Dental Traumatology

Dental Traumatology 2009; **25**: 176–180; doi: 10.1111/j.1600-9657.2008.00644.x

Effect of different bleaching systems on the ultrastructure of bovine dentin

Laise Daniela Carrasco-Guerisoli[1], Renato Jonas dos Santos Schiavoni[1], Juliana Machado Barroso[1], Danilo Mathias Zanello Guerisoli[2], Jesus Djalma Pécora[1], Izabel Cristina Fröner[1]

[1] Ribeirão Preto Dental School, University of São Paulo, Ribeirão Preto, São Paulo; [2] Faculty of Dentistry, Federal University of Mato Grosso do Sul, Campo Grande, Mato Grosso do Sol, Brazil

Correspondence to: Prof. Danilo M. Zanello Guerisoli, Departamento de Odontologia Clínica, Faculdade de Odontologia, Universidade Federal do Mato Grosso do Sul, 79070-900, Campo Grande, Mato Grosso do Sul, Brazil
Tel.: +55 67 3345 7383
Fax: +55 67 3345 7380
e-mail: zanello@nin.ufms.br

Accepted 28 August, 2007

Abstract – The aim of this study was to evaluate *in vitro* the effect of different in-office bleaching systems on the surface morphology of bovine dentin. Thirty tooth fragments measuring 4 × 4mm, containing enamel and dentin, were obtained from the crowns of extracted bovine incisors. Samples were subjected to simulated intracoronal bleaching techniques using conventional (Opalescence Endo® and Whiteness Super Endo®) and light-activated systems (Opalescence Xtra® and Whiteness HP Maxx®). Controls were treated with either sodium perborate mixed with 10% hydrogen peroxide or no bleaching agent. The samples were observed under SEM and the recorded images were evaluated for topographic alterations. The ultrastructural alterations of dentin observed in this study varied greatly between groups according to the products used. Higher pH products (Whiteness HP Maxx® and Opalescence Xtra®) associated with in-office techniques yielded better maintenance of dentin ultrastructure. Apparently, both low pH and hydrogen peroxide oxidation play a role in altering the ultrastructure of dentin during internal dental bleaching. The use of alkaline products with reduced time of application (in-office techniques) may decrease such morphological alterations.

Internal dental bleaching is an established, simple, conservative and cost-effective method of improving the colour of discoloured teeth that have received root canal treatment (1–3). The discolouration of the dental tissues is reversed by an oxidation reaction between the bleaching agent (a peroxide) and the substrate, normally an organic molecule (4, 5).

The demand for faster results in dental bleaching procedures, for either endodontically treated teeth or not, led to the development of products specifically designed for in-office use. Such products, containing hydrogen peroxide or carbamide peroxide at various concentrations, may use light as a catalyst to accelerate the chemical reaction and, thus, produce the desired change in tooth colour faster than the walking bleach technique.

Although such bleaching agents are highly effective in lightening tooth colour, concern has been expressed regarding their use, especially hydrogen peroxide, because of the associated post-bleaching complications. These include alteration in the surface morphology of dentin (6), lower microhardness values (7, 8), changes in chemical composition of dentin (9), increased dentin permeability (10) and external cervical root resorption (11, 12).

The effects of peroxide-based products on dentin ultrastructure are still controversial, and while some authors report no adverse effects (5, 13), others found significant changes (6, 14).

The mechanism by which bleaching agents affect dentin is still unknown, but studies reported that hydrogen peroxide may cause dissolution of inorganic material (15), reduction in calcium-phosphorus ratio (9), reduction in the organic components of dentin by protein oxidation (16) and dentin denaturation (17).

The aim of this study was to contribute to the topic, by evaluating *in vitro* the effects of different in-office bleaching systems on the ultrastructure of bovine dentin.

Materials and methods

The buccal portions of 30 crowns of freshly extracted bovine incisors were cut using a water-refrigerated, hard tissue microtome (Minitron Struers, Copenhagen, Denmark), 1mm from the cement-enamel junction, producing the same number of blocks measuring 4 × 4 × 2mm, containing enamel and dentin.

Both surfaces of the blocks were polished successively using a polishing machine (Minitron Struers) with 400, 600 and 1200 grit silicon carbide paper (3M Brazil, Sumaré, SP, Brazil), followed by 0.3 and 0.05 μm alumina suspension (Arotec, São Paulo, SP, Brazil) on felt cloth (Arotec). The blocks were then washed in running water for 2 h, and immersed in distilled,

deionized water under ultrasonic agitation for 5 min to remove possible residues.

Silicon frames were prepared to accommodate the dental blocks without gaps in the sides, with sufficient height (5 mm) to allow space for the bleaching agent, as shown in Fig. 1. This device also provided, when necessary, a hermetic sealing with the use of glass plates over and under it.

The samples were randomly divided randomly into six groups, according to the bleach system to be tested and each group was placed in position in the silicon frames, with the dentin portion facing the bleaching product. Group I was treated with Whiteness Super Endo® (37% carbamide peroxide; FGM Produtos Odontológicos, Joinville, SC, Brazil), receiving the bleaching gel in contact with the dentin. The silicon frame was covered by dark glass plates fixed with metal clamps, simulating the 'walking bleach' technique, and the device was stored at 37°C, 100% humidity. The bleaching agent was replaced every 5 days, for a total of four applications. Group II received the same treatment as group I, but the bleaching agent used was Opalescence Endo® (35% hydrogen peroxide; Ultradent, South Jordan, UT, USA). Group III received Whiteness HP Maxx® (35% hydrogen peroxide; FGM Produtos Odontológicos), a light-activated bleaching product. Activation was performed with LEDs (Brightness LaserLight; Kondortech, São Carlos, SP, Brazil) for 30 s, at a distance of 5 mm. After 5 min, the bleaching gel was removed from the samples, and the process was repeated until 6 applications were performed. Group IV was treated with Opalescence Xtra® (35% hydrogen peroxide; Ultradent), following the same method used for group III. Group V (positive control) received tetra-hydrated sodium perborate (Merck KGaA, Darmstadt, Germany) mixed with 10% hydrogen peroxide (Merck KGaA) (1:1, by weight), following the same technique as group I. Group VI (negative control) received the same treatment as group I, but no bleaching agent was used.

During the experimental procedures, bleaching products were removed by suction and washed thoroughly with distilled, deionized water. After the bleaching procedures, samples were fixed with 2% glutaraldehyde, washed in running water for 24 h, washed in distilled, deionized water, critical point dried and sputter coated with gold (120 s, ~70 nm) for SEM analysis. Photomicrographs (3500× magnification, 15 kV) were obtained from the samples and compared with group VI by a single assessor, evaluating qualitatively the dentin surface for alterations.

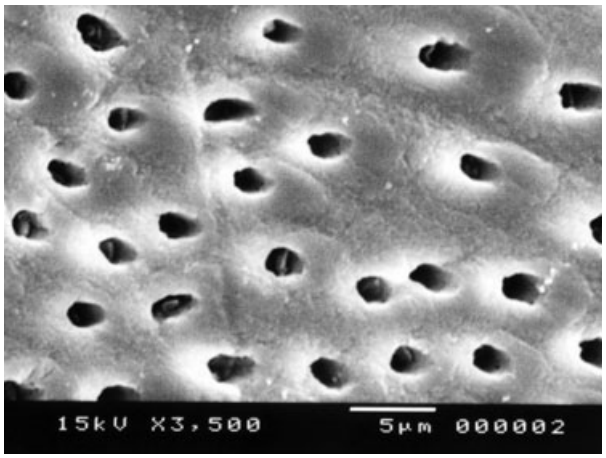

Fig. 2. Photomicrograph of a dentin sample treated with Whiteness Super Endo® (group I).

Results

Representative photomicrographs of each group are presented in Figs 2–7. Samples presented mostly free of smear layer or smear plug, with open dentinal tubules. Some debris was present, probably from the abrasives used during sample preparation, and different patterns of dentin erosion were observed among the groups.

Whiteness Super Endo®-treated samples (group I) presented a rough surface, with even areas of fair erosion affecting intertubular dentin (Fig. 2). Peritubular areas seem to be less affected by the bleaching agent.

Opalescence Endo® (group II) produced localized, well delineated eroded areas on dentin (Fig. 3). The depth of such erosions seems to be higher than that observed in any other group.

Samples treated with Whiteness HP Maxx® (group III) generated surfaces with little or no erosion at all (Fig. 4). The eventual uneven areas recorded were localized in intertubular dentin, while the peritubular dentin seems to be smoother.

Opalescence Xtra®-treated samples (group IV) showed to be irregular, with shallow, constant erosion covering the dentin surface (Fig. 5). No perceptible

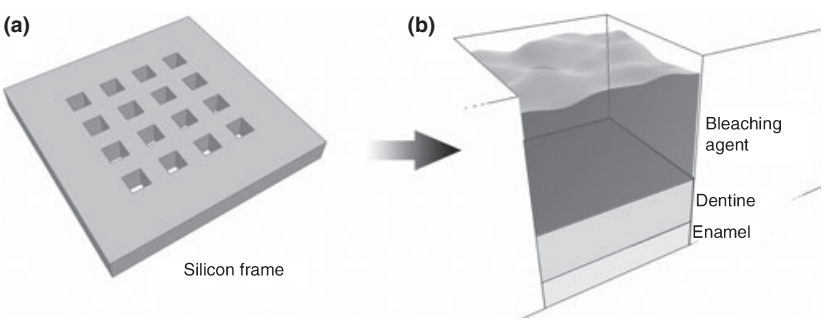

Fig. 1. Schematic drawing of (a) the silicon frame and (b) exploded view of one of the spaces of the silicon frame, with sample placed in position and the bleaching agent applied over dentin.

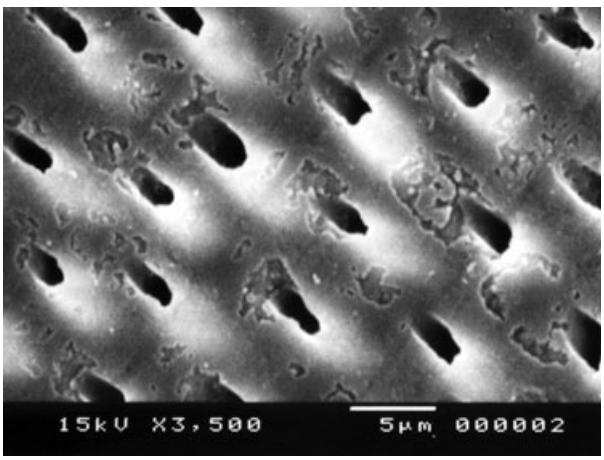

Fig. 3. Photomicrograph of a dentin sample treated with Opalescence Endo® (group II).

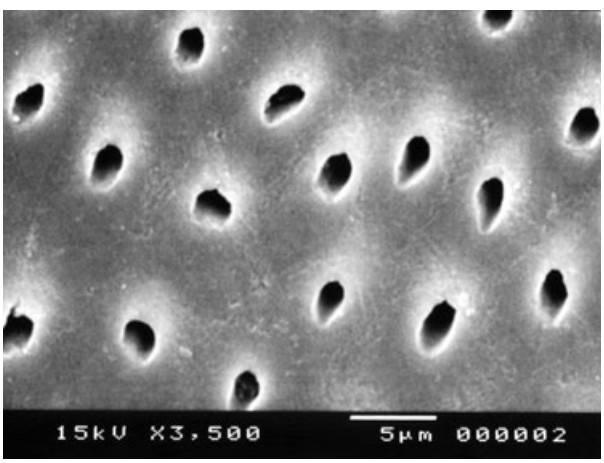

Fig. 4. Photomicrograph of a dentin sample treated with Whiteness HP Maxx® (group III).

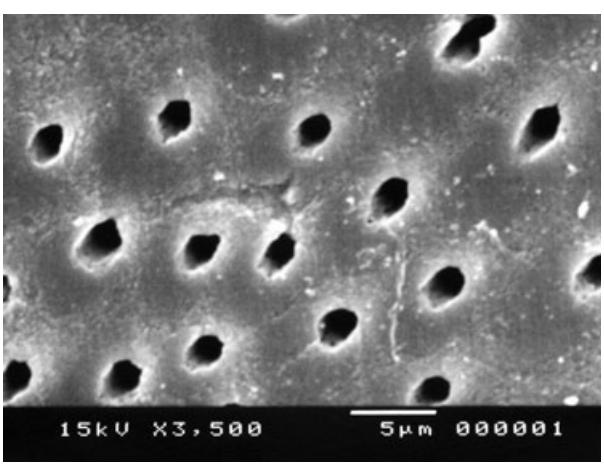

Fig. 5. Photomicrograph of a dentin sample treated with Opalescence Xtra® (group IV).

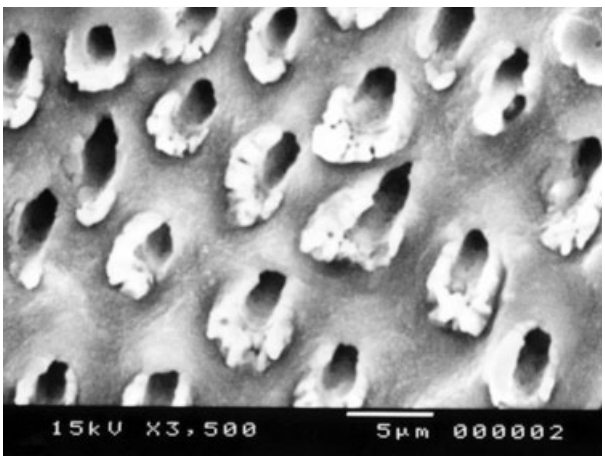

Fig. 6. Photomicrograph of a dentin sample treated with sodium perborate mixed with 10% hydrogen peroxide (group V).

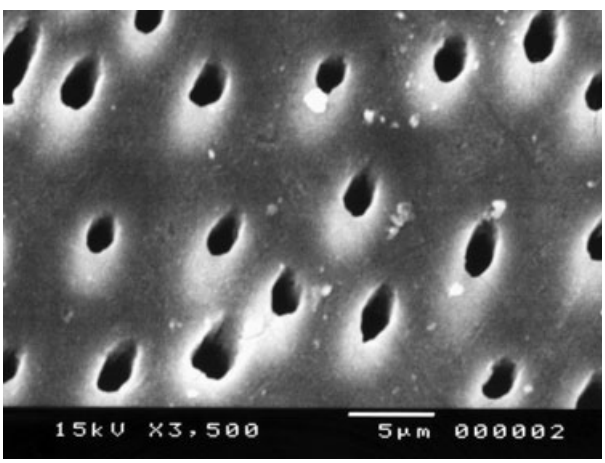

Fig. 7. Photomicrograph of a dentin sample which did not undergo bleaching procedures (negative control, group VI).

differences could be noticed between intertubular and peritubular dentin regarding the erosive pattern.

Dentin exposed to sodium perborate and 10% hydrogen peroxide (group V) presented a regular, smooth erosive pattern affecting intertubular dentin's ultrastructure (Fig. 6). The peritubular dentin appears to be projected from the dentin tubule, apparently suffering less the effects of the bleaching agent.

Control group samples (group VI) presented a smooth surface, with no visible signs of erosion (Fig. 7).

Discussion

Truman (18) is believed to be the first to describe bleaching of discoloured, pulpless teeth and, since then, a variety of medicaments and techniques have been used to achieve safe and predictable aesthetic excellence (19). Currently, the most common techniques utilize peroxides as bleaching agents (20), but the side-effects and implications of this cosmetic treatment are still controversial (21).

Recently, dental bleaching agents activated by lasers, LEDs or halogen lamps are routinely used by clinicians, but the correct understanding of their implications on dental hard tissues still needs more consideration.

In this study, two light-activated products (Whiteness HP Maxx® and Opalescence Xtra®) and two 'walking bleach' type products (Whiteness Super Endo® and Opalescence Endo®) were evaluated. Whiteness Super Endo® contains 37% carbamide peroxide, while the others contain 35% hydrogen peroxide as active principle. Sodium perborate mixed with 10% hydrogen peroxide was used according to the 'walking bleach' technique proposed by Nutting and Poe (22) as positive control group, whereas samples with no bleaching procedure performed were used as negative control.

The manufacturer of Whiteness Super Endo® recommends three to four repetitions of the bleaching procedure, whereas the instructions for Opalescence Endo® requires a repeat of the procedure every 3 to 5 days until the desired color change is achieved. Dahl and Pallesen (19) also recommend the repetition of the 'walking bleach' technique until acceptable lightening is achieved. Other authors used bleaching regimens similar to this study (10, 23). In this study, a protocol of four repetitions of the procedure was followed.

The ultrastructural alterations of dentin observed in this study varied greatly between groups, even when the tested products reportedly carried the same formula. While treatment with Whiteness HP Maxx® produced little or no morphological alterations on dentin, the other tested products caused more noticeable modifications. A previous study on the effects of Whiteness HP Maxx® on enamel, however, shows severe erosion and structure dissolution, as well as an increase in surface roughness (24). Another study with a similar product, used according to the walking bleach technique, reports increased dentin permeability (10).

Opalescence Xtra®, which is also a 35% peroxide-based bleaching agent, produced a more irregular pattern on dentin, with shallow erosion areas covering the sample surface. A 22% reduction of microhardness for dentin, using this product, is reported by another study (25).

Opalescence Endo® produced localized, well delineated eroded areas on dentin. This may be because of the longer exposure period as it is intended to be left inside the pulp chamber for a period (normally 3–5 days, according to the manufacturer). In this study, it was left in contact with dentin for four periods of 5 days each.

Even though Whiteness HP Maxx®, Opalescence Endo® and Opalescence Xtra® are 35% hydrogen peroxide-based products, they have different pH values. The first one is alkaline (26), whereas the last is highly acidic, with a pH of 3.67 (27). Opalescence Endo®, according to the manufacturer, has a pH of 5.0. This may have a major impact on the dentin ultrastructure, according to Sulieman et al. (28), who found the pH, and not hydrogen peroxide, to cause alterations on dentin surface morphology during bleaching procedures. Apparently, the more acidic nature of Opalescence Xtra was the main factor responsible for the differences in ultrastructure observed when this group was compared to the group where Whiteness HP Maxx® was used.

Other studies, however, imply that hydrogen peroxide affects not only the inorganic components of the dental hard tissues through acidic demineralization but also attacks the relatively rich organic substance of the dentin. This effect of hydrogen peroxide on the organic substance might be because of collagen and amino acid denaturation, as proposed by some authors (15, 20, 29). This may explain the pattern of erosion found in some groups, with the intertubular dentin being more aggressively attacked by the products than peritubular dentin, as the first is less mineralized than the last. The remarkable ultrastructure alteration found for group V illustrates the action of hydrogen peroxide on the organic matrix of dentin and preservation of the mineral rich peritubular dentin.

Chng et al. (8) reported the peritubular dentine to be more resistant than intertubular dentine, indicating that it is the strong oxidizing effect of hydrogen peroxide on the organic matrix of intertubular dentine, rather than its acidic pH, that plays a predominant role in the changes observed in intertubular dentine. This is in agreement with the present observations.

Carbamide peroxide-based product Whiteness Super Endo® caused mild eroded areas on dentin, even with an application regimen similar to Opalescence Endo®, which was more aggressive. However, according to Joiner (30), degradation of carbamide peroxide would yield a maximum of 36% (w/w) of hydrogen peroxide, thus significantly reducing the final concentration of the active principle. Moreover, ammonia is among the subproducts of degradation of carbamide peroxide, which increases the pH of the medium (19); according to the manufacturer, Whiteness Super Endo® has a neutral pH.

According to the present study, apparently both low pH and oxidation by hydrogen peroxide play a role in altering the ultrastructure of dentin during simulated internal bleaching. The use of alkaline products with reduced time of application (in-office techniques) may decrease the morphological alterations of dentin. However, whether these alterations are clinically significant or not is still to be researched.

Conclusions

The results of this study indicate that both low pH and hydrogen peroxide oxidation play a role in altering the ultrastructure of dentin during internal dental bleaching. The use of alkaline products with reduced time of application (in-office techniques) may decrease such morphological alterations.

References

1. Lee GP, Lee MY, Lum SO, Poh RS, Lim KC. Extraradicular diffusion of hydrogen peroxide and pH changes associated with intracoronal bleaching of discoloured teeth using different bleaching agents. Int Endod J 2004;37:500–6.
2. Lim MY, Lum SO, Poh RS, Lee GP, Lim KC. An in vitro comparison of the bleaching efficacy of 35% carbamide

peroxide with established intracoronal bleaching agents. Int Endod J 2004;37:483–8.
3. Carrasco LD, Guerisoli DM, Rocha MJ, Pécora JD, Fröner IC. Efficacy of intracoronal bleaching techniques with different light activation sources. Int Endod J 2007;40:204–8.
4. Smigel I. Laser tooth whitening. Dent Today 1996;15:32–6.
5. Sulieman M. An overview of bleaching techniques: History, chemistry, safety and legal aspects. Dent Update 2004;31: 608–16.
6. Titley K, Torneck CD, Smith DC. Effect of concentrated hydrogen peroxide solution on the surface morphology of cut human dentin. Endod Dent Traumatol 1988;4:32–6.
7. Pecora JD, Cruzfilho AM, Sousaneto MD, Silva RG. In vitro action of various bleaching agents on the microhardness of human dentin. Braz Dent J 1994;5:129–34.
8. Chng HK, Ramli HN, Yapa AU, Limb CT. Effect of hydrogen peroxide on intertubular dentin. J Dent 2005;33:363–9.
9. Rotstein I, Dankner E, Goldman A, Heling I, Stabholz A, Zalkind M. Histochemical analysis of dental hard tissues following bleaching. J Endod 1996;22:23–5.
10. Carrasco LD, Zanello Guerisoli DMZ, Pécora JD, Fröner IC. Evaluation of dentin permeability after light activated internal dental bleaching. Dent Traumatol 2007;23:30–4.
11. Madison S, Walton R. Cervical root resorption following bleaching of endodontically treated teeth. J Endod 1990;16:570–4.
12. Heithersay GS. Invasive cervical resorption: an analysis of potential predisposing factors. Quintessence Int 1999;30:83–95.
13. White DJ, Kozak KM, Zoladz JR, Duschner HJ, Gotz H. Effects of tooth-whitening gels on enamel and dentin ultrastructure – a confocal laser scanning microscopy pilot study. Compend Contin Educ Dent Suppl 2000;29:S29–34.
14. Zalkind M, Arwaz JR, Goldman A, Rotstein I. Surface morphology changes in human enamel, dentin and cementum following bleaching: a scanning electron microscopy study. Endod Dent Traumatol 1996;12:82–8.
15. Rotstein I, Lehr Z, Gedalia I. Effect of bleaching agents on inorganic components of human dentin and cementum. J Endod 1992;18:290–3.
16. Chng HK, Palamara JEA, Messer HH. Effect of hydrogen peroxide and sodium perborate on biomechanical properties of human dentin. J Endod 2002;28:62–7.
17. Lado EA, Stanley HR, Weisman MI. Cervical resorption in bleached teeth. Oral Surg Oral Med Oral Pathol 1983;55:78–80.
18. Truman J. Bleaching of non-vital discoloured anterior teeth. Dent Times 1864;1:69–72.
19. Dahl JE, Pallesen U. Tooth bleaching – a critical review of the biological aspects. Crit Rev Oral Biol Med 2003;14:292–304.
20. Farmer DS, Burcham P, Marin PD. The ability of thiourea to scavenge hydrogen peroxide and hydroxyl radicals during the intra-coronal bleaching of bloodstained root-filled teeth. Aust Dent J 2006;51:146–52.
21. Lewinstein I, Hirschfeld Z, Stabholz A, Rotstein I. Effect of hydrogen peroxide and sodium perborate on the microhardness of human enamel and dentin. J Endod 1994;20:61–3.
22. Nutting EB, Poe GS. A new combination for bleaching teeth. J Calif Dent Assoc 1963;31:289–91.
23. de Oliveira DP, Teixeira EC, Ferraz CC, Teixeira FB. Effect of intracoronal bleaching agents on dentin microhardness. J Endod 2007;33:460–2.
24. Pinto CF, Oliveira R, Cavalli V, Giannini M. Peroxide bleaching agent effects on enamel surface microhardness, roughness and morphology. Braz Oral Res 2004;18:306–11.
25. Lewinstein I, Fuhrer N, Churaru N, Cardash H. Effect of different peroxide bleaching regimens and subsequent fluoridation on the hardness of human enamel and dentin. J Prosthet Dent 2004;92:337–42.
26. Castello RR, Monnerat AF. Avaliação das alterações estruturais em esmalte submetido ao clareamento com peróxido de hidrogênio a 35%. Rev Bras Odontol 2004;61:160–4.
27. Price RB, Sedarous M, Hiltz GS. The pH of tooth-whitening products. J Can Dent Assoc 2000;66:421–6.
28. 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.
29. Kawamoto K, Tsujimoto Y. Effects of the hydroxyl radical and hydrogen peroxide on tooth bleaching. J Endod 2004;30:45–50.
30. Joiner A. The bleaching of teeth: a review of the literature. J Dent 2006;34:412–9.