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Effective tooth-bleaching protocols capable of reducing H_2O_2 diffusion through enamel and dentine

Diana G. Soares [a], Fernanda G. Basso [b], Elaine C.V. Pontes [a], Lucas da F.R. Garcia [c], Josimeri Hebling [d], Carlos A. de Souza Costa [c,*]

[a] Department of Dental Materials and Prosthodontics, Araraquara School of Dentistry, Univ Estadual Paulista, Araraquara, SP, Brazil
[b] Department of Oral Diagnosis, Piracicaba Dental School, State University of Campinas – UNICAMP, Piracicaba, SP, Brazil
[c] Department of Physiology and Pathology, Araraquara School of Dentistry, Univ Estadual Paulista, Araraquara, SP, Brazil
[d] Department of Orthodontics and Pediatric Dentistry, Araraquara School of Dentistry, Univ Estadual Paulista, Araraquara, SP, Brazil

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ABSTRACT

Objectives: To evaluate the effects of experimental protocols on bleaching effectiveness and hydrogen peroxide (HP) diffusion through enamel and dentine.
Methods: Enamel/dentine discs were subjected to six bleaching sessions, consisting of 1 or 3 applications of 17.5% or 35%-HP gel for 5/15 min, or 37% carbamide peroxide (CP) gel for 10/20 min. Discs undergoing the regular protocol (35%-HP; 3 × 15 min) constituted the positive control group. Colour change (ΔE) was assessed (CIE $L^*a^*b^*$ system) after each session. HP diffusion was quantified (sessions 1, 3, and 6) in enamel/dentine discs adapted to artificial pulp chambers. Data were analysed by Pillai's Trace and Bonferroni test, or by one-way ANOVA and SNK/Tamhane's test ($\alpha = 5\%$).
Results: All tooth-bleaching protocols significantly increased the ΔE values. A reduction in HP diffusion and no significant difference in ΔE compared with the positive control were observed for the following bleaching protocols: 17.5%-HP 3 × 15 min, at the 4th session; and 35%-HP 1 × 15 and 3 × 5 min, at the 5th session. HP diffusion in the 37%-CP 3 × 20 min bleaching protocol was statistically similar to that in the positive control. The other experimental bleaching protocols significantly decreased HP diffusion through enamel/dentine discs, but the ΔE values were statistically lower than those observed in the positive control, in all sessions.
Conclusion: Shortening the contact time of a 35%-HP gel or reducing its concentration produces gradual tooth colour change and reduced HP diffusion through enamel and dentine.
Clinical significance: A reduction in HP concentration, from 35% to 17.5%, in a bleaching gel or shortening its application time on enamel provides a significant tooth-bleaching improvement associated with decreased HP diffusion across hard dental tissues. Therefore, these protocols may be an interesting alternative to be tested in the clinical situation.

* *Corresponding author at*: Department of Physiology and Pathology, Univ Estadual Paulista – UNESP, Araraquara School of Dentistry, Humaitá Street 1680, 14801-903 Araraquara, SP, Brazil. Tel.: +55 16 3301 6477; fax: +55 16 3301 6488.
E-mail address: casouzac@foar.unesp.br (C.A. de Souza Costa).
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1. Introduction

An at-home tooth-bleaching procedure with 10% carbamide peroxide (CP) gel has been considered the safest method for bleaching teeth with minimal adverse effects.[1–5] However, since this tooth-bleaching modality is patient-applied, there is a risk of gel application over exposed dentine in patients with gingival recession and abfraction/abrasion lesions. Also, the inadequate use of the tray may result in gel overflow, with extended soft-tissue exposure and likely material ingestion.[3,6] Therefore, it seems evident that this kind of aesthetic therapy should be performed entirely under professional supervision.

For the in-office tooth-bleaching technique, in which the procedure is performed by clinicians, bleaching gels with high concentrations (35–38%) of hydrogen peroxide (HP) are applied in a 30- to 45-min chair-side session.[1] This therapy allows for a clinically perceptible dental colour improvement even after one clinical appointment.[6–10] However, tooth sensitivity is a side-effect commonly reported in the literature when this technique is applied to vital teeth.[7–11] The low molecular mass of HP and its sub-products favours its diffusion through mineralized dental tissues to reach the pulp chamber.[12–14] The contact of pulp cells with these reactive oxygen species (ROS) results in oxidative stress generation, due, at least in part, to an imbalance between the amount of ROS and endogenous/exogenous antioxidants.[10,11] It has been shown that HP and its sub-products can reduce cell viability, as well as cause cell membrane damage and proteolytic enzyme activation, extracellular-matrix degradation, tissue inflammatory reaction, and even partial pulp necrosis.[11,15–22]

Previous studies demonstrated that the higher the concentration and the length of time of the bleaching agent application on enamel, the higher the HP penetration of the pulp chamber, and the more intense are the adverse effects to pulp cells.[15] Thus, changes in in-office tooth-bleaching techniques may provide an interesting alternative for maintaining bleaching effectiveness, while preventing or at least minimizing the negative effects of this kind of aesthetic therapy on pulp tissue. Therefore, the present study aimed to assess and correlate the bleaching effectiveness of different experimental in-office tooth-bleaching protocols and their capacity for HP diffusion through enamel and dentine.

2. Materials and methods

2.1. Sample preparation

Enamel/dentine discs (5.6 mm diameter, 3.5 mm thick) were obtained from the buccal surfaces of sound bovine central incisors (24–30-month-old bullocks).[9] An intrinsic stain model was carried out as described by Sulieman et al.[23] The dentine surfaces of experimental and control discs were etched with 37% phosphoric acid (3M ESPE, St. Paul, MN, USA) for 60 s. The acidic product was then rinsed with distilled water spray. The discs remained in contact with a standardized solution of black tea (Leão Jr S.A., Fazenda Rio Grande, PR, Brazil), produced by filtration of 2 g of tea in 100 mL of boiling water for 5 min, for 6 days at 37 °C. The enamel was polished with a low-speed rubber cup (KG Sorensen, Barueri, SP, Brazil) and pumice stone/water solution. This procedure was performed to remove any undesirable external staining caused by the 6-day immersion of the sample in black tea. The enamel/dentine discs were then immersed in distilled water for 6 days to remove any non-adhering dentine pigments.

2.2. Bleaching procedure

Three bleaching gels were evaluated: a 37% CP gel (Whiteness super; FGM, Joinville, SC, Brazil); a 35% HP gel (Whiteness HP; FGM); and a 17.5% HP gel, which was obtained by dilution of the 35%-HP gel in distilled water immediately before the bleaching procedure.[24] The gels were applied according to different protocols (Table 1). For each bleaching protocol, 6 sessions were performed within a 7-day interval. At the end of each session, the enamel surface was kept in contact with artificial saliva (3.9% monobasic potassium phosphate; 3.6% potassium chloride; 2% sodium chloride; 2% potassium chloride; 3.7% magnesium chloride; 0.2% phenochem; 10% natrosol gel; distilled water qsp), and the dentine surface was kept in a humid environment to prevent dehydration.[19]

2.3. Colour analysis

A colour readout was performed by a UV-VIS spectrophotometry (Spectro Guide 45/0, BYK-Gardner GmbH, Geretsried, Germany), following the CIE $L^*a^*b^*$ system, which consists of two axes, a^* and b^*, which have right angles and represent the dimension of colour. The third axis (L^*) represents lightness, corresponding to the amount of light reflected from the object, and is expressed in numeric values ranging from 0 (black) to 100 (white). ΔL represents the difference in lightness. The closer to zero, the darker the colour of the object. In turn, lighter samples present numeric values close to 100. Positive ΔL values indicate that the sample is lighter than the standard (baseline readout), with negative values indicating that the sample is darker than the standard. Discs with similar initial

Table 1 – Experimental groups description according to the gel formulation and application protocol.

Groups	Bleaching gel	Application protocol
G1 (negative control)	None	None
G2 (positive control)	35%-HP gel	3× 15 min
G3	35%-HP gel	1× 15 min
G4	35%-HP gel	3× 5 min
G5	35%-HP gel	1× 5 min
G6	17.5%-HP gel	3× 15 min
G7	17.5%-HP gel	1× 15 min
G8	17.5%-HP gel	3× 5 min
G9	17.5%-HP gel	1× 5 min
G10	37%-CP gel	3× 20 min
G11	37%-CP gel	1× 20 min
G12	37%-CP gel	3× 10 min
G13	37%-CP gel	1× 10 min

L* values were randomly distributed into the control and experimental groups ($n = 8$) to obtain standardized samples. The discs were adapted in a white silicone matrix, with only the enamel surface left exposed.[15] The spectrophotometer was positioned over the enamel surface, and colour readouts were performed before bleaching (baseline), 24 h after each session, and 30 days after the last session. The mean values of each coordinate were calculated, and the colour change (ΔE) between values obtained in the baseline and subsequent readouts was calculated according to the following equation: $\Delta E = ((\Delta L)^2 + (\Delta a)^2 + (\Delta b)^2)^{1/2}$, where: ΔE = colour change; $\Delta L = L_{Final} - L_{Initial}$; $\Delta a = a_{Final} - a_{Initial}$ and $\Delta b = b_{Final} - b_{Initial}$. $\Delta E \geq 3.3$ were considered perceptible to the naked eye.[25]

2.4. *HP diffusion quantification*

To quantify the amount of HP capable of diffusing through enamel and dentine substrates for each bleaching protocol, we mounted enamel/dentine discs in artificial pulp chambers (APC).[15] Each disc was placed between 2 silicon o-rings in the upper compartment of the APC, which was individually positioned in a well of a 24-well plate containing 1 mL of acetate buffer. The dentine surface was maintained in direct contact with the buffer, and the enamel was exposed to receive the bleaching agents. Immediately after the bleaching procedures, an aliquot (100 μL) of the buffer was transferred to tubes containing 250 μL of leucocrystal violet (0.5 mg/mL, Sigma–Aldrich Corp., St. Louis, MO, USA) and 50 μL of horseradish peroxidase enzyme solution (1 mg/mL, Sigma–Aldrich Corp.). The final volume of the reaction was adjusted to 3 mL with distilled water. Then, three 100-μL aliquots of each tube were transferred to 96-well plates, and the optical density of the solutions was measured at a 600-nm wavelength in an ELISA plate reader (Tp Reader, Thermoplate, Nanshan District, Shenzhen, China). A standard curve of known HP concentrations was used for conversion of the optical density obtained in the samples into μg of HP, and the data were related to μg *per* mL of acetate buffer solution.

2.5. *Statistical analysis*

To analyse the effects of the bleaching protocols through the different time-points (sessions), we performed a Profile Analysis. When the flatness and parallelism hypotheses were rejected, Pillai's Trace supplemented by the Bonferroni test (pairwise comparison) was used. The mean values of the bleaching protocols in each session were analysed by one-way ANOVA supplemented by SNK or Tamhane's test. All analyses were performed at the 5% significance level. SPSS 19.0 software (SPSS, Inc., Chicago, IL, USA) was used to run the statistical analysis.

3. Results

3.1. *Bleaching effectiveness*

The graphic representation of Δa, Δb, ΔL, and ΔE according to the different tooth-bleaching sessions is shown in Fig. 1. When each experimental group was analysed during the sessions, a significant reduction in Δa values was observed for G2 (sessions 3–6), G4 (session 6), G3 (session 6), and G6 (sessions 5–6) ($p < 0.05$). No significant difference was observed in Δb through the sessions for all experimental groups ($p > 0.05$). All groups presented a significant increase in mean ΔL values, which mainly governed the overall colour change (ΔE). Such a gain in lightness may be considered as 'test specimen bleaching', since positive ΔL values mean a trend towards white.

The ΔL and ΔE results are described in detail in Table 2. The positive control group (G2) presented the highest ΔE and ΔL values. When this group was analysed through the sessions, significant increases in ΔE and ΔL were observed until the 4th session ($p < 0.05$). Otherwise, the experimental protocols promoted a gradual colour change through all sessions (Table 2, rows). When the groups were compared in each session (Table 2, columns), no significant difference with G2

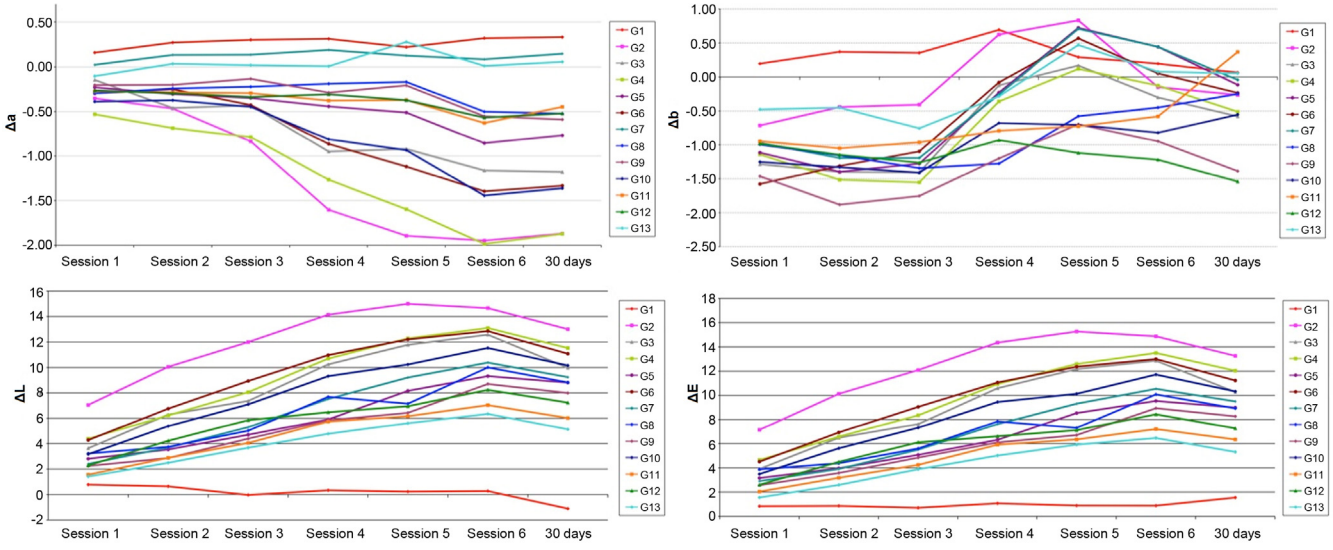

Fig. 1 – Graphic representation of variations in Δa, Δb, ΔL, and ΔE mean values through the different sessions and 30 days thereafter.

Table 2 – Mean values and standard deviation for ΔE and ΔL of the different bleaching protocols at each session (S) and 30 days after the last session.

Protocol	S1	S2	S3	S4	S5	S6	30 d	S1	S2	S3	S4	S5	S6	30 d
	ΔE							ΔL						
(G1) negative control	0.84 (0.14)^{Aa}	0.86 (0.37)^{ABa}	0.70 (0.42)^{ABa}	1.07 (0.48)^{ABa}	0.89 (0.40)^{ABa}	0.88 (0.41)^{ABa}	1.55 (0.41)^{Ba}	0.78 (0.17)^{Aa}	0.64 (0.29)^{Aa}	−0.03 (0.63)^{Aa}	0.33 (0.43)^{Aa}	0.23 (0.48)^{Aa}	0.27 (0.76)^{Aa}	−1.11 (0.80)^{Aa}
(G2) 35%-HP gel 3× 15 min	7.14 (0.83)^{Ab}	10.13 (1.09)^{Bb}	12.09 (1.40)^{Cb}	14.36 (1.36)^{Db}	15.27 (1.27)^{DEb}	14.88 (1.30)^{DEFb}	13.26 (1.62)^{CDFb}	7.05 (0.81)^{Ab}	10.05 (1.07)^{Bb}	12.00 (1.40)^{Cb}	14.15 (1.32)^{Db}	15.00 (1.23)^{Db}	14.67 (1.30)^{Db}	13.01 (1.57)^{Db}
(G3) 35%-HP gel 1× 15 min	3.91 (0.98)^{Ac}	6.49 (0.96)^{Bcd}	7.62 (0.96)^{Cc}	10.59 (1.19)^{Dc}	12.17 (1.78)^{Ebc}	12.86 (1.06)^{Ebc}	10.28 (1.24)^{Dbc}	3.64 (1.03)^{Acd}	6.29 (0.88)^{Bceg}	7.37 (0.96)^{Bcf}	10.24 (0.94)^{CEc}	11.78 (1.54)^{CDbc}	12.58 (0.97)^{Dbd}	9.97 (1.14)^{Ecd}
(G4) 35%-HP gel 3× 5 min	4.65 (1.12)^{Ac}	6.57 (1.43)^{Bcd}	8.35 (1.87)^{Cc}	10.94 (2.10)^{Dbc}	12.59 (1.72)^{Ebc}	13.50 (1.03)^{DEbc}	12.03 (1.04)^{DEb}	4.38 (0.92)^{Ac}	6.23 (1.24)^{Bceg}	8.06 (1.73)^{Ccd}	10.70 (1.91)^{Dce}	12.29 (1.61)^{Ebc}	13.10 (1.00)^{DEb}	11.53 (1.07)^{DEc}
(G5) 35%-HP gel 1× 5 min	3.16 (0.46)^{Ad}	3.97 (0.44)^{Ag}	5.07 (0.25)^{Bdf}	6.32 (0.39)^{Cdf}	8.54 (1.37)^{CDd}	9.54 (1.36)^{Dd}	8.99 (1.20)^{Dcdg}	2.81 (0.45)^{Adef}	3.54 (0.22)^{Ad}	4.70 (0.29)^{Bde}	5.91 (0.69)^{Cdf}	8.16 (1.14)^{CEde}	9.32 (1.20)^{Ece}	8.81 (1.12)^{Edef}
(G6) 17.5%-HP gel 3× 15 min	4.51 (0.97)^{Ac}	6.93 (0.92)^{Bc}	9.05 (1.15)^{Cc}	11.06 (1.67)^{Dbc}	12.36 (1.69)^{Ebc}	13.00 (0.96)^{DEbc}	11.23 (1.25)^{DEbd}	4.28 (0.78)^{Ac}	6.76 (0.94)^{Beg}	8.93 (1.17)^{Cc}	10.97 (1.61)^{bc}	12.21 (1.61)^{Ebc}	12.86 (0.88)^{DEb}	11.08 (1.19)^{DEc}
(G7) 17.5%-HP gel 1× 15 min	2.90 (0.86)^{Adf}	3.91 (0.47)^{Ag}	5.50 (0.57)^{Bdf}	7.62 (1.20)^{Cedf}	9.31 (1.24)^{Dc}	10.54 (0.94)^{Dcd}	9.50 (0.76)^{CDcdg}	2.40 (0.30)^{Aef}	3.69 (0.42)^{Bd}	5.29 (0.53)^{Cde}	7.52 (1.22)^{Ddef}	9.21 (1.27)^{Ecdf}	10.38 (0.81)^{Ece}	9.24 (0.68)^{Ede}
(G8) 17.5%-HP gel 3× 5 min	3.27 (0.70)^{Acd}	4.38 (0.78)^{Aeg}	5.60 (0.87)^{Bdf}	7.84 (1.51)^{Ccdf}	8.31 (1.17)^{BCDde}	10.08 (1.39)^{Ede}	8.92 (1.20)^{Dcdf}	3.22 (0.36)^{Ade}	3.75 (0.64)^{Ad}	5.02 (0.74)^{Bde}	7.69 (1.50)^{Ccdf}	7.15 (1.13)^{CEde}	10.00 (1.38)^{Dcde}	8.82 (1.19)^{Edef}
(G9) 17.5% HP-gel 1× 5 min	2.56 (0.39)^{Af}	3.58 (1.18)^{Afg}	4.84 (1.47)^{Bdf}	6.10 (0.92)^{BDdf}	7.70 (0.61)^{BDde}	8.94 (0.95)^{Ce}	8.27 (0.80)^{CDcef}	2.24 (0.67)^{Afg}	2.86 (1.33)^{Aadf}	4.39 (1.48)^{Be}	5.84 (0.96)^{Bdf}	6.43 (0.76)^{BCef}	8.70 (0.84)^{CDce}	7.99 (0.83)^{BCef}
(G10) 37%-CP gel 3× 20 min	3.49 (0.81)^{Ade}	5.61 (0.64)^{Bcd}	7.32 (1.48)^{Ccd}	9.45 (2.10)^{CDcd}	10.13 (2.11)^{Dc}	11.72 (1.84)^{Ebde}	10.33 (1.71)^{Dbde}	3.17 (0.64)^{Ade}	5.38 (0.53)^{Befg}	7.10 (1.31)^{Ccde}	9.31 (2.09)^{CDcd}	10.23 (2.05)^{Dcde}	11.53 (1.77)^{Ebc}	10.16 (1.71)^{Dcd}
(G11) 37%-CP gel 1× 20 min	2.04 (0.62)^{Afg}	3.18 (0.83)^{Bfg}	4.24 (1.79)^{ABCDf}	5.91 (1.51)^{Cdf}	6.35 (1.71)^{CDde}	7.20 (2.08)^{Cde}	6.35 (1.19)^{CDf}	1.56 (0.43)^{Agh}	2.88 (0.79)^{Bd}	4.05 (1.80)^{Bef}	5.74 (1.56)^{Cdf}	6.16 (1.76)^{CDef}	7.04 (2.13)^{De}	6.02 (1.36)^{CDgh}
(G12) 37%-CP gel 3× 10 min	2.60 (0.58)^{Af}	4.49 (1.02)^{Beg}	6.11 (1.80)^{BCcdf}	6.61 (2.63)^{BCcdf}	7.12 (2.92)^{BCde}	8.43 (2.20)^{Ccde}	7.27 (1.47)^{Cefg}	2.28 (0.62)^{Afg}	4.22 (1.01)^{Bcdg}	5.85 (1.82)^{BCcde}	6.48 (2.67)^{BCcdf}	6.95 (2.98)^{CDcdf}	8.24 (2.27)^{Dcde}	7.23 (1.64)^{CDfg}
(G13) 37%-CP gel 1× 10 min	1.56 (0.66)^{Ag}	2.59 (0.73)^{Bf}	3.88 (1.59)^{BCDfg}	5.01 (1.66)^{BCDf}	5.92 (2.32)^{BCDde}	6.47 (2.48)^{Cde}	5.31 (2.38)^{ACDafg}	1.41 (0.55)^{Aah}	2.49 (0.69)^{Ad}	3.68 (1.43)^{BCDde}	4.77 (1.56)^{BCDf}	5.61 (2.19)^{BCDde}	6.35 (2.40)^{Ce}	5.12 (2.34)^{Dh}

Values represent mean (standard deviation), $n = 8$.
Different uppercase letters in lines (Bonferroni test) and lowercase letters in columns (SNK or Tamhane's test) indicate statistically significant difference among groups ($p < 0.05$).

Table 3 – Data of HP diffusion quantification through enamel and dentine discs for the different bleaching protocols at the sessions (S).

Protocol	S1	S3	S6
(G1) Negative Control	n.d.	n.d.	n.d.
(G2) 35%-HP gel 3× 15 min	1.84 (0.20)^{a}	1.82 (0.39)^{a}	2.26 (0.65)^{a}
(G3) 35%-HP gel 1× 15 min	0.79 (0.21)^{b}	0.75 (0.18)^{b}	0.95 (0.09)^{b}
(G4) 35%-HP gel 3× 5 min	0.77 (0.25)^{b}	0.68 (0.34)^{bd}	0.84 (0.22)^{b}
(G5) 35%-HP gel 1× 5 min	0.28 (0.05)^{ce}	0.35 (0.12)^{cde}	0.58 (0.08)^{c}
(G6) 17.5%-HP gel 3× 15 min	0.73 (0.06)^{b}	0.72 (0.27)^{b}	0.98 (0.27)^{b}
(G7) 17.5%-HP gel 1× 15 min	0.35 (0.11)^{ce}	0.25 (0.09)^{cd}	0.32 (0.08)^{df}
(G8) 17.5%-HP gel 3× 5 min	0.36 (0.09)^{ce}	0.30 (0.10)^{cde}	0.36 (0.06)^{df}
(G9) 17.5% HP-gel 1× 5 min	0.10 (0.05)^{d}	0.15 (0.03)^{df}	0.13 (0.01)^{e}
(G10) 37%-CP gel 3× 20 min	1.53 (0.31)^{a}	1.39 (0.28)^{a}	1.36 (0.37)^{a}
(G11) 37%-CP gel 1× 20 min	0.73 (0.04)^{b}	0.70 (0.23)^{b}	0.68 (0.22)^{bd}
(G12) 37%-CP gel 3× 10 min	0.79 (0.18)^{b}	0.85 (0,. 4)^{b}	0.76 (0.12)^{b}
(G13) 37%-CP gel 1× 10 min	0.21 (0.06)^{de}	0.24 (0.06)^{cf}	0.23 (0.07)^{ef}

n.d. = not detected.
Values represent mean (standard derivation), $n = 8$.
Different lowercase letters in lines (SNK or Tamhane's test) indicate significant difference among groups ($p < 0.05$).

for ΔE and ΔL values was observed for G6, at the 4–6th sessions; for G3 and G4, at the 5–6th sessions; and for G10, at the 6th session ($p > 0.05$). The other experimental groups presented ΔE and ΔL values significantly lower than those of G2 in each session. Colour change perceptible to the naked eye ($\Delta E \geq 3.3$) was observed after the 1st session for G2, G3, G4, G6, and G10; after the 2nd session for G5, G7, G8, G9, and G12; and after the 3rd session for G11 and G13. Analysis 30 days after the last session demonstrated decreased ΔE and ΔL values for all groups, which were significantly lower than those of the 6th session only for ΔL in the groups G3, G8, G10, and G13 ($p < 0.05$).

3.2. *HP diffusion quantification*

The results for HP diffusion quantification are presented in Table 3. No HP was detected in the negative control group, which was disregarded for statistical analysis. There were no significant differences in HP diffusion among the sessions in all groups ($p > 0.05$). However, significant differences were found among the bleaching protocols ($p < 0.05$). The highest values of HP diffusion through enamel and dentine were observed in G2 and G10, with no significant difference between them ($p > 0.05$). The experimental groups presented significantly lower HP diffusion than did groups G2 and G10. Considering the positive control group (G2) as having 100% of HP diffusion, groups G3, G4, G6, G11, and G12 presented around 60% less HP diffusion, with no significant differences among them ($p > 0.05$). Similar results were also found among groups G7, G8, and G13, which presented around 85% less diffusion than the positive control group. The smallest HP values were found for G9 (around 95% less than G2) and G13 (around 88% less than G2).

4. Discussion

Currently, post-operative sensitivity claimed by patients subjected to tooth-bleaching therapies is believed to be a consequence, at least in part, of HP diffusion through enamel and dentine into the pulp chamber. This phenomenon results in pulp inflammation, with release of inflammatory mediators and pulp sensory nerve stimuli.[16,18] In some cases, the sensitivity is so intense that treatment is discontinued.[7,11] Therefore, a reduction in enamel/dentine-HP diffusion and, consequently, its penetration into the pulp chamber is required to prevent, or at least decrease, the oxidative damage caused by HP and its toxic sub-products to pulp tissue. To prevent or minimize transenamel and transdentinal HP diffusion, in the present study, the authors decreased the bleaching gel-HP concentration from 35% to 17.5%, as well as reduced the application time of the product on enamel. The data obtained were compared with those from the condition in which a bleaching gel with a high concentration of CP was used. Colour analysis was performed to demonstrate the bleaching potential for each tested protocol through 6 sessions.

The traditional in-office protocol (35%-HP gel; 3× 15 min) was used in the present study as the positive control, since tooth-bleaching effectiveness[7,8,10] and transenamel and transdentinal toxicity[15,19,20] have been well-demonstrated in the literature. Despite the rapid saturation of dentine chromophores and a significant visual impact noted immediately after the first bleaching session, which corroborated the results from previous studies,[7,8,10] the highest HP diffusion occurred in this positive control group. Therefore, it may be suggested that, *in vivo*, the large number of non-reacted toxic molecules present in dentine is capable of reaching the pulp space[12,13] to cause tooth sensitivity[7–10] associated with intense inflammatory pulp reaction[18] or even partial necrosis in this specialized connective tissue.[21]

When the contact time of the 35%-HP gel with enamel was reduced to 15 min (1× 15 min; 3× 5 min), a bleaching pattern similar to that of the traditional protocol (positive control) was obtained at the 5th session, with the advantage of reducing HP diffusion across enamel and dentine by 60%. Likewise, when the 5-min protocol was performed, HP diffusion decreased by around 85%; however, even after 6 sessions, the colour alteration did not reach the positive control parameters. In a recent study, Soares et al.[15] demonstrated that shortening the contact time of a 35%-HP gel with enamel to 15 min

(1× 15 min; 3× 5 min) caused significantly less HP diffusion through enamel/dentine discs, with a consequent decrease in toxicity to cultured odontoblast-like cells compared with that of the traditional in-office bleaching procedure (3× 15 min). The 35%-HP bleaching gel applied for only 5 min to enamel increased ALP activity, which is an enzyme that plays an important role in dentine matrix deposition. This finding indicates that a low concentration of HP in contact with pulp cells may stimulate differentiation and deposition of reactionary dentine matrix protein.

The reduction of HP concentration in the bleaching agent from 35% to 17.5%, which was applied to enamel for 45 min (3× 15 min – 4 sessions), caused the same bleaching pattern of colour change as observed in the positive control group. Also, a 60% reduction in HP diffusion through dental structure was observed. So, it may be assumed that by subjecting tooth structure gradually to less HP, the amount of non-reacted HP on tooth structure would be reduced, with a consequent decrease in HP and its sub-products capable of diffusing across enamel and dentine substrates. The effectiveness of gels with low concentrations of HP was previously evaluated by Sulieman et al.,[24] who used teeth subjected to the same darkness process as used in the present study. Those authors reported that 1, 2, 4, 7, and 12 bleaching sessions (3× 10 min) were required to obtain an optimal shade outcome (B1-VITA Classic Shade Guide) when gels with 35, 25, 15, 10, and 5% HP concentrations were used, respectively. Many current clinical studies have also demonstrated that bleaching gels with 15–20% of HP in their composition, when applied to enamel for 45–60 min, were capable of promoting a significant colour change, similar to that caused by a 35%-HP bleaching gel. In these studies, the incidence of tooth sensitivity ranged from 24% to 78%, which was considered as mild in severity.[9,26,27]

Gels with high concentrations of CP are encouraged for bleaching teeth in a chair-side session, combining the advantages of both in-office and at-home tooth-bleaching techniques, chiefly, slow and gradual release of HP under a controlled environment.[28] In the present investigation, a 37%-CP gel was evaluated following the manufacturer's instructions (3× 20 min), and an aesthetic outcome similar to that in the positive control group was observed only after 6 bleaching sessions. These data are similar to those provided previously by Patel et al.,[28] who observed a slight colour alteration after a single 30-min session using a 35%-CP gel. It is known that 37%-CP gel has an HP concentration similar to that of the 17.5%-HP gel used in the present study. When both bleaching gels were applied following the traditional protocols (G6 and G10), no significant difference between them was observed until the 5th session. However, components released from 17.5%-HP resulted in higher ΔE values compared with those achieved from the 37%-CP gel. Previous studies showed that CP and HP gels with similar final concentrations applied to enamel for the same period yielded similar whitening effects.[29,30] However, in these studies, a long-time treatment (up to 14 days) was carried out, which may explain the better results found for the CP gels compared with those of the present study. In this way, in the present investigation, the method of whitening gel delivery (CP × HP) influenced the tooth-bleaching effect. Other *in vitro* and *in vivo* studies also demonstrated significant colour alteration when gels with high concentrations of CP were applied daily for up to 2 weeks.[31,32] Therefore, it seems that, to be effective, the gels with high concentrations of CP should be applied to enamel for long periods of time, as required for at-home bleaching therapies. However, since the increased CP concentration causes higher toxic effects in the pulp cells,[2,4,5] with no benefits in the final aesthetic outcome,[31] it appears that there is no advantage in performing at-home bleaching therapy with this kind of gel. Additionally, the use of this product for in-office tooth-bleaching therapy may be considered unsuitable, since a long-time application of the gel to enamel is necessary to produce only a slight colour alteration.

To assess colour stability over time, we evaluated the tooth bleaching achieved after the last session for each group 30 days later. The colour rebound observed in all the experimental and control groups were neither significant nor clinically perceptible ($\Delta E \leq 3.3$). Moreover, L^* coordinate variation appeared to be the most significant parameter in global colour change, because the increase in lightness (whitening) may be interpreted as dental structure bleaching, as previously demonstrated.[23,24,31] Therefore, it may be speculated that the quantity of oxidized molecules present in dentine structure after bleaching was reduced, since previous researchers showed that organic substances in artificial saliva may contribute to this process.[33]

In the present investigation, the application of 35%-HP gel to enamel for only 15 min, or 17.5%-HP gel for 45 min (3× 15 min), caused a gradual colour alteration through the sessions. In both conditions, the optimal shade outcome was achieved with a few more bleaching sessions, which reduced by 60% the transenamel and transdentinal HP diffusion. As the amount of HP that diffuses through enamel and dentine is directly related to the toxic effects to the pulp cells,[15] one can expect that these bleaching protocols would be less toxic to the pulp cells. However, since this study used an *in vitro* model, the data obtained must be interpreted with caution. Vital teeth present a continuous outward dentine fluid movement through dentinal tubules. Conversely, extracted teeth devoid of dentinal fluid allow for a rapid inward movement of HP and its sub-products through enamel and dentine, which favours the tooth-bleaching outcome as well as intensifies HP diffusion.[24] Also, in this study, the intrinsic stain model used only black tea, which constitutes a limitation to this *in vitro* study, because, in clinical situations, the intrinsic discolouration of teeth is caused by a combination of different chromogens that have been accumulated over a long period with different binding characteristics to the enamel and dentine, which, in turn, may result in diverse responses to the bleaching procedure.[23] In addition, it is known that human teeth are more permeable to HP than are bovine teeth.[12] Therefore, one can suggest that the same bleaching protocols evaluated in the present study may result in a greater HP diffusion in human teeth, increasing the risks of damage to pulp tissue. Thus, *in vivo* studies in vital human teeth are needed to assess tooth-bleaching effectiveness as well as pulp responses after the application of the bleaching protocols successfully evaluated in the present study.

5. Conclusions

Based on the data obtained in the present study, it can be concluded that the application of 35%-HP gel to enamel for only 15 min or 17.5%-HP gel for 45 min (3× 15 min) produces gradual tooth colour change and increases its lightness. Both protocols, which presented bleaching performance similar to that of the traditional in-office protocol (35%-HP gel – 3× 15 min), significantly reduced HP diffusion through enamel and dentine. The 37%-CP gel presented the worst tooth-bleaching performance, requiring up to 6 sessions (60 min each) to achieve a tooth-bleaching effect similar to that of the in-office protocol, with no reduction in HP diffusion.

Conflict of interest

The authors have no conflict of interest.

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