

THE GEL CYTOTOXICITY IN RELATION TO THE DENTAL PULP

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Received: 02/28/2014; Accepted: 03/28/2014

ABSTRACT

The technique of tooth whitening has evolved, with new techniques brought comfort and perspective to patients, therefore this study is to evaluate the response of vital teeth pulp, bleached with the technique in practice in peroxide concentration 35% hydrogen. We selected patients with indications for extractions of mandibular central incisors by orthodontic reasons. In two sessions were applied teeth bleaching gel for 45 minutes, according to manufacturer's guidelines. After 1 week, the teeth were extracted. Afterward, the teeth were placed in a jar with formaldehyde, keeping up the dehydration and clearing. Later, it was embedded in paraffin and then cuts and staining of slides. In the vestibular region, where the bleaching agent was applied there was a sharp reaction of odontoblasts disorganized, losing their character and palisade layer juxtaposed. We noted that the core of odontoblasts are pyknotic. In lingual region where no bleaching agent was applied, there is the organization of odontoblasts. Therefore, changes occurred in the tooth-pulp, but the pulp tissue is compatible with normality.

KEYWORDS: Whitening. Hydrogen peroxide. Dental pulp.

1. INTRODUCTION

The vital teeth whitening can be performed in a dental office or at home. In the dental office, the dentist applies the whitening gel based on hydrogen peroxide in high concentration (15% -40%). The home whitening is done with the aid of milder bleaching agents such as carbamide peroxide (10 -% 16%) equivalent to hydrogen peroxide (3.5-5.5%)¹.

The whitening occurs because the tooth structure is permeable to bleaching agents able of diffusing into the enamel and dentin oxidation occurring², consisting in a chemical process where organic materials are converted into carbon dioxide and water. The pig-

ments are composed of large amounts of carbon molecules. These are broken and converted into intermediate compounds (smaller carbon chains) that are whiter. This chemical reaction changes the type, number and relative position of the atoms that compose these molecules. During the whitening the carbon chains are transformed into CO₂ and H₂O, being gradually released along with the nascent oxygen³.

Currently there is a great quest for the "white smile", with extensive media coverage and, consequently, an increased interest by patients in relation to treatment, with the advantage of being a conservative technique without wear of dental elements⁴. However, there is controversy about the biocompatibility relative to pulp organ⁵⁻¹¹.

The realization clinical research using the whitening gel is important because the bleaching agents can reach the pulp chamber and cause damage to the cellular membrane, leading to the death of pulp cells by apoptosis or necrosis in lower incisors, probably due to minor thickness enamel and dentin^{10,12}. Thus, the goal of this research was to evaluate the pulpal cytotoxicity with the use of hydrogen peroxide whitening gel to 35% in incisors extracted after 1 week of whitening technique.

2. MATERIAL AND METHODS

Three patients were selected with pre-established criteria: teeth free of cracks, caries, fractures, developmental abnormalities and periodontal disease. The patient was informed about the research procedures and, after agreeing, signed "Statement of Consent", authorizing the use of extracted teeth in this study, in accordance with Resolution No. 196, of October 10, 1996, of the National Board of Health / Ministry of Health - Brasília / FD. The selected volunteer donors had indicated for extraction of mandibular central in-

cisors by orthodontic reason.

The whitening gel technique, has used a gel based on hydrogen peroxide (H_2O_2) at 35%, in a dental office; two clinical whitening sessions and dental elements extracted one week after the bleaching treatment were performed (Table 1).

Table 1. The established routine in research

SESSIONS	WHITENING PRODUCT	EXTRACTION
02 - 45 min.	Pola Office + (SDI)	After 1 week

The bleaching agent used is composed of two attached needles containing hydrogen peroxide and the other the thickener. The manipulation of whitening gel followed the manufacturer's instructions. The three teeth underwent two sessions of office bleaching with hydrogen peroxide at 35%. Two applications of the teeth whitening gel were performed for 45 minutes¹³ at each clinic session of whitening.

At the end of clinic session, the gel was removed with an endodontic sucking and washed with water/air using a triple syringe for complete removal of the gel. After this procedure was applied colorless neutral fluoride for 10 minutes in order to prevent tooth sensitivity. The interval between bleaching sessions was 7 days. The extractions were performed one week later under local anesthesia.

After tooth extraction performed by extraction alveolar route with forceps, each tooth was kept wrapped in gauze soaked in 0.9% saline solution, and the final suture procedures were performed in "X" external. The patient was monitored by the researcher, in the healing process, during the period of 45 days after the surgical procedure. After the extraction of each dental element, all gingival and periodontal tissue were scraped using a scalpel handle (Golgran, São Paulo, Brazil) fitted with blade number 12 (Lamedid and Commercial Services Ltda., Barueri, São Paulo, Brazil), cleaned with a rubber cup, attached to a hand-piece (Duratec 23D, Kavo, Germany).

The teeth were immediately placed into individual vials properly identified solution containing 10% formalin buffered at neutral pH during the first 48 hours and washed in running water for a period of 5 hours to remove any fixing solution.

After fixation, the specimens were demineralized in a solution of 4% formaldehyde. After about three months, it was considered finalized the process of demineralization. This was controlled as a fine needle could be introduced into the specimen without any resistance. At that time, the pieces presented with rubbery consistency, without the shear blade. Subsequently, the teeth were washed with water, dehydrated

in alcohol, diaphanized in xylene and embedded in paraffin. The orientation at inclusion allowed for tissue sections lengthwise. Once contained, the parts semi-serial sections were cut with six mm thick, made in a microtome.

For each specimen studied, took advantage of the tissue sections with evidence of dental pulp in its long axis. For each specimen ($n = 3$), 10 glass slides were prepared and stained with hematoxylin and eosin (H/E). Each of them possessed 5 tissue sections. All tissue sections were microscopically analyzed, tabulated and subjected to statistical analysis.

In all sections were evaluated by a calibrated examiner blinded to the groups. Using a light microscope (Diastar, Cambridge Instruments, Buffalo, NY) adapted to a video camera (DXC-107A/107AP; Sony Electronics, Tokyo, Japan), 3 linear measurements of enamel junction / dentin to the pulp chamber were performed to determining the thickness of dentin (DT - thickness). The video images were loaded into a computer and processed using standard software (Mocha; Jondel Scientific, San Rafael, CA). The methodology used in this study to measure the DT, was similar to that previously used to determine the thickness of the remaining dentin between the cavity floor and the underlying pulp tissue. The three measures were made to obtain an average DT of each tooth. The data on DT were submitted to the nonparametric Kruskal-Wallis test, complemented by the Mann-Whitney test with a significance level of 5% (SPSS, Chicago, IL).

3. RESULTS

Changes in the pulp tissue were observed and exemplified in Figure 1.

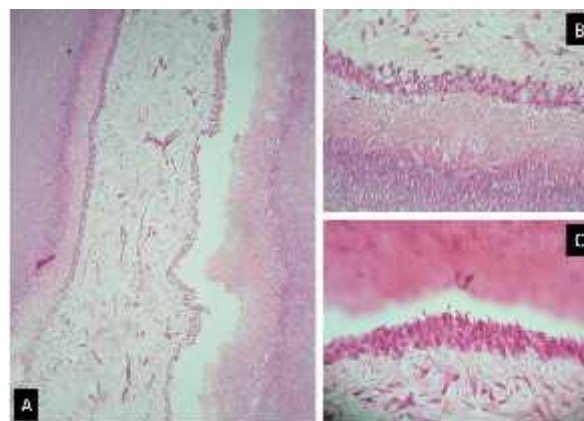


Figure 1. Microscopic appearance of the pulp-dentin of a tooth subjected to treatment with whitening gel complex. **A:** Overview (40x magnification); **B:** (400x magnification), Region Lingual: organization of odontoblasts; **C:** (400x magnification), Vestibular Region: disruption of odontoblasts in relation to the application of the bleaching agent region, being possible to observe pyknotic nuclei.

The analysis showed Lingual Region odontoblasts found themselves neatly juxtaposed with feature layer and palisade (Figure 1B). Already in the vestibular region, there were irregularities in the layer of odontoblasts, dentin in the pre-and dentin layer. It was observed that the odontoblasts lost their characteristic juxtaposed layer and palisade. This made it become a disorganized reaction and this is shown where the production of pre-dentin and dentin is decreased (Figure 1C).

4. DISCUSSION

The bleaching agents act mainly by oxidation of organic compounds. These agents are highly unstable and, when in contact with the tissue, release free (especially nascent oxygen) radicals that oxidize the pigments^{1,3,5}.

Although tooth enamel is a mineral structure, studies have reported that both the hydrogen peroxide and carbamide peroxide penetrates the enamel and dentin. Subsequently, they enter the pulp chamber at various rates and widespread amount is dependent on its concentration, the length of time that the agent is in contact with the dentin, the presence of cracks or wear and presence of restorations^{7,11,15,16}.

Other laboratory studies, *in vitro* and *in situ* were performed to evaluate the effects of practice on the technique of tooth structure, showing that the technique does not affect tissues and dental structures¹⁷. Yet other studies have shown the diffusion of hydrogen peroxide (H₂O₂) for enamel and dentin⁵ due to its ability to denature proteins and low molecular weight, penetration is facilitated¹⁰ this are thus demonstrated cytopathic effects in the cells even after single application of this product in enamel⁶, or multiple sessions of the application¹¹.

In this *in vivo* study was evaluated the presence of histological changes in the pulp-dentin complex due to the use of H₂O₂ to 35% on the buccal surfaces of the lower incisors selected as the study of Gökay *et al.* (2005)¹⁸, but in a research *in vitro*. Those authors reported in the literature several studies that assessed the laboratory level different topics related to bleaching and pulp organ⁶⁻¹¹, yet few *in vivo* studies as performed in this study. It is especially difficult to find intact teeth incisors with orthodontic indication for extraction.

The use of bleaching agents causes the rupture of the protein matrix that occurs by increasing the porosity of the enamel can induce side effects such permeability and dental tissues may lead to post-operative sensitivity, and also allowing for the promotion of morphologic changes tissue and in dental structures^{6,10}.

In the lingual region of the teeth receiving the whitening gel showed normal histological characteristics of the pulp tissue. There were irregularities in the

odontoblasts, dentin and pre-dentine layer in the region where the whitening gel is applied. In this case, there was no light activation pulpal temperature changes occur, when they are associated with the light source; in other words, enhanced by the bleaching gel apparatus raises the temperature of the bleaching gel and result in increased temperature intrapulpal, causing damage to the pulp⁷. It is noteworthy that the light source is not responsible for activity whitening bleaching material, it acts as an accelerator of the reaction, making the procedure quicker. However, it is known that even in situations where no light is applied, the oxidation reaction is usually responsible for the whitening¹².

The Professor Costa's *et al.* (2010)⁶ group evaluated the whitening in a dental office of 6 premolars and 4 incisors, under hydrogen peroxide at 35%, and subsequently analyze the pulp response, using the same methodology of this study and proved irreversible changes only in lower incisors, corroborated this result. The premolars met irregularities in the odontoblast layer, predentin and dentin but without necrosis. The root pulp such teeth showed dilation of blood vessels, and no change was observed in premolars. It was shown in that research with the thickness of enamel and dentin is crucial in the occurrence of irreversible pulp damage. This is the grounds for the selection of the lower incisors by placing the test the biocompatibility of the bleaching agent and its effect on pulp level.

The diffusion of bleaching material through enamel and dentin has been observed in laboratory studies. In these studies, regardless of the light source used, the hydrogen peroxide was able to cross the entire thickness of enamel and dentin, reaching the layer of cultured cells and causing reduction in metabolism and significant morphological changes, this finding is corroborated by other studies^{7,9,10,11}.

The contact time of the bleaching agent to the tooth surface influences the distribution of the components of bleaching agent to reach the pulp, the greater the space time of contact of the whitening gel with the largest dental element is its penetration, independent of the concentration of the bleaching gel⁹, although this study did not evaluate different concentrations. Finally, it should be considered that could provide selected patients anatomical differences between the teeth, variable in thickness enamel and dentin, as this plays an important role in protecting the pulp tissue against the toxic products released by bleaching agents, which diffuse into the tooth surface.

5. CONCLUSION

Based on the scope of this study we suggest that tooth bleaching with hydrogen peroxide at 35% may produce irregularities in the odontoblast layer, preden-

tin and dentin in incisors, but without necrosis.

REFERENCES

- [1] Baratieri, I.n. Clareamento de dentes vitais. In: BARATIERI, L.N. Clareamento Dental. 1.ed. São Paulo: Santos/Quintessence Books. 1996; 6:31- 88.
- [2] Costa CAS, *et al.* Human pulp responses to in-office toothbleaching. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2010; 109:e59-e64.
- [3] Elfallah HM, Swain MV. A review of the effect of vital teeth bleaching on the mechanical properties of tooth enamel. N Z Dent J. 2013; 109(3):87-96.
- [4] Haywood VB, Heymann HO. Nightguard vital bleaching. Quintessence Int., Berlin. 1989; 20(3):173-6.
- [5] Costa CAS, *et al.* Cytotoxic effect sand biocompatibility of bleaching agents used in dentistry. A literature review. ROBRAC. 2006; 15(39):3-14.
- [6] Dias Ribeiro AP, *et al.* Cytotoxic effect of a 35% hydrogen peroxide bleaching gel on odontoblast-like MDPC-23 cells. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2009; 108:458-64.
- [7] Trindade FZ, *et al.* trans-enamel and trans-dentinal cytotoxic effects of a 35% H₂O₂ bleaching agent on an odontoblasts cell in after consecutive applications. Int Endod J. 2009; 42:516-24.
- [8] Soares DG, Ribeiro AP, da Silveira Vargas F, Hebling J, de Souza Costa CA. Efficacy and cytotoxicity of a bleaching gel after short application times on dental enamel. Clin Oral Investig. 2013; 17(8):1901-9.
- [9] Ferreira VG, Nabeshima CK, Marques MM, Paris AF, Gioso MA, dos Reis RS, Machado ME. Tooth bleaching induces changes in the vascular permeability of rat incisor pulps. Am J Dent. 2013; 26(5):298-300.
- [10] de Oliveira Duque CC, Soares DG, Basso FG, Hebling J, de Souza Costa CA. Bleaching effectiveness, hydrogen peroxide diffusion, and cytotoxicity of a chemically activated bleaching gel. Clin Oral Investig. 2013.
- [11] Cintra LT, Benetti F, da Silva Facundo AC, Ferreira LL, Gomes-Filho JE, Ervolino E, Rahal V, Briso AL. The number of bleaching sessions influences pulp tissue damage in rat teeth. J Endod. 2013; 39(12):1576-80.
- [12] Soares DG, Basso FG, Hebling J, de Souza Costa CA. Concentrations of and application protocols for hydrogen peroxide bleaching gels: effects on pulp cell viability and whitening efficacy. J Dent. 2014; 42(2):185-98.
- [13] Marson FC, Conceição EN. Clareamento Dental. In: Ewerton Nocchi Conceição. (Org.). Visão horizontal: Odontologia Estética para Todos. 1ed. Maringá: Dental Press. 2012; 1; 70-90.
- [14] Spalding M, Taveira LA, Assis GF. Scanning electron microscopy study of dental enamel surface exposed to 35% hydrogen peroxide: alone, with saliva, and with 10% carbamide peroxide. J. Esthetic Restorative Dent. Philadelphia. 2003; 15(3):154-64.
- [15] Benetti AR, Valera MC, Mancini MNG, Miranda CB, Balducci I. In vitro penetration of bleaching agents into the pulp chamber. IEJ. 2004; 37:120-24.
- [16] Gaurav P, Yoshaskam A, Saketh RR, Namratha L, Sambarta D. An in Vitro Spectrophotometric Analysis of the Penetration of Bleaching Agent into the Pulp Chamber of Intact and Restored Teeth. J Clin Diagn Res. 2013; 7(12):3057-9.
- [17] Haywood VB. Nightguard vital bleaching: current concept sand research. J Am Dent Assoc. Chicago. 1997; 128; 19S-25S.
- [18] Gökay O, Mújdeci A, Algin E. In vitro peroxide penetration into pulp chamber from newer bleaching products. Int Endod J. 2005; 38:516-20.

