxide gel for one hour without replenishment was as effective as three 20-minute applications. Similar results were obtained in an *in vitro* pilot study by Marson and others.[11] They reported no difference in lightness change after bleaching between a single 45-minute and three 15-minute applications. In their chemical analysis to quantify

Table 3: *Correlations of Color Change Parameters With Tooth Thickness, HPP, and baseline Color Measurements*[a]

	Tooth Thickness	HPP	L_1^*	a_1^*	b_1^*
ΔL	−0.03 (0.8169)	0.16 (0.1544)	−0.32 (0.0038)	0.41 (0.0001)	0.07 (0.5551)
Δa	0.37 (0.0009)	−0.02 (0.8935)	0.26 (0.0187)	−0.42 (<0.0001)	−0.22 (0.0469)
Δb	0.25 (0.0249)	0.09 (0.4135)	−0.04 (0.7388)	−0.02 (0.8477)	−0.17 (0.1325)
ΔE	−0.19 (0.0840)	0.12 (0.2757)	−0.29 (0.0104)	−0.39 (0.0004)	0.15 (0.1824)

Abbreviation: HPP, hydrogen peroxide penetration.
[a] *Pearson correlation coefficients, n=80, Prob>|r| under H_O:Rho=0,* p *value in parentheses.*

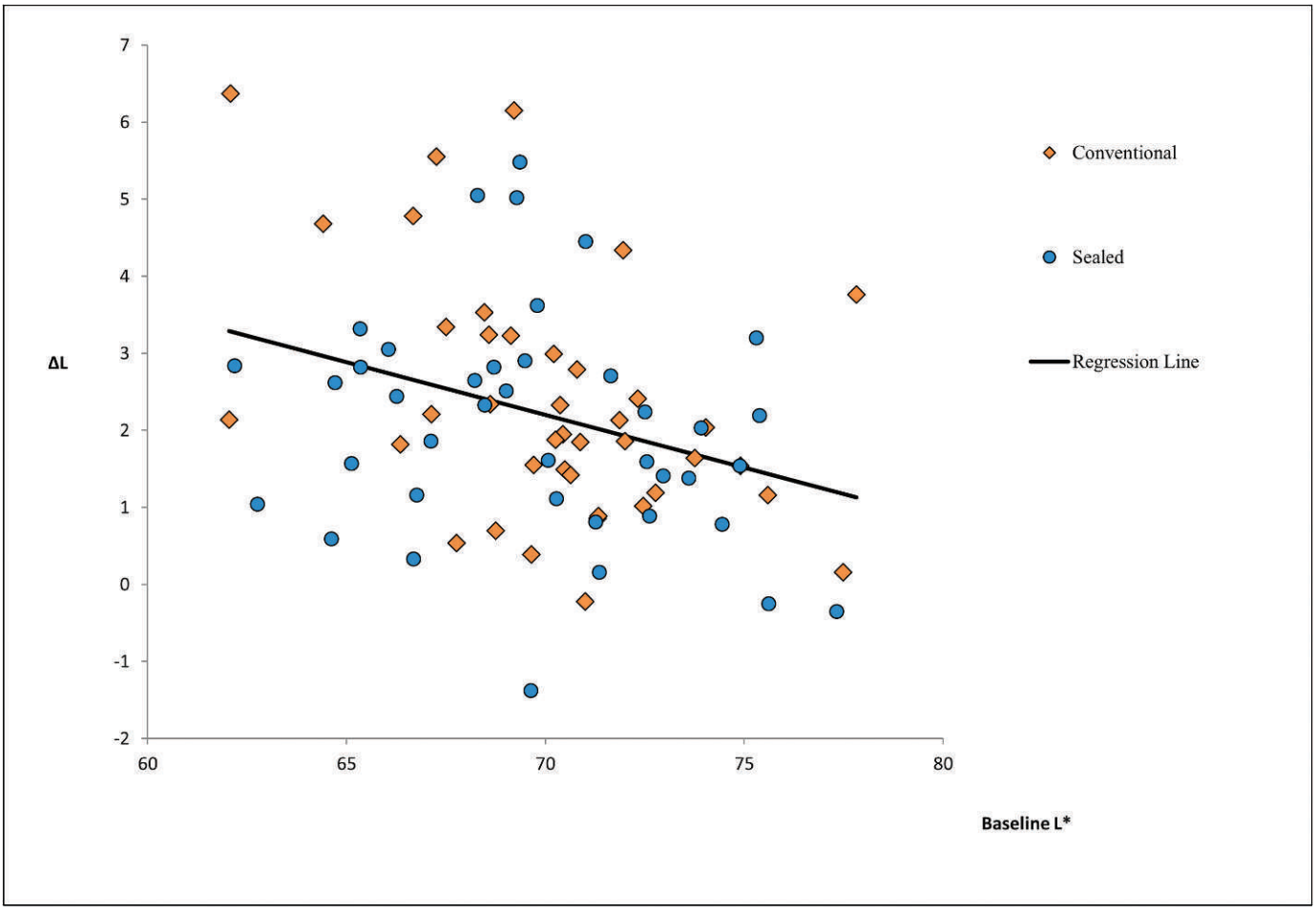

Figure 4. *Linear regression of change in lightness (ΔL) on baseline L*.*

the concentration of hydrogen peroxide as a function of time, they showed only a minor change from 34% at baseline to 29% after 40 minutes. This may suggest the rationale of not having to replenish the gel during a single in-office bleaching session.

However, Reis and others[12] reported contradictory results in their recent clinical trial. In-office bleaching was performed in 30 participants with 35% hydrogen peroxide. They reported superior bleaching results and less sensitivity with replenishment of the bleaching gel compared with a single prolonged application. The difference of these results may be explained by the use of the LLDPE used in our study as advocated in the sealed bleaching technique. The LLDPE wrap may prevent dehydration of the gel and rapid degradation of active agents in the gel, thus making replenishment less critical.

The two in-office bleaching methods were found to differ significantly in the level of hydrogen peroxide penetration following bleaching. In the conventional bleaching group, the mean level of H_2O_2 penetration was higher (ie, 0.54 μg). In the sealed bleaching group, the mean level of penetration was significantly lower, 0.33 μg. The mean hydrogen peroxide penetration levels of both groups are much lower than in a previous study by Bowles and Ugwuneri,[4] who applied 30% hydrogen peroxide for 15 minutes at 37°C and detected hydrogen peroxide levels of 25.4 ± 8.5 μg. This difference can be explained by the difference of cavity preparation, tooth selection, hydrogen peroxide delivery method, and temperature settings. First, the cavity preparation was more conservative in our study by encompassing a smaller amount of acetate buffer and confining the enlargement of the pulp cavity to the lingual side. Second, central and lateral incisors were used in the study by Bowles and Ugwuneri,[4] whereas bulkier canines were selected in this study. Third, the facial surfaces of teeth were immersed in liquid hydrogen peroxide at 37°C, which might have created hydrogen peroxide penetration by capillary action directly into the

pulp cavity rather than from diffusion from the outer surface in Bowles and Ugwuneri's study.

There are many factors affecting the hydrogen penetration level, and the levels seem to differ according to the experimental protocol employed. It is important to understand the clinical significance of hydrogen peroxide penetration into the pulp cavity and the possible risk associated with significant levels of hydrogen peroxide penetration. The threshold for pulpal enzyme inhibition, which was calculated to be in the range of 50 mg, explains why pulpal damage resulting from the clinical use of in-office bleaching procedures has been remarkably low.[4] However, considering the lack of knowledge of the effect of hydrogen peroxide penetration at the molecular level within the cell and connective tissue of the pulp,[13] it is challenging to assess the actual comparative clinical difference between the small values of recovered hydrogen peroxide in our study.

Bleaching involves a series of complex changes that alter a set of separate color parameters, of which L* is generally regarded as the primary one and also the most used to assess the effectiveness of a bleaching procedure.[14] Modeling of ΔL showed that it was not affected by other covariates except for initial lightness values, which seem to make it a consistent measure for evaluating bleaching efficacy. It is also noteworthy to point out the importance of taking initial baseline values into consideration when comparing different treatment groups in bleaching studies since they affect the amount of change in lightness (ΔL).

This *in vitro* model is representative of the *in vivo* process, although it is not known how closely it would compare to the *in vivo* absorption of hydrogen peroxide in teeth with vital pulps exhibiting positive pulpal pressure during the bleaching process.[4]

Another limitation of this study was that it did not consider the cumulative effect of color change with repeated conventional vs sealed bleaching technique. Although an *in vitro* study by Rosenstiel and others[15] has shown that color changes beyond the first in-office bleaching treatment were small, repeated bleaching with a different bleaching regimen might result in other findings.

This study explored the tooth color change and the amount of hydrogen peroxide penetration levels into the pulp cavity by comparing two different in-office bleaching treatments, and the findings supported the null hypothesis that the color change would not differ between the two techniques. However, there was a significant difference between hydrogen peroxide penetration levels, so the second null hypothesis had to be rejected. There was no correlation between penetration levels and tooth color change, which led to the acceptance of the third null hypothesis.

Based on these findings, further studies should be employed to evaluate the significance of hydrogen peroxide penetration levels at the molecular level of pulpal cells and the clinical significance of these penetration levels. Different bleaching agent concentrations and various delivery methods should be assessed regarding hydrogen peroxide penetration levels as well as color change and ultimately suggest a bleaching regimen with minimal hydrogen peroxide penetration and maximum bleaching efficacy.

CONCLUSION

Within the limitation of this study, the sealed bleaching technique compared with the conventional in-office technique exhibited lower hydrogen peroxide penetration levels without compromising bleaching efficacy in terms of all parameters in color change. Change in lightness was not affected by hydrogen peroxide penetration levels or bleaching techniques after adjustment for initial L*.

Acknowledgements

The authors would like to thank Ultradent Products Inc for kindly providing all bleaching materials used in this study.

Conflict of Interest

The authors of this article certify that they have no proprietary, financial, or other personal interest of any nature or kind in any product, service, and/or company that is presented in this article.

(Accepted 23 March 2012)

REFERENCES

1. 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.
2. Sulieman M (2005) An overview of bleaching techniques: 3. in-surgery or power bleaching *Dental Update* **32(2)** 101-108.
3. Kwon S (2007) The sealed bleaching technique *Aesthetic Dentistry Today* **1(3)** 18-22.
4. Bowles WH, Ugwuneri Z (1987) Pulp chamber penetration by hydrogen peroxide following vital bleaching procedures *Journal of Endodontics* **13(8)** 375-357.
5. Rotstein I, Torek Y, Lewinstein I (1991) Effect of bleaching time and temperature on the radicular pene-

tration of hydrogen peroxide *Endodontics and Dental Traumatology* **7(5)** 196-198.

6. Camargo SE, Cardoso PE, Valera MC, de Araujo MA, Kojima AN (2009) Penetration of 35% hydrogen peroxide into the pulp chamber in bovine teeth after LED or nd:YAG laser activation *European Journal of Esthetic Dentistry* **4(1)** 82-88.
7. Benetti AR, Valera MC, Mancini MN, Miranda CB, Balducci I (2004) *In vitro* penetration of bleaching agents into the pulp chamber *International Endodontic Journal* **37(2)** 120-124.
8. Camps J, de Franceschi H, Idir F, Roland C, About I (2007) Time-course diffusion of hydrogen peroxide through human dentin: clinical significance for young tooth internal bleaching *Journal of Endodontics* **33(4)** 455-459.
9. Mottola HA, Simpson BE, Gorin G (1970) Absorptiometric determination of hydrogen peroxide in submicrogram amounts with leuco crystal violet and peroxidase as catalyst *Analytical Chemistry* **42** 410-411.
10. Vichi A, Ferrari M, Davidson CL (2004) Color and opacity variations in three different resin-based composite products after water aging *Dental Materials* **20(6)** 530-534.
11. Marson FC, Sensi LG, Strassler H, Miraziz L, Riehl HL (2008) In-office bleaching gel-application time evaluation (3x15min X 1x45min): pilot studies *Journal of Dental Research* **87(Special Issue B)** Abstract #1027.
12. Reis A, Tay LY, Herrera DR, Kossatz S, Loguercio AD (2011) Clinical effects of prolonged application time of an in-office bleaching gel *Operative Dentistry* **36(5)** 1-7.
13. Cooper JS, Bokmeyer TJ, Bowles WH (1992) Penetration of the pulp chamber by carbamide peroxide bleaching agents *Journal of Endodontics* **18(7)** 315-317.
14. Wetter NU, Branco EP, Deana AM, Pelino JE (2009) Color differences of canines and incisors in a comparative long-term clinical trial of three bleaching systems *Lasers in Medical Science* **24(6)** 941-947.
15. Rosenstiel SF, Gegauff AG, McCafferty RJ, Johnston WM (1991) *In vitro* tooth color change with repeated bleaching *Quintessence International* **22(1)** 7-12.