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ORIGINAL RESEARCH

Dynamic Model of Hydrogen Peroxide Diffusion Kinetics into the Pulp Cavity

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ABSTRACT

Aim: To measure the time course hydrogen peroxide penetration into the pulp cavity and evaluate short-term tooth color changes after bleaching.

Materials and methods: Twenty extracted human canines were sectioned, pulp tissue removed and the cavity enlarged. Teeth were painted with nail varnish to leave a 6-mm diameter circle on the buccal surface. Baseline color was measured spectrophotometrically.

Teeth were randomized into a control group (n = 10) treated with 30 µl of glycerin base and a bleaching group (n = 10) exposed to 30 µl of 40% hydrogen peroxide for 1 hour. A linear low density polyethylene wrap was placed to prevent evaporation of the material. Acetate buffer was placed into the cavity and replenished every 10 minutes and placed into plastic tubes. Hydrogen peroxide amount was estimated spectrophotometrically using leukocrystal violet and horseradish peroxidase. Specimen color was remeasured immediately after bleaching, 1 hour, 1 day 1, 2 and 6 weeks postbleaching. Color change was measured per Commission Internationale de l'Eclairage methodology.

Mann-Whitney procedure was used to assess baseline color measurements and total hydrogen peroxide penetration amount. Friedman's test was used to assess within group differences for color change and hydrogen peroxide penetration.

Results: There was significantly greater hydrogen peroxide penetration in the bleaching group ($p < 0.05$). Hydrogen peroxide penetration levels were constant throughout the 1-hour evaluation period in the bleaching group. The groups showed no difference at baseline with respect to any of L*a*b color measurements ($p > 0.05$). The postbleaching color measurement showed an increase of change in overall color (ΔE) and lightness (ΔL) up to 1 week followed by a gradual stabilization up to 6 weeks.

Conclusion: This dynamic model provided information about the time course diffusion kinetics into the pulp cavity, demonstrating constant penetration of hydrogen peroxide into the pulp cavity during a 1-hour bleaching session.

Clinical significance: A prolonged application of 40% hydrogen peroxide bleaching material for 1 hour produces constant penetration of hydrogen peroxide into the pulp cavity *in vitro.*

Keywords: Bleaching, Color change, Diffusion, Hydrogen peroxide.

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INTRODUCTION

Tooth bleaching is a conservative and economic treatment option to enhance the esthetics of discolored teeth that has been practiced in dentistry for over 100 years. Thus, the safety and efficacy of this procedure has been well-established.[1]

In-office bleaching generally is preferred by both the dentist and patient in that less compliance is required on the patient's side.[2] This preference is reflected by the number of products and variation in techniques available on the market. Despite manufacturers' claims and some marketing statements, the comparison of results with regard to bleaching efficacy and color stability is difficult to assess.

Bleaching efficacy is related to the interaction of the bleaching material with the stain molecule and its ability to diffuse into the tooth structure. It has been proposed that oxygen and hydroxyl radicals released from hydrogen peroxide degrade and oxidize the complex ring structure of stain molecules to more simple chain structures, thus altering the light reflectance of the tooth.[3] Tooth structure is semipermeable and it has been shown that water soluble, low molecular weight molecules readily penetrate from the outer tooth surface into the pulp cavity through interprismatic spaces in the enamel and via dentinal tubules in the dentin.[4] The diffusion aspect of hydrogen peroxide

into the dentin has been investigated and suggested to follow Fick's second law of diffusion,[5] which is proportional to the surface area available for diffusion, diffusion coefficient, concentration and inversely proportional to the diffusion distance.

Significant penetration of hydrogen peroxide were detected in the pulp cavity after exposure to hydrogen peroxide in an *in vitro* model by Bowles and Ugwuneri.[6] Many studies adopting this model confirmed that diffusion of hydrogen peroxide follows Fick's second law. Penetration of hydrogen peroxide into the pulp cavity was affected by higher concentrations of hydrogen peroxide,[6] heat and prolonged bleaching time,[7] light activation,[8] and large open dentinal tubules of young teeth.[9]

However, the time course kinetics of hydrogen peroxide penetration into the pulp cavity has not been demonstrated and there is no standardized time protocol for the proper determination of postbleaching color measurement *in vitro*.

Thus, the purpose of this study was to introduce a dynamic *in vitro* model of hydrogen peroxide diffusion kinetics into the pulp cavity and determine the best time for postbleaching color measurements. The null hypotheses to be tested were that first, hydrogen peroxide penetration levels would not be constant during the 1 hour in-office bleaching regimen and second, there would be no significant time for color stabilization during the 6 weeks evaluation period.

MATERIALS AND METHODS

Sample Selection and Preparation

Twenty extracted human maxillary canines were collected prior to the study and stored in 0.1% thymol at 4ºC and transferred to vials with artificial saliva containing porcine gastric mucin, calcium chloride, potassium phosphate and *tris*-hydrochloride, at the time of the experiment.

All teeth were cleaned and observed for the absence of developmental anomalies, caries, existing restorations and deep crack lines. The roots were trimmed 3 mm apical to the cementoenamel junction and a standardized cavity prepared with a pointed taper diamond bur (NeoDiamond, Microcopy, Kennesaw, Georgia, USA) to encompass 25 µl of acetate buffer.

With the purpose of limiting the color reading area and creating a standardized bleaching area, a circular adhesive label 6 mm in diameter was adhered to the center of the buccal surface. The remaining tooth was painted with grey nail varnish (Sally Hansen, New York, NY, USA) and the adhesive label removed after drying, leaving a 6 mm diameter window on the tooth surface.

Bleaching Protocol

The canines were randomly assigned into a control group (CG) treated with 30 µl of a carbopol (Carbopol 940, Acros Organics, NJ, USA) and glycerin (Glycerin, Fisher Scientific, Fair Lawn, NJ, USA) mixture (0.1gm/10 ml), and a bleaching group (BG) exposed to 30 µl of 40% hydrogen peroxide gel (Opalescence Boost, Ultradent Products Inc South Jordan, UT, USA). A jig was fabricated by gently placing the lingual surface of each tooth into a polyvinyl siloxane putty impression material (Exaflex, GC America Inc., Alsip, IL, USA) at a 30 degrees angle from the base (Fig. 1).

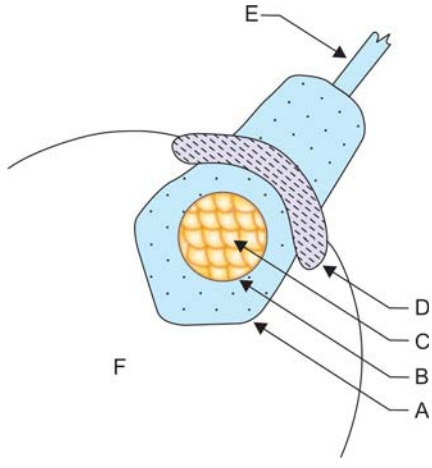

Fig. 1: Schematic representation of the dynamic model: (A) Nail varnish painted tooth, (B) standardized 6 mm diameter window for color measurement and bleaching, (C) bleaching material, (D) resin barrier, (E) hamilton syringe for retrieval and replenishment of acetate buffer, (F) polyvinyl siloxane jig

The bleaching material was applied onto the buccal window and covered with a linear low density polyethylene wrap (Saran Wrap, SC Johnson & Son, Inc. Racine, WI, USA) during the treatment 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.

Color Measurements

Instrumental color measurements were performed with a contact-type intraoral spectrophotometer (Vita Easyshade Compact, Vita Zahnfabrik, Bad Säckingen, Germany) with a 5-mm diameter probe. The Easyshade was calibrated according to manufacturer's instruction and mean L*, a*, b* values of each specimen was determined after three measurements.

Color measurements were taken at baseline (T_0), immediately after bleaching (T_1), 1 hour (T_2), 1 day (T_3),

1 week (T_4), 2 weeks (T_5) and 6 weeks (T_6) postbleaching. The color difference at different time intervals was measured as ΔE from the Commission Internationale de l'Eclairage. It was calculated from the following equation: $\Delta E = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$.

Measurement of Hydrogen Peroxide Penetration Levels

Hydrogen peroxide penetration levels were estimated according to the method of Mottola et al.[10] The acetate buffer was retrieved every 10 minutes and placed into plastic tubes. The pulp cavities were thoroughly rinsed twice with 25 µl of distilled water and the washes were added to the tubes. The pulp cavity was then replenished with fresh acetate buffer.

One milliliter of leucocrystal violet solution (0.5 mg/ml), 0.5 ml of horseradish peroxidase solution (1 mg/ml), and 4 ml of acetate buffer were added to the tubes and the total volume adjusted to 10 ml with distilled water. The final color intensity of the mixed solution was measured in an UV/visible spectrophotometer (Ultrospec 1100 pro, GE Healthcare, Waukesha, USA) at a wavelength of 596 nm. 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.

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 dependent measures of interest included hydrogen peroxide penetration levels. The nonparametric Mann-Whitney procedure was used to assess whether the two treatment groups differed at baseline with respect to L*, a*, and b* values and to evaluate group differences in color change and H_2O_2 penetration following treatment. The within group differences were evaluated with the Friedman's two-way analysis of variance by ranks and appropriate posthoc comparisons adjusting for multiple testing. All tests of hypotheses were conducted at a level of significance set at $\alpha = 0.05$. Analysis was conducted with SAS v 9.2 (SAS Institute, Cary, NC, USA).

RESULTS

Hydrogen peroxide penetration levels by group retrieved at 10 minutes interval overtime are shown in Table 1. The control group exposed to a carbopol and glycerin mixture gave only a barely detectable amount and based upon the Mann-Whitney U-test, the two groups significantly differed in the level of penetration at all time intervals ($p < 0.05$). There was no difference in the amount of penetration within the groups (Friedman's test $p > 0.05$), suggesting constant penetration of hydrogen peroxide during the 1 hour bleaching session in the bleaching group (Fig. 2).

Color change data (ΔE, ΔL and Δb) by group at different time intervals is given in Table 2.

The two groups were similar at baseline with respect to the L_1^*, a_1^* and b_1^* color values (Mann-Whitney U-test, $p > 0.05$).

Based upon the Mann-Whitney U-test, the groups differed for all color change measurements at all time points (T_1-T_6; $p < 0.05$) except for delta E immediately after bleaching ($p > 0.05$). Change in overall color (ΔE) and lightness (ΔL) tended to stabilize at 1 week after bleaching (Figs 3 and 4). This was indicated by the absence of statistical significance in comparison with 2 and 6 weeks postbleaching. Noteworthy was the increase of these changes up to 1 week after bleaching. Pairwise comparisons showed there were no significant differences among overall color changes (ΔE) at week 1, 2 and 6 ($p > 0.05$).

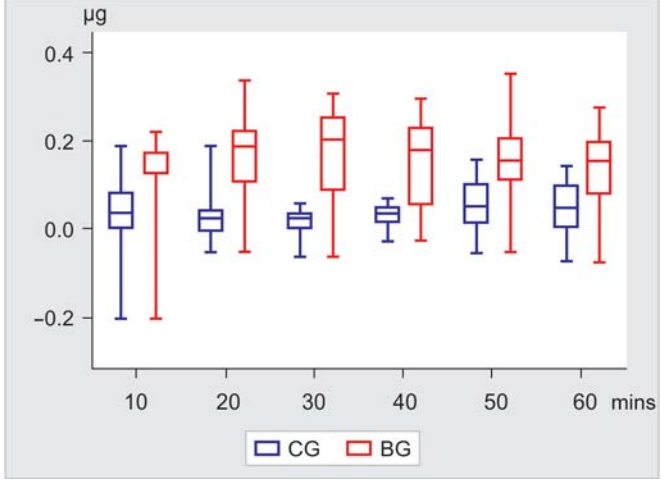

Fig. 2: Box plots showing hydrogen peroxide penetration by group overtime

Table 1: Hydrogen peroxide penetration level [mean/median (SD)] by group overtime

	10 mins	*20 mins*	*30 mins*	*40 mins*	*50 mins*	*60 mins*	*p-value***
CG	0.02/0.02 (0.1)	0.02/0.01 (0.06)	0/0.01 (0.03)	0.01/0.02 (0.03)	0.04/0.03 (0.06)	0.03/0.03 (0.06)	0.119
BG	0.12/0.11 (0.04)	0.17/0.16 (0.08)	0.18/0.17 (0.06)	0.16/0.17 (0.08)	0.16/0.16 (0.08)	0.14/0.16 (0.07)	0.07
p-value*	0.009	<0.001	<0.001	<0.001	0.002	0.002	

*Mann-Whitney U-test; ** Friedman's test; SD: Standard deviation

Table 2: Color change data (ΔE, ΔL and Δb), [mean/median (SD)] by group overtime

	T_1	T_2	T_3	T_4	T_5	T_6	*p-value***
CG (ΔE)	2.25/2.23 (0.90)	1.49/0.99 (1.10)	1.34/0.89 (1.07)	1.48/1.21 (1.24)	1.12/0.63 (1.21)	0.86/0.41 (1.27)	0.004
BG (ΔE)	2.51/2.41 (0.87)	2.99/2.69 (1.57)	3.68/3.31 (1.74)	4.06/3.61 (1.78)	3.74/3.15 (1.83)	4.13/3.76 (1.53)	<0.001
p-value*	0.579	0.035	0.001	0.002	0.001	<0.001	
CG (ΔL)	0.06/0.40 (1.71)	0.14/0.51 (1.02)	–0.47/–0.42 (0.94)	0.41/0.58 (0.66)	–0.13/0.07 (0.73)	–0.17/–0.05 (0.55)	0.069
BG (ΔL)	1.82/1.72 (1.11)	2.31/2.17 (1.47)	3.17/2.72 (1.72)	3.55/3.13 (1.79)	2.91/2.40 (1.99)	3.27/2.97 (1.80)	<0.001
p-value*	0.029	0.001	< 0.001	<0.001	<0.001	<0.001	
CG (Δb)	1.18/1.17 (1.07)	0.77/0.35 (1.12)	–0.02/–0.1 (1.29)	0.19/0.28 (1.75)	0.07/0.3 (1.45)	–0.47/0.17 (1.29)	0.003
BG (Δb)	–0.91/–0.70 (1.06)	–0.73/–0.85 (1.05)	–1.12/–0.98 (0.99)	–1.32/–1.52 (0.82)	–1.81/–1.85 (0.67)	–1.93/–1.97 (0.89)	<0.001
p-value*	0.001	0.009	0.015	0.009	0.002	0.002	

*Mann-Whitney U-test; **Friedman's test; SD: Standard deviation

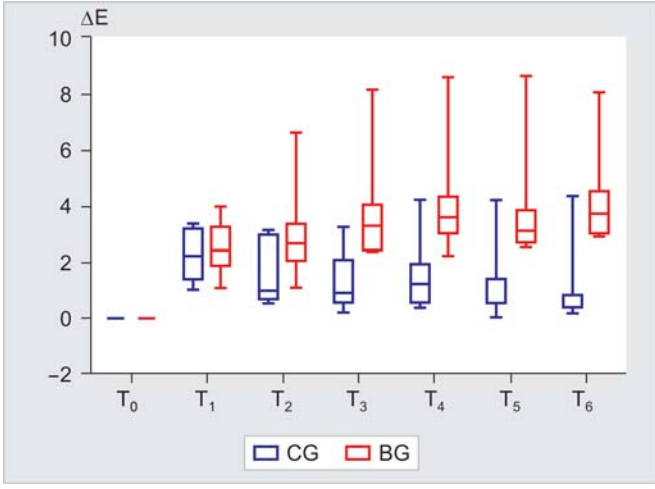

Fig. 3: Box plots showing overall color change (ΔE) by group overtime

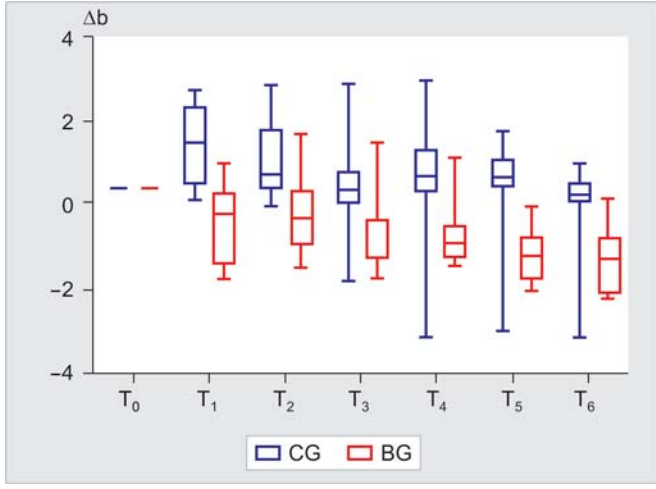

Fig. 5: Box plots showing change in the yellow-blue dimension (Δb) by group overtime

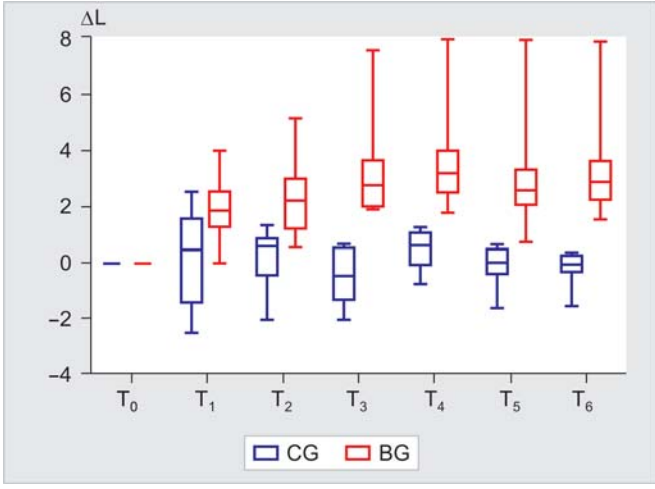

Fig. 4: Box plots showing change in lightness (ΔL) by group overtime

A statistically significant decrease in Δb was observed from baseline to 6 weeks postbleaching ($p < 0.05$). However, the amount of Δb for this time interval (Fig. 5) may not be detectable clinically (median: –1.15).

DISCUSSION

This study introduced a dynamic model to measure the time course diffusion kinetics of hydrogen peroxide into the pulp cavity and determine the short-term time needed for the color to stabilize after bleaching. This model is representative of the *in vivo* process, although it is partly limited by the absence of positive pressure with vital pulps.

There have been several *in vitro* studies assessing the hydrogen peroxide penetration levels and comparing these penetration levels to thresholds for pulpal enzyme inhibition.

The amount for pulpal enzyme inhibition was calculated to be 50,000 µg, which explains why the clinical use of in-office bleaching procedures is unlikely to cause significant damage to pulp tissues.[6] However, further *in vivo* studies are needed to address the effect of hydrogen peroxide penetration into the vital pulp cavity.

A recent *in vitro* study correlated hydrogen peroxide penetration amounts into the pulp cavity to tooth color change and reported that there was no correlation between the two factors.[11] Therefore, it seems prudent to minimize hydrogen peroxide penetration without compromising the bleaching efficacy.

The results of this study showed constant hydrogen peroxide penetration levels with a prolonged application of 40% hydrogen peroxide gel for 1 hour. This may suggest the rationale of not having to replenish the gel during a single in-office bleaching session as suggested by other colleagues.[11,12]

Numerous studies have assessed the efficacy of bleaching products, however, comparison between these data is difficult due to different study protocols, type of teeth, and the time of postbleaching measurements.[13]

The time for postbleaching measurement is important, since it can influence the proper interpretation of bleaching efficacy. As was shown in our study color measurements of the control and bleaching group did not differ immediately after bleaching ($p > 0.05$), which may be attributed to a dehydration component in the control group.

According to the ADA professional product review[14] on 'In-office whitening agents laboratory testing methods', tooth bleaching efficacy is determined by using a standardized staining broth to stain teeth and the postbleaching measurement is taken 24 hours after bleaching.

The International Standard (ISO 28399: 2011),[15] test method for laboratory assessment of tooth bleaching efficacy does not provide a specific postbleaching measurement time, but states that delta E after bleaching treatment should be two or greater for the product to be regarded as acceptable.

Considering the lack of standardized time protocol for the reporting of *in vitro* postbleaching measurements there seemed to be a need for establishing a cutoff time to report postbleaching measurements. Our model suggested that 1 week is the cutoff point for overall color change stabilization. Change in lightness, which is generally regarded as the most used parameter to assess bleaching efficacy also stabilized at 1 week after bleaching.

This study showed that there was a constant penetration of hydrogen peroxide during the 1 hour bleaching procedure, which led to the rejection of the first the null hypothesis. Change in overall color and lightness exhibited stabilization at 1 week so that the second null hypothesis was also rejected.

CONCLUSION

Within the limitation of this study, the dynamic model provided information about the time course diffusion kinetics of hydrogen peroxide into the pulp cavity. The following conclusions could be drawn:

1. There was constant penetration of hydrogen peroxide into the pulp cavity during a 1-hour bleaching session.
2. The postbleaching color measurements showed an increase in ΔE and ΔL up to 1 week followed by a gradual stabilization up to 6 weeks.

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