In vitro peroxide penetration into the pulp chamber from newer bleaching products

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Abstract

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Aim To investigate peroxide penetration from newer bleaching products into the pulp chamber.

Methodology Fifty extracted human maxillary central incisor teeth were separated into five groups ($n = 10$). All the teeth were sectioned 3 mm apical to the cemento-enamel junction; the intracoronal pulp tissue was removed, and the pulp chamber filled with acetate buffer. Buccal crown surfaces of teeth in the experimental groups were subjected to either a whitening strip (containing 5.3% hydrogen peroxide-G1) or one of three paint-on liquid whiteners (containing 19% sodium percarbonate peroxide-G2, 18% carbamide peroxide-G3 and 8.7% hydrogen peroxide-G4). The teeth in the control group (G5) were exposed only to distilled water. The acetate buffer solution in each tooth was then transferred to a glass test tube after 30 min and leuco-crystal violet and enzyme horseradish peroxidase were added, producing a blue solution. The optical density of the resultant blue colour in the tubes was measured by a UV-visible spectrophotometer at a wavelength of 596 nm. The values were converted into microgram equivalents of HP using a spectrophotometric calibration curve. Data were analysed statistically using the Kruskal–Wallis Analysis of Variance and the Mann–Whitney *U*-test.

Results Statistically significant differences were found between all of the groups ($P < 0.05$). Pulpal peroxide was not observed in the control group. The amount of hydrogen peroxide (μg) found in the pulp chamber of G1 (0.726 ± 0.024) > G4 (0.443 ± 0.017) > G3 (0.231 ± 0.011) > G2 (0.175 ± 0.012).

Conclusions The peroxides from the whitening strip and paint-on whiteners penetrated into the pulp chamber to varying degrees.

Keywords: bleaching gel, carbamide peroxide, hydrogen peroxide peroxide penetration, paint-on whiteners, whitening strip.

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Introduction

Patient demand for whiter teeth is growing, resulting in a rapid development of tooth whitening products and procedures (Nathoo *et al.* 2002). Tooth bleaching is now more commonly carried out at home rather than in the office. A soft, custom-made plastic tray filled with a bleaching gel containing carbamide peroxide (CP) has been successfully used for home bleaching since 1989 (Greenwall 2001). Today, there are many options for home tooth bleaching. One of these involves the use of whitening strips, which are applied directly onto the vestibular tooth surfaces. These strips contain various concentrations of hydrogen peroxide (HP) uniformly distributed on a polyethylene strip with concentrations of 5.3, 6.5 and 14% HP and eliminate the need to custom fabricate a tray for each patient. It has been reported that whitening strips are easy to use, more comfortable and require shorter duration of wear compared with tray bleaching systems (Sagel *et al.* 2000). However, Nathoo *et al.* (2002) reported that the use of both tray-based and strip-based products had some drawbacks such as difficulty in adapting to malposed teeth. A more recent option is paint-on whiteners which may be used either in the day or at

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night. These products are applied to the buccal surface of a tooth with an applicator brush. They have been formulated to release peroxide slowly onto the tooth surface until they are removed by normal tooth brushing (Slezak *et al.* 2002, Date *et al.* 2003, Gambarini *et al.* 2003, Garcia-Godoy *et al.* 2004). They contain one or other of the various peroxide derivatives such CP, HP or sodium percarbonate peroxide (NPP) [also known as solid hydrogen peroxide]. The effects of CP and NPP are similar to HP, because CP ultimately breaks down into urea and HP (Haywood & Heymann 1991), and NPP dissociates into sodium carbonate and HP (Kaneko *et al.* 2000). HP breaks down into free radicals which eventually combine to form molecular oxygen and water. The oxygen oxidizes the stained areas (Haywood & Heymann 1991).

Bowles & Thompson (1986) have shown that hydrogen peroxide can dramatically inhibit pulpal enzyme activity in direct contact with cells. It was reported that peroxide penetration into the pulp may result in different levels of tooth sensitivity or bleaching efficacy (Thitinanthapan *et al.* 1999). A number of previous studies (Bowles & Ugwuneri 1987, Cooper *et al.* 1992, Benetti *et al.* 2004) have shown that after the application of bleaching gels and solutions containing HP or CP, peroxides diffused through the coronal surface of teeth into the pulp chamber. Gökay *et al.* (2004) also reported that HP from the whitening strips diffused into the pulp chamber. However, information about diffusion of HP from paint-on whiteners is not available.

Therefore, the aim of this *in vitro* study was to evaluate and compare hydrogen peroxide penetration from a whitening strip, containing 5.3 % HP, and various paint-on whiteners, containing 19% NPP, 18% CP and 8.7% HP, into the pulp chamber of extracted teeth.

Materials and methods

Fifty extracted maxillary central incisor teeth were used in this study. The teeth were approximately of the same size and free of caries or other defects. After cleaning, they were stored in distilled water.

The teeth were separated into five groups, each containing ten teeth. Apices of all the teeth were cut approximately 3 mm apical to the cemento-enamel junction, the pulpal tissue was removed with a round bur and the pulp chamber was washed with distilled water. A 25 μL aliquot of 2 M acetate buffer was placed into the pulp chamber of each tooth to absorb and stabilize any peroxide that might penetrate into the pulp chamber. The vestibular crown surfaces of the teeth in group 1–4 (test groups) were treated by one of the following bleaching products according to manufacturers' instructions; respectively: whitening strip containing 5.3% HP (Crest Whitestrips; Procter & Gamble, Cincinnati, OH, USA), night-use paint-on whitener containing 19% NPP-equivalent to 5.3% HP (Crest Night Effects), day-use paint-on whitener containing 18% CP-equivalent to 6.5% HP (Colgate Simply White; Colgate Palmolive Company, New York, NY, USA), and night-use paint-on whitener containing 8.7% HP (Colgate Simply White Night). The bleaching products were left on the tooth surfaces for 30 min at 37 °C. The teeth in group 5 were used as a control and were exposed to distilled water only (Table 1).

The acetate buffer solution in the pulp chamber of each tooth was removed by means of a Pasteur pipette after treatment and transferred to a glass tube. The pulp chamber of each tooth was rinsed twice with a 100 μL portion of distilled water and was also transferred to the glass tube. Distilled water (2775 μL) was then added to the glass tube together with 100 μL, 0.5 mg mL^{-1} of leuco-crystal violet (Aldrich; Sigma-Aldrich Chemie GmbH, Steinheim, Germany), and 50 μL of enzyme horseradish peroxidase (Sigma; Sigma Chemical Co., St Louis, MO, USA) 1 mg mL^{-1} according to the method described by Mottola *et al.* (1970). This procedure was repeated separately for each tooth.

The optical density of the resultant blue colour in the tubes was measured by a UV-visible spectrophotometer (Shimadzu UV 1601; Kyoto, Japan) at the wavelength

Table 1 The bleaching products and groups used in this study

Groups	Bleaching products	Type	Active ingredient
1	Crest White Strips (Procter & Gamble)	Whitening strip	5.3% HP gel
2	Crest Night Effects (Procter & Gamble)	Paint-on whitener (night-use)	19% NPP (equivalent to 5.3% HP)
3	Colgate Simply White (Colgate Palmolive Company)	Paint-on whitener (day-use)	18% CP (equivalent to 6.5% HP)
4	Colgate Simply White Night (Colgate Palmolive Company)	Paint-on whitener (night-use)	8.7% HP gel
5	Control (distilled water)	–	–

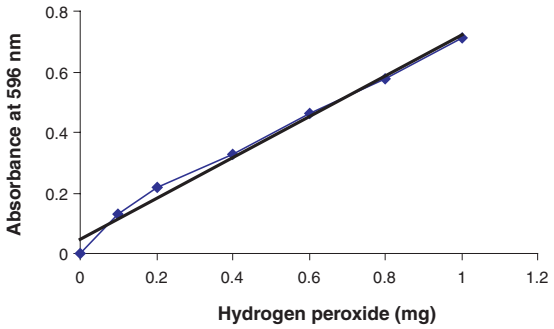

Figure 1 Spectrophotometric calibration curve used in this study.

of 596 nm (at room temperature). The values were converted into microgram equivalents of HP using a spectrophotometric calibration curve (Fig. 1) obtained through dilutions of the 30% HP (Merck KGaA, Darmstadt, Germany) stock solution with distilled water.

The results of measurements were evaluated statistically by using the Kruskal–Wallis Analysis of Variance and non-parametric Mann–Whitney *U*-test.

Results

The Kruskal–Wallis Analysis of Variance ($P < 0.001$) and the Mann–Whitney *U*-tests showed statistically significant differences between all of the groups ($P < 0.05$) (Table 2).

Pulpal peroxide was not observed in the control group (G5). G1, the whitening strip containing 5.3 % HP showed the highest pulpal peroxide penetration. The differences in peroxide penetration were also observed within the paint-on whitener groups. Amounts of hydrogen peroxide found in the pulp chamber of G4 (8.7% HP) was significantly higher when compared with G2 (19% NPP) and G3 (18% CP) specimens. G3 showed the higher pulpal peroxide than G2.

Table 2 Penetration of hydrogen peroxide (37 °C, 30′)

Groups ($n = 10$)	Absorbance (minimum–maximum)	Pulpal peroxide (μg), mean ± SE
1	0.480–0.640	0.726 ± 0.024*
2	0.145–0.215	0.175 ± 0.012*
3	0.170–0.240	0.231 ± 0.011*
4	0.310–0.400	0.443 ± 0.017*
5	0	0

*Statistically significant differences ($P < 0.05$).

Discussion

The recommended application period for home bleaching gels with a tray is given as a minimum of 4 h day^{-1}, and 30 min twice a day for whitening strips. Application period for paint-on whiteners dependent upon the type of product. According to their instructions, night-use paint-on whiteners are applied usually once a day prior to bedtime and usually left *in situ* overnight (approximately 8 h), whilst the day-use paint-on whiteners are usually used for 30 min twice a day. In the studies of peroxide penetration into the pulp chamber, experimental time periods between 15 and 60 min are generally used (Bowles & Ugwuneri 1987, Thitinanthapan *et al.* 1999, Gökay *et al.* 2000a,b, Benetti *et al.* 2004). In this study, a time of 30 min was selected for each of the products evaluated.

Numerous methods are available for the spectrophotometric determination of HP in microgram amounts. The experimental method selected in the current study was considered to be an accurate, convenient, selective and sensitive (Mottola *et al.* 1970). It has been used other by the researchers in a number of other studies (Bowles & Ugwuneri 1987, Cooper *et al.* 1992, Gökay *et al.* 2000a,b).

It is indicated that various factors such as contact time, positive pulpal pressure and osmotic pressure of the gels (Hanks *et al.* 1993), application of heat during bleaching procedure (Bowles & Ugwuneri 1987) might have influence on the pulpal penetration of peroxides. Thitinanthapan *et al.* (1999) also reported that the pulpal peroxide penetration of commercial bleaching products was different even though the product was labelled as having the same concentration of peroxide. As with previous studies (Cooper *et al.* 1992, Gökay *et al.* 2000a,b), teeth were not stored in saliva during the test period in this study. Absence of a water source like saliva may affect the release of HP and the level of penetration of peroxide into the pulp chamber.

This study revealed that peroxides from both whitening strip and paint-on whiteners penetrated into the pulp chamber of an extracted tooth. A great amount of pulpal peroxide was found in the whitening strip group than the paint-on whitener groups. A difference in peroxide penetration was also observed within the paint-on whitener groups.

It is generally argued that use of higher HP containing bleaching products (gel, strip) cause higher pulpal peroxide penetration (Cooper *et al.* 1992, Benetti *et al.* 2004, Gökay *et al.* 2004). In this study, the

correlation between the concentration of HP and peroxide penetration was observed only amongst paint-on whitener groups. Although HP concentration of the whitening strip (5.3%) was similar to or lower than paint-on whiteners (5.3, 6.5 and 8.7%), the highest pulpal penetration was observed in the whitening strip group. This may have resulted from differences in the composition of the bleaching products (paint-on, strip). Paint-on products have been reported as to release HP slowly (Nathoo *et al.* 2002, Date *et al.* 2003). On the other hand, the active ingredient of bleaching products might have affected peroxide penetration. Amongst the bleaching products used in this study, one of the paint-on whiteners and strip contained hydrogen peroxide, whereas the other paint-on products contained either CP or NPP as active ingredients. For bleaching to occur CP and NPP must be broken down firstly into the form of active HP (Haywood & Heymann 1991, Kaneko *et al.* 2000). Therefore, paint-on whitener groups containing 19% NPP and 18% CP have been thought to demonstrate lower pulpal peroxide penetration than the whitening strip group, although initial HP concentrations released from these two paint-on whiteners were reported as similar to or higher than strip (Slezak *et al.* 2002, Date *et al.* 2003, Mahony *et al.* 2003).

The findings of this study with respect to whitening strips are consistent with a previous study (Gökay *et al.* 2004) that had reported pulpal penetration with strips containing 6.5% HP (1.10 ± 0.06 μg) and 14% HP (1.95 ± 0.08 μg). However, with the lower concentration of the whitening strip (5.3% HP) used in this study, lower peroxide penetration amounts (0.72 ± 0.02 μg) were noted.

Since information about pulpal peroxide penetration from paint-on whiteners is not available in the literature, no comparison can be made. It was reported that 10% CP gels were accepted as the safe and tolerable bleaching agents (Haywood & Heymann 1991, Haywood *et al.* 1994) and had been selected as the reference standards for comparison of vital bleaching (Karpinia *et al.* 2002). Cooper *et al.* (1992) and Gökay *et al.* (2000a) used 10 % CP gels in their studies and 3.3 μg of peroxide was recorded in pulp chambers in both. All of the paint-on gels and the whitening strips used in this study had lower pulpal peroxide penetration than 10 % CP gels reported in previous studies.

In vitro studies are limited as they can only simulate the clinical conditions. In the healthy pulp, the pulpal fluid pressure is capable of reducing inward diffusion of chemicals (Matthews & Pashley 1992, Matthews & Vongsavan 1994). The defense mechanism of a healthy pulp tissue would significantly reduce available levels of free HP (Marshall *et al.* 1995), therefore, the amount of HP perfusing the healthy pulp may be less than the levels measured under laboratory conditions. Considering the defense mechanisms of a healthy pulp and the amount of peroxide found in the pulp chamber in this study, it can be assumed that these products are safe, and that peroxides penetrating into the pulp should not be hazardous to the healthy pulp. Gerlach & Zhou (2001) and Gerlach & Barker (2004) also reported that the side effects associated with whitening strips were minor, did not interrupt the treatment and resulted in successful whitening. Similarly, Gambarini *et al.* (2003) and Gerlach & Barker (2003) observed that paint-on whiteners were well-tolerated and provided safe and effective tooth whitening.

Conclusions

On the basis of these results and within the limitations of this *in vitro* study, it may be concluded that HP from paint-on whiteners and whitening strips readily penetrates into the pulp chamber. On the other hand, it is assumed that the degree of penetration of peroxides observed in this *in vitro* study does not affect treatment negatively. However, further studies are necessary to evaluate the clinical behaviour and other properties of these newer bleaching products.

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