

Influence of different hydrogen peroxide neutralizer solutions on the dental root morphology

Influência de diferentes soluções neutralizadoras do peróxido de hidrogênio na morfologia da raiz dentária

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ABSTRACT

The consequences of the application of antioxidant agents on the root morphology are yet to be fully known. **Objective:** Evaluate through scanning electron microscopy (SEM) the dental root morphology after the application of different neutralizer solutions, which were submitted to internal tooth bleaching with hydrogen peroxide 35%. **Material and Methods:** Eighteen bovine incisor teeth were randomly selected and prepared. Then, they were distributed into 6 groups: CG:without bleaching; B:only bleaching; BSA:bleaching + 10% of sodium ascorbate solution for 3 hours; BCH:bleaching + calcium hydroxide paste for 7 days; BA:bleaching + 10% alpha-tocopherol for 3 hours; BC:bleaching + catalase based gel for 2 minutes. The roots were vertically sectioned and analyzed under SEM at 2000x and 5000x magnification. Two photomicrographs, obtained from the cervical third, were randomly selected from each sample using SEM and subsequently evaluated. The images were verified based on a scoring system for smear removal. The Kruskal-Wallis test and Dunn post hoc with Bonferroni correction was performed. **Results:** It was observed that B presented dentin tubules with larger diameter, BSA presented smaller and partially obliterated tubules, while BCH and BA presented higher formation of precipitates with irregular surface and obliterated tubules. The BC agent showed a cleaner surface, with opening of the tubules similar to the CG group. Groups B, BC and CG were statistically similar. **Conclusion:** The different neutralizer substances promoted the deposition of precipitate and obliteration of the dentin tubules, being BC the neutralizer substance which generated less interference on the dental root morphology.

KEYWORDS

Antioxidants; Dentin; Hydrogen peroxide; Microscopy; Tooth bleaching.

RESUMO

As consequências da aplicação de agentes antioxidantes na morfologia radicular ainda não são totalmente conhecidas. **Objetivo:** Avaliar por meio de microscopia eletrônica de varredura (MEV) a morfologia das raízes dentárias, submetidas ao clareamento interno com peróxido de hidrogênio 35%, após a aplicação de diferentes soluções neutralizadoras. **Material e Métodos:** Foram selecionados e preparados aleatoriamente dezoito incisivos bovinos. Em seguida, foram distribuídos em 6 grupos: GC:sem clareamento; B:somente clareamento; BSA:clareamento + solução de ascorbato de sódio 10% por 3 horas; BCH:clareamento + pasta de hidróxido de cálcio por 7 dias; BA:clareamento + alfatocoferol 10% por 3 horas; BC:clareador + gel à base de catalase por 2 minutos. As raízes foram seccionadas verticalmente e analisadas em MEV com aumento de 2.000x e 5.000x. Duas fotomicrografias, obtidas dos terços cervicais de cada amostra, foram selecionadas aleatoriamente e posteriormente avaliadas. As imagens foram verificadas com base em um sistema de pontuação para remoção de esfregaços. Foi realizado o teste de Kruskal-Wallis e post hoc de Dunn com correção de Bonferroni. **Resultados:** Observou-se que B apresentou

túbulos dentinários de maior diâmetro, BSA apresentou túbulos menores e parcialmente obliterados, enquanto BCH e BA apresentaram maior formação de precipitados com superfície irregular e túbulos obliterados. O agente BC apresentou superfície mais limpa, com abertura dos túbulos semelhante a GC. Os Grupos B, BC e CG foram estatisticamente idênticos. **Conclusão:** As diferentes substâncias neutralizadoras promoveram a deposição de precipitados e obliteração dos túbulos dentinários, sendo o BC a substância neutralizadora que gerou menor interferência na morfologia da raiz dentária.

PALAVRAS-CHAVE

Antioxidantes; Dentina; Peróxido de hidrogênio; Microscopia; Clareamento dental.

INTRODUCTION

Tooth bleaching is a commonly approach with verified effectiveness that has been increasingly by patients who aim to improve the aesthetic results of darkened dental teeth, with an even greater challenge for situations of devitalized. Among the different whitening agents that can be considered, we can highlight the peroxides, such as hydrogen peroxide [1,2]. The root dentin microhardness reduces considerably after the internal tooth bleaching with hydrogen peroxides due to the attack to its inorganic components, denaturation of the collagen fiber, and reduction of the calcium-phosphate concentration [3].

Antioxidant agents are substances capable of delaying or inhibiting the upcoming and progress of oxidation, by inactivating the free radicals through an interaction or preventing them from being formed [1,4]. The use of these antioxidant agents can be considered between the bleaching and restorative procedures with the purpose of reversing the deleterious effects caused by the action of the hydrogen peroxide on the dental structure and, consequently, improving the quality of the adhesion in restorations manufactured immediately after the bleaching procedure [3,5].

Currently, different antioxidant agents can be used for this purpose, among them, the 10% of sodium ascorbate solution that re-establishes the union of the bleaching dentin to the resin [6,7]. The calcium hydroxide, through its use as curative after the bleaching procedure, and can reduce the damaging effects of the bleaching agent [8]. The α -Tocopherol, main active component of vitamin E, that promotes the increase of the bond resistance [5,9]. In addition, the Catalase that is also considered one of the main antioxidant enzymes acting against the toxic oxygen radicals [10].

Several studies have investigated the influence of antioxidant substances on the bond resistance. However, few studies evaluated the consequences of the use of antioxidant agents on the root micro-morphology [11]. Therefore, the purpose of this study was to evaluate the influence of neutralizer solutions of hydrogen peroxide on the dental root canal morphology through scanning electron microscopy (SEM) evaluation.

MATERIAL AND METHODS

Bovine incisors (n=18) had their crowns removed and were submitted to root canal preparation using endodontic K-fillers and gates glidden (Dentsply/Maillefer, Petrópolis, Brazil) with irrigation of 2.5% of sodium hypochlorite. After preparation the root canals were root filling with gutta-percha and zinc oxide eugenol cement (Endofill, Dentsply Maillefer, Petrópolis, RJ, Brasil) with lateral condensation technique. After filling the root canals were temporary sealed with glass ionomer (Ketac™ Cem, 3M, ESPE, St Paul, MN, USA) to avoid root canal contamination.

The roots were placed in a dental surveyor (B2, BioArt, São Carlos, SP, Brazil) attached to the vertical axis, perpendicular to avoid any inclination. The root canal preparation removing of 4 mm of gutta-percha was performed using gates glidden (n° 02), followed by application of EDTA and washing for 60 seconds to clean the pulp chamber. A 2 mm cervical barrier was performed using glass ionomer cement (Maxxion R, FGM, Joinville, SC, Brasil) [12], followed by application of 37% phosphoric acid (15x) and washing (60s). The root canal was bleaching with 35% hydrogen peroxide (Whiteness HP, FGM, Joinville, SC, Brazil).

The bleaching agent was applied inside the 2mm of the root canal during 15min, totaling

3 applications, with 5 min interval between each application and washing (60s). After the preparation, the samples were randomly divided into 6 groups: CG: without bleaching (control group); B: only bleaching; BSA: bleaching + 10% of sodium ascorbate solution for 3 hours; BCH: bleaching + calcium hydroxide paste for 7 days; BA: bleaching + 10% alpha-tocopherol for 3 hours; BC: bleaching + catalase based gel for 2 minutes.

The solution of alpha-tocopherol was prepared through vitamin E in powder added to the solvent of 0.9% physiological saline, until a paste-like consistence was reached and applied on the intracoronary surface. The solution of sodium ascorbate was prepared through vitamin C in powder and saline. Just as well, the calcium hydroxide paste was composed by saline. On the other hand, the catalase was used from the neutralizer vial present on the 35% Hydrogen Peroxide kit (Whiteness HP, FGM, Joinville, SC, Brazil).

The roots were then cleaved into two halves (mesial and distal) through a chisel. To be submitted to high vacuum SEM analysis, the specimens were dehydrated with crescent series of alcohol (70%, 80%, 90% and absolute) and gold-sputtered (EMITECH SC7620, USA) for 150 seconds, and followed the samples were submitted to the analysis under Scanning Electron Microscopy (SEM), using a tungsten Scanning Electron Microscope (Inspect S50 – FEI, Czech Republic) at 2000x and 5000x magnification.

Two photomicrographs, obtained from the cervical thirds, were randomly selected from each sample using SEM and subsequently evaluated by two blinded and previously calibrated observers (Kappa = 0.912). The images were analyzed based on a scoring system for smear removal: Score 0: not present; Score 1: detectable in $\leq 25\%$ of the surface area; Score 2: detectable in 25-50% of the surface area; Score 3: detectable in 50-75% of the surface area; Score 4: detectable in $> 75\%$ of the surface area [13]. The Kruskal-Wallis test was performed to investigate differences in scores between groups (JASP, version 0.18.3, Amsterdam, Netherlands)

RESULTS

From the SEM analysis, images at 2000x and 5000x zoom were obtained from the cervical,

medium, and apical third throughout the canal of one of each specimen of the 6 groups. The application of different neutralizers showed differences on the dentin micro-morphology when compared to the CG. The CG presented wide open dentin tubules with a surface free of debris. The B group presented tubules with larger diameter. The BSA group presented smaller and partially obliterated tubules in all evaluated thirds. The BCH group presented in the cervical almost full obliteration of the tubules by the formation of precipitates. In addition, in the cervical of BA group also presented significant formation of precipitates generating an irregular surface and with obliteration of tubules. On the other hand, the BC cervical presented a surface free of debris, with the opening of the dentin tubules similar to the control group. SEM images at 2000x were obtained from the dentin wall of the root canal of such elements, and the vertical view of the tubules, differences among the groups were not found (Figure 1).

The application of the antioxidant agents occurred on the cervical third. However, for the analysis of the medium and apical third, it was aimed at identifying whether the action of the antioxidants would also be extended to those thirds. In such findings, only debris were found, remains of the endodontic cement and gutta-percha, occluding the tubules of the medium and apical thirds of each group, which act as contaminants and diminish the dentin permeability.

The description of the scores for each group can be seen in Figure 2. The Kruskal-Wallis test was statistically significant ($H(5) = 66.792, p < 0.001$), . Pairwise comparisons using Dunn's post hoc with Bonferroni correction were performed. The results can be seen in Table I.

DISCUSSION

The decomposition of the bleaching agent during the bleaching process results in the liberation of residual oxygen, which inhibits the curing of the resin, reducing the adhesion and the resistance of the restorative material to the dentin substrate. Such deleterious effects are reversed throughout the time, thus, several authors recommend that after the external bleaching process, 7 to 14 days are waited [5,14] and at least 4 weeks after the endogenous bleaching [15], to start the definitive restorations.

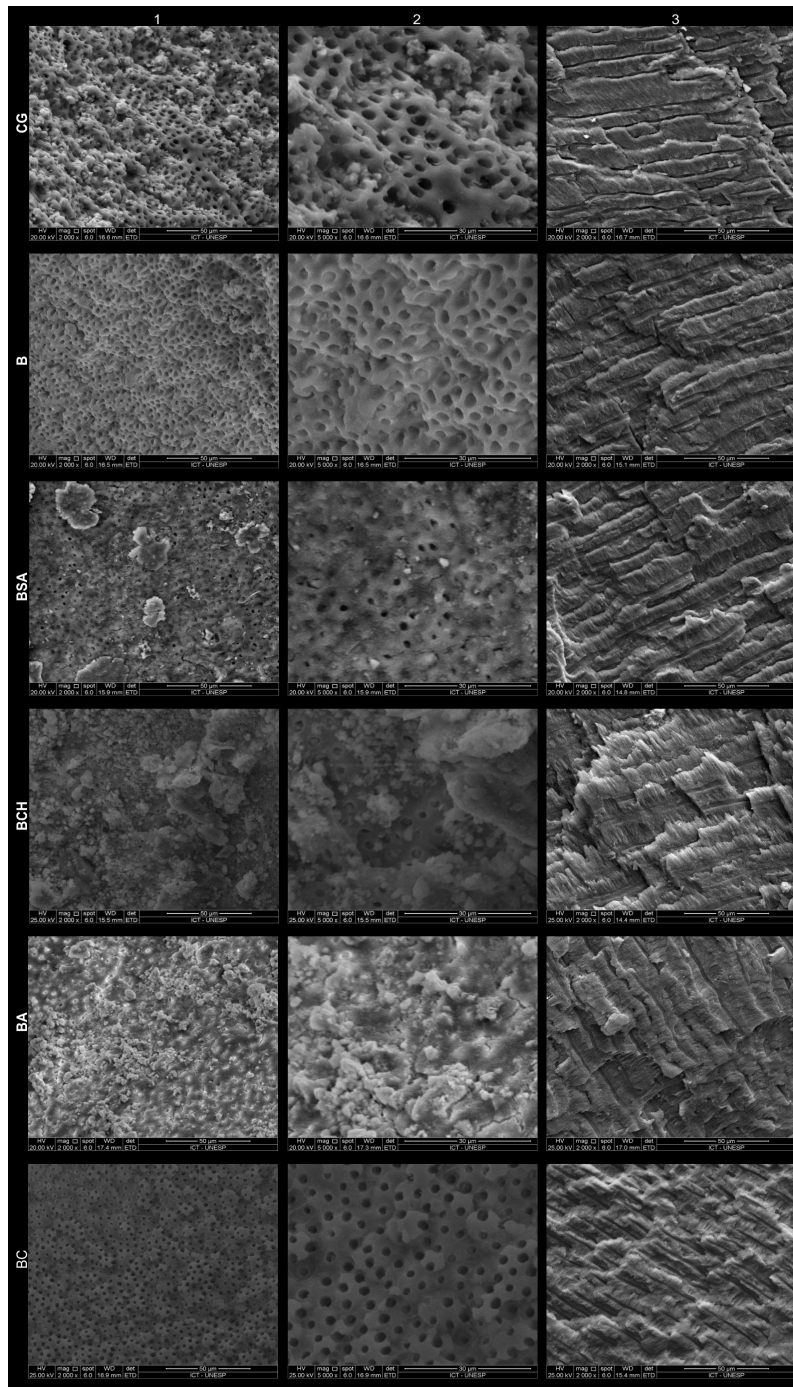


Figure 1 - Column 1 represents images of the cervical third at 2,000X; Column 2 represents images of the cervical third at 5,000X; Column 3 represents images of vertical view of the cervical tubules at 2,000X.

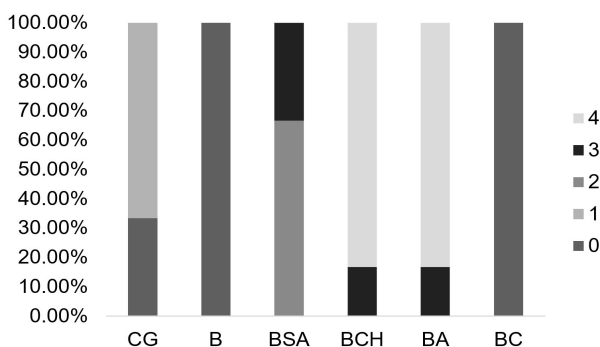


Figure 2 - Frequency of scores by group.

However, periods of waiting are not well-accepted in clinical practice. In endodontically treated teeth, the higher number of clinical sessions generate more costs to the patient and time may lead to failures in the restorative treatment, micro-leakage in temporary restorations and recurrence of discoloration in recently bleached teeth [16].

Several studies evaluate the effects of bleaching agents in the adhesion resistance to enamel, but where there is much exposure of dentin, this is also

Table 1 - Dunn's post hoc with Bonferroni correction

Comparison	P_{bonf}	
B - BA	< .001	**
B - BC	1.000	
B - BCH	< .001	**
B - BSA	0.007	*
B - CG	1.000	
BA - BC	< .001	**
BA - BCH	1.000	
BA - BSA	0.564	
BA - CG	< .001	**
BC - BCH	< .001	**
BC - BSA	0.007	*
BC - CG	1.000	
BCH - BSA	0.564	
BCH - CG	< .001	**
BSA - CG	0.623	

* $p < 0.01$; ** $p < 0.001$.

important [17]. The restorative strategies for the adhesion between the glass fiber post and the root dentin still generate discussions [18]. In addition, dentin adhesion is a more challenging process than enamel adhesion, due to the humidity and the architecture of the collagen fibers, which may influence the results [3]. Endodontically treated teeth, often, present insufficient coronary structure to retain the restorative materials, needing the employ of glass fiber posts [19-21].

The integrity of the resin-dentin interface is essential to the adhesion resistance of glass fiber posts and the longevity of the adhesive cementation. Nonetheless, some factors such as the presence of smear layer with reminiscence of endodontic cement, obturation material, micro-organisms and infected dentin may degrade it. Therefore, different chemical treatment in intraradicular dentin aim at unblocking the dentin tubules, cleaning the substrate and improving the surface that will receive the cement [19]. During the endodontic treatment, the use of sodium hypochlorite degrades the organic component of the dentin, mainly collagen, preventing the formation of the hybrid layer. In addition, the dissociation of such irrigating substance in sodium chloride and oxygen compromises the photo-curing of adhesives. Therefore, it is possible that the association of sodium hypochlorite and hydrogen peroxide may also influence the action of antioxidants [5]. According to the recommendations for the execution of SEM, the samples were immersed in 2.5%

sodium hypochlorite, which may have generated alterations on the surface.

Dentin tubules are permeable to the bleaching agents and, therefore, they act as reservoirs of oxygen free radicals. In higher levels on the adhesive interface, the leakage of resin materials in the tubules is harmed, generating a diminishing of the number, size and quality of resin tags [22]. By comparing the surface of a non-bleached tooth with a bleached one, it is possible to observe a reduction of approximately two thirds of penetrability from the resin markers on the surface of the bleached tooth [23].

Although it was not evaluated in the present study, the morphological factor is related to the diminishing of the adhesion resistance [24]. The literature demonstrates that the number of dentinal tubules present in the cervical third is higher, and their diameter is larger when compared to the apical third. This particular anatomy may influence the effectiveness of restorative procedures in endodontically treated teeth, as the filling of dentinal tubules with adhesive and core material is significantly affected by the location within the root canal, being more pronounced in the cervical region [25,26]. This reflects a general trend of decreasing bond strength from the cervical to the apical region [27].

Moreover, the cervical portion is closer to the access point for the curing light, which likely impacts the degree of conversion of the resin cement [28]. Therefore, the cervical third of intraradicular dentin should be considered when analyzing adhesion. Although the present study only evaluated the morphology in the 2 mm of the cervical third, this region should be taken into account during clinical interventions, as its structure may affect permeability and the interaction with bleaching and restorative agents. Thus, although the focus of this work was not to evaluate the adhesion of fiberglass posts, the analysis of dentin morphology is essential to understanding the challenges and implications of bleaching and restorative techniques in endodontically treated teeth, necessitating further investigation on the subject.

The bleaching agent in addition to inhibiting the adhesive curing also provides changes in the morphology of the bleached dentin, such as reduction of the organic components, through the influence on the collagen net, resulting in the denaturation and instability of the matrix, which are

essential to the adhesion procedures, in addition to diminishing its micro-hardness [29,30]. The sodium ascorbate is capable of reversing the denaturation of the acid etching, sodium hypochlorite or hydrogen peroxide on the dentin's collagen and also a strong inhibitor of metalloproteinases of the matrix (MMPs), providing protection against the long term degradation of the adhesive-dentin interface [31]. The 10% AS promotes the closing of the dentin tubules compared to the surface treated with α -tocopherol soon after the bleaching, which was observed in the present study.

Applying a solution of 75% ethanol for 3 minutes on the dentin surface after the bleaching of non-vital teeth may be effective in eliminating the adverse effects of bleaching on the dentin adhesion [32]. Also, the presence of alcohol in the antioxidant may contribute to a better diffusion of the adhesive throughout the dentin. However, there are no studies comparing their different formulations after the bleaching [33]. The association of acetone as solvent for the alpha-tocopherol enables the reduction in the time of application due to the synergistic effect of removing the residual oxygen from the tooth structure [34]. Nonetheless, with the purpose of standardizing the groups and observing the action of the antioxidant separately from the effects of the alcohol, the physiological saline was used as solvent for the antioxidants of the groups CAS, CHC and CA.

The SEM analysis by Jung et al. [35] presented that the application of the solution of 10% AS revealed crystals on the dentin surface. Thus, the longer the time of application, the higher the tendency of those crystals to present themselves increased in size and amount. In a way that, after the application for over 24 hours, the majority of the dentin surface in both groups was covered with crystals. Despite the washing of the dentin surface with running water for 30 seconds before the adhesion procedures, the crystals still remained on the dentin surface. The presence of such crystals on the dentin-restoration interface may be responsible for the diminishing of the adhesion resistance between the composite resin and the bleached dentin. In the present study such crystals were not highlighted by the SEM images, in the group submitted to bleaching, followed by the application of 10% AS for 3 hours.

The almost complete obliteration of the dentin tubules in CHC may be explained by the

calcium hydroxide being a non-demineralizing agent. The cleaning agents can be demineralizing and non-demineralizing [36]. The demineralizing agents remove the layer of debris resulting from the cavity prep, unblocking the dentin tubules aiming at penetrating the adhesive, when the non-demineralizing agents keep the cavity aseptic [37].

In the study of Moura et al. [38] the SEM analysis showed that the healthy dentin surface treated with calcium hydroxide solution with 37% acid etching presented practically all dentin tubules obliterated. The procedures of etching and washing do not ensure the elimination of the oxygen residues from the surface [39]. The presence of smear layer and smear plug visualized in the groups treated with antioxidants means partial inhibition of the residual oxygen, which makes the penetration of adhesives harder, affecting, consequently, the duration of the adhesive restorations [29].

The specimen treated with catalase presented a more similar surface to CG and B. These findings were confirmed by statistical analysis. Clean dentin surfaces can be seen in the CG group after using EDTA and in the B group after using 35% Hydrogen Peroxide. The application of catalase after the intra-coronary bleaching generates a complete elimination of the harmful residual hydrogen peroxide [40]. Thakur et al. [41] in their study stated that the enamel bleached with 37% hydrogen peroxide, with catalase and sodium fluoride presented meaningful clear surface under SEM and increased micro-hardness, meaning that such treatment may improve the morphology and the hardness. Therefore, it is suggested that more in vivo studies clinically evaluate the efficiency of the catalase and topic fluoride application over dentin.

The main limitation of this in vitro study consisted in being a quantitative and qualitative analysis of the morphology. Considerations were performed regarding changes occurred in the intraradicular substrate, however, it was not possible to affirm whether the formation of the precipitates observed makes the surface more or less appropriate to the adhesion of the restorative materials. For such, new studies regarding adhesion testing shall be performed. Such studies may also evaluate beyond the potential of the antioxidants, by determining the stability of the antioxidant activity, the proper concentration and the time to a better result on the dentin.

CONCLUSION

The catalase generated less interference in the root morphology. On the other hand, the application of different neutralizing substances on the intra-coronary surface of the bovine elements provided the deposition of precipitates and obliteration of the dentin tubules in the cervical third.

Author's Contributions

RFC: Conceptualization. AVTR, NRR: Methodology. NRR: Investigation. CSCR: Data Curation. TCP: Formal Analysis. AVTR: Writing – Original Draft Preparation. AVTR, TJAPJ: Writing – Original Draft Preparation. TCP, CSCR, RFC: Writing – Review & Editing. TJAPJ: Supervision, Methodology. TJAPJ, RFC: Project Administration.

Conflict of Interest

The authors have no conflicts of interest to declare.

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Regulatory Statement

This study was conducted in accordance with ethical guidelines governing the use of animal materials in research. The research utilized animal teeth obtained as discarded materials. An official notice was submitted to the Ethics Committee on the Use of Animals (CEUA) of the Federal University of Juiz de Fora to provide documentation of the materials' origin and inform the committee about the study.

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