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Influence of intracoronal bleaching agents on the ultimate strength and ultrastructure morphology of dentine

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Abstract

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Objective To evaluate the effects of intracoronal bleaching on ultimate tensile strength (UTS) of sound and etched dentine and its ultrastructure morphology.

Methodology Bovine dentine specimens with (e) or without previous etching with 37% phosphoric acid for 15 s were used for the intracoronal bleaching experiments. Teeth were randomly assigned to five treatments (n = 10): (C) control – no bleaching, (SP) sodium perborate, (CP) 35% carbamide peroxide, (25% HP) 25% hydrogen peroxide and (35% HP) 35% hydrogen peroxide. Bleaching was performed four times within a 72 h interval and afterwards, dentine pulp chamber blocks were obtained. The blocks were sectioned in 0.7 mm-thick slices and these were trimmed to reduce the inner dentine to a dumbbell shape with a cross-sectional area of 0.8 mm^2. Specimens were tested with the microtensile method (0.5 mm min^{-1}) and data were analysed (two-way ANOVA-Tukey test, $P < 0.05$). Additional teeth were prepared for transmission electron microscopy (TEM) to evaluate dentine ultramorphology.

Results The mean values of the UTS (SD) in MPa for sound dentine were: C = 48.3(8.5)a, SP = 34.6 (8.2)b, CP = 32.9 (8.9)b, 25% HP = 28.0(4.6)b, 35% HP = 26.4(6.6)b, and pre-etched dentine: Ce = 38.9(13.8)a, SPe = 31.3 (9.3)ab, CPe = 28.4 (6.2)ab, 25% HPe = 30.0 (7.9)ab, 35% HPe = 19.9(4.6)b. Significant differences between the means are indicated by the letters. TEM observations exhibited demineralization areas for all bleaching treatments.

Conclusion Bleaching decreased dentine UTS after treatment. Pre-etched not-bleached dentine (Ce) presented UTS similar to pre-etched bleached dentine, except for 35% HPe. The decrease of UTS of bleached dentine could be attributed to ultrastructural alterations such as loss of inorganic components.

Keywords: dentine, intracoronal bleaching, ultimate tensile strength, ultrastructure.

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Introduction

Root filled teeth may discolour due to the incorporation of degradation products from haemorrhage, incomplete removal of pulpal remnants, or the presence of filling materials and sealers after endodontic procedures

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(Watts & Addy 2001). Thus, numerous intracoronal bleaching procedures, such as the walking bleach technique, have been proposed to reverse tooth discolouration (Chng *et al.* 2004).

The walking bleach technique involves the application of a bleaching agent to the dentine of the pulp chamber between dental visits (Nutting & Poe 1967). A mixture of sodium perborate and distilled water has been extensively used as an effective agent for intracoronal bleaching. In order to enhance bleaching efficacy, 30% hydrogen peroxide was suggested as a substitute for water (Ho & Goerig 1989). Although concentrated hydrogen peroxide (25–35%) is efficient for bleaching teeth with or without vital pulp, it has been associated with undesirable complications such as changes in surface morphology and hardness (Zalkind *et al.* 1996), decreased ultimate tensile strength (UTS) (Chng *et al.* 2002), and reduced bond strength of composite resins to dental structures (Timpawat *et al.* 2005). However, the most devastating known complication of intracoronal bleaching is cervical root resorption (Chng *et al.* 2004).

Another bleaching agent, 35–37% carbamide peroxide, has emerged as a popular and effective agent for both in-office and intracoronal bleaching techniques (Gokay *et al.* 2000, Cavalli *et al.* 2004a, Chng *et al.* 2004, Lim *et al.* 2004). Although considered effective, some reports have shown that even 35% carbamide peroxide may cause adverse effects, such as decreased dentine microhardness (Pécora *et al.* 1994, Chng *et al.* 2004) and decreased dentine ultimate strength (Piemjai & Surakompontorn 2006).

Removal of the smear layer with 37% phosphoric acid has been proposed to improve and hasten intracoronal bleaching (Horn *et al.* 1998), assuming that this procedure would result in more permeable dentine and, in turn, faster and more effective bleaching (Casey *et al.* 1989). However, some reports demonstrated that removal of the smear layer did not improve bleaching effectiveness (Casey *et al.* 1989, Horn *et al.* 1998). Yet, it is unknown if smear layer removal affects the ultimate strength of dentine following bleaching. In order to access dentine alterations and establish a comparison amongst bleaching agents currently used for teeth with nonvital pulps, this study tested the null hypotheses: (i) the UTS of dentine was not altered after different intracoronal bleaching treatments, (ii) the removal of smear layer will not affect dentine UTS, and (iii) intracoronal bleaching agents will not affect the ultrastructure of dentine.

Materials and methods

Selection and tooth preparation

A total of one hundred bovine incisors were collected and stored in 0.1% thymol solution (pH 7.0). The teeth were thoroughly cleaned, and conventional endodontic access cavities were prepared in each tooth using a diamond bur (3018HL, KG Sorensen, Barueri, SP, Brazil) in a high-speed hand piece (Kavo do Brasil, Joinville, SC, Brazil) under water cooling. The pulp tissue was removed, and, during subsequent preparations, care was taken to prevent dehydration of the specimens by wrapping all teeth in gauze moistened with water (Timpawat *et al.* 2005). The roots were removed 4 mm apical to the cement–enamel junction (CEJ) with a diamond saw (7020, Kavo do Brasil) under water cooling, and the root orifice was sealed with glass–ionomer cement (Vidreon R, SS White Duflex, Rio de Janeiro, RJ, Brazil).

Half of the teeth (n = 50) used in the experiment were etched with a 37% phosphoric acid gel (3M ESPE, St Paul, MN, USA) for 15 s and rinsed for 15 s under a water spray. The phosphoric acid etching was applied intracoronally to the dentine pulp chamber. The preetched, referred to as (e) and nonetched bovine incisors were randomly assigned to five treatments (n = 10):

- Control (C, Ce) – no bleaching. A cotton pellet soaked with distilled water was inserted into the pulp chamber (Chng *et al.* 2004).
- Sodium perborate (SP, SPe) (Vitale Pharmaceutics, Limeira, SP, Brazil) – SP was mixed with distilled water to a consistency of wet sand (0.01g 0.5 mL^{-1}).
- 35% Carbamide peroxide gel (CP, CPe) (Vitale Pharmaceutics).
- 25% Hydrogen peroxide gel (25% HP, 25% HPe) (Vitale Pharmaceutics).
- 35% Hydrogen peroxide gel (35% HP, 35% HPe) (Vitale Pharmaceutics).

The bleaching agents (0.01 g) were applied to the pulp chamber, and the access cavity was sealed with temporary cement (Cavit, 3M ESPE, Seefeld, Germany). Each tooth was stored in an individually labelled, capped plastic vial (Injeplast, São Paulo, SP, Brazil) containing gauze soaked with distilled water. After a 3-day period, the temporary restoration was removed, the bleaching agent was rinsed out via flushing with water, and fresh bleaching agent was placed into the access cavity (Oliveira *et al.* 2007). This procedure was repeated four times (Attin *et al.* 2003). After the fourth

bleaching session, the temporary cement was removed from the access cavity and teeth were stored 24 h in water for the complete removal of intracoronal agents.

Microtensile testing

The crown was cut vertically, mesial to distal, with a diamond saw mounted in a slow-speed hand piece (Kavo do Brasil) under water cooling, and the labial pulp chamber dentine was obtained. The labial segments were cleaned with distilled water to remove debris, and each block was sectioned into approximately four slices 0.7 mm thick. Enamel was ground and removed with 400 grit-wet silicon carbide. Each slice was trimmed with a fine diamond bur (1040, KG Sorensen) to reduce the inner dentine to a dumbbell shape. The area of the trimmed inner dentine subjected to microtensile strength test was of approximately 0.8 mm^2 (Fig. 1). The specimens were stored in deionized water for 24 h at 37 °C, prior to testing. Each specimen was fixed to the 'grips' of a microtensile test device, and tension was evaluated (EZ Test – Shimadzu, Tokyo, Japan) at a crosshead speed of 0.5 mm min^{-1} until failure. After testing, specimens were carefully removed from the fixtures with a scalpel blade, and the cross-sectional area at the site of fracture was measured with a digital caliper (727–6/150, Starret, Itu, SP, Brazil) to calculate the UTS, which was expressed in MPa (Cavalli *et al.* 2004b). The mean bond strengths were statistically analysed by two-way analysis of variance (ANOVA) and Tukey test ($\alpha = 0.05$). A *P*-value of <0.05 was considered to be significant.

Transmission electron microscopy

Specimens of each group that had not been subjected to microtensile strength assessment were used for transmission electron microscopy (TEM) analysis. Two slices per group approximately 0.7 mm thick obtained from labial pulp chamber dentine were fixed in Karnovsky's solution, post-fixed in osmium tetroxide, dehydrated in an ascending ethanol series (30–100%), and embedded in epoxy resin. Propylene oxide was used as a transitional fluid. Representative 60 nm-thick ultra-thin sections were prepared with an ultramicrotome (Sorvall-Porter-Blum MT 2b, Newtown, CT, USA) using a diamond knife and collected onto 100-mesh carbon/formvar-coated cooper grids. The ultra-thin sections were placed on the grids, stained with phosphotungstic acid (PTA) and observed at TEM (Philips CM12; Philips, Eindhoven, The Netherlands) operated at 80 kV (Reis *et al.* 2007). Staining of the ultra-thin sections with PTA was necessary in order to observe collagen fibrils banding in case of exposure. Representative images were analysed qualitatively at 5600×, 25 000× and 31 000× magnification.

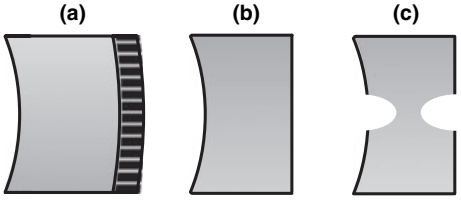

Figure 1 Schematic drawing of the labial segments sliced (a); enamel removal (b) and trimming of the inner dentine to a dumbbell shape (c).

Results

The effects of the various intracoronal bleaching agents on sound (nonetched) and pre-etched pulp chamber dentine are shown in Table 1. The UTS mean for sound dentine that was not subjected to the intracoronal bleaching procedure (C) was 48.3 ± 8.5 MPa, which was significantly higher than dentine subjected to all intracoronal bleaching procedures ($P < 0.05$). There were no discernable differences amongst bleached groups ($P > 0.05$). The UTS mean of pre-etched, nonbleached dentine (Ce) was statistically similar to pre-etched SPe, CPe and 25% HPe treatments, though it was higher than 35% HPe. There were no statistical differences amongst pre-etched dentine bleaching groups ($P > 0.05$). The comparison between sound and pre-etched dentine revealed that sound dentine treated with 35% HP presented higher UTS than pre-

Table 1 Mean microtensile strength (MPa) to sound and pre-etched pulp chamber dentine after different intracoronal bleaching treatments

Intracoronal bleaching agents	Sound dentine	Pre-etched dentine
	Mean (SD)	Mean (SD)
Control	48.3 (8.5) Aa	38.9 (13.8) Aa
Sodium perborate	34.6 (8.2) Ba	31.3 (9.3) ABa
35% Carbamide peroxide	32.9 (8.9) Ba	28.4 (6.2) ABa
25% Hydrogen peroxide	28.0 (4.6) Ba	30.0 (7.9) ABa
35% Hydrogen peroxide	26.4 (6.6) Ba	19.9 (4.6) Bb

The Tukey test compared differences amongst bleaching treatments on sound and pre-etched pulp chamber dentin ($P = 0.084$). Mean followed by different letters are significantly different. Capital letters indicate mean values that are considered in the horizontal direction and lower case letters are considered in the vertical direction.

etched bleached dentine (35% HPe). There were no difference between sound and pre-etched dentine for all other bleached groups as well as the C group.

TEM observations

The ultrastructural sound dentine images demonstrated that the mineral content of the C group was intact with no indications of demineralization, as collagen fibrils remained unaffected (Fig. 2). Conversely, sound (not-etched) bleached groups presented complete exposure of collagen fibrils, indicating tissue demineralization (Fig. 3). The images of pre-etched dentine for the Ce group (Fig. 4) were similar to those of the bleached groups (Figs 3 and 5). A perceptible loss of dentine mineral content was found for all groups (bleached or not) due to the bleaching agent exposure and previous acid etchings. Bleached specimens (Figs 3 and 5) presented areas of partially demineralized dentine and mineralized areas surrounding collagen fibrils (Fig. 5). The demineralized areas contiguous to collagen fibrils exhibit remnant apatite crystallites (Fig. 5).

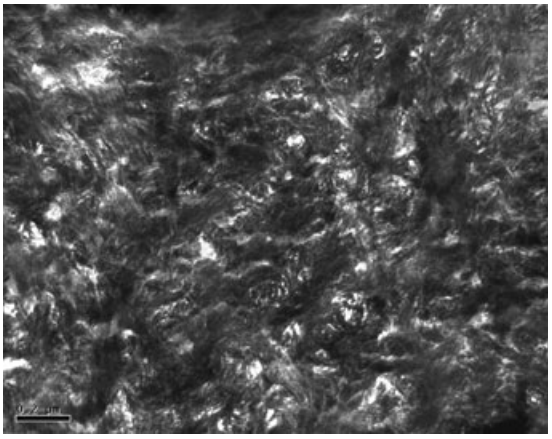

Figure 2 TEM representative micrograph of sound not bleached dentine (C) revealing a compact matrix without traces of collagen fibrils (31 000×).

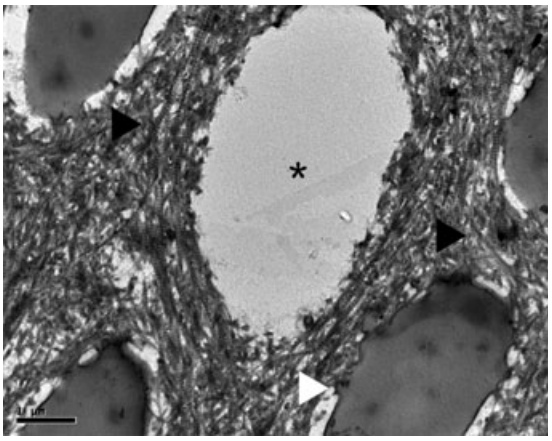

Figure 3 TEM representative micrograph of 35% hydrogen peroxide bleached dentine. Image demonstrates the exposure of collagen fibrils after bleaching (black arrowhead). Intertubular demineralization possibly promoted by bleaching (white arrowhead) and visualization of dentine tubules (asterisks) (5600×).

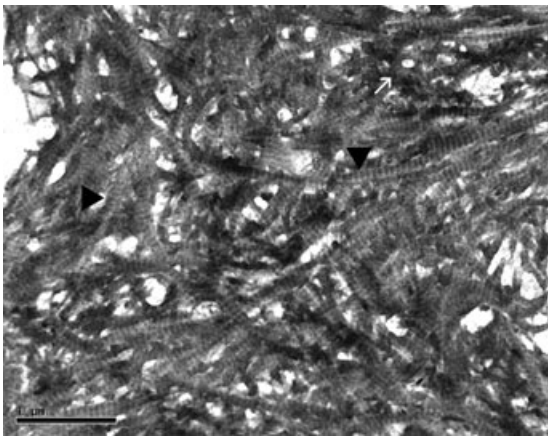

Figure 4 TEM micrograph of pre-etched nonbleached dentine (Ce). Collagen fibrils exposure is observed (black arrowhead) and partially mineralized dentine around the collagen fibrils (white arrow) (25 000×)

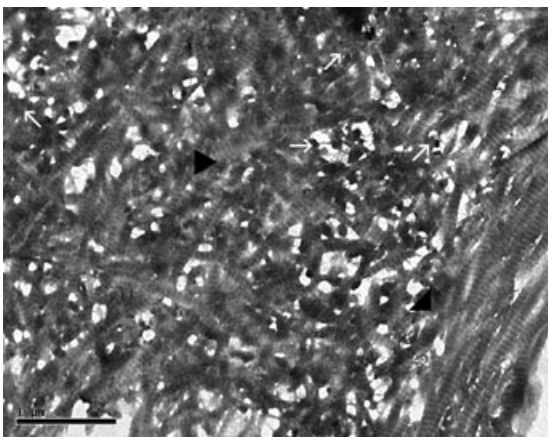

Figure 5 TEM micrograph of pre-etched dentine treated with 35% hydrogen peroxide (35% HPe). The demineralized areas exhibit collagen fibrils (black arrowhead) and these fibrils are also surrounded by mineralized sites (25 000×). The mineralized zone is constituted of remnants of apatite crystallites (arrow).

Discussion

Intracoronal bleaching treatment is performed on root filled teeth through the 'walking bleach' technique, in which the bleaching agent, usually sodium perborate associated with water or hydrogen peroxide, is placed into the pulp chamber and sealed with temporary restorative material for 4–7 days (Attin *et al.* 2003). As an alternative, 35% carbamide peroxide and relatively low concentrations of hydrogen peroxide (25%) have been used intracoronally, to bleach discoloured teeth without compromising hard and soft tissues (Chng *et al.* 2004).

The UTS of sound, nonbleached dentine (C) was significantly higher than intracoronal bleached dentine (SP, CP, 25% HP and 35% HP). There were no significant differences amongst bleached groups. The ultrastructural TEM image of sound, nonbleached dentine (C, Fig. 2) revealed a normal solid matrix pattern of intracoronal, sound mineralized dentine. The observed structural characteristics indicate that the organic matrix (composed mostly of type I collagen fibrils and proetoglycans) (Dahl *et al.* 1998) remained associated and connected with inorganic, mineral substrates. Conversely, sound bleached dentine (Fig. 3) was partially demineralized, as collagen fibrils were observed. It is important to notice that no previous acid etching treatment was performed in these groups, except for bleaching. Demineralization and exposure of collagen fibrils were most likely caused by the bleaching treatments, especially due to the acidity of 25% and 35% HP bleaching agents (with pH values of 4.5 and 3.6 respectively). Previously, it has been observed that exposure to high concentrations of hydrogen peroxide decreased dentine microhardness and caused alterations in its chemical structure (Rotstein *et al.* 1996, Kawamoto & Tsujimoto 2004, Oliveira *et al.* 2007). A reduction in the hardness of treated dentine indicates mineral dissolution and degradation (Saleh & Ettman 1999). Dissolution and degradation of the inorganic matrix was the primary reason for decreased UTS of bleached dentine as observed in this study.

Collagen fibrils were also exposed after intracoronal bleaching to sodium perborate mixed with water and 35% carbamide peroxide gel, which are both alkaline compounds (pH values of 10 and 6.5 respectively) (Rotstein & Friedman 1991, Frysh *et al.* 1995, Price *et al.* 2000). In these particular cases, it is likely that dentine demineralization and decreased UTS were not related to pH values but instead due to the redox reaction that is a common characteristic for all bleaching agents (Lai *et al.* 2002, Kashima-Tanaka *et al.* 2003). The chemical reaction is based on the ability of peroxide to produce hydroxyl radicals, which are oxygen-derived free radicals known to accumulate in dentine (Timpawat *et al.* 2005). As hydroxyl radicals are highly reactive and unstable, they may react continuously and reduce the UTS (Kawamoto & Tsujimoto 2004). The released hydrogen peroxide can generate different radicals or ions dependent on pH value, light influence, temperature, existence of co-catalysts and metallic reaction partners (Feinman 1991, Goldstein & Garber 1995).

Other components of bleaching agents are also related to the consequential adverse effects on enamel and dentine. It has been observed that carbopol (carboxypolymethlene polymer), which is present in most carbamide peroxide bleaching gels to provide thickness, is able to decrease enamel microhardness after carbamide peroxide bleaching at a neutral pH (Basting *et al.* 2005). Carboxypolymethylene is an acidic vinyl polymer with active carboxyl groups and dissociates in the presence of water, resulting in a pH close to 3.0 (Hosmani 2006). The low pH of carbopol could explain dentine demineralization of CP bleached specimens. It is also hypothosized that urea, a significant component of carbamide peroxide that is commonly used in the laboratory as a protein denaturing agent, is able to promote changes in the organic matrix of enamel and mainly dentine during bleaching (Tam *et al.* 2005).

Prior to bleaching procedures, smear layer removal and demineralization of pulp chamber dentine has been proposed as a method to improve dentine permeability and hasten diffusion of bleaching products (Horn *et al.* 1998). The results showed that the UTS of pre-etched control group (Ce) was similar to pre-etched bleached groups (SPe, CPe, 25% HPe), with the exception of the pre-etched, 35% HP-bleached group. The TEM images of pre-etched dentine indicate that the control group (shown in Fig. 4) presented higher collagen fibrils exposure than sound control dentine (shown in Fig. 2). This exposure clearly demonstrates the effects of dentine acid etching. Therefore, it is evident that pre-etching was responsible for the decrease in dentine UTS for the control group, regardless of bleaching action.

When sound and pre-etched dentine were compared, the results revealed that UTS was exclusively lower after pre-etched and bleached 35% HP dentine. It is possible that the double exposure of dentine to 37% phosphoric acid and 35% HP was the reason for the

lowest dentine tensile strength values. The image of pre-etched sound dentine (Ce, Fig. 4) was similar to morphological alterations produced by bleaching agents (Fig. 5), due to the removal of inorganic matrix and consequent collagen fibrils exposure.

High concentrations of HP have been associated with cervical root resorption (Madison & Walton 1990, Heller *et al.* 1992) as well as biochemical alterations of dentine (Rotstein *et al.* 1996, Chng *et al.* 2002). *Ex vivo* studies revealed that 35% HP diffuses through cement dentine, especially in the presence of root defects (Koulaouzidou *et al.* 1996). Furthermore, *in vivo* studies confirm reports of such diffusion with cervical root resorption (Madison & Walton 1990, Heller *et al.* 1992). It is speculated that hydrogen peroxide released from bleaching agents creates an adequate acidic environment for osteoclastic activity and bone resorption (Vaes 1968). The results of this study elucidated that sound and pre-etched 35% HP-bleached dentine presented low UTS and ultra structural analysis indicated removal of inorganic content (Fig. 5). Due to these results, as well as complications associated with the use of 35%HP, this treatment should be carefully advised or substituted with another agent during intracoronal bleaching. The preferable substitutes for 35% HP are typically carbamide peroxide and sodium perborate mixed with water, since these compounds are considered to be as effective as 35% HP, albeit with lower peroxide concentrations (Madison & Walton 1990, Weiger *et al.* 1994, Dahl & Pallesen 2003). Sodium perborate (available in the mono-, tri-, or tetra-hydrated salts) has been successfully used as an oxidiser as perborate decomposes in hydrogen peroxide (H_2O_2) (Ari & Üngor 2002) whereas 35% carbamide peroxide decomposes into approximately 12% hydrogen peroxide (Lim *et al.* 2004). Both agents have been successfully used to lighten teeth, and no reports have associated these treatments with cervical root resorption, most likely due to the low concentration of hydrogen peroxide that is released (Chng *et al.* 2004, Lee *et al.* 2004). Hence, sodium perborate and/or 35% carbamide peroxide should be the agents of choice when intracoronal bleaching is clinically performed.

The results suggest that removal of the smear layer compromises the UTS of 35% HP bleached dentine and support the hypothesis that pre-etching procedures should be considered unnecessary, as most agents are of low molecular weight and thus fully diffuse into enamel and dentine (Cavalli *et al.* 2004b). Moreover, removing the smear layer could lead to an undesirable increase in bleaching diffusion to the periodontium (Fuss *et al.* 1989).

Based on the results of this study, the null hypotheses were rejected: (i) dentine presents lower UTS after bleaching, (ii) smear layer removal does affect dentine, and (iii) intracoronal bleaching agents affect dentine ultrastructure morphology.

The mechanical properties of dentine are of significant interest as dentine provides the base for both enamel and cementum and is largely responsible for the structural integrity of the entire tooth (Tam *et al.* 2005). Therefore, further clinical and laboratory investigations concerning the effects of different bleaching agents on dentine are necessary.

Conclusions

Intracoronal bleaching agents (sodium perborate and water, 35% carbamide peroxide, as well as 25% and 35% hydrogen peroxide) were able to decrease UTS after treatment and pre-etching dentine was considered unnecessary and of concern as UTS of nonbleached dentine (C) was similar to that of pre-etched bleached dentine. Clinicians should consider the use of low hydrogen peroxide agents (such as sodium perborate and 35% carbamide peroxide) as high-concentrated hydrogen peroxide is related to undesirable cervical root resorption.

Acknowledgements

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