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Evaluation of morphological and chemical alterations in enamel, dentin and cementum after internal bleaching technique using different bleaching agents

Avaliação das alterações químicas e morfológicas em esmalte, dentina e cemento após clareamento interno usando diferentes clareadores

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ABSTRACT

Objective: The aim of this study was to evaluate the morphological and chemical alterations in enamel, dentin and cementum after internal bleaching using scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS). **Material and Methods:** Seventy-two bovine incisor teeth were prepared, cut and bleached for 7 days as follows: HP: 35% hydrogen peroxide gel; HP+SP: 35% hydrogen peroxide gel + sodium perborate; CP: 37% carbamide peroxide gel; CP+SP: 37% carbamide peroxide gel + sodium perborate; SP: sodium perborate + water; and control: deionized water. The specimens were sectioned and prepared for morphological analysis under SEM and analysis of calcium, phosphorus, oxygen and carbon levels using EDS. **Results:** A significant reduction was found in the calcium levels in enamel after treatment with CP + SP and CP ($p < 0.05$). Carbon (organic part) was hardly altered in enamel. A significant reduction in the calcium levels was found in dentin in Groups HP+SP, CP and CP+SP. Phosphorus levels increased after SP+H20 ($p < 0.05$) and CP ($p < 0.05$). Carbon levels showed little variation and the largest amount was found in Groups CP and CP+SP ($p < 0.05$); in the other groups there was no alteration. A significant reduction in the calcium levels was found in the cementum in Group CP+SP ($p < 0.05$). **Conclusion:** Alterations in the enamel, dentin and cementum compositions occurred after bleaching and these alterations showed to be less significant with sodium perborate and water.

RESUMO

Objetivo: O objetivo deste estudo foi avaliar as alterações químicas e morfológicas em esmalte, dentina e cemento após clareamento interno utilizando microscopia eletrônica de varredura e espectrometria de energia dispersiva. **Materiais e Métodos:** Setenta e dois incisivos bovinos foram preparados, cortados e clareados por 7 dias com os seguintes agentes clareadores: PH: gel de peróxido de hidrogênio 35%; PH + PS: gel de peróxido de hidrogênio 35% + perborato de sódio; PC: gel de peróxido de carbamida 37%; PC + PS: gel de peróxido de carbamida 37% + perborato de sódio; PS: perborato de sódio + água; e Controle: água deionizada. Os espécimes foram seccionados e preparados para análise em microscopia eletrônica de varredura e análise dos níveis de cálcio, fósforo, oxigênio e carbono usando espectrometria de energia dispersiva. **Resultados:** Foi observada uma redução significante nos níveis de cálcio em esmalte após clareamento com PC + PS e PC ($p < 0,05$). Os níveis de carbono (parte orgânica) foram alterados em esmalte. Uma redução significante nos níveis de cálcio foi encontrada na dentina nos grupos PH + PS, PC e PC + PS. Os níveis de fósforo aumentaram após o uso de PS ($p < 0,05$) e PC ($p < 0,05$). Níveis de carbono mostraram pequena variação e a maior quantidade foi encontrada nos grupos PC e PC + PS ($p < 0,05$), nos outros grupos não houve alteração. Uma redução significante nos níveis de cálcio foram encontradas no cemento no grupo PC + PS ($p < 0,05$). **Conclusão:** Alterações em esmalte, dentina e cemento ocorreram após o clareamento e essas alterações foram menos significantes quando utilizado perborato de sódio e água.

KEYWORDS

Carbamide peroxide; Hydrogen peroxide; Scanning electron microscopy; Sodium perborate; Tooth bleaching.

PALAVRAS-CHAVE

Peróxido de carbamida; Peróxido de hidrogênio; Microscopia eletrônica de varredura; Perborato de sódio; Clareamento dental.

INTRODUCTION

Bleaching of discolored and endodontically treated teeth is a widely used esthetic option that preserves the dental tissues when compared with other esthetic treatments [1]. The bleaching agents used for dental bleaching may be sodium perborate, carbamide and hydrogen peroxide and their associations [2]. The action mechanism of bleaching agents consists of an oxidation reaction of hydrogen peroxide [3] from the bleaching agents that are decomposed in water and oxidative radicals. During oxidation reaction, the organic components of the pigments donate electrons to the bleaching agent and the compounds with pigmented carbon rings are opened and converted into intermediate lighter-colored chains, promoting dental bleaching [4].

Penetration of the bleaching agent into the tooth structures occurs due to its low molecular weight and capacity to denature proteins, which increases movement of ions through the tooth structure [5] causing alterations in the chemical composition of the tooth, reducing the calcium and phosphate ratio of enamel and dentin [6] and consequently changing the texture of bleached teeth, decreasing microhardness [7], as well as increasing permeability of enamel and penetration of dyes and oxidative radicals [8,9] and bonding failures of dentin and enamel [2,10,11].

However, few studies have evaluated the effects of bleaching agents used in internal bleaching and the chemical composition of hard dental tissues. Therefore, the aim of this study was to evaluate the morphological and chemical alterations in enamel, dentin and cementum after internal bleaching using scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS).

MATERIAL AND METHODS

After extraction, the 72 bovine incisor teeth were frozen in physiological saline solution up to the time they were used. The teeth were evaluated under a stereomicroscopy at 20x magnification (Zeiss Stemi C 2000) to detect possible cracks or fissures.

After coronal openings, the pulp chamber was irrigated with a physiological saline solution and the roots were cut horizontally 5 mm from the amelocemental junction in the apical direction. The remaining pulp tissue was completely removed with the H-type file #15 (Dentisply Maillefer – Ballaigues – Switzerland), followed by irrigation with physiological saline solution. A 2 mm-thick barrier was fabricated with glass ionomer cement (Vidrion R, SS White) and placed 2 mm from the cementoenamel junction in the apical direction, sealing the root canal entrance. The apical extremity of the specimens was sealed externally with light-curing resin composite (TPH – Dentsply – USA).

The teeth were divided into five experimental groups and one control group (n = 12), according to the bleaching agent used, as follows: Group HP: teeth beached with 35% hydrogen peroxide gel (Opalescence Endo, Ultradent – USA); Group HP+SP: teeth bleached with 35% hydrogen peroxide gel + sodium perborate (Byofórmula Pharmacy– Brazil); Group CP: teeth bleached with 37% carbamide peroxide (Whiteness Super Endo - FGM Products, Brazil); Group CP+SP: teeth bleached with 37% carbamide peroxide gel + sodium perborate; Group SP: sodium perborate + deionized water; and Control Group: deionized water.

In the groups in which the association of sodium perborate and gel or deionized water was used, the consistency obtained was sandy.

The bleaching agents were applied, filling the pulp chamber, and the bleaching material was protected with pieces of paper filter (paper filter 103 – Carrefour – Celupa Industrial Celulose e Papel Guaíba – Brazil). Afterwards the access of cavities were sealed with a layer of resin composite (TPH - Dentsply – USA). After polymerization of the first layer, etching with 37% phosphoric acid was performed (Alpha Etch gel – DFL), adhesive system (Single Bond Adhesive – 3M) and a new layer of the same resin composite was inserted to seal the cavity completely.

The teeth were stored for 7 days in a microbiological oven at 37 ± 1ºC and 100% relative humidity. Afterwards, the sealing of the coronal opening was removed and the pulp chamber was irrigated for 30 s with deionized water. The teeth were sectioned in three slices in transversal direction and the incisal third was discarded. The two remaining slices were longitudinally cut separating the vestibular and lingual faces, which were used to analyze enamel, dentin and cementum.

The specimens were fixed in 2.5% glutaraldehyde in a 0.1M sodium cacodylate buffer at pH 7.5 for one hour, with three changes, immersed in distilled water for one minute and then dehydrated. They were dehydrated in ethanol concentrations (25%, 50%, 75%, 90%) for 20 min in each one and in absolute ethanol (100%) for one hour. The specimens were kept overnight in an oven at 37˚C and mounted on aluminum stubs. Metallization was performed with a thin gold-palladium coat (200Å) in a high-pressure vaporizer (DV-502-Denton, NJ, USA).

The analysis by scanning electron microscopy (SEM) and energy dispersive spectrometry (EDS) were performed using scanning electron microscope (Scanning microscope – JSM – 840A Jeol, Japan). The EDS evaluations quantified the carbon, calcium, phosphorous and oxygen levels and the SEM evaluations, under 500x and 2000x magnification, were descriptive and illustrative in order to correlate the morphological alterations with the alterations of chemical elements found in EDS.

The changes in the levels of chemical elements obtained in different groups were compared using the Kruskal-Wallis test (5%). When a statistical difference was found, the Dunn multiple comparison test was used to verify which groups differed among them.

RESULTS

Tables 1, 2 and 3 shows the mean and standard deviation of percentages of calcium, carbon, phosphorus and oxygen found in different groups in enamel, dentin and cementum, respectively.

A significant reduction was found in the calcium levels in enamel after treatment with CP + SP and CP ($p < 0.05$). The phosphorous levels remained similar in CP + SP ($p < 0.05$); in the other groups these levels significantly increased with HP and CP ($p < 0.05$). Carbon (organic part) was hardly altered in enamel.

A significant reduction in the calcium levels was found in dentin in Groups HP+SP, CP and CP+SP. An increase in the calcium levels occurred in Group SP+H20, but these differences were not significant ($p > 0.05$). Phosphorus levels increased after SP+H20 ($p < 0.05$) and CP ($p < 0.05$). Carbon levels showed little variation and the largest amount was found in Groups CP and CP+SP ($p < 0.05$); in the other groups there was no alteration.

A significant reduction in the calcium levels was found in the cementum in Group CP+SP ($p < 0.05$). In the other groups, this element remained similar (CP: $p > 0.05$); (SP+H2O: $p > 0.05$); (HP: $p > 0.05$) or higher (HP+SP: ($p < 0.05$). The phosphorous levels were similar in CP + SP ($p > 0.05$) or higher but with no statistically significant differences in Groups HP ($p > 0.05$), CP ($p < 0.05$) and HP+SP ($p > 0.05$). Carbon levels showed little variation and the largest amount was found in Group HP+SP ($p > 0.05$) and the lowest amount in Group HP ($p > 0.05$); but with no statistically significant differences.

Figure 1 shows the SEM images of the representative specimens in which alterations occurred in enamel, dentin and cementum in the different experimental groups.

DISCUSSION

In the present study, the groups in which CP was used showed reduction in the calcium levels in the enamel, dentin and cementum. Although release of lower percentages of hydrogen peroxide from carbamide peroxide occurs, it is less aggressive than the other bleaching agents, particularly when used in association with SP [12]. According to Rotstein et al. [13], the association of SP with CP has more

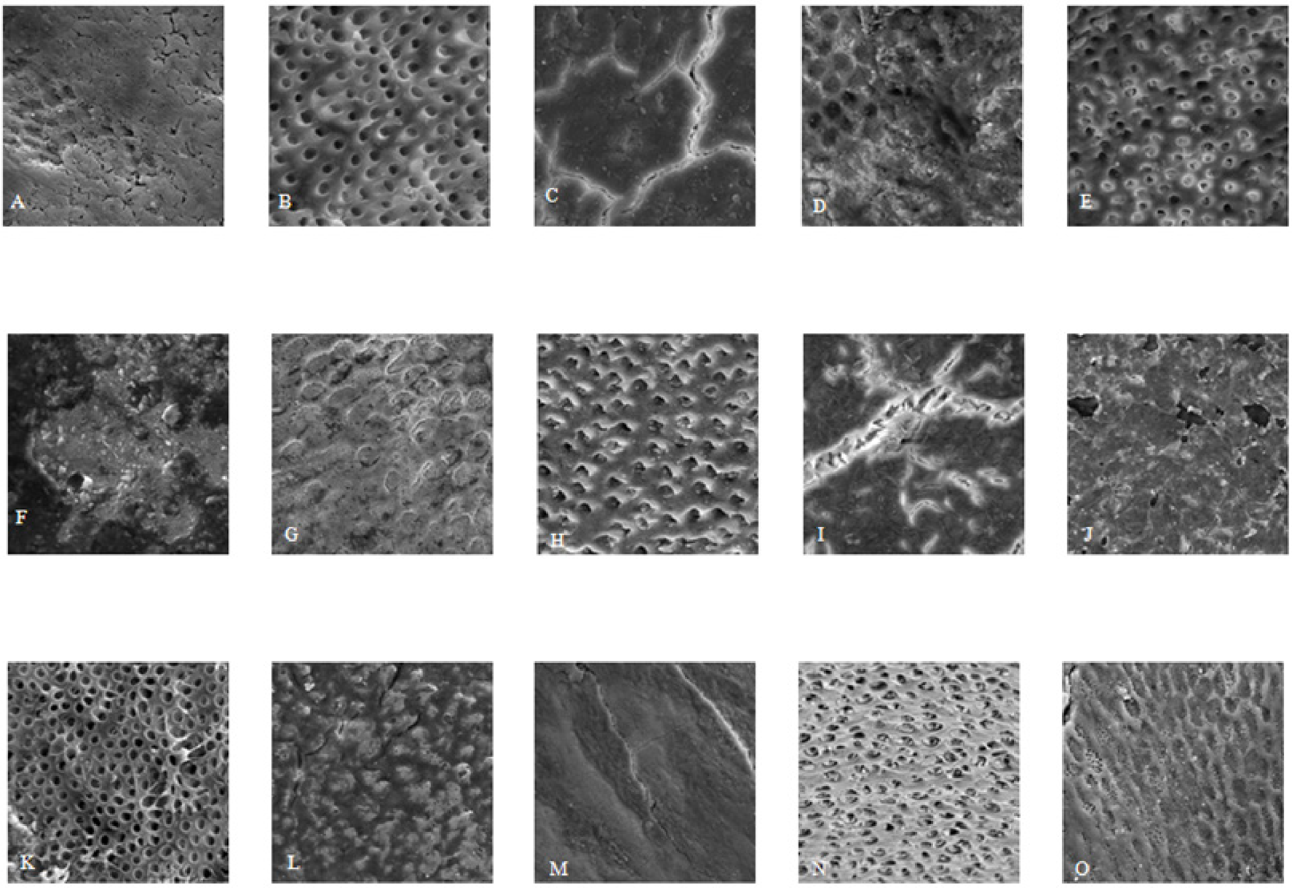

Figure 1 - Photomicrographs obtained by SEM of the representative specimens of the groups (2000x magnification). Group HP: A) irregularities and areas with exposed enamel prisms; B) wide dentinal tubules and with an aspect of having been "attacked" by the product; C) smooth cementum with some cracks. Group HP+SP: D) regular enamel with spots of erosion; E) normal intertubular dentin and tubules. Some tubules obstructed by the sodium perborate may be seen; F) large areas of cementum erosion. Group CP: G) irregular enamel; loss of enamel prisms exposing organic material; H) normal dentin, but some areas of irregularity may be seen close to the tubule entrance; tubules with residues of bleaching agent; I) smooth cementum with irregular areas. Group CP+SP: J) areas of enamel with loss of structure; K) dentin with an aspect of erosion due to the chemical product; L) irregular cementum. Group SP: M) regular enamel with no alterations; N) large amount of bleaching agent obstructing tubules and walls; O) regular cementum with no alterations.

Table I – Descriptive statistics analysis: mean and standard deviation of percentages (%) of the elements calcium, carbon, phosphorus and oxygen found in the enamel in different groups

Element	Enamel											
	HP		HP + SP		CP		CP + SP		SP		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ca	19.89	11.88	28.14	9.06	11.62	7.81	12.84	11.78	26.79	14.69	27.06	7.52
O	74.23	15.53	72.96	12.80	83.25	10.29	86.12	13.05	59.12	17.99	68.13	7.58
P	6.37	4.99	3.89	4.13	6.56	5.56	1.949	2.978	4.63	4.07	1.968	3.358
C	38.94	10.75	57.10	15.47	53.59	6.84	43.12	16.39	44.57	14.71	44.05	12.27

Table I I – Descriptive statistics analysis: mean and standard deviation of percentages (%) of the elements calcium, carbon, phosphorus and oxygen found in dentin in different groups

Element	Dentin											
	HP		HP + SP		CP		CP + SP		SP		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ca	23.05	12.85	19.40	8.30	13.21	8.79	14.84	18.16	33.26	8.13	20.19	5.73
O	67.27	24.60	78.03	9.90	77.82	7.70	83.64	23.06	58.95	11.68	77.60	6.93
P	2.50	3.51	4	2.599	4.142	2.045	3.05	4.48	6.56	4.38	0.196	0.675
C	37.15	10.20	36.51	4.76	43.10	9.24	38.94	17.51	37.19	12.36	37.52	9.90

Table III – Descriptive statistics analysis: mean and standard deviation of percentages (%) of the elements calcium, carbon, phosphorus and oxygen found in cementum in different groups

Element	Cementum											
	HP		HP + SP		CP		CP + SP		SP		Control	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Ca	18.19	12.45	26.29	13.68	12.53	8.18	7.20	6.00	19.37	7.08	14.61	7.87
O	75.78	16.90	72.22	17.89	83.04	7.47	92.46	6.15	74.90	10.29	84.45	7.10
P	6.15	5.05	3.866	2.933	4.789	2.757	0.0505	0.1121	1.767	1.648	0.0417	0.0793
C	36.04	12.77	53.64	9.74	48.742	3.355	42.52	14.70	44.64	13.96	44.39	16.85

oxidative power than the association of SP with HP. Furthermore, urea released by CP is highly diffusible, it has a smaller molecular weight, it is capable of inhibiting some substances from the tooth structure and it penetrates into healthy or carious enamel and dentin [14], it may also destroy or weaken the peptide structure, especially the interprismatic structure, altering the union of hydrogen bonds in proteins and therefore, modifying the bond between the enamel matrix and mineral components [15]. These alterations in the mineralized structure caused by urea in association with the effects of hydrogen peroxide may be responsible for the morphological alterations found in the mineralized tissues when carbamide peroxide was used as the bleaching agent.

All the bleaching agents tested decompose to form hydrogen peroxide, which may cause protein denaturing [9] increasing the amount of inorganic material after bleaching, which may be a result of protein oxidation [13]. The purpose of this study was to assess the morphological alterations and the composition of the tooth structures in order to prove that the bleaching agent penetrates into the pulp chamber up to the periodontium, significantly altering the structures, as proved by the analysis under SEM and EDS. All the teeth may have small openings or gaps with micro exposure of dentin into the periodontal tissues, which enables communication between the pulp chamber and the cervical tooth surface [16] in the cementoenamel junction. However, Palo et al. [12], found that oxidative agents of the bleaching agents cross dentin and cementum, reaching the external root surface even when there are no failures in the junction. Therefore, the bleaching agent that penetrates into the dentinal tubules reaches the periodontium and this may cause an inflammatory process initiating external cervical resorption [17]. In the present study, particularly the dentin that suffered the direct action of bleaching agents, the calcium levels, which may indicate higher demineralization, were higher in Group SP+H2O; but enamel and cementum of this tooth was not altered in Group SP+H2O.

Cases of external root resorption related to bleaching reported in the literature have been associated with the use of hydrogen peroxide alone or with sodium perborate [18-21]. However, Holmstrup et al. [22] and Valera et al. [23] believe that sodium perborate associated with water is an effective bleaching agent and it does not seem to be associated with external root resorption, which makes it a safer bleaching agent because it releases a lower amount of oxidative radicals [12] and has proven bleaching effect [23,24] causing few chemical alterations in the tooth structures, as seen in this study.

CONCLUSION

The results obtained in this study showed that alterations in the chemical compounds and the morphology of hard dental tissues always occur after internal bleaching, but these alterations depend on the bleaching agent. Therefore, the smaller effects in enamel, dentin and cementum in this study occurred in the group treated with sodium perborate and water. This product is the safest bleaching agent for bleaching endodontically treated teeth.

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