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Research Article

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Effect of medium or high concentrations of in-office dental bleaching gel on the human pulp response in the mandibular incisors

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ABSTRACT

Objectives: The present study evaluated the pulp response of human mandibular incisors subjected to in-office dental bleaching using gels with medium or high concentrations of hydrogen peroxide (HP).

Materials and Methods: The following groups were compared: 35% HP (HP35; $n = 5$) or 20% HP (HP20; $n = 4$). In the control group (CONT; $n = 2$), no dental bleaching was performed. The color change (CC) was registered at baseline and after 2 days using the Vita Classical shade guide. Tooth sensitivity (TS) was also recorded for 2 days post-bleaching. The teeth were extracted 2 days after the clinical procedure and subjected to histological analysis. The CC and overall scores for histological evaluation were evaluated by the Kruskal-Wallis and Mann-Whitney tests. The percentage of patients with TS was evaluated by the Fisher exact test ($\alpha = 0.05$).

Results: The CC and TS of the HP35 group were significantly higher than those of the CONT group ($p < 0.05$) and the HP20 group showed an intermediate response, without significant differences from either the HP35 or CONT group ($p > 0.05$). In both experimental groups, the coronal pulp tissue exhibited partial necrosis associated with tertiary dentin deposition. Overall, the subjacent pulp tissue exhibited a mild inflammatory response.

Conclusions: In-office bleaching therapies using bleaching gels with 20% or 35% HP caused similar pulp damage to the mandibular incisors, characterized by partial necrosis, tertiary dentin deposition, and mild inflammation.

Keywords: Hydrogen peroxide; In-office bleaching; Odontoblasts

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Conflict of Interest

No potential conflict of interest relevant to this article was reported.

Author Contributions

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INTRODUCTION

The increase in aesthetic demands, influenced by social media [1], has caused tooth bleaching to gain popularity, as this procedure is effective at solving tooth color [2]. Therefore, when patients visit dentists in search of treatments with quick results, in-office bleaching is well indicated [3]. Despite the efficacy of in-office dental bleaching [4,5], tooth sensitivity is one of the most common side effects associated with this treatment [6]. Clinical studies have estimated that 40% to 100% of patients experience bleaching-induced tooth sensitivity with mild to severe intensity [4,5,7,8].

Although the etiology of this side effect has not yet been fully established, it seems likely to result from hydrogen peroxide diffusion through the enamel and dentin into the pulp during dental bleaching [2,9]. Within 15 minutes of the application of bleaching gel, hydrogen peroxide can be found in the pulp tissue [9]. This molecule is capable of causing negative effects on the pulp, such as an inflammatory reaction and even partial tissue necrosis [10-13]. Several *in vitro* studies have shown damage to pulp cells stemming from the trans-enamel and trans-dentin diffusion of high concentrations of hydrogen peroxide [14,15].

The side effects of professional dental bleaching have led some manufacturers to release low-concentration (< 20%) in-office bleaching gels [16-18]. In-office dental bleaching gels have been developed that considerably reduce the concentration of hydrogen peroxide in the pulp, which may be related to the low reported intensity of tooth sensitivity in clinical studies [16-18]. However, these gels require more bleaching sessions, because they do not produce the bleaching effect as efficiently as medium-concentration (20%–30%) and high-concentration (> 30%) formulations [19]. In contrast, high-concentration dental bleaching gels are the opposite of low-concentration gels [19]. For this reason, the medium-concentration gels seem to be interesting, since they are capable of promoting a bleaching effect similar to that achieved using high concentrations, but with a considerable reduction in tooth sensitivity after dental bleaching [7,8]. Laboratory investigations demonstrated no significant difference concerning the diffusion of hydrogen peroxide from medium-concentrations gels in comparison with high-concentration products [19]. However, low-concentration gels pose a lower risk of tooth sensitivity [20], although their effect on the pulp tissue still needs to be evaluated.

Therefore, the aim of this study was to evaluate the response of the pulps of human mandibular sound incisors subjected to in-office dental bleaching with products containing medium or high hydrogen peroxide concentrations. The color change and risk of tooth sensitivity were also evaluated as secondary outcomes.

MATERIALS AND METHODS

Ethics approval

This clinical investigation was approved under protocol number 09171/10 by the Ethics Committee of the State University of Ponta Grossa, PR, Brazil. Based on pre-established criteria, 11 participants with lower incisors scheduled to be extracted for orthodontic reasons were selected for this study. After reading and receiving all necessary explanations including the experimental rationale, clinical procedures, and possible risks, the participants signed a consent form explaining the research protocol, which was previously approved by the Ethics Committee.

Inclusion and exclusion criteria

The participants included in this clinical study were required to have good general health (American Society of Anesthesiologists [ASA] I, a normal healthy patient; or ASA II, a patient with mild systemic disease without substantive functional limitations) and have an acceptable level of oral hygiene according to the Simplified Oral Hygiene Index. The participants were also required to have at least 1 caries-free mandibular incisor without restorations, with shade A2 or darker by comparison with a value-oriented shade guide (Vita Lumin; Vita Zahnfabrik, Bad Säckingen, Germany) that needed extraction due to orthodontic reasons. Participants that had undergone dental bleaching procedures, were pregnant/lactating, had severe internal tooth discoloration [tetracycline stains, fluorosis, pulpless teeth], or had bruxism habits or any gross pathology in the mouth were excluded from the study. Participants regularly using any drug with anti-inflammatory and antioxidant action were also excluded. The participants were asked about tooth sensitivity the week before the beginning of the bleaching, and those who reported any degree of spontaneous tooth sensitivity were not included in the study sample.

Two weeks before the dental bleaching procedures, all participants received a dental screening. Furthermore, all participants underwent a prophylaxis and oral hygiene guidance procedure prior to the bleaching procedure. The diagnosis of pulp vitality was confirmed by a cold test application (Endo-Frost, Roeko, Germany). After 10 seconds of application, all patients answered positively, indicating a slight and not lingering pain response.

Study intervention

The participants were randomly divided into 3 groups (the control group or 1 of 2 groups that received in-office dental bleaching therapy). After placement of a lip retractor (Arcflex; FGM, Joinville, SC, Brazil), the gingival tissue of the teeth to be bleached was isolated with a light-cured gingival barrier (Top Dam; FGM). A 35% hydrogen peroxide gel (Whiteness HP Maxx; FGM) or 20% hydrogen peroxide gel (Whiteness HP Maxx FGM) was used according to the instructions described in **Table 1**. The bleaching gel was left on the tooth surface for 15 minutes, and this procedure was repeated 3 times. The 35% hydrogen peroxide gel was commercially available, and the 20% hydrogen peroxide gel was specifically formulated by the manufacturer for this study. All participants were instructed to brush their teeth regularly using fluoridated toothpaste (Sorriso Fresh; Colgate-Palmolive, São Paulo, Brazil).

Table 1. Product composition, technique (code) and application protocols

Product composition	Technique (code)	Application protocols
35% hydrogen peroxide, thickeners, dye mixture, glycol, inorganic load, and deionized water	Three 15-min applications (HP35)	1. For the bleaching of each tooth, 3 drops of phase 1 (hydrogen peroxide) should be mixed with 1 drop of phase 2 (thickener). 2. A thin layer of gel, approximately 0.5 mm thick, is applied to the buccal surface of all teeth undergoing treatment. 3. The bleaching gel should be left on the surface of the teeth for 15 min and then removed with an aspirator. 4. This procedure should be repeated 3 times.
20% hydrogen peroxide, thickeners, dye mixture, glycol, inorganic load, and deionized water*	Three 15-min applications (HP20)	1. For the bleaching of each tooth, 3 drops of phase 1 (hydrogen peroxide) should be mixed with 1 drop of phase 2 (thickener). 2. A thin layer of gel, approximately 0.5 mm thick, is applied to the buccal surface of all teeth undergoing treatment. 3. The bleaching gel should be left on the surface of the teeth for 15 min and then removed with an aspirator. 4. This procedure should be repeated 3 times.

*Formulated by the manufacturer especially for this study.

Color evaluation

The color change was registered at baseline and 2 days after the dental bleaching session using a value-oriented shade guide (Vita Lumin, Vita Zahnfabrik). The shade guide's 16 tabs were arranged from highest (B1) to lowest (C4) value (B1, A1, B2, D2, A2, C1, C2, D4, A3, D3, B3, A3.5, B4, C3, A4, C4). Although this scale is not linear in the truest sense, we treated the changes as representing a continuous and approximately linear ranking for the purpose of analysis. The color was measured at the baseline and 2 days after the dental bleaching procedure. The shade changes from baseline (before) to after bleaching were used to calculate the change in the number of shade guide units (ΔSGU) that occurred toward the lighter end of the value-oriented list of shade tabs. A single calibrated and experienced evaluator recorded the shade of each subject's teeth at baseline and 2 days after the bleaching procedure. The measurement area of interest for shade matching was the middle third of the facial surface of the teeth.

Tooth sensitivity evaluation

Two days after the procedure, and before extraction, the participants were asked if they had experienced any tooth sensitivity in the bleached tooth. No attempt was made to record the tooth sensitivity intensity—instead, we only recorded whether or not the participants experienced any degree of tooth sensitivity.

Histologic procedure and evaluation

This protocol followed the description of Costa *et al.* [11]. Two days after the bleaching procedure, the teeth were extracted under local anesthesia. The roots were immediately sectioned midway between the cementoenamel junction and the root tip with a high-speed handpiece under water spray. The teeth were stored for 48 hours in formalin fixative solution at pH 7.2, decalcified in buffered Morse's solution, dehydrated, vacuum-infiltrated with wax paraffin, and finally embedded in paraffin. Six-micrometer-thick serial sections were cut ("820" Spencer Microtome, Carson, CA, USA), mounted on glass slides and stained with hematoxylin and eosin. Three teeth were lost during the laboratory processing. Then, based on previous *in vivo* studies, all sections obtained from 11 out of 14 teeth were subjected to an evaluation by a calibrated examiner blinded to the groups [21,22]. Using a light microscope (62774; Carl Zeiss, Oberköchen, Germany) the histopathological characteristics described in **Table 2** were assessed and classified through a descriptive analysis.

Table 2. Scores used during the histological examination: inflammatory cell response, tissue disorganization, and reactionary dentin formation

Score	Characterization		
	Inflammatory cell response	Tissue disorganization	Reactionary dentin formation
0	None or a few scattered inflammatory cells present in the pulp area corresponding to the buccal surface of the tooth in which the bleaching gel was applied	Normal pulp tissue	Absence
1	Mild inflammatory cell infiltrate with polymorphonuclear or mononuclear leukocytes	Pulp zones (odontoblast, acellular, and cell-rich) disorganization in the buccal surface of the tooth in which the bleaching gel was applied	Modest hard tissue deposition in the coronal pulp of the tooth in which the bleaching gel was applied
2	Moderate inflammatory cell infiltrate	Coronary pulp tissue disorganization with partial local necrosis	Moderate hard tissue deposition in the coronal pulp tissue of the tooth in which the bleaching gel was applied
3	Severe inflammatory cell infiltrate	Intense pulp disorganization with necrosis in the coronal and root pulp tissue	Intense hard tissue deposition in the coronal and root pulp tissue

Statistical analysis

The overall median value of ΔSGU was calculated and compared using the non-parametric Kruskal-Wallis test and Mann-Whitney *U*-test. The absolute risk of tooth sensitivity among groups was evaluated using the Fisher exact test. Regarding the pulp response, 3 established histopathological characteristics were evaluated (**Table 2**) in such a way that 3 scores per tooth were considered for statistical analysis. The overall comparison of the groups was performed with the non-parametric Kruskal–Wallis statistical test. Comparisons between groups were performed using the Mann-Whitney *U*-test. All tests were performed with appropriately computed critical values (α = 0.05) in the MedCalc software (MedCalc Version 11.3.8.0, 2010, Mariakerke, Belgium).

RESULTS

The age of the participants in this study varied between 12 and 30 years old (mean, 16.9 ± 5.7 years). The means and standard deviations of color change are shown in **Table 3**. The color scale allowed the calculation of changes in ΔSGU, which reflected the difference in color between baseline and the assessment periods after treatment. The ΔSGU values of all dental bleaching techniques were statistically similar ($p > 0.49$). However, the 35% hydrogen peroxide (HP35) treatment showed a significant difference from the non-bleached control group ($p = 0.03$).

A significantly higher absolute risk of tooth sensitivity was observed for the HP35 group ($p = 0.007$; **Table 3**). The group bleached with 20% hydrogen peroxide (HP20) did not show statistically significant differences from the other groups ($p > 0.28$).

The scores for every criterion determined by the histological assessment of the specimens according to groups are shown in **Table 4**. Overall, the pulp tissue observed in the control group was quite different from that of the experimental groups, in which the teeth were bleached ($p = 0.003$). Teeth from the control group showed pulp tissue with normal histological characteristics, the lack of an inflammatory response, and no tissue disorganization (**Figure 1**).

Table 3. Means ± standard deviations and medians of shade guide units and ΔSGU (change in shade guide units) for the experimental groups as well as the number of patients with tooth sensitivity for each group

Groups	Shade guide unit				ΔSGU*		Sensitivity
	Baseline		After bleaching				
	Mean ± SD	Median (1st/3rd interquartile ranges)	Mean ± SD	Median (1st/3rd interquartile ranges)	Mean ± SD	Median (1st/3rd interquartile ranges)	Total number of patients/ patients with tooth sensitivity†
Control	6.0 ± 3.0	5 (5/9)	6.0 ± 3.0	5 (5/9)	0.0 ± 0.0	0 (5/9)^{A}	2/0^{a}
HP35	5.8 ± 1.8	5 (5/5)	3.2 ± 1.3	2 (2/2)	2.6 ± 1.5	3 (3/3)^{B}	5/5^{b}
HP20	5.0 ± 1.2	5 (5/5)	3.8 ± 2.0	3 (0/5)	1.2 ± 1.6	1.5 (0/3)^{A,B}	4/2^{a,b}

*Similar uppercase letters indicate statistically similar groups for ΔSGU (Mann-Whitney test, $p < 0.05$). †Similar lower-case letters indicate statistically similar groups for tooth sensitivity (Fisher exact test, $p < 0.05$).

Table 4. Number of specimens for each group in each criterion as well as multiple comparisons among the experimental conditions

Groups	Criteria														Overall median*
	Inflammatory cell response					Tissue disorganization					Reactionary dentin formation				
	0	1	2	3	Median	0	1	2	3	Median	0	1	2	3	Median	
Control	2	0	0	0	0	2	0	0	0	0	2	0	0	0	0	0^{B}
HP35	0	5	0	0	1	0	1	4	0	2	0	0	4	1	2	2^{A}
HP20	0	3	1	0	1	0	1	3	0	2	0	0	4	0	2	2^{A}

*Overall medians for each group. Different letters mean significant differences ($p < 0.05$).

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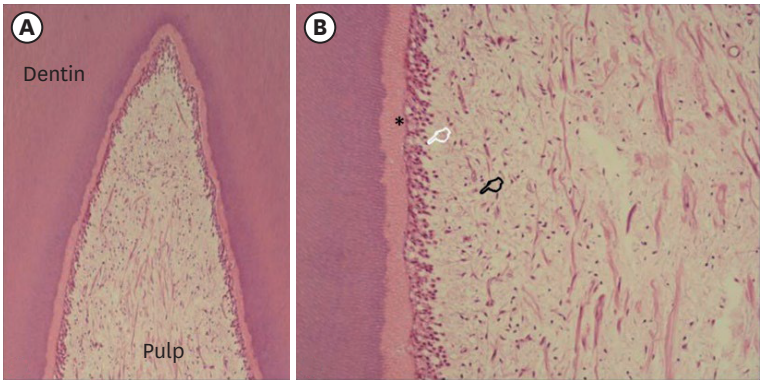

Figure 1. (Control group) Dentin-pulp complex of a sound mandibular tooth. (A) A general view of coronal pulp tissue that exhibits normal histological features. (B) (High magnification of Figure 1A) The pulp tissue exhibits all defined histological zones. A continuous odontoblast layer (white finger point) is underlying the pre-dentin layer (asterisk). The non-inflamed subjacent pulp tissue shows pulp cells dispersed among a number of small blood vessels (black finger point) (hematoxylin and eosin., ×32 and ×125).

Four and 3 human incisors bleached with gels containing 35% (HP35) or 20% (HP20) hydrogen peroxide, respectively, showed a wide zone of coagulation necrosis in the coronal pulp tissue associated with deposition of reactionary dentin (**Figure 2A, 2B, 2E, and 2F**). In 1 of those 5 teeth bleached with HP35, the reactionary dentin observed adjacent to the coronal necrotic tissue was continuous to that deposited in the lateral walls of the root pulp tissue (**Figure 2A and 2C**). In 8 of those 9 bleached teeth, a mild inflammatory response mediated by mononuclear cells among dilated and congested blood vessels was observed (**Figure 2C, 2D, 2G, and 2H**).

DISCUSSION

In this study, the response of human pulp following dental bleaching with products containing medium or high concentrations of hydrogen peroxide was evaluated under light microscopy. Additionally, color change and tooth sensitivity were assessed in an attempt to verify whether the dental bleaching protocols were effective for producing a bleaching effect without causing side effects.

Only a few investigations have compared, in a single study design, the efficacy and tooth sensitivity of bleaching gels containing 20% or 35% of hydrogen peroxide [7,8]. In a recent study, Maran *et al.* [19] showed that medium-concentration gels used to perform in-office dental bleaching caused a similar color change, as well as a lower risk and intensity of tooth sensitivity, when compared with high-concentration gels. However, the certainty of the evidence was considered to be low for objective and subjective evaluation outcomes. Thus, more studies should be carried out on the topic.

In the present study, lower degrees of dental bleaching and less tooth sensitivity were observed for the 20% hydrogen peroxide gel than for the 35% hydrogen peroxide gel. This was somewhat expected, as the concentration of hydrogen peroxide in a bleaching gel has a marked effect on the number of applications required to produce an optimal shade outcome, which Reis *et al.* [3] reported. The authors showed that bleaching gels with medium hydrogen peroxide concentrations required more applications to produce similar tooth bleaching effects [3]. However, differences in clinical symptoms and signs between hydrogen peroxide

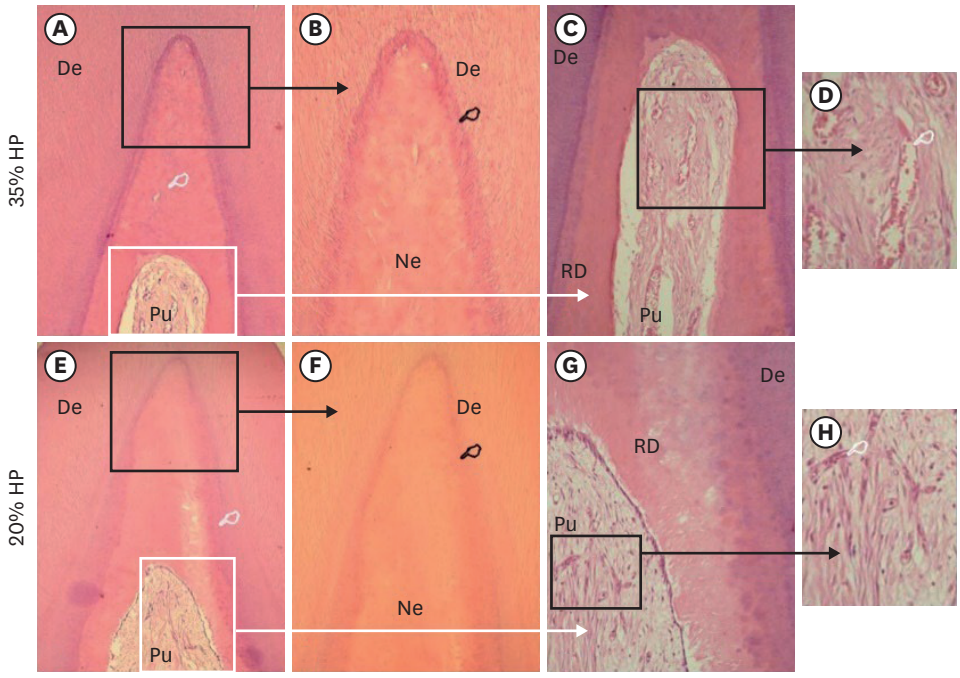

Figure 2. Dentin-pulp complex of mandibular incisors subjected to in-office bleaching treatment ("A-D" = HP35 and "e-h" = HP20). (A) The coronal pulp tissue exhibits a wide area of necrosis (white finger point) associated with notable deposition of tertiary dentin (arrows) (H/E [hematoxylin and eosin], ×40). (B) High magnification of Figure 2A. Note the transition border (black finger point) between the tubular dentin (De) and the necrotic pulp tissue (Ne) (×84). (C) The coronal and root pulp tissue exhibit continuous deposition of reactionary dentin (RD) (H/E, ×125). (D) Detail of the pulp area demonstrated in Figure 2C. The residual pulp tissue shows only a few inflammatory mononuclear cells among dilated and congested blood vessels (white finger point) (H/E, ×250). (E) RD (white finger point) adjacent to the partial coronal pulp necrosis. Note the residual pulp (Pu) tissue (H/E, ×40). (F) High magnification of the tip of pulp horn with necrotic tissue (Ne). Note the transition zone (black finger point) between the tubular dentin (De) and the necrotic tissue (H/E, ×84). (G) Detail of Figure 2E. Subjacent to the tubular dentin (De), a wide layer of RD is observed. Note the residual coronal pulp tissue (Pu) (H/E, ×125. (H) Detail of Figure 2G. Only a few mononuclear inflammatory cells among blood vessels can be seen (H/E, ×250).

concentrations were not observed in a comparison of the histopathological features of the pulp bleached with 20% or 35% hydrogen peroxide. This indicates that the amount of hydrogen peroxide that reaches the pulp after the application of a 20% hydrogen peroxide gel is enough to produce similar damage to the pulp tissue when compared with the 35% hydrogen peroxide [20].

The application of a 35% hydrogen peroxide in-office dental bleaching gel was shown to result in trans-enamel and trans-dentinal cytotoxic effects characterized by direct damage to cultured odontoblast-like cells and a decrease in their metabolic activity [14,15]. Previous studies have also demonstrated that hydrogen peroxide and the products of its degradation, such as reactive oxygen species, may act as free radicals that cause oxidative stress in the pulp cells [23-25]. The increase in reactive oxygen species levels triggers deleterious effects on several cell components, such as mutagenesis, carcinogenesis, cell membrane damage by lipid peroxidation, and protein fragmentation, which may reduce cell proliferation and result in cell necrosis or apoptosis [24,25].

It has been shown that the amount of hydrogen peroxide that reaches the pulp tissue is directly proportional to the hydrogen peroxide concentration and the contact time of the product with the enamel [26,27]. This may partially explain the lack of agreement between

the clinical symptoms of the participants in this study and the pulp response to gels with different hydrogen peroxide concentrations. Mena-Serrano *et al.* [20] demonstrated similar hydrogen peroxide diffusion across enamel and dentin for groups treated with the same bleaching gels used in the present study, which is in accordance with the data found in the present work. However, clinical studies have not been able to demonstrate positive correlations among clinical symptomatology after bleaching, hydrogen peroxide penetration, and pulp response. Perhaps the expression of inflammatory mediators, such as substance P (a nerve-released vasoactive peptide), and arachidonic acid metabolites, such as prostaglandins, which play a role in triggering and subsequently potentiating nociceptive impulses that are transmitted to the central nervous system for the perception of pain, may be proportional to the amount of hydrogen peroxide that reaches the pulp [28,29]. However, this issue still merits further investigation.

It is worth mentioning that the pulp response of bleached teeth in the present investigation, as well as in the studies by Costa *et al.* [11] and Roderjan *et al.* [10,13], does not agree with previous literature findings. Earlier *in vivo* studies demonstrated insignificant pulp damage after in-office bleaching. For instance, Cohen [30] applied 35% hydrogen peroxide combined with heat for three 30-minute applications and showed no histological evidence of pulpal damage. Another study reported only mild inflammation after 2 applications of 5 minutes of a 35% hydrogen peroxide product [31]. Kina *et al.* [32] also showed no pulpal damage after the use of 38% hydrogen peroxide gel 3 times, with 10 minutes of application each, with or without light activation. The main difference between the previous studies and the present one is the type of tooth subjected to bleaching procedures. In the present study, mandibular incisors, instead of the premolars used in the aforementioned studies [30-32], had their pulp tissue evaluated after in-office dental bleaching therapy. Therefore, it seems that the thickness of enamel and dentin play a fundamental role in the pulp response of teeth bleached with high-concentration gels. Previous laboratory studies demonstrated that the thickness of the enamel and dentin, as well as the time of the application of bleaching gels on the enamel, are directly related to the intensity of the damage caused to the pulp cells [14,15,27]. In fact, in a review of the literature, Haywood [33] reported that bleaching-induced tooth sensitivity usually affects the smaller teeth, such as the maxillary laterals and the mandibular incisors. Costa *et al.* [11] also proved this when they evaluated the histological pulp responses of incisors and premolars subjected to in-office dental bleaching. The authors showed necrosis in the coronal pulp tissue of lower incisors, but not in premolars, when a bleaching gel with high-concentration hydrogen peroxide (38%) was used. Thus, the thinner layers of enamel and dentin in the mandibular incisors compared with the premolars may reduce the speed of hydrogen peroxide diffusion across the hard tooth tissues to reach the pulp.

From a clinical standpoint, the results of this study should be interpreted with caution. Whether the pulp damage resulting from hydrogen peroxide is reversible has yet to be addressed. In addition, to the extent of the authors' knowledge, no clinical/histopathological study has reported complete pulp death after in-office dental bleaching; thus, further studies evaluating long-term pulp vitality after in-office dental bleaching should be conducted. Studies of other dental bleaching therapies that deliver lower amounts of hydrogen peroxide to the enamel surface should also be conducted [5].

Caution is needed in the interpretation of histological studies like these, mainly because the present study evaluated a small sample size. However, previous clinical studies with a larger number of participants that evaluated different concentrations of bleaching gels showed

lower risk and intensity of tooth sensitivity for 20% HP than for higher concentrations [7,8]. Therefore, it seems that 20% HP could be considered as a good option for in-office bleaching for young patients. However, future clinical studies evaluating low-concentration HP applied in young patients need to be done to confirm this hypothesis.

One limitation of this *in vivo* study is that the participants were relatively young. It is known that young patients' teeth present dentinal tubules with wide inner diameters, and the dentin substrate is thin because no more than a small layer of secondary dentin is present. Based on this fact, one may expect that a higher amount of hydrogen peroxide or its toxic decomposition products diffuse faster through the enamel and dentin to cause damage to the pulp tissue. Thus, further *in vivo* studies are needed to evaluate whether similar pulpal damage, as demonstrated in the present investigation, also takes place in the teeth of older patients who undergo in-office dental bleaching therapies.

CONCLUSIONS

According to the methodology employed in the present *in vivo* study, and based on the data obtained, it may be concluded that bleaching gels with 20% or 35% hydrogen peroxide applied to lower human incisors in young participants cause a similar degree of pulp damage as, characterized by partial necrosis of the coronal pulp tissue combined with reactionary dentin deposition and a mild inflammatory response, as well as similar color changes.

REFERENCES

1. Theobald AH, Wong BK, Quick AN, Thomson WM. The impact of the popular media on cosmetic dentistry. N Z Dent J 2006;102:58-63.
PUBMED
2. Kwon SR, Wertz PW. Review of the mechanism of tooth whitening. J Esthet Restor Dent 2015;27:240-257.
PUBMED | CROSSREF
3. Reis A, da Silva LM, Martins L, Loguercio A. In-office tooth whitening. Clin Dent Rev 2018;2:1-8.
4. Maran BM, Vochikovski L, Hortkoff DR, Stanislawczuk R, Loguercio AD, Reis A. Bleaching sensitivity with a desensitizing in-office bleaching gel: a randomized double-blind clinical trial. Quintessence Int 2020;51:788-797.
PUBMED
5. Vaez SC, Correia A, Santana TR, Santana M, Peixoto AC, Leal PC, Faria-E-Silva AL. Is a single preliminary session of in-office bleaching beneficial for the effectiveness of at-home tooth bleaching? A randomized controlled clinical trial. Oper Dent 2019;44:E180-E189.
PUBMED | CROSSREF
6. de Geus JL, Wambier LM, Kossatz S, Loguercio AD, Reis A. At-home vs in-office bleaching: a systematic review and meta-analysis. Oper Dent 2016;41:341-356.
PUBMED | CROSSREF
7. Mena-Serrano AP, Garcia E, Luque-Martinez I, Grande R, Loguercio AD, Reis A. A single-blind randomized trial about the effect of hydrogen peroxide concentration on light-activated bleaching. Oper Dent 2016;41:455-464.
PUBMED | CROSSREF
8. Reis A, Kossatz S, Martins GC, Loguercio AD. Efficacy of and effect on tooth sensitivity of in-office bleaching gel concentrations: a randomized clinical trial. Oper Dent 2013;38:386-393.
PUBMED | CROSSREF
9. Cooper JS, Bokmeyer TJ, Bowles WH. Penetration of the pulp chamber by carbamide peroxide bleaching agents. J Endod 1992;18:315-317.
PUBMED | CROSSREF

10. Roderjan DA, Stanislawczuk R, Hebling J, Costa CA, Reis A, Loguercio AD. Response of human pulps to different in-office bleaching techniques: preliminary findings. Braz Dent J 2015;26:242-248.
PUBMED | CROSSREF

11. Costa CA, Riehl H, Kina JF, Sacono NT, Hebling J. Human pulp responses to in-office tooth bleaching. Oral Surg Oral Med Oral Pathol Oral Radiol Endod 2010;109:e59-e64.
PUBMED | CROSSREF

12. Vaz MM, Lopes LG, Cardoso PC, Souza JB, Batista AC, Costa NL, Torres ÉM, Estrela C. Inflammatory response of human dental pulp to at-home and in-office tooth bleaching. J Appl Oral Sci 2016;24:509-517.
PUBMED | CROSSREF

13. Roderjan DA, Stanislawczuk R, Hebling J, da Souza Costa CA, Soares DG, Reis A, Loguercio AD. Histopathological features of dental pulp tissue from bleached mandibular incisors. J Mater Sci Eng B 2014;4:178-185.

14. de Oliveira Duque CC, Soares DG, Basso FG, Hebling J, de Souza Costa CA. Influence of enamel/dentin thickness on the toxic and esthetic effects of experimental in-office bleaching protocols. Clin Oral Investig 2017;21:2509-2520.
PUBMED | CROSSREF

15. 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;17:1901-1909.
PUBMED | CROSSREF

16. Bersezio C, Martín J, Angel P, Bottner J, Godoy I, Avalos F, Fernández E. Teeth whitening with 6% hydrogen peroxide and its impact on quality of life: 2 years of follow-up. Odontology 2019;107:118-125.
PUBMED | CROSSREF

17. Ferraz NK, Nogueira LC, Neiva IM, Ferreira RC, Moreira AN, Magalhães CS. Longevity, effectiveness, safety, and impact on quality of life of low-concentration hydrogen peroxides in-office bleaching: a randomized clinical trial. Clin Oral Investig 2019;23:2061-2070.
PUBMED | CROSSREF

18. Carneiro TS, Favoreto MW, Bernardi LG, Bandeca MC, Borges C, Reis A, Loguercio AD. Application tip and concentration of a self-mixing bleach: hydrogen peroxide inside the pulp chamber, color change, and amount of bleaching gel used. Oper Dent. Forthcoming 2023;
PUBMED | CROSSREF

19. Maran BM, Matos TP, de Castro AD, Vochikovski L, Amadori AL, Loguercio AD, Reis A, Berger SB. In-office bleaching with low/medium vs. high concentrate hydrogen peroxide: a systematic review and meta-analysis. J Dent 2020;103:103499.
PUBMED | CROSSREF

20. Mena-Serrano AP, Parreiras SO, do Nascimento EM, Borges CP, Berger SB, Loguercio AD, Reis A. Effects of the concentration and composition of in-office bleaching gels on hydrogen peroxide penetration into the pulp chamber. Oper Dent 2015;40:E76-E82.
PUBMED | CROSSREF

21. de Lourdes Rodrigues Accorinte M, Reis A, Dourado Loguercio A, Cavalcanti de Araújo V, Muench A. Influence of rubber dam isolation on human pulp responses after capping with calcium hydroxide and an adhesive system. Quintessence Int 2006;37:205-212.
PUBMED

22. de Souza Costa CA, Lopes do Nascimento AB, Teixeira HM, Fontana UF. Response of human pulps capped with a self-etching adhesive system. Dent Mater 2001;17:230-240.
PUBMED | CROSSREF

23. Kawamoto K, Tsujimoto Y. Effects of the hydroxyl radical and hydrogen peroxide on tooth bleaching. J Endod 2004;30:45-50.
PUBMED | CROSSREF

24. Cintra LT, Benetti F, Ferreira LL, Gomes-Filho JE, Ervolino E, Gallinari MO, Rahal V, Briso AL. Penetration capacity, color alteration and biological response of two in-office bleaching protocols. Braz Dent J 2016;27:169-175.
PUBMED | CROSSREF

25. Benetti F, Gomes-Filho JE, Ferreira LL, Ervolino E, Briso AL, Sivieri-Araújo G, Dezan-Júnior E, Cintra LT. Hydrogen peroxide induces cell proliferation and apoptosis in pulp of rats after dental bleaching in vivo: Effects of the dental bleaching in pulp. Arch Oral Biol 2017;81:103-109.
PUBMED | CROSSREF

26. Marson FC, Gonçalves RS, Silva CO, Cintra LT, Pascotto RC, Santos PH, Briso AL. Penetration of hydrogen peroxide and degradation rate of different bleaching products. Oper Dent 2015;40:72-79.
PUBMED | CROSSREF

27. 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;42:185-198.
PUBMED | CROSSREF

28. Caviedes-Bucheli J, Ariza-García G, Restrepo-Méndez S, Ríos-Osorio N, Lombana N, Muñoz HR. The effect of tooth bleaching on substance P expression in human dental pulp. J Endod 2008;34:1462-1465.
PUBMED | CROSSREF

29. Huynh MP, Yagiela JA. Current concepts in acute pain management. J Calif Dent Assoc 2003;31:419-427.
PUBMED

30. Cohen SC. Human pulpal response to bleaching procedures on vital teeth. J Endod 1979;5:134-138.
PUBMED | CROSSREF

31. Robertson WD, Melfi RC. Pulpal response to vital bleaching procedures. J Endod 1980;6:645-649.
PUBMED | CROSSREF

32. Kina JF, Huck C, Riehl H, Martinez TC, Sacono NT, Ribeiro AP, Costa CA. Response of human pulps after professionally applied vital tooth bleaching. Int Endod J 2010;43:572-580.
PUBMED | CROSSREF

33. Haywood VB. Treating sensitivity during tooth whitening. Compend Contin Educ Dent 2005;26 Supplement 3:11-20.
PUBMED