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Original Article

Surface morphology alterations in bovine dentin exposed to different bleaching agents

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Abstract

Aim: This study evaluated the morphological changes caused by internal bleaching agents on dentin surface. **Methods:** Twenty crowns of bovine incisors were cut into slabs that were randomly distributed in six experimental groups (n = 5), according to the bleaching agent used: G1 – sodium perborate + water, G2 – sodium perborate + 2% chlorhexidine gel, G3 – sodium perborate + 30% hydrogen peroxide, G4 – 30% hydrogen peroxide, G5 – 37% carbamide peroxide and G6 – gel base without carbamide. Two Control Groups were used: C1 with distilled water and C2 with 2% chlorhexidine gel. The specimens were immersed in the respective test bleaching agent and incubated at 37 °C for seven days. Following, they were prepared for scanning electron microscopy and five images from each tooth segment were recorded and analyzed for surface morphological alterations, by three previously calibrated examiners. Inter-examiner agreement was verified using the Kappa test. The rank averages obtained for the groups were subjected to Kruskal-Wallis analysis of variance at 5% significance level. **Results:** The analysis of the scores obtained indicated that all tested materials caused some morphological alteration on dentin, except for sodium perborate + water (G1) and Control Groups 1 and 2. Hydrogen peroxide and carbamide peroxide caused significantly more severe alterations ($p < 0.05$) to dentin structure, than the other bleaching agents evaluated. **Conclusions:** Sodium perborate-based pastes seemed to be the most harmless agent to dentin structures in non-vital tooth bleaching, while hydrogen peroxide solutions and carbamide peroxide agents caused the greatest alterations.

Keywords: tooth bleaching, dentin, dental pulp cavity.

Introduction

Color changes may occur in teeth that had undergone endodontic treatment, consisting in an important esthetic problem. Bleaching is one of the procedures that can be used to treat discoloration, and it is commonly performed in non-vital teeth[1].

The walking bleach technique is considered the safest and most accepted intracoronal bleaching technique[1-2]. It consists on the placement of bleaching agents in the pulp chamber of root-filled teeth. Traditionally, intracoronal bleaching is achieved with the use of 30% hydrogen peroxide and sodium perborate, which can be used either separately or in combination[3]. Carbamide peroxide formulations, commonly used for nightguard vital bleaching, have also shown to be effective as an intracoronal bleaching agent[4]. Recently, a gel base containing 2% chlorhexidine was introduced as a vehicle for sodium perborate, being an alternative to water or hydrogen peroxide in order to prevent coronal microleakage during the walking bleach technique[5].

However, some side effects resulting from the use of internal bleaching agents have been reported. Thirty-percent hydrogen peroxide has been associated to the development of ex-

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ternal cervical root resorption[6]. Chng et al.[7] found that intracoronal bleaching with 30% hydrogen peroxide and sodium perborate used, either alone or in combination, weakened dentin. A significant alteration on the levels of inorganic components of dentin[8] and surface morphological changes[9] were reported after treatment with carbamide peroxide.

During intracoronal bleaching, the materials are placed in direct contact with dentin, and subsequently the access cavity should be readily sealed to minimize coronal leakage[10]. Being the major constituent of tooth structure, any changes on the surface morphology of dentin are likely to affect the outcomes of dentin bonding[2].

The aim of this study was to determine *in vitro*, the effects of different bleaching agents on dentin surface morphology using scanning electron microscopy.

Material and methods

Twenty bovine mandibular incisors were obtained from slaughtered cattle at the age of one to two years, and stored in 0.2% thymol to be used within six months after extraction. The coronal portion of each tooth was cut longitudinally from mesial to distal into two equal segments with a water-cooled diamond disk (KG Sorensen, Barueri, SP, Brazil). The buccal segment was sectioned in "x" and "y" directions and serial coronal slabs were obtained. The exposed pulp chamber dentin was cleaned, by immersing the slabs in 5.25% NaOCl for ten minutes, and sequentially in 17% EDTA for the same time in order to remove the smear layer formed during sectioning[11]. The pooled coronary slabs were rinsed in running water for 12 hours, dried, and then randomly assigned to eight groups of five specimens each.

Each experimental group was treated with one of the following bleaching agents: G1 – sodium perborate ($NaBO_3.4H_2O$; Proderma, Piracicaba, SP, Brazil) mixed with water, G2 – sodium perborate mixed with 2% chlorhexidine gel (Proderma), G3 – sodium perborate mixed with 30% hydrogen peroxide, G4 – 30% hydrogen peroxide, G5 – 37% carbamide peroxide (Whiteness, Porto Alegre, RS, Brazil), and G6 – gel base without carbamide (Whiteness). The following control solutions were used: C1– distilled water and C2 – 2% chlorhexidine gel. The bleaching agent was combined with the designated vehicle for each group to maintain a 2:1 ratio in solution. The specimens were immersed in the respective test material and incubated at 37 °C for seven days in 100% humidity, as previously described by Zalkind et al.[9]. The samples were, then, rinsed in an ultrasonic bath during one hour, dehydrated in ascending alcohol concentrations, dried and sputter coated with gold (Denton Vaccum Desk II, Moorestown, NJ, USA)[9]. Dentin surface morphology was analyzed with a scanning electron microscope (JEOL – JSM 5,600 LV, Noran Instruments, Tokyo, Japan) operated at 15 KV. The five most representative images from different regions of each tooth segment were captured at × 2000 magnification, and the surface morphological alterations were analyzed by three previously calibrated examiners. Morphological changes were classified as absent (score 0), moderate (score 1) and severe (score 2), according to pre-established parameters (**Figure 1**).

Inter-examiner agreement was verified using the Kappa test. The rank averages obtained for the groups were subjected to Kruskal-Wallis analysis of variance at 5% significance level.

Results

The effects of the various bleaching agents on dentin surface morphology are expressed in ranks in **Table 1**. The Kappa value for inter-examiner agreement was 0.9. Most tested materials caused some morphological alteration on dentin, except for sodium perborate mixed with water (G1), the control solutions distilled water and 2% chlorhexidine gel (C1 and C2) (**Figure 2**).

Specimens treated with sodium perborate mixed with 2% chlorhexidine gel (G2) and sodium perborate mixed with 30% hydrogen peroxide (G3) showed more evident surface alterations than Groups G1, C1 and C2, as a moderate flattening of dentin surface and a mild erosion of intertubular dentinal matrix could be detected. The dentinal tubules remained opened but not widened (**Figure 3**).

Severe morphological changes could be observed for the groups treated with 30% hydrogen peroxide (G4), 37% carbamide peroxide (G5) and the gel base without carbamide peroxide (G6), which differed significantly ($p < 0.05$) from the other groups. These agents gave

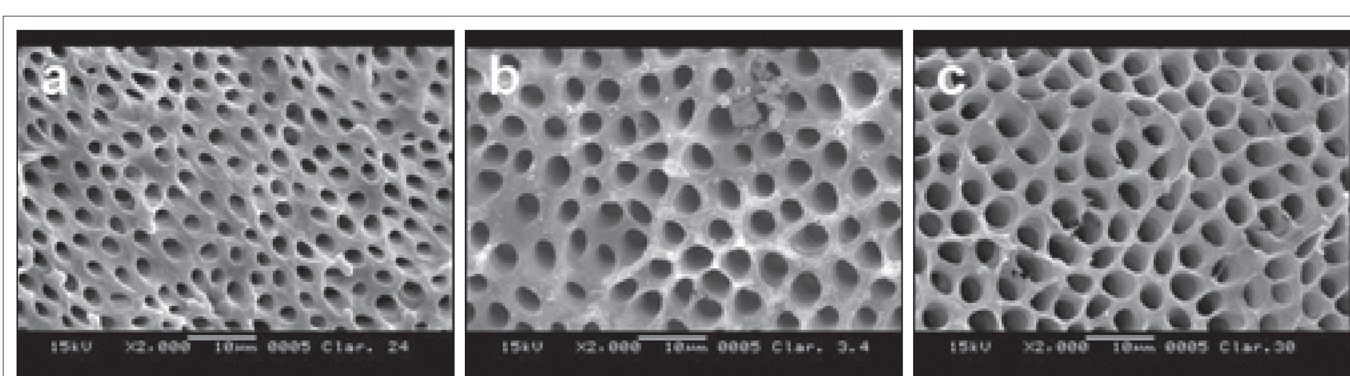

Figure 1. Scanning electron microscope micrographs representing score rates of dentin surface alterations. A: score 0 – absent morphological changes. B: score 1 – moderate morphological changes. C: score 2 – severe morphological changes.

to the dentin a flattening and an etching-like appearance on intertubular area. The dentinal tubules were wider, showing demineralization of intratubular dentin (**Figure 4**).

Discussion

The results of this study indicated that the materials commonly used for internal bleaching may have an effect on dentin surface morphology. The alterations observed showed a strong relationship between the morphological changes and the bleaching agent used.

It is quite relevant to test the effects of the bleaching materials on dentin, since a close contact occurs during internal bleaching procedures. Most morphological studies have evaluated external bleaching agents and their effects on enamel[12-13], whilst information concerning the influence of materials used for internal bleaching on dental tissues is rather limited. Structural changes of dentin substrate may have an important role on the performance of dental restorations. In fact, a variety of studies have shown a detrimental effect of the bleaching procedures on the bond strength and sealing ability of composite restorations[10,14-16] to dentin, thus the authors suggest morphological alterations as one of the reasons for that.

The most significant morphological changes were caused by 30% hydrogen peroxide, 37% carbamide peroxide and the gel base without carbamide peroxide. The adverse effects of hydrogen peroxide on dental hard tissues in external and internal bleaching has been previously reported on the literature and include alterations in the chemical structure of dentin[17] and a reduction on the calcium/phosphorous (Ca/P) ratio[8-9], which could be responsible for the alterations observed. In addition, hydrogen peroxide was found to increase dentin solubility and cause protein oxidation of organic matrix[8], this may explain the severe etching-like appearance and surface flattening observed in this study. The acidity provided by the low pH of hydrogen peroxide solutions[2] might have contributed for the enlargement of dentinal tubules. The above-mentioned complications, allied to a frequent occurrence of external root resorption, made some researchers recommend avoidance of highly concentrated hydrogen peroxide solutions for intracoronal bleaching[18-19].

Bleaching agents containing carbamide peroxide are commonly used in the treatment of discolored vital teeth. These materials started to be considered for intracoronal bleaching since satisfactory esthetic results were reached[4] and no association with external root resorption was found[20]. However, the severe dentin surface alterations observed in this study indicate that carbamide peroxide agents may have an adverse effect on the organic and inorganic components of dentin. Moderate surface alterations have already been reported for 10 and 15% carbamide peroxide[9,21]. Since a highly concentrated (37%) formulation was used in the present study, more severe mor-

Table 1. Dentin surface morphological changes caused by different bleaching agents

Groups	Bleaching agent	Ranks
G1	Sodium perborate + water	55.24[c]
G2	Sodium perborate + 2% chlorhexidine gel	73.34[bc]
G3	Sodium perborate + 30% hydrogen peroxide	104.98[b]
G4	30% hydrogen peroxide	161.60[a]
G5	37% carbamide peroxide	143.28[a]
G6	Gel base without carbamide peroxide	133.36[ab]
C1	Distilled water	62.48[c]
C2	2% chlorhexidine gel	69.72[c]

Different superscript letters indicate statistically significant differences between the groups.

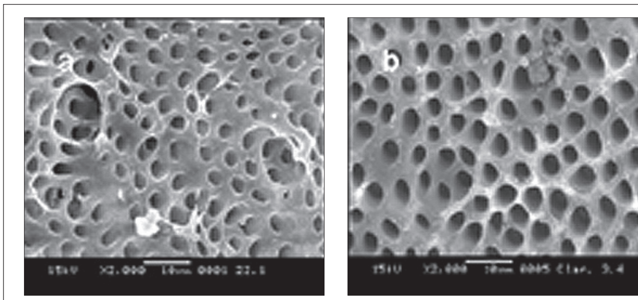

Figure 3. Scanning electron microscope micrographs showing moderate alterations of dentin surface. A: specimen treated with sodium perborate mixed with chlorhexidine (Group 2). B: specimen treated with sodium perborate mixed with hydrogen peroxide (Group 3).

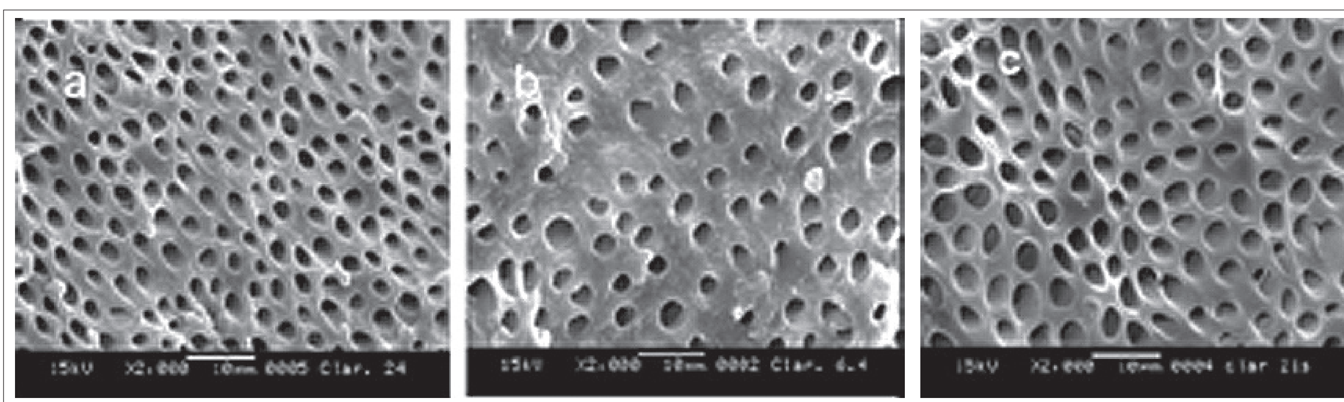

Figure 2. Scanning electron microscope micrographs showing dentin surface without morphological alterations. A: specimen treated with water (Control Group 1). B: specimen treated with chlorhexidine gel (Control Group 2). C: specimen treated with sodium perborate mixed with water (Group 1).

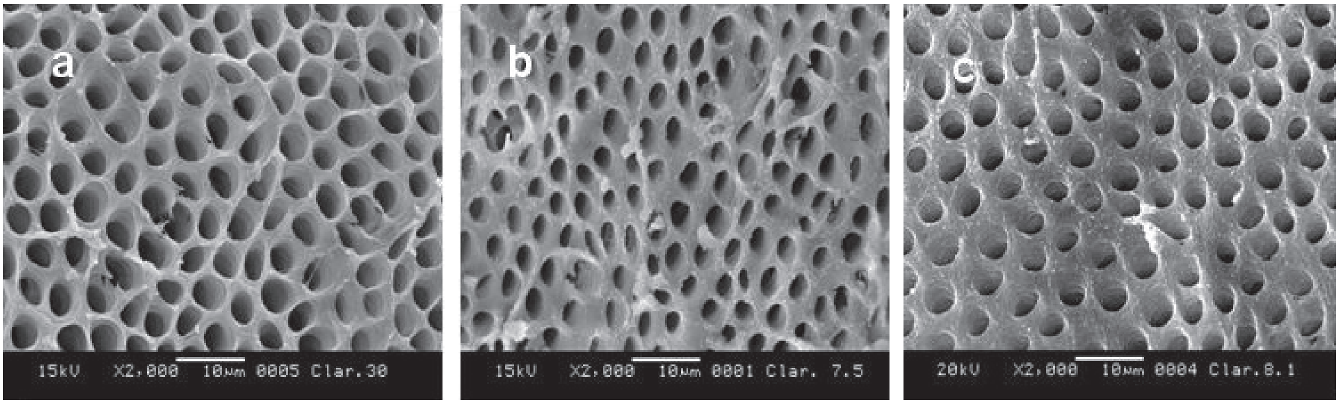

Figure 4. Scanning electron microscope micrographs of severe altered dentin surface. A: specimens treated with hydrogen peroxide (Group 4). B: specimens treated with carbamide peroxide (Group 5). C: specimens treated with the gel base without carbamide peroxide (Group 6).

phological changes would be expected. Another study[20] showed a decrease on dentin microhardness similar to that caused by hydrogen peroxide-based solutions. As carbamide peroxide breaks down into urea and hydrogen peroxide, the previously reported adverse effects of hydrogen peroxide bleaching may be applicable for carbamide peroxide. Even though an acidic pH is attributed to hydrogen peroxide solutions, the pH value of carbamide peroxide is almost neutral[9], indicating that the surface morphological alterations were not strictly related to pH variations among the bleaching agents. Another important consideration is the fact that the gel base depleted of carbamide peroxide also caused severe morphological changes on dentin surface. Although the effects of carbamide peroxide's vehicle have not been tested before, these findings indicate that other constituents of the bleaching formulation may also cause some kind of structural alterations on dental hard tissues.

Specimens treated with sodium perborate mixed with water did not show any morphological changes and were similar to the Control Groups C1 and C2. This result confirms a previous report of the absence of dentin alterations related to the use of sodium perborate as a bleaching agent[9]. In addition, it has been stated that the solubility and chemical composition of dentin remained undisturbed[3], and the biomechanical properties were reduced only to a small extent[7]. These findings could be explained by the lower amount of hydrogen peroxide released from this formulation. However, significant surface changes were observed when sodium perborate was mixed with hydrogen peroxide, probably as a result of the increase in hydrogen peroxide concentration. The association of sodium perborate with hydrogen peroxide results in an alkalinization of the pH of the latter, which may explain why the morphological changes were less severe than those found for the specimens treated with hydrogen peroxide alone.

A preparation of sodium perborate and 2% chlorhexidine gel was also tested on this experiment. Chlorhexidine digluconate in a gel base has a broad antimicrobial activity spectrum and substantivity[22]. It showed a good potential to increase the antimicrobial properties of the bleaching agents when used as a vehicle[23], since the bleaching effect of sodium perborate associated with chlorhexidine gel was not decreased[5]. The morphological changes caused by this formulation were very slight and occupied an intermediary classification, when compared to the results obtained with the other preparations containing sodium perborate. It is likely that the use of a gel based vehicle – a hydroxyethyl cellulose – would allow a slower ionization of sodium perborate molecule than the other liquid vehicles used in the present study, thus lowering hydrogen peroxide concentration[24-25].

In the present study, tooth slabs were exposed to bleaching agents for seven days before the analysis of surface morphological alterations. Clinically, the bleaching procedure is usually repeated for additional seven days until achieving a satisfactory esthetic outcome. Based on the present results, it is possible to anticipate more intense alterations on dentin surface with repeated applications of the bleaching agents. Nevertheless, this hypothesis should be further investigated.

This study confirmed the general concern about the hazardous effects of 30% hydrogen and carbamide peroxide on dental hard tissues, and indicated that the use of these materials as an intracoronal bleaching agent should be carefully considered. Sodium perborate-based formulations caused slight or even absent alterations on dentin surface morphology and seemed to be the safest agent for use in non-vital tooth bleaching. The association of a gel-based chlorhexidine with sodium perborate showed satisfactory results concerning dentin surface integrity and should; therefore, be further investigated.

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References

1. Nutting EB, Poe G. A new combination for bleaching teeth. J S Calif Assoc. 1963;41:289-91.
2. Attin T, Paqué F, Ajam F, Lennon AM. Review of the current status of tooth whitening with the walking bleach technique. Int Endod J. 2003;36:313-29.
3. Rotstein I, Lehr Z, Gedalia I. Effect of bleaching agents on inorganic components of human dentin and cementum. J Endod. 1992;18:290-3.

4. Perrine GA, Reichl RB, Baisden MK, Hondrum SO. Comparison of 10% carbamide peroxide bleaching and sodium perborate for intracoronal bleaching. Gen Dent. 2000;48:264-70.
5. Oliveira DP, Gomes BPFA, Zaia AA, Souza-Filho FJ, Ferraz CC. In vitro assessment of a gel base containing 2% chlorhexidine as a sodium perborate's vehicle for intracoronal bleaching of discolored teeth. J Endod. 2006;32:672-4.
6. Heithersay GS, Dahlstrom SW, Marin PD. Incidence of invasive cervical resorption in bleached root-filled teeth. Aust Dent J. 1994;39:82-7.
7. 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.
8. 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.
9. 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.
10. Shinohara MS, Rodrigues JA, Pimenta LAF. In vitro microleakage of composite restorations after nonvital bleaching. Quintessence Int. 2001;32:413-7.
11. Perez F, Rochd T, Lodter JP, Calas P, Michel G. In vitro study of the penetration of three bacterial strains into root dentine. Oral Surg Oral Med Oral Pathol. 1993;76:97-103.
12. Titley KC, Torneck CD, Smith DC, Adibfar A. Adhesion of composite resin to bleached and unbleached bovine enamel. J Dent Res. 1988;67:1523-8.
13. McGuckin RS, Babin JF, Meyer BJ. Alterations in human enamel surface morphology following vital bleaching. J Prosthet Dent. 1992;68:754-60.
14. Teixeira EC, Hara AT, Turssi CP, Serra MC. Effect of non-vital tooth bleaching on microleakage of coronal access restorations. J Oral Rehabil. 2003;30:1123-7.
15. Elkhatib H, Nakajima M, Hiraishi N, Kitasako Y, Tagami J, Nomura S. Surface pH and bond strength of a self-etching primer/adhesive system to intracoronal dentin after application of hydrogen peroxide bleach with sodium perborate. Oper Dent. 2003;28:591-7.
16. Attin T, Hannig C, Wiegand A, Attin R. Effect of bleaching on restorative materials and restorations – a systematic review. Dent Mater. 2004;20:852-61.
17. Lai YL, Yang ML, Lee SY. Microhardness and color changes of human dentin with repeated intracoronal bleaching. Oper Dent. 2003;28:786-92.
18. Madison S, Walton R. Cervical root resorption following bleaching of endodontically treated teeth. J Endod. 1990;16:570-4.
19. Friedman S. Internal bleaching: long-term outcomes and complications. J Am Dent Assoc. 1997;128 Suppl:51S-55S.
20. Chng HK, Yap AUJ, Wattanapayungkul P, Sim CP. Effect of traditional and alternative intracoronal bleaching agents on microhardness of human dentine. J Oral Rehabil. 2004;31:811-6.
21. Cobankara FK, Unlü N, Altinöz HC, Füsun O. Effect of home bleaching agents on the roughness and surface morphology of human enamel and dentine. Int Dent J. 2004;54:211-8.
22. Ferraz CC, Gomes BP, Zaia AA, Teixeira FB, Souza-Filho FJ. In vitro assessment of the antimicrobial action and the mechanical ability of chlorhexidine gel as an endodontic irrigant. J Endod. 2001;27:452-5.
23. Amaechi BT, Higham SM. Dental erosion: possible approaches to prevention and control. J Dent. 2005;33:243-52.
24. Stevens RH, Grossman LI. Evaluation of the antimicrobial potential of calcium hydroxide as an intracanal medicament. J Endod. 1983;9:372-4.
25. Stuart KG, Miller CH, Brown CE Jr, Newton CW. The comparative antimicrobial effect of calcium hydroxide. Oral Surg Oral Med Oral Pathol. 1991;72:101-4.