

Penetration of 38% Hydrogen Peroxide into the Pulp Chamber in Bovine and Human Teeth Submitted to Office Bleach Technique

Samira Esteves Afonso Camargo, MSc,* Marcia Carneiro Valera, MSc, PhD,[†]
 Carlos Henrique Ribeiro Camargo, MSc, PhD,[†] Maria Nadir Gasparoto Mancini, MSc, PhD,[‡]
 and Marcia Maciel Menezes, MSc, PhD[§]

Abstract

This study evaluated the pulp chamber penetration of peroxide bleaching agent in human and bovine teeth after office bleach technique. All the teeth were sectioned 3 mm apical of the cement-enamel junction and were divided into 2 groups, A (70 third human molars) and B (70 bovine lateral incisors), that were subdivided into A1 and B1 restored by using composite resin, A2 and B2 by using glass ionomer cement, and A3 and B3 by using resin-modified glass ionomer cement; A4, A5, B4, and B5 were not restored. Acetate buffer was placed in the pulp chamber, and the bleaching agent was applied for 40 minutes as follows: A1–A4 and B1–B4, 38% hydrogen peroxide exposure and A5 and B5, immersion into distilled water. The buffer solution was transferred to a glass tube in which leuco crystal violet and horseradish peroxidase were added, producing a blue solution. The optical density of the blue solution was determined by spectrophotometer and converted into microgram equivalents of hydrogen peroxide. Data were submitted to analysis of variance and Dunnett, Kruskal-Wallis, and Tukey tests (5%). A higher level of hydrogen peroxide penetrated into the pulp chamber in resin-modified glass ionomer cements in bovine ($0.79 \pm 0.61 \mu\text{g}$) and human ($2.27 \pm 0.41 \mu\text{g}$) groups. The bleaching agent penetration into the pulp chamber was higher in human teeth for any experimental situation. The penetration of the hydrogen peroxide depends on restorative materials, and under the conditions of this study human teeth are more susceptible to penetration of bleaching agent into the pulp chamber than bovine teeth. (*J Endod* 2007;33:1074–1077)

Key Words

Bovine, dental pulp, human, hydrogen peroxide, tooth bleaching

Tooth bleaching has been reaching great popularity as a result of the esthetic demands imposed by society; it has been indicated for teeth discolored by aging, trauma, endodontic treatment, ingestion of colored foods and beverage, tobacco, and naturally discolored teeth (1). Tooth bleaching can be performed in vital or non-vital teeth. In vital teeth, hydrogen peroxide and carbamide peroxide might be used in the dental office or by patients under the dentist's supervision (1, 2).

One of the greatest concerns about tooth bleaching refers to peroxide penetration through enamel and dentin, reaching the pulp; however, the effects of peroxide penetration are still contradictory (3–8). Pulp reactions as inflammatory processes are common, although most studies consider these reactions reversible when adequate bleaching agents and techniques are used (9, 10).

Some studies report that peroxide penetration into the pulp chamber of restored and bleached teeth is higher than in intact teeth and therefore causes greater pulp sensitivity (6, 8). Polymerization shrinkage, thermal stress, and water absorption of restorative materials are some factors that can lead to microcracks in the tooth restoration margin and facilitate peroxide penetration into the pulp chamber (11).

Studies quantifying the hydrogen peroxide penetrating through enamel and dentin report that this penetration is different among the available products, depending on the concentration and time of application, besides the presence or absence of restorations (3, 5, 7, 8, 12). Furthermore, the tooth and its structural and morphologic characteristics can also influence the penetration of bleaching agents into the pulp cavity.

The purpose of this study was to evaluate the amount of peroxide inside the pulp chamber after bleaching with 38% hydrogen peroxide. This evaluation was performed in bovine and human unrestored or restored teeth.

Materials and Methods

This project was developed in accordance with the Research Ethics Code (approved under no. 042/2004-PH/CEP).

Human teeth (Group A) and bovine teeth (Group B) were used in this study. Groups A and B were subdivided into subgroups of unrestored and restored teeth and according to the restorative material (composite resin, glass ionomer cement, resin-modified glass ionomer cement).

Group A comprised 70 human third molars extracted for orthodontic reasons from patients 25–30 years of age. The teeth were immersed in 10% formalin for 24 hours after extraction and then immersed in saline and frozen saline solution at -18°C . Group B was composed of 70 bovine lateral incisors that received the same initial treatment as human teeth.

The roots were ground with discs up to 3 mm from the cement-enamel junction. The pulp tissue was removed by using Hedström files (Maillefer, Romulus, MI), and the pulp chamber was irrigated with saline. The pulp cavities were widened with a round bur (no. 1016; KG Sorensen, Ltda, Barueri, SP, Brazil) to allow the introduction of a micropipette inside the pulp chamber. Teeth with similar sizes were selected for achievement of standard pulp chamber size to allow application of $50 \mu\text{L}$ of solution in human teeth and $100 \mu\text{L}$ of solution in bovine teeth. The enamel and dentin thickness in the buccal area was measured with a caliper (Golgran, São Paulo, Brazil) to standardize this thickness (3–3.5 mm in human teeth and 3.5–4 mm in bovine teeth).

*From the Department of Oral Pathology, School of Dentistry, [†]School of Dentistry, Department of Endodontics, [‡]School of Dentistry, Department of Biochemistry, and [§]Paulista State University—UNESP, São José dos Campos, São Paulo, Brazil.

Address requests for reprints to Samira Esteves Afonso Camargo, Av. Dr. Jorge Zarur, 330 apto.56, Jd. Apolo, São José dos Campos/SP, Brazil, Cep 12243-081. E-mail address: samiraafonso@uol.com.br.

0099-2399/\$0 - see front matter

Copyright © 2007 by the American Association of Endodontists.

doi:10.1016/j.joen.2007.04.014

TABLE 1. Tukey Test for the Main Restorative Material Effect

Restorative material	Mean (± standard deviation)	Homogeneous groups*	
Resin-modified glass ionomer cement	1.5344 ± 0.915	A	
Unrestored	1.2275 ± 0.966	A	B
Glass ionomer cement	1.2188 ± 0.984	A	B
Composite resin	0.9380 ± 0.930		B

*Mean values followed by different letters correspond to statistically different data.

Standardized class V preparations with 4-mm mesiodistal width, 2-mm cervical-occlusal width, and 2-mm depth were prepared on the buccal side of teeth by using cylindrical diamond burs (no. 2094; KG Sorensen Ind Ltda). The enamel margins were beveled in 0.5 mm by using fine grit, pencil-shaped diamond burs (no. 3195F; KG Sorensen Ind Ltda) at low speed.

Human and bovine teeth were subdivided into groups A1–A5 and groups B1–B5, respectively. Groups A1 and B1 were restored with composite resin Esthetic-X (Dentsply, Ballaigues, Switzerland); groups A2 and B2 were restored with glass ionomer cement (Vidrión R; SS White, Rio de Janeiro, Brazil); and groups A3 and B3 were restored with resin-modified glass ionomer cement (Vitremér; 3M Dental Products, Two Harbors, MN) according to the manufacturers' instructions. Groups A4, A5, B4, and B5 were not restored.

Teeth were isolated by using 2 layers of nail polish, leaving a standardized buccal area exposed for application of the bleaching agent. For restored teeth, this exposed area corresponded to 1 mm beyond the limits of the restoration.

The pulp chambers were dried, and 100 μ L and 50 μ L of 2 mol/L acetate buffer (pH 4.5) were placed into the pulp chamber of the bovine and human teeth, respectively. The acetate buffer was necessary to stabilize the hydrogen peroxide that might penetrate into the pulp chamber for later quantification. Then all teeth were vertically fixed on a wax plate to allow application of the treatment agent.

In subgroups A5 and B5, the teeth were immersed in a flask containing distilled water until the coronal portion of teeth was covered. For bleaching treatment in the other subgroups (A1, A2, A3, A4, B1, B2, B3, B4), the 38% hydrogen peroxide gel was applied (Opalescence Xtra Boost; Ultradent, South Jordan, UT).

The time of exposure to the bleaching agent was 40 minutes. After this period, the acetate buffer solution was removed with a microsyringe (Terumo Micro Syringe MS-100; Terumo Corporation, Tokyo, Japan) and transferred to a glass tube. The pulp chamber of each tooth was rinsed twice with distilled water that was placed into the same glass tube. One hundred microliters of 0.5 mg mL⁻¹ leuco crystal violet (Sigma Chemical Co, St Louis, MO) and 50 μ L of 1 mg mL⁻¹ enzyme horseradish peroxidase (Sigma Chemical Co) were also added to each tube, and the solution was diluted to 3 mL with distilled water.

The optical density of the resulting blue color in the tubes was measured with a spectrophotometer (UV Spectrophotometer UV-1203; Shimadzu, Kyoto, Japan) at a wavelength of 596 nm. A standard curve of known amounts of hydrogen peroxide was used to convert the optical density values obtained from the samples into microgram equivalents of hydrogen peroxide.

Fourteen replicates were made for each experimental condition. An exploratory data analysis was performed to determine the most appropriate statistical test. The values obtained for penetration were analyzed by 2-way analysis of variance (ANOVA) considering a factorial 4 × 2 (treatments × types of teeth) and 2 additional treatments (controls). The Dunn test was applied for comparison between each treatment and the respective control. The significance level for all statistical tests was $P < .05$. The Dunn test was per-

formed with the Minitab for Windows (2004, version 14.12.0; Minitab Inc, State College, PA); the Tukey test for multiple comparisons for statistically significant effects was conducted on Statistix (2003, version 8.0; Analytical Software, Tallahassee, FL).

Results

Descriptive statistics data (μ g) for bovine teeth for the following 4 experimental conditions were B1 (0.28 ± 0.15 μ g), B2 (0.30 ± 0.20 μ g), B3 (0.79 ± 0.61 μ g), and B4 (0.30 ± 0.20 μ g). Descriptive statistics data (μ g) for human teeth for the following 4 experimental conditions were A1 (1.59 ± 0.92 μ g), A2 (2.04 ± 0.62 μ g), A3 (2.27 ± 0.41 μ g), and A4 (2.15 ± 0.22 μ g). Descriptive statistics data (μ g) for control groups were human teeth (0.134 ± 0.016 μ g) and bovine teeth (0.229 ± 0.039 μ g).

For bovine and human teeth, a comparison was performed between the control group and 4 experimental conditions by the Dunn test after evaluation of different distributions of values. Dispersion of the control data was smaller than the others. This analysis was performed by a macro of the Minitab software.

The test indicated that for bovine teeth, only the distribution of values of B3 differed from the control group (B5) ($P < .005$); for human teeth, all groups (A1, A2, A3, and A4) differed from the control (A5) ($P < .005$). These conclusions might be seen by graphic representations on a column graph (Fig. 1).

For evaluation of types of teeth and restorative material, data were submitted to the statistical model of two-way ANOVA. Statistical assumptions were evaluated before statistical analysis. The results indicated that the residues were normally distributed, and uniformity was checked by plotting against predicted values; thus, none of the analysis of assumptions was violated.

The 2-way ANOVA test revealed that the interaction effect was not significant ($F_{df(3,104)} = 1.50$; $P = .2191 > .05$), yet there was statistically significant difference between the main effects restorative material ($F_{df(3,104)} = 6.50$; $P = .0004 < .05$) and types of teeth ($F_{df(1,104)} = 272.50$; $P = .0001 < .05$). Therefore, hydrogen peroxide penetration in human teeth (2.0178 ± 0.6457 μ g) was higher than in bovine teeth (0.4415 ± 0.4219 μ g).

Comparison of experimental groups, except for the control group, for human and bovine teeth separately with the Tukey test (5%) demonstrated that A3 and B3 groups presented statistically significant difference in relation to A1 and B1 groups, showing higher values of hydrogen peroxide penetration in bovine and human teeth ($P < .005$) (Table 1).

Discussion

Studies reported that during tooth bleaching, hydrogen peroxide can penetrate through enamel and dentin and reach the pulp tissue (3, 5, 6, 8, 13–15). Bowles and Ugwuneri (14) and Cooper et al (15) verified higher peroxide penetration levels in extracted human teeth treated with 30% hydrogen peroxide. On the other hand, some studies reported that 10% or 6% carbamide peroxide, respec-

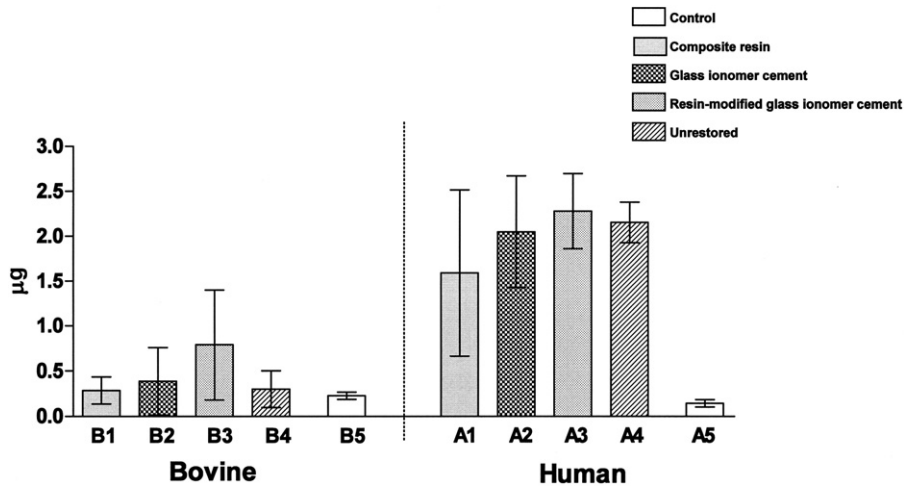


Figure 1. Column graph (mean ± standard deviation) for penetration data according to the experimental conditions.

tively, was diffused into the pulp chamber of intact teeth in lower concentrations (5, 16).

The penetration of bleaching agents occurs mainly as a result of their low molecular weight and ability to denature proteins, which increases the ion movement through the tooth structure (17). The present study revealed that all groups submitted to bleaching with 38% hydrogen peroxide showed peroxide penetration in the pulp chamber in bovine and human teeth. These results are in agreement with several previous studies that demonstrated that significant peroxide amounts can be diffused through dentin after bleaching agent application (3, 5, 6, 8).

Dental enamel presents 0.6% of organic content; the hydrogen peroxide, as a result of its low molecular weight, penetrates through the enamel, promoting increased porosity and loss of substances of enamel matrix as a result of free radicals oxidation (3, 4, 8, 18–20). In addition, pores created on the enamel surface are deeper when higher concentrations of bleaching agents are used (4).

In the present study, a high concentration of hydrogen peroxide (38%) was used and might induce alterations on the enamel surface, which possibly contributed to bleaching agent penetration into the pulp chamber. According to previous studies, high concentrations of bleaching agents are potentially harmful to the pulp tissue (10), possibly as a result of alterations that these substances cause in dental tissue.

On the other hand, in vivo studies demonstrated little or insignificant damage after clinical bleaching treatment with high concentrations of bleaching agents (3). Cohen and Chase (21) reported that human premolars exposed to 35% hydrogen peroxide did not present histologic evidence of pulp damage, presenting few alterations as moderate vasodilatation and imprisonment of odontoblast nuclei inside the dentinal tubules. Robertson and Melfi (22) verified only little inflammatory pulpal response after 2 applications of 35% hydrogen peroxide during 4 days.

However, Haywood (23) reported that 35% hydrogen peroxide reaches the pulp more quickly, and in 15 minutes of exposure, 12 times more peroxide penetration occurred compared with the amount of peroxide penetration obtained from 10% carbamide peroxide. Nevertheless, such aggression did not cause irreversible pulpal damage.

This study verified that in bovine teeth, peroxide penetration into the pulp chamber was smaller than in human teeth. This higher penetration in human teeth can be explained by the smallest dentin thickness in these teeth.

According to Gomez et al (24), the amount of bleaching agent that penetrates through the tooth structure is influenced by enamel and dentin thickness and by bleaching agent concentration. These authors verified an

increase in dentin permeability when bleaching agents are used on dentin and reported that the degree of pulpal response is associated with dentin thickness. Nathanson (10) reported that the higher the dentin thickness, the better will be the pulp protection against bleaching agents.

In this study, dentin thickness and surface area in contact with bleaching agent were standardized. The dentin thickness cannot be precisely measured; however, teeth presenting similar thickness were chosen.

The highest peroxide penetration in human teeth, verified in the present study, can be explained by dentin thickness and morphologic differences between these teeth. When bovine teeth are used to simulate procedures of dentin hybridization in areas close to the pulp, there is small permeability because the diameter of dentin tubules in bovine teeth is smaller and the intertubular dentin area is larger than in human teeth (25).

Another factor that can influence the differences in peroxide penetration between human and bovine teeth can be the alterations that bleaching agents promote in the tooth structure. Calcium and phosphate are present in hydroxyapatite, and any change in the quantity of these ions leads to alterations in inorganic components of dental tissues (26). The hydrogen peroxide can promote chemical alterations in the composition of the tooth, reducing the quantity of calcium and phosphate in enamel and dentin (27, 28). These alterations might be different in human and bovine dentin.

Recently, studies have been evaluating the bleaching agent penetration into the pulp chamber of restored teeth (8, 11, 12, 29). Hydrogen peroxide penetration into the pulp chamber of restored teeth occurred as a result of microleakage around restorative materials, because no material prevented microleakage completely (6, 11). Crim (30) evaluated the effect of carbamide peroxide on microleakage of class V restorations with composite resin in human teeth, verifying that carbamide peroxide affected the marginal sealing of resin restorations. Also, Gokay et al (12) and Benetti et al (8) evaluated peroxide penetration into the pulp chamber, verifying a high penetration of bleaching agents into the pulp chamber of restored teeth. Therefore, restorations margins can be considered a possible way for hydrogen peroxide penetration into the pulp chamber.

In the present study, it was verified that human and bovine teeth restored with resin-modified glass ionomer cement presented statistically higher hydrogen peroxide penetration in relation to teeth restored with composite resin. The smallest penetration into teeth restored with composite resin can be related to the marginal sealing ability of this

material, according to Attin et al (31) and Mair and Joiner (32), who demonstrated that teeth restored with composite resins presented smaller infiltration than teeth restored with resin-modified glass ionomer cement (Vitremer). Gokay et al (6) investigated the bleaching agent penetration into the pulp chamber in human teeth restored with several restorative materials, verifying that peroxide penetration was significantly smaller in teeth restored with composite resin, in agreement with the present results. Contrarily, Owens et al (11) evaluated the effect of carbamide and hydrogen peroxide bleaching agents on microleakage of class V restorations in human teeth and verified that teeth restored with composite resin presented higher microleakage values when compared with resin-modified glass ionomer and compomer.

Some studies verified that teeth restored with resin-modified glass ionomer cement presented higher bleaching agent penetration than teeth restored with other materials (5, 11, 33).

However, results obtained *in vitro* cannot correspond to *in vivo* hydrogen peroxide penetration (3, 5, 7, 13–15, 34). Vongsavan and Matthews (35) studied dye penetration into the dentin surface and obtained results that sustain this hypothesis. They verified that dye penetration *in vivo* was smaller than *in vitro*. According to these authors, there is a flux inside dentin tubules running from the pulp toward the external surface, which might avoid diffusion of substances from the oral cavity to the pulp chamber. Sulieman et al (34, 36) related that in the oral cavity, teeth present a continuous movement of fluids through dentinal tubules and enamel porosities. The use of teeth *in vitro*, without dentin fluid, probably allows bleaching agents to penetrate into teeth more quickly than in clinical situations. Therefore, the amount of hydrogen peroxide that reaches the vital pulp can be smaller than *in vitro* conditions (7).

With the results of the present study, we can report that bleaching agents might have caused morphologic alterations in enamel and dentin surface and penetrated through the dental tissues, reaching the pulp space in extracted intact and restored teeth. Despite the *in vitro* nature of this study and the literature reports limited to reversible effects caused by defensive mechanisms of pulp against irritation caused by bleaching agents, the data obtained in this study reinforce the care needed in the use of bleaching agents.

Conclusions

According to the methodology and results of this study, the following can be suggested:

- Independent of the presence of restoration, all teeth submitted to bleaching presented peroxide penetration into the pulp chamber;
- The human teeth presented higher peroxide penetration than the bovine teeth in all experimental situations;
- Peroxide penetration into the pulp chamber of restored teeth depends on the type of restorative material; it is higher in teeth restored with resin-modified glass ionomer cement.

Acknowledgments

We thank FAPESP (Fundação de Amparo à Pesquisa de São Paulo) for financial support offered for the acquisition of the materials, which were essential for the development of this research project.

References

1. Haywood VB. Current status of nightguard vital bleaching. *Compend Cont Educ Dent* 2000;28:S10–7., S48.
2. Amato M, Serena Scaravilli M, Farella M, Riccitiello F. Bleaching teeth treated endodontically: long-term evaluation of a case series. *J Endod* 2006;32:376–8.
3. Hanks CT, Fat JC, Wataha JC, Corcoran JF. Cytotoxicity and dentin permeability of carbamide peroxide and hydrogen peroxide vital bleaching materials *in vitro*. *J Dent Res* 1993;72:931–8.
4. Hegedüs C, Bistey T, Flora-Nagy E, Keszthelyi G, Jenei A. An atomic force microscopy study on the effect of bleaching agents on enamel surface. *J Dent* 1999;27:509–15.
5. Thitinthapan W, Satamanont P, Vongsavan N. *In vitro* penetration of the pulp chamber by three brands of carbamide peroxide. *J Esthet Dent* 1999;11:259–64.
6. Gokay O, Yilmaz F, Akin S, Tuncbilek M, Ertan R. Penetration of the pulp chamber by bleaching agents in teeth restored with various restorative materials. *J Endod* 2000;26:92–4.
7. Gokay O, Müjdeci A, Algn E. Peroxide penetration into the pulp from Whitening Strips. *J Endod* 2004;30:887–9.
8. Beneti AR, Valera MC, Mancini MN, Miranda CB, Balducci I. *In vitro* penetration of bleaching agents into the pulp chamber. *Int Endod J* 2004;37:120–4.
9. Seale NS, Wilson CF. Pulpal response to bleaching of teeth in dogs. *Pediatr Dent* 1985;7:209–14.
10. Nathanson D. Vital tooth bleaching: sensitivity and pulpal considerations. *J Am Dent Assoc* 1997;128:41S–4S.
11. Owens BM, Rowland CC, Brown DM, Covington JS. Postoperative dental bleaching: effect of microleakage on Class V tooth colored restorative materials. *J Tenn Dent Assoc* 1998;78:36–40.
12. Gokay O, Tuncbilek M, Ertan R. Penetration of the pulp chamber by carbamide peroxide bleaching agents on teeth restored with a composite resin. *J Oral Rehabil* 2000;27:428–31.
13. Griffin RE Jr, Grower MF, Ayer WA. Effects of solutions used to treat dental fluorosis on permeability of teeth. *J Endod* 1977;3:139–43.
14. Bowles WH, Ugwuneri Z. Pulp chamber penetration by hydrogen peroxide following vital bleaching procedures. *J Endod* 1987;13:375–7.
15. Cooper JS, Bokmeyer TJ, Bowles WH. Penetration of the pulp chamber by carbamide peroxide bleaching agents. *J Endod* 1992;18:315–7.
16. Joiner A, Thakker G. *In vitro* evaluation of a novel 6% hydrogen peroxide tooth whitening product. *J Dent* 2004;32:19–25.
17. Goldstein CE, Goldstein RE, Feinman RA, Garber DA. Bleaching vital teeth: state of the art. *Quint Int* 1989;20:729–37.
18. Bitter NC. A scanning electron microscope study of the long-term effect of bleaching agents on the enamel surface *in vivo*. *Gen Dent* 1998;46:84–8.
19. Sulieman M, Addy M, MacDonald E, Rees JS. A safety study *in vitro* for the effects of an in-office bleaching system on the integrity of enamel and dentine. *J Dent* 2004;32:581–90.
20. Kwon YH, Huo MS, Kim KH, Kim SK, Kim YJ. Effects of hydrogen peroxide on the light reflectance and morphology of bovine enamel. *J Oral Rehabil* 2002;29:473–7.
21. Cohen SC, Chase C. Human pulpal response to bleaching procedures on vital teeth. *J Endod* 1979;5:134–8.
22. Robertson WD, Melfi RC. Pulpal response to vital bleaching procedures. *J Endod* 1980;6:645–9.
23. Haywood VB. Nightguard vital bleaching: current concepts and research. *J Am Dent Assoc* 1997;128:19S–25S.
24. Gomez APA, Giraldo GMH, Arango LGH. Peroxide de hidrogeno al 35% y peroxide de carbamido al 10% para el blanqueamiento dental. *Univ Odontol* 1999;19:14–20.
25. Tonami K, Takahashi H. Effects of aging on tensile fatigue strength of bovine dentin. *Dent Mat* 1997;16:156–69.
26. McCracken MS, Haywood VB. Demineralization effects of 10 percent carbamide peroxide. *J Dent* 1996;24:395–8.
27. Rotstein I, Dankner E, Goldman A, Helling I, Stabholz A, Zalkind M. Histochemical analysis of dental hard tissues following bleaching. *J Endod* 1996;22:23–5.
28. Kawamoto K, Tsujimoto Y. Effects of the hydroxyl radical and hydrogen peroxide on tooth bleaching. *J Endod* 2004;30:45–50.
29. Price RB, Sedarous M, Hiltz GS. The pH of tooth-whitening products. *J Can Dent Assoc* 2000;66:421–6.
30. Crim GA. Post-operative bleaching: effect on microleakage. *Am J Dent* 1992; 5:109–12.
31. Attin T, Hannig C, Wiegand A, Attin R. Effect of bleaching on restorative materials and restorations: a systematic review. *Dent Mat* 2004;20:852–61.
32. Mair L, Joiner A. The measurement of degradation and wear of three glass ionomers following peroxide bleaching. *J Dent* 2004;32:41–5.
33. Yap AU, Wattanapayungkul P. Effects of in-office tooth whiteners on hardness of tooth-colored restoratives. *Oper Dent* 2002;27:137–41.
34. Sulieman M, Addy M, MacDonald E, Rees JS. The bleaching depth of a 35% hydrogen peroxide based in-office product: a study *in vitro*. *J Dent* 2005;33:33–40.
35. Vongsavan N, Matthews B. The permeability of cat dentine *in vivo* and *in vitro*. *Arch Oral Biol* 1991;36:641–6.
36. Sulieman M, Addy M, MacDonald E, Rees JS. The effect of hydrogen peroxide concentration on the outcome of tooth whitening: an *in vitro* study. *J Dent* 2004;32:295–9.