In vitro penetration of bleaching agents into the pulp chamber

A. R. Benetti[1], M. C. Valera[1], M. N. G. Mancini[2], C. B. Miranda[1] & I. Balducci[3]

[1]Restorative Dentistry, [2]Physiological Sciences and [3]Social Science and Pediatric Dentistry, São José dos Campos School of Dentistry UNESP, São Paulo, Brazil

Abstract

Benetti AR, Valera MC, Mancini MNG, Miranda CB, Balducci I. *In vitro* penetration of bleaching agents into the pulp chamber. *International Endodontic Journal*, **37**, 120–124, 2004.

Aim To investigate pulp chamber penetration of bleaching agents in teeth following restorative procedures.

Methodology Bovine lateral incisors were sectioned 3 mm apical to the cemento-enamel junction and the coronal pulpal tissue was removed. Teeth were divided into six groups ($n = 10$): G1, G2 and G3 were not submitted to any restorative procedure, while G4, G5 and G6 were submitted to Class V preparations and restored with composite resin. Acetate buffer was placed in the pulp chamber and treatment agents were applied for 60 min at 37 °C as follows: G1 and G4, immersion into distilled water; G2 and G5, 10% carbamide peroxide (CP) exposure; G3 and G6, 35% CP bleaching. The buffer solution was removed and transferred to a glass tube where leuco crystal violet and horseradish peroxidase were added, producing a blue solution. The optical density of the blue solution was determined spectrophotometrically at 596 nm. A standard curve made with known amounts of hydrogen peroxide was used to convert the optical density values of the coloured samples into microgram equivalents of hydrogen peroxide. Data were submitted to ANOVA and Tukey's test (5%).

Results Amounts of hydrogen peroxide found in the pulp chamber of G2 and G5 specimens (0.1833 ± 0.2003 μg) were significantly lower ($P = 0.001$) when compared to G3 and G6 specimens (0.4604 ± 0.3981 μg). Restored teeth held significantly higher ($P = 0.001$) hydrogen peroxide concentrations in the pulp chamber than intact teeth.

Conclusion Higher concentrations of the bleaching agent produced higher levels of hydrogen peroxide in the pulp chamber, especially in restored teeth.

Keywords: carbamide peroxide, dental bleaching, pulp.

Received 2 May 2003; accepted 15 September 2003

Introduction

There are many bleaching agents commercially available with various constituents, such as hydrogen peroxide and carbamide peroxide (CP). CP gels have progressively replaced concentrated hydrogen peroxide solutions (Cooper *et al.* 1992) because of their effectiveness in obtaining whiter teeth.

CP readily decomposes to produce hydrogen peroxide (Haywood & Heymann 1991), which may be considered as the active ingredient of choice for bleaching because of its low molecular weight and its ability to denature proteins (McEvoy 1989). These properties allow hydrogen peroxide molecules to penetrate the enamel and dentine to produce the whitening effect.

Tooth permeability to bleaching agents has been described previously by Bowles & Ugwuneri (1987), Cooper *et al.* (1992), Hanks *et al.* (1993) and Thitinanthapan *et al.* (1999), and this may explain side-effects, such as tooth sensitivity, that result from a reversible pulpitis because of chemical irritation produced by hydrogen peroxide (Haywood 2000). Gokäy *et al.* (2000a) investigated the penetration of bleaching agents into the pulp chamber in teeth restored with various restorative materials. They concluded that the amount of penetration of hydrogen peroxide into the pulp chamber might be

Correspondence: Ana Raquel Benetti, Rua Nicarágua, 2181 apto.51, Bacacheri, Curitiba/PR, Brazil, Cep 82515-260 (Tel.: +55 41 356 9044; fax: +55 41 236 2366; e-mail: anaraquel@rocketmail.com).

affected by the type of restorative material, and was significantly lower for the group restored with composite resin. The authors suggested that composite resins resulted in less microleakage because of the advanced adhesive technology.

As composite resin is the material commonly used for aesthetic restorative procedures of anterior teeth and as most of the patients seeking whither teeth have restorations, the purpose of this *in vitro* study was to evaluate the penetration of bleaching agents in the pulp chamber of restored and intact teeth.

Materials and methods

This project was developed in accordance with the Code of Ethics in Research (approved – n.026/2001-PA/CEP). Bovine lateral incisors were extracted and cleaned of all periodontal tissues. The roots of all teeth were sectioned 3 mm apical to the cemento-enamel junction, and the pulp tissue was removed. The pulp cavities were widened using a round bur and washed with saline solution. All teeth were examined under a stereomicroscope in order to select those without surface defects.

Sixty teeth were divided into six groups, each containing 10 teeth. Groups 1, 2 and 3 were not submitted to cavity preparation. Standardized Class V preparations, 2 mm deep and 4 mm in diameter, were prepared on groups 4, 5 and 6, 3 mm coronal to the cemento-enamel junction. The cavity margins were beveled with fine grit pencil-shaped diamond burs. The teeth were restored with Scotchbond Multipurpose (3M Dental Products; Two Harbors, MN, USA) and composite resin Durafill (Heraeus Kulzer, Hanau, Germany) according to the manufactures' instructions. Twenty-four hours after polymerization, the restorations were finished with Sof-Lex discs (3M Dental Products; Two Harbors, MN, USA) and stored in distilled water. All teeth were subjected to thermocycling between 5 and 55 °C for 500 cycles.

The pulp chambers were dried and 100 μL of 2 M acetate buffer was placed into the pulp chamber of each tooth. The acetate buffer was necessary to stabilize the hydrogen peroxide that might penetrate into the pulp chamber for later quantification.

Teeth were isolated using two layers of nail polish, leaving a standardized buccal area exposed to the bleaching agents. For restored teeth, this area corresponded to 2 mm beyond the limits of the restoration; the intact teeth were treated in the same way. All teeth were fixed vertically to a wax plaque so that the treatment agents could be applied. Groups 1 and 4 were

Table 1 Test groups

Buccal surface	Groups	Testing agent	Exposure period (min)
Intact	1	Distilled water	60
	2	10% CP	60
	3	35% CP	60
Restored (composite resin)	4	Distilled water	60
	5	10% CP	60
	6	35% CP	60

immersed into distilled water, groups 2 and 5 were exposed to 10% CP (Opalescence, Ultradent, South Jordan, UT, USA), while groups 3 and 6 were submitted to 35% CP bleaching (Opalescence Quick, Ultradent) for 60 min at 37 °C (Table 1).

After the exposure period, the acetate buffer solutions in the pulp chamber of each tooth were removed using a microsyringe and transferred to a glass tube. The pulp chamber of each tooth was rinsed two times with 100 μL aliquots of distilled water that were placed into the same glass tube. One hundred microlitres of 0.5 mg mL^{-1} leuco crystal violet (Sigma Chemical Co.) and 50 μL of 1 mg mL^{-1} enzyme horseradish peroxidase (Sigma Chemical Co.) were also added to each tube according to the method described by Mottola *et al.* (1970), and the solution was diluted to 3 mL with distilled water.

The optical density of the resultant blue colour in the tubes was measured by a spectrophotometer (UV-Vis Spectrophotometer UV-1203; Shimadzu, Kyoto, Japan) at the 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.

The statistical variables studied were the concentration of bleaching agent (three levels) and the condition of the buccal surface of the teeth (two levels), which could be analyzed through the optical density of the solutions tested. Descriptive statistics of measurements, two-way ANOVA and Tukey's test were performed at the level of significance of 5%.

Results

Data obtained were submitted to two-way ANOVA under two conditions: original data and logarithmically transformed data. For both situations, no difference was observed in the results.

Statistically significant values for hydrogen peroxide were found depending on the condition of the buccal

Table 2 Homogeneous grouping after Tukey's test (5%) for the interaction effect of the condition of the buccal surface and the concentration of the bleaching agent

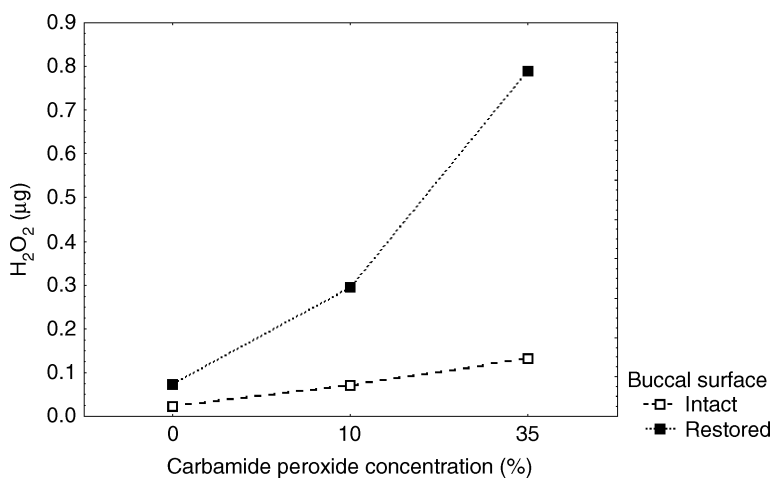| Interaction		Mean (µg) $\pm$ SD	Homogeneous grouping[*]		
Restored	35% CP	0.7897 $\pm$ 0.3003	A		
Restored	10% CP	0.2954 $\pm$ 0.2354		B	
Intact	35% CP	0.1310 $\pm$ 0.0579		B	C
Restored	Water	0.0737 $\pm$ 0.0239			C
Intact	10% CP	0.0712 $\pm$ 0.0368			C
Intact	Water	0.0218 $\pm$ 0.0145			C

CP, carbamide peroxide.

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

surface ($F_{df1;54} = 57.83$; $P = 0.001$), the concentration of the bleaching agent ($F_{df2;54} = 35.13$; $P = 0.001$) and the interaction of both of these factors ($F_{df2;54} = 19.41$; $P = 0.001$). Restored teeth allowed greater hydrogen peroxide penetration (mean $\pm$ SD = 0.3863 ± 0.3715 µg H_2O_2) when compared to intact teeth (mean $\pm$ SD = 0.0747 ± 0.0599 µg H_2O_2).

Tukey's test (5%) indicated that the higher the concentration of the bleaching agent, the higher was the penetration of hydrogen peroxide into the pulp chamber (35% CP, 0.4604 ± 0.3981 µg; 10% CP, 0.1833 ± 0.2003 µg; water, 0.0478 ± 0.0321 µg).

Homogeneous grouping for the interaction effect demonstrated three groups of similar behaviour (Table 2). Greater hydrogen peroxide penetration into the pulp chamber was found in restored teeth exposed to 35% CP (0.7897 ± 0.3003 µg), followed by restored teeth and 10% carbamide bleaching (0.2954 ± 0.2354 µg) and intact teeth after 35% CP exposure (0.1310 ± 0.0579 µg). Intact or restored teeth immersed into distilled water and intact teeth submitted to 10% CP bleaching were not statistically different from each other.

Results of the interaction effect are provided in Fig. 1.

Discussion

Results showed significant amounts of hydrogen peroxide in the pulp chamber for the higher CP concentration. This finding was also demonstrated by Bowles & Ugwuneri (1987) and Hanks *et al.* (1993). According to Hanks *et al.* (1993), the diffusion of hydrogen peroxide through dentine depends on the original concentration of the bleaching agent and the length of time the agent is in contact with the dentine.

Higher hydrogen peroxide levels were found in the pulp chambers of restored teeth, which were also observed by Gökay *et al.* (2000b). The greater penetration of hydrogen peroxide into the pulp chamber of restored teeth is because of the microleakage around restorative materials, as none of the materials are able to completely prevent microleakage (Owens *et al.* 1998, Gokäy *et al.* 2000a).

These results are of concern, especially as hydrogen peroxide affects living tissues. Bowles & Thompson (1986) and Bowles & Burns (1992) have shown that pulpal enzymes, especially the concentrated agents, were inhibited to some degree by hydrogen peroxide. Hoffman & Meneghini (1979) demonstrated that hydrogen peroxide

Figure 1 Graphic representation of the interaction effect.

was toxic to cultured human fibroblasts with loss of colony-forming ability because of single-strand breaks in DNA. Hanks *et al.* (1993) verified that it took as little as 15 min for bleaching agents to diffuse through dentine disks and reach a level capable of causing harmful biological effects on fibroblast cultures.

On the other hand, *in vivo* studies demonstrated little or insignificant damage after bleaching. Cohen & Chase (1979) reported that human premolars exposed to 35% hydrogen peroxide at 54 °C during three sessions of 30 min did not produce any subsequent histological evidence of pulpal damage. Robertson & Melfi (1980) found only mild inflammatory pulpal response after two 35% hydrogen peroxide applications 4 days apart. Clinical trials have found observations of sporadic and reversible reactions, either for in-office or at-home techniques (Matis *et al.* 2000, Leonard *et al.* 2001).

This *in vitro* model is representative of the *in vivo* process, although it is not known how closely it compares to the *in vivo* absorption of hydrogen peroxide in teeth with vital pulps during the bleaching processes (Bowles & Ugwuneri 1987). One possible mechanism by which the pulp may protect itself from damage by hydrogen peroxide is through enzymatic breakdown of the molecule by peroxidase and catalase (Bowles & Burns 1992). Anderson *et al.* (1999) demonstrated also that pulp cells produce haeme-oxygenase 1, an important defensive enzyme under conditions of oxidative stress, specially found in odontoblasts and endothelial cells subjacent to the areas of bleached enamel. According to the authors, these findings indicate that the pulp responds to oxidative stress at the molecular level. There are also at least two forces that might work against the diffusive flux of molecules of the bleaching agents towards the pulp: the positive pulpal pressure and osmotic pressure of the gels (Hanks *et al.* 1993, Thitinanthapan *et al.* 1999).

Although there are defensive mechanisms against the irritation caused by the bleaching agents and only reversible side-effects have been reported, the data obtained in this study reinforce the need for caution in the use of bleaching agents. As different concentrations of solutions eventually yield the same colour change, although following different rates (Leonard *et al.* 1998), bleaching agents of lower concentrations are preferred in order to minimize the side-effects produced by hydrogen peroxide.

Conclusion

In this *in vitro* study, a higher concentration of bleaching agent produced higher levels of hydrogen peroxide in the pulp chamber, especially in restored teeth. Although further research is essential to investigate the penetration of bleaching agents *in vivo*, the results from this study suggest that the use of lower concentration bleaching agents may be safer in restored teeth.

Acknowledgements

References

Anderson DG, Chiego DJ Jr, Glickman GN, McCauley LK (1999) A clinical assessment of the effects of 10% carbamide peroxide gel on human pulp tissue. *Journal of Endodontics* **25**, 247–50.

Bowles WH, Burns JRH (1992) Catalase/peroxidase activity in dental pulp. *Journal of Endodontics* **18**, 527–9.

Bowles WH, Thompson LR (1986) Vital bleaching: the effects of heat and hydrogen peroxide on pulpal enzymes. *Journal of Endodontics* **12**, 108–12.

Bowles WH, Ugwuneri Z (1987) Pulp chamber penetration by hydrogen peroxide following vital bleaching procedures. *Journal of Endodontics* **13**, 375–7.

Cohen SC, Chase C (1979) Human pulpal response to bleaching procedures on vital teeth. *Journal of Endodontics* **5**, 134–8.

Cooper JS, Bokmeyer TJ, Bowles WH (1992) Penetration of the pulp chamber by carbamide peroxide bleaching agents. *Journal of Endodontics* **18**, 315–7.

Gökay O, Yilmaz F, Akin S, Tunçbilek M, Rahmiye E (2000a) Penetration of the pulp chamber by bleaching agents in teeth restored with various restorative materials. *Journal of Endodontics* **26**, 92–4.

Gökay O, Tunçbilek M, Ertan R (2000b) Penetration of the pulp chamber by carbamide peroxide bleaching agents on teeth restored with a composite resin. *Journal of Oral Rehabilitation* **27**, 428–31.

Hanks CT, Fat JC, Wataha JC, Concoran JF (1993) Cytotoxicity and dentin permeability of carbamide peroxide and hydrogen peroxide vital bleaching materials *in vitro*. *Journal of Dental Research* **72**, 931–8.

Haywood VB (2000) Current status of nightguard vital bleaching. *Compendium Continuing Education in Dentistry* **21** (Suppl.), S10–7.

Haywood VB, Heymann HO (1991) Nightguard vital bleaching: how safe is it? *Quintessence International* **22**, 515–23.

Hoffmann ME, Meneghini R (1979) Action of hydrogen peroxide on human fibroblasts in culture. *Photochemistry and Photobiology* **30**, 151–5.

Leonard RH, Sharma A, Haywood VB (1998) Use of different concentrations of carbamide peroxide for bleaching teeth: an *in vitro* study. *Quintessence International* **28**, 503–7.

Leonard RH, Bentley C, Eagle JC, Garland GE, Knight MC, Phillips C (2001) Nightguard vital bleaching: a long-term study on efficacy, shade retention, side effects, and patient's perceptions. *Journal of Esthetic and Restorative Dentistry* **13**, 357–69.

Matis BA, Mousa HN, Cochran MA, Eckert GJ (2000) Clinical evaluation of bleaching agents of different concentrations. *Quintessence International* **31**, 303–10.

McEvoy SA (1989) Chemical agents for removing intrinsic stains from vital teeth. Part II. Current techniques and their clinical application. *Quintessence International* **20**, 379–84.

Mottola HA, Simpson BE, Gorin G (1970) Absorptiometric determination of hydrogen peroxide in submicrogram amounts with leuco crystal violet and peroxidase as catalyst. *Analytical Chemistry* **42**, 410–1.

Owens BM, Halter TK, Brown DM (1998) Microleakage of tooth-colored restorations with a beveled gingival margin. *Quintessence International* **29**, 356–61.

Robertson WD, Melfi RC (1980) Pulpal response to vital bleaching procedures. *Journal of Endodontics* **6**, 645–9.

Thitinanthapan W, Stamanont P, Vongsavan N (1999) *In vitro* penetration of the pulp chamber by three brands of carbamide peroxide. *Journal of Esthetic and Restorative Dentistry* **11**, 259–64.