
Extraradicular diffusion of hydrogen peroxide and pH changes associated with intracoronal bleaching of discoloured teeth using different bleaching agents

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Abstract

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Aim To evaluate the extraradicular pH and hydrogen peroxide (HP) diffusion when either 35% carbamide peroxide (CP), 35% HP or sodium perborate (SP) is used for intracoronal bleaching of artificially discoloured teeth.

Methodology Single rooted extracted human premolars were stained with whole blood cells. After shaping and cleaning, they were root filled and a base cement placed 1 mm below the buccal cemento-enamel junction (CEJ). Four cemental defects were prepared just below the CEJ on each root surface. The teeth were randomly divided into four groups of 11 specimens, and intracoronally bleached using CP, HP, SP or distilled water (CL). Each tooth was suspended in a vial of distilled water and bleached for 7 days. The pH of the

extraradicular distilled water was tested at 0, 1, 2 and 7 days and the HP that diffused through the root quantified using the Ferrous Oxidation–Xylenol Orange 2 Assay. The results were analysed using the one-way ANOVA and Scheffe tests.

Results Carbamide peroxide produced the greatest increase and HP the least pH change ($P < 0.05$ except day 1), SP was intermediate. From day 1 onwards, radicular diffusion of HP was greatest with HP and least with CP ($P < 0.01$), again SP was intermediate. There was no significant difference between CP and SP.

Conclusions Carbamide peroxide had very low levels of extraradicular diffusion of HP, in the presence of cemental defects. It could be an alternative to the other intracoronal bleaching agents.

Keywords: carbamide peroxide, dentine diffusion, hydrogen peroxide, sodium perborate, tooth bleaching, tooth discolouration.

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Introduction

Root filled teeth may discolour because of the incorporation of breakdown products from pulpal haemorrhage, incompletely removed pulpal remnants (Hattab *et al.* 1999, Watts & Addy 2001) or from root canal

filling materials and sealers containing eugenol or silver salts (Boksman *et al.* 1983, van der Burgt & Plasschaert 1986). In the anterior region, this discolouration could detract from the appearance of the teeth. Fortunately, this can be corrected simply and economically using intracoronal bleaching in the appropriate circumstances. The biological aspects of intracoronal bleaching (Dahl & Pallesen 2003) and its current status (Attin *et al.* 2003) have been reviewed recently.

For some time, the most popular intracoronal bleaching technique has been thermocatalytic bleach-

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ing using 30–35% hydrogen peroxide (HP). However, since initial reports of cervical root resorption (Harrington & Natkin 1979), clinicians have been more circumspect in recommending intracoronary bleaching. Where it is indicated, it is generally considered safer to use sodium perborate (SP) as the intracoronary bleaching agent (Attin *et al.* 2003). Laboratory studies have demonstrated that intracoronary HP can diffuse through the root, and this diffusion is greater in the presence of cemental root defects (Rotstein *et al.* 1991a, Koulaouzi-dou *et al.* 1996). Animal studies have confirmed the association of intracoronary bleaching with cervical root resorption (Madison & Walton 1990, Heller *et al.* 1992). There is speculation that diffusion of hydrogen ions from intracoronary bleaching agents may provide an acidic environment that is optimal for osteoclastic activity and bone resorption (Vaes 1968), resulting in external cervical root resorption. Price *et al.* (2000) investigated the pH of some bleaching agents and found that the in-office bleaching products were acidic. Kehoe (1987) reported that the Superoxol-SP combination is acidic, although both Fuss *et al.* (1989) and Rotstein & Friedman (1991) determined that the combination is alkaline. The difference observed is probably because of variation in the proportion of the mixture used in the three studies, as Superoxol is acidic whereas SP is alkaline.

Hydrogen peroxide is a ubiquitous molecule produced by a range of oxidase enzymes including glycolate and monoamine oxidases as well as by the peroxisomal pathway for beta-oxidation of fatty acids. Therefore, most or all human cells are exposed to some level of HP, and levels below $20 \mu\text{mol L}^{-1}$ are considered safe. Millimolar levels of HP can contribute to Fenton chemistry (Fenton chemistry is the term used to describe the reaction of iron with HP that results in hydroxyl radicals) by not only providing itself as one of the substrates but by also liberating iron from haem proteins (Halliwell *et al.* 2000). Therefore, to simulate the clinical situation, evaluation of radicular penetration of HP should be performed in discoloured teeth as haem proteins causing the discolouration will alter the pharmacodynamics.

Rotstein *et al.* (1991b) used an *in vitro* model utilizing nondiscoloured, extracted teeth to quantify HP penetration through the root during intracoronary bleaching. They demonstrated increased radicular penetration of HP with both bleaching time and temperature. Weiger *et al.* (1994) used different forms of SP and found radicular penetration of HP in at least 83% of each group of artificially discoloured extracted teeth.

Most studies investigating the pH or radicular penetration of HP during intracoronary bleaching have been performed using nondiscoloured teeth. As carbamide peroxide (CP), which has more recently recommended for use in intracoronary bleaching (Vachon *et al.* 1998), has not been evaluated, the study was performed to evaluate the pH changes and quantify the radicular penetration of HP when using three different intracoronary bleaching agents, 35% CP, 35% HP and SP to bleach artificially discoloured extracted human teeth.

Materials and methods

Premolar teeth extracted for orthodontic reasons from patients under the age of 21 years were used. Soft tissue covering the root surface was removed with gauze soaked with 2.5% sodium hypochlorite and any calculus was removed gently with scalers. The teeth were then stored in thymol saline and kept in an incubator at 37 °C throughout the experiment.

All teeth were stained with whole human blood cells obtained from the blood bank following the method described by Freccia & Peters (1982). The teeth were immersed in whole blood without the serum, and centrifuged at 3200 rpm for 20 min twice a day for three consecutive days to encourage penetration of the haemolysed red blood cells into the dentinal tubules. At the end of 3 days, the precipitate were removed and the remaining haemolysate containing the haemoglobin was used to immerse the teeth for a further 3 days, and centrifuged twice daily as previously described. The discoloured teeth were subsequently washed and cleaned with distilled water.

The apical portion of each root was removed to leave 5 mm of the root from the buccal cemento-enamel junction (CEJ). After standard access cavity preparation, the root canals were cleaned and shaped with files, using 2.25% sodium hypochlorite for irrigation. The coronal root opening was prepared with a Gates-Glidden number 4 bur. The root canal was filled with thermoplasticized gutta-percha (Obtura II; Obtura Corporation, Fenton, MO, USA) and root canal sealer (Roth sealer, Chicago, IL, USA). Sufficient gutta-percha was removed to allow a 2 mm thick layer of Cavit (3M ESPE, Seefeld, Germany) to be placed 1 mm apical to the labial CEJ, to isolate the bleaching agent within the pulp chamber. Any remnants of gutta-percha or cement covering the walls of the access cavity were completely removed with a small carbide bur followed by thorough rinsing.

The root surface was coated with a double layer of a nail varnish to seal potential superficial defects. To

simulate standardized breaks in the cemental covering, four cemental–dentinal defects were prepared just below the CEJ on each root surface – mesial, distal, buccal and lingual aspects. The hemispherical defects (diameter 1.0 mm, depth 0.5 mm) were created using a round diamond bur (diameter 1.0 mm) in a high-speed handpiece. The smear layer created in the defects was removed with 15% EDTA, then thoroughly washed with distilled water.

The teeth were randomly divided into four groups of 11 specimens each, and intracoronally bleached for 7 days using:

Group CP: 35% CP gel (Opalescence Quick; Ultradent Products, Inc., South Jordan, UT, USA).

Group HP: 35% HP gel (Opalescence Endo; Ultradent Products, Inc.).

Group SP: SP (Roth International Ltd) mixed with distilled water in the ratio 2 : 1 to form a paste.

Group CL: Distilled water only (control).

After 0.04 mL of the respective bleaching agent was syringed into the access cavity, it was sealed with Cavit. Each tooth was suspended in a plastic vial of distilled water using laboratory sealing film (Whatman plc, Maidstone, UK). The sealing film was cut to fit each tooth at the level of the CEJ and stabilized with sticky wax to achieve a tight seal (Fig. 1). The pH of the surrounding distilled water and the amount of HP that leached out into the distilled water were evaluated at 0, 1, 2 and 7 days.

pH testing

The pH meter (Orion PrepHect Log R meter; Allometrics Inc., Baton Rouge, LA, USA) was calibrated with

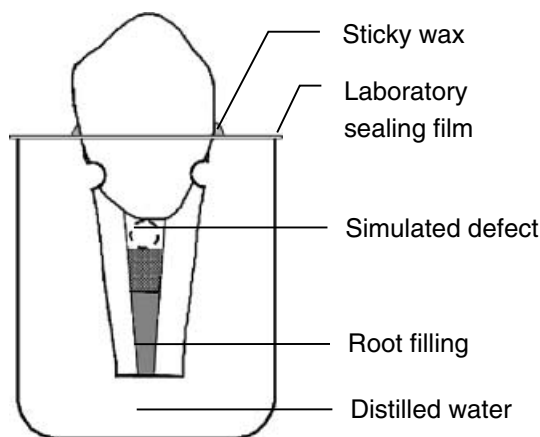


Figure 1 Diagrammatic view of experimental set-up.

standard pH solutions before each experiment. For each sample, the average of two readings was utilized. The test tip was washed with distilled water and dried with laboratory wipes to prevent any contamination between tests.

Measurement of hydrogen peroxide

Quantification of HP that diffused into the surrounding distilled water was determined using the Ferrous Oxidation–Xylenol Orange 2 (FOX 2) Assay (Jiang *et al.* 1992), in duplicate. Test solution (90 µL) was placed into four test tubes. Two test tubes contained 10 µL of methanol and the other two tubes contained 10 µL of catalase; 900 µL of the FOX 2 reagent was added to all four tubes. The tubes were vortexed and incubated at room temperature for 10 min, then centrifuged (Eppendorf, Schonenbuch, Switzerland) at 14 000 rpm at room temperature to ensure that the samples were supernatant. The samples were then placed into a spectrophotometer (Model: DU 6408; Beckman, Fullerton, CA, USA) using a wavelength of 560 nm and blanked with methanol, to determine their absorbance value. FOX 2 reagent was subsequently used in calibrating eight different known values of HP to obtain a standard calibration graph to determine the concentration of HP in the test solutions. The results were analysed using the one-way ANOVA and the Scheffe *post-hoc* test.

Results

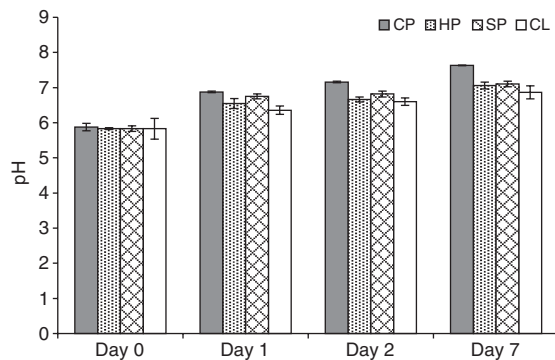
The mean pH of each group is summarized in Table 1 and illustrated graphically in Fig. 2. The between-group comparisons are shown in Table 2. The mean levels of HP detected are summarized in Table 3 and illustrated graphically in Fig. 3. The between-group comparisons are shown in Table 4.

Discussion

In this study, intact, single-rooted mandibular premolars extracted for orthodontic reasons from patients below 21 years were used. They were selected because of the availability of such teeth as well as most reported cases of cervical root resorption associated with intracoronally bleaching were in young patients (Heithersay *et al.* 1994). It is probable that the wide and patent dentinal tubules in young teeth would favour ionic diffusion of the bleaching agent through dentine.

Table 1 Summary of pH of extraradicular solution

Group	Day 0		Day 1		Day 2		Day 7	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CP	5.876	0.105	6.876	0.024	7.159	0.082	7.632	0.294
HP	5.832	0.024	6.550	0.141	6.661	0.074	7.059	0.121
SP	5.831	0.024	6.750	0.070	6.818	0.084	7.100	0.104
CL	5.828	0.010	6.356	0.098	6.602	0.079	6.866	0.183

**Figure 2** Graph of pH changes.**Table 2** Between-group comparisons of pH using Sheffe *post hoc* analysis

Groups	Day 0	Day 1	Day 2	Day 7
CP × HP	<0.05	NS	<0.05	<0.05
CP × SP	<0.05	NS	NS	NS
CP × CL	NS	NS	NS	NS
HP × SP	NS	NS	NS	<0.05
HP × CL	<0.05	NS	<0.05	<0.05
SP × CL	<0.05	NS	<0.05	NS

Over the 7 days evaluated, the pH of all groups increased with time. The test system itself was acidic and this was attributed to the protective varnish applied onto the root surface, which was later determined to have a value of pH 4 before setting. The extraradicular solution of all test groups had a higher pH than the control group. When the effect of the control is taken into account, the rate of change of pH

in the test groups becomes lower, but it nevertheless still increases. Rotstein & Friedman (1991) also observed a similar increase in their evaluation of the changes in pH of HP and SP stored in plastic vials over a period of 14 days. They attributed the increasing pH to the decomposition of acidic HP to oxygen and water. However, in addition, CP also yields urea, which further breaks down to ammonia and carbon dioxide (Dahl & Pallesen 2003). This was probably responsible for group CP showing the largest pH change besides being the most alkaline of all the groups.

The pH of 35% HP gel and 35% CP gel is 3.7 and 6.5 respectively (Price *et al.* 2000), while SP is pH 9.9 (Rotstein & Friedman 1991). When comparing the pH of the extraradicular solution of the different intracoronal bleaching agents, even at day 1 where the difference in extraradicular HP levels between the test groups was greatest, their mean pH was recorded within a narrow band between pH 6.6 and pH 6.9 and there were no significant differences between the groups. This demonstrates the poor diffusivity of hydrogen ions through dentine, as a result of the buffering capacity of the hydroxyapatite (Wang & Hume 1988). Hence the results support the view of Rotstein & Friedman (1991) that it is unlikely that cervical root resorption is the result of an acidic extraradicular pH environment produced by the bleaching agent. Bleaching agents cause superficial structural changes to dentine (Rotstein *et al.* 1996), and the acid pH probably produces an acid-etch effect on dentine, opening up the smear layer covered cut surface of dentinal tubules, increasing its permeability (Carrasco *et al.* 2003). This then probably permits

Table 3 Summary of levels of extraradicular hydrogen peroxide $\times 10^{-2}$ (nmol L⁻¹)

Group	Day 0		Day 1		Day 2		Day 7	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
CP	39.8157	14.0031	39.1728	16.5006	28.2836	16.6394	7.6669	3.7484
HP	156.7824	63.3409	288.2197	276.1737	254.9897	190.4632	58.9618	29.4727
SP	138.6831	41.0157	50.3047	27.5842	177.6824	43.7664	20.2228	9.8998
CL	3.8509	2.4733	7.8487	1.9196	9.2629	2.0712	8.2731	1.8818

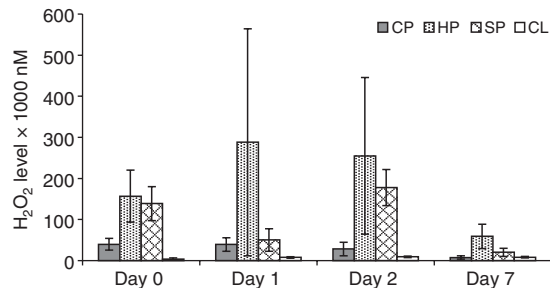


Figure 3 Graph of extraradicular H_2O_2 levels detected (nmol L^{-1}).

Table 4 Between group comparisons of extraradicular hydrogen peroxide detected using Scheffe *post hoc* tests

Group	Day 0	Day 1	Day 2	Day 7
CP vs. HP	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
CP vs. SP	$P < 0.01$	NS	$P < 0.01$	NS
CP vs. CL	NS	NS	NS	NS
HP vs. SP	NS	$P < 0.01$	NS	$P < 0.01$
HP vs. CL	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$
SP vs. CL	$P < 0.01$	NS	$P < 0.01$	NS

greater diffusion of HP through the dentinal tubules, particularly at cemental defects that may be present at the CEJ (Koulaouzidou *et al.* 1996). Perhaps if the level of HP goes beyond the critical level, then destructive cervical root resorption is seen.

The pH of bleaching agents is important as it determines the rate of reaction of the bleaching process. More free radicals are produced the higher the pH. According to a review of the bleaching reaction (Sun 2000), optimal ionization occurs when HP is buffered in the range of pH 9.5–10.8. In this range, the bleaching effect could be 50% better than when it is more acidic. However, most commercial bleaching agents are acidic (Price *et al.* 2000) as this results in longer shelf life. Thus, it would seem that most commercial products are optimized for shelf life rather than optimal bleaching action.

To simulate cemental defects, which enhance extraradicular HP diffusion during intracoronal bleaching (Rotstein *et al.* 1991a), four identical defects were created on each tooth surface at the CEJ. Clinically, a defect can be found between the cementum and enamel at the level of the CEJ in about 10% of all teeth, thus exposing dentine (Ten Cate 1985). Higher penetration results were measured in teeth with a gap between cementum and enamel while minimal values were measured in teeth with cementum overlap and/or edge-to-edge contact of the two tissues (Koulaouzidou *et al.*

1996). As the depth of the defects was made relative to the external root surface, the thickness of dentine through which the bleaching agent had to traverse was not standardized.

The control group CL showed that the test system had very low levels of HP. This was attributed to the phenomena autophoto-oxidation, a process that naturally liberates HP as long as hydrogen and oxygen is present in any form. As HP is a ubiquitous molecule (Halliwell *et al.* 2000), it is difficult to totally exclude extraneous HP from the test system.

Thirty-five per cent HP showed the greatest levels of extraradicular diffusion, while 35% CP showed the lowest, SP was intermediate. The peak amount of extraradicular diffusion of HP was detected from the samples in group HP at day 1; with the highest sample producing a level of $0.7095 \text{ nmol L}^{-1}$. When using 30% HP for 24 h of intracoronal bleaching of premolars without an intermediate base, Koulaouzidou *et al.* (1996) detected 7350 nmol L^{-1} HP in the extraradicular solution. Rotstein (1991) evaluated the diffusion of hydrogen with thermocatalytic intracoronal bleaching using 30% HP solution without an intermediate base. The maximum quantity of extraradicular HP detected was even higher at $83\,348 \text{ nmol L}^{-1}$, as heat increases the rate of diffusion. Another factor to consider is that in our study the 35% HP used was in gel form, whereas other studies used liquid form. The gel could possibly retard the release of the active ingredient from it by acting as a controlled release depot. However, it is unlikely that it would have such a large effect.

On the whole, the levels of HP detected in our study in all three experimental groups were low. According to Halliwell *et al.* (2000), levels of HP under $20 \mu\text{mol L}^{-1}$ should be safe as when it exceeds $50 \mu\text{mol L}^{-1}$, it is cytotoxic to most living cells. As the levels of HP quantified in our study were below this critical level for all three experimental groups, these intracoronal bleaching agents should be safe to use when an intermediary base is placed. However, as the present study did not have an experimental group without an intermediate base, it was not possible to quantify the effectiveness of an intermediate base in reducing extraradicular diffusion of HP. However, as mentioned previously, the levels of HP detected in the present study was far lower than those of two previous studies where an intermediate base was not utilized (Rotstein 1991, Koulaouzidou *et al.* 1996). Taking these three studies together, it would seem that an intermediate base is an effective means of reducing the diffusion of

HP into the extraradicular environment. This also seems to corroborate MacIsaac & Hoen's (1994) conclusion from their extensive literature review of intracoronary bleaching that the common thread through all reported cases of external cervical root resorption was that an intermediate base was not used.

As 35% CP decomposes on contact with moisture to yield approximately 12% HP, it is understandable that the levels detected in group CP were significantly less than in group HP ($P < 0.01$) throughout the 7-day period. However, it was surprising that the levels detected were not significantly different from the control group (CL). Like group HP, CP was in gel form and this may act as a depot and slowly release CP. More likely it is because CP does not diffuse through dentine as well as HP (Cooper et al. 1992). Therefore, it can be surmised that as CP traverses dentine more slowly, together with the rise in pH rise aided by the resultant ammonia, the deionization of the HP is facilitated (Sun 2000). Therefore, little unreacted HP is left to diffuse through the root dentine into the extraradicular environment.

Hydrogen peroxide may be used as an inter and intracellular signalling molecule in some cells. On its own it is poorly reactive; and the body has evolved mechanisms for dealing with it (Halliwell et al. 2000). However, in the presence of inflammation, proinflammatory agents activate NADPH oxidase, which produces superoxides that could react with HP. It is speculated that the resultant hypochlorous acid, N-chloroamines and reactive hydroxyl ions may initiate some disease processes (Grisham 1994). This is probably the reason why in the quest to identify predisposing factors associated with intracoronary bleaching induced external cervical root resorption, a history of trauma or orthodontics which can cause inflammatory changes or heat which increases the diffusivity of the bleaching agent have been amongst the most frequently associated factors after the absence of an intermediate base (MacIsaac & Hoen 1994, Heithersay 1999).

In the present study, it is possible to specifically relate the changes in pH of the extraradicular medium to the quantity of HP. It appears that the quantity of HP is inversely proportional to the pH of the surrounding medium. Thirty-five per cent CP gel was the least acidic and also showed the lowest diffusion of HP into the extraradicular environment. In another study, it has been reported that 35% CP was equally effective as 35% HP for intracoronary bleaching of artificially discoloured teeth, both of which were more efficient than SP for intracoronary bleaching (Lim et al., submit-

ted). Together with these very low levels of extraradicular diffusion detected in this study, 35% CP could very well be the intracoronary bleaching agent of choice.

Conclusions

The extraradicular diffusion of HP was inversely proportional to the increase in external root pH. The quantity of HP detected in the extraradicular medium in group CP was significantly lower than group HP, and not significantly different from group SP. Therefore, 35% CP gel could be an alternative to SP or 30–35% HP presently used for intracoronary tooth bleaching.

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