

# Effect of Fluoride-Treated Enamel on Indirect Cytotoxicity of a 16% Carbamide Peroxide Bleaching Gel to Pulp Cells

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The aim of this study was to evaluate the possibility of fluoride solutions applied to enamel to protect pulp cells against the trans-enamel and transdental cytotoxicity of a 16% carbamide peroxide (CP) bleaching gel. The CP gel was applied to enamel/dentin discs adapted to artificial pulp chambers (8 h/day) during 1, 7 or 14 days, followed by fluoride (0.05% or 0.2%) application for 1 min. The extracts (culture medium in contact with dentin) were applied to MDPC-23 cells for 1 h, and cell metabolism (MTT assay), alkaline phosphatase (ALP) activity and cell membrane damage (flow cytometry) were analyzed. Knoop microhardness of enamel was also evaluated. Data were analyzed statistically by ANOVA and Kruskal-Wallis tests ( $\alpha=0.05$ ). For the MTT assay and ALP activity, significant reductions between the control and the bleached groups were observed ( $p<0.05$ ). No statistically significant difference occurred among bleached groups ( $p>0.05$ ), regardless of fluoride application or treatment days. Flow cytometry analysis demonstrated 30% of cell membrane damage in all bleached groups. After 14 days of treatment, the fluoride-treated enamel presented significantly higher microhardness values than the bleached-only group ( $p<0.05$ ). It was concluded that, regardless of the increase in enamel hardness due to the application of fluoride solutions, the treated enamel surface did not prevent the toxic effects caused by the 16% CP gel to odontoblast-like cells.

## Introduction

Nearly two-thirds of the patients subjected to tooth bleaching techniques complained about postoperative tooth hypersensitivity (1). This negative side effect may be explained, at least in part, by the inward hydrogen peroxide (HP) movement through enamel and dentin, which, in turn, causes inflammatory pulp reaction when in contact to the pulp cells (2,3). This fact has driven a number of researchers to evaluate and understand the effects of tooth bleaching on pulp tissue (2-9).

Some studies have shown that the application of bleaching gels on enamel causes protein matrix disruption by free radical oxidation as well as mineral loss (10). These superficial defects increase enamel porosity, which enhances trans-enamel diffusion of HP to reach deep areas of the dentin and pulp chamber (11). Current *in vitro* studies have demonstrated that high concentrations of toxic components released from 35% HP bleaching gels used for in-office treatment are capable of diffusing through enamel and dentin and decrease significantly the metabolism of pulp cells (4,5). Moreover, human mandibular incisors subjected to an in-office bleaching therapy with a gel containing 38% HP exhibited large coagulation necrosis in their coronal pulp tissue (3). Therefore, at-home tooth bleaching using low concentrations of carbamide peroxide

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(CP) gels, such as 10% or 16%, has been considered as a more friendly treatment.

It has been demonstrated that 10% CP bleaching gel caused low toxic effects to cultured pulp cells (7,8). Clinical trials also showed that esthetic outcomes after tooth bleaching with a 10% CP gel are similar to those obtained with 16% CP gel, even after long-term tooth color evaluation (12). However, Soares et al. (8) reported that 16% CP gel resulted in a significantly higher cytotoxicity than 10% CP gel, which was probably caused by the intense diffusion of HP across enamel and dentin. Therefore, in spite of the faster esthetic outcomes obtained when tooth bleaching therapy is performed with 16% CP gel compared with 10% CP gel, the higher concentration may be more toxic to pulp cells (8).

Since clinical application of fluoride immediately after tooth bleaching remineralizes enamel (13-16) and consequently reduces post-bleaching tooth sensitivity (17-19), and considering that CP gels are actually an interesting alternative to obtain esthetic outcomes with reduced toxic effects to pulp cells, this study evaluated the protective effects of low fluoride concentrations applied on enamel after tooth bleaching to prevent, or at least reduce, the trans-enamel and transdental cytotoxicity of a 16% CP-based bleaching gel.

## Material and Methods

### Artificial Pulp Chambers

One hundred seventy-six enamel/dentin discs were obtained from bovine incisors, as previously described (8). The diameters and thicknesses of the discs were standardized at 5.2 mm and 3.5 mm, respectively. The dentin surface was treated with a 0.5 M ethylenediaminetetraacetic acid (EDTA) solution (pH 7.2) for 30 s to remove the smear layer. Each enamel/dentin disc was adapted individually to artificial pulp chambers (ACP) and the APC/disc sets were autoclaved at 121°C for 15 min in a receptacle with water. Each APC/disc set was placed in sterile 24-well plates (Costar Corp., Cambridge, MA, USA) containing 1 mL of serum-free Dulbecco's Modified Eagle's Medium (DMEM; Sigma-Aldrich, St. Louis, MO, USA). The enamel surface of the discs was facing upwards and the dentin surface was maintained in direct contact with DMEM. A 5-mm stainless steel matrix (Injecta Products Odontológicos, Diadema, SP, Brazil) was adapted in the upper compartment and an additional seal with red wax was provided.

### Cell Culture

MDPC-23 cells were seeded in the wells of 24-well plates at a density of  $3 \times 10^4$  cells/cm<sup>2</sup> in DMEM supplemented with antibiotics (penicillin, streptomycin and glutamine; Gibco, Grand Island, NY, USA) and fetal bovine serum (10% FBS; Gibco). The cells were maintained in a humidified incubator with 5% CO<sub>2</sub> and 95% air at 37°C for 72 h before the experiment.

### Experimental Procedure

The ACP/discs sets were distributed into groups according to treatment of enamel (n=22) as demonstrated in Table 1. The bleaching procedure was performed on

the enamel surface of the discs (facing upwards) with a bleaching gel containing 16% CP (Whiteness HP, FGM, Joinville, SC, Brazil). The gel was left in contact with the enamel for 8 h/day during the periods of 1, 7 and 14 days, and those groups were identified as G2, G3, and G4, respectively. After each 8-h bleaching time in a humidified incubator, the gel was aspirated, the enamel surface rinsed with sterile deionized water, and a 100-μL quantity of artificial saliva was applied (8). The saliva remained in contact with the enamel for 16 h in the incubator. For the fluoridated groups, 100-μL quantities of fluoride solutions with 0.05% (G5 and G6) or 0.2% (G7 and G8) were applied for 1 min before saliva application. This procedure was repeated during the periods of 7 and 14 days (Table 1). In control group (G1), the enamel surface remained in contact with deionized water for 8 h, followed by artificial saliva for 16 h during 14 days. At 7- and 14-day treatment periods, before the last application of the bleaching gel, the ACP was positioned in a new well of a 24-well plate with fresh DMEM (without SFB), and then the bleaching gel was finally applied (7<sup>th</sup> or 14<sup>th</sup> day) to obtain the extracts (culture medium containing products released from the last application of the bleaching gel that diffused through the enamel/dentin disc). Aliquots of the extracts (500 μL) were collected and applied to the previously cultured MDPC-23 cells for 1 h in the humidified incubator.

### Analysis of Cell Metabolism

In each group, 10 wells (n=10 ACP discs/sets) were used for the analysis of cell metabolism by the cytochemical demonstration of succinic dehydrogenase (SDH) activity, as performed in previous studies (4,5). Cell metabolism, evaluated by spectrophotometry as being proportional to the absorbance measured at 570 nm wavelength with an ELISA microplate reader (Tp Reader; Thermoplate, Nanshan District, Shenzhen, China), was calculated in percentages for each group, with the negative control (G1) representing 100% of cell metabolism. The optical density (570 nm) data were analyzed by the Kruskal-Wallis and Mann-Whitney tests ( $\alpha=0.05$ ). Two independent experiments were undertaken at different times to demonstrate the reproducibility.

### Analysis of Cell Membrane Damage

Cell membrane damage was determined with propidium iodide (PI) staining by flow cytometry into 6 wells per group (n=6 ACP discs/sets). After treatment, 200 μL of trypsin was added to each well, and the cells were centrifuged (2000 rpm for 2 min). The supernatant was discarded, and a 300-μL quantity of ligation buffer (10 mM HEPES, pH 7.4, 150 mM NaCl, 5 mM KCl, 1 mM MgCl<sub>2</sub> and 1.8 mM CaCl<sub>2</sub>) was added. The cells were treated with 1 μg/mL of PI, and

Table 1. Distribution of artificial pulp chambers (ACP)/discs sets into experimental and control groups for cytotoxicity analysis

Group	Treatment	MTT assay	Flow cytometry	ALP activity
G1	Control (artificial saliva)	10	6	6
G2	CP (1 day)	10	6	6
G3	CP (7 days)	10	6	6
G4	CP (14 days)	10	6	6
G5	CP + 0.05% F (7 days)	10	6	6
G6	CP + 0.05% F (14 days)	10	6	6
G7	CP + 0.2% F (7 days)	10	6	6
G8	CP + 0.2% F (14 days)	10	6	6

CP: 16% carbamide peroxide gel. F: Fluoride. ALP: Alkaline phosphatase.

the intensity of stained cells was determined immediately by means of a FACS Canto System (Becton Dickinson, San Jose, CA, USA). As a positive control (Cpos), a 0.06% HP solution was applied to the cells during 30 min. Data were analyzed statistically by ANOVA and Tukey's test for pairwise comparisons ( $\alpha=0.05$ ). Two independent experiments were undertaken at different times to demonstrate the reproducibility.

### Alkaline Phosphatase (ALP) Activity

In each group, 6 wells ( $n=6$  ACP discs/sets) were used for the analysis of ALP activity, by a colorimetric endpoint assay (ALP Kit; Labtest Diagnóstico S.A., Lagoa Santa, MG, Brazil) with a thymolphthalein monophosphate substrate. After the contact time of the extracts with the cells, the extract was replaced for serum-free DMEM and the cells were incubated at 37°C and 5% CO<sub>2</sub> for an additional period of 24 h. After that, 0.1% sodium lauryl sulfate (Sigma-Aldrich, St. Louis, MO, USA) was added to the wells for 30 min to produce cell lysis. In a buffered environment (pH 10.1) at 37°C, thymolphthalein monophosphate (22 mM/L) was added, which is hydrolyzed in the presence of ALP, releasing thymolphthalein. Next a color reagent (sodium carbonate 94 mM/L and sodium hydroxide 250 mM/L) was added, which reacts with the hydrolyzed thymolphthalein, changing the color of the final product. The aliquots obtained from each experimental and control groups were placed in wells of 96-well plate, and the absorbance was measured at 590-nm wavelength with an ELISA microplate reader. ALP activity was calculated by a standard curve constructed with various amounts of ALP (standard solution), and the final values were normalized with the values obtained for total protein dosage. Total protein dosage was performed according to the Read and Northcote (20) protocol, with the same sample as used for ALP activity. One milliliter of Lowry reagent solution (Sigma Chemical Co.) was added to the samples that remained in the wells. After 20 min at room temperature, 500  $\mu$ L of Folin-Ciocalteu's phenol reagent solution (Sigma Chemical Co.) were added to each well. Twenty min later, three 100- $\mu$ L aliquots of each well were transferred to a 96-well plate, and the absorbance was measured at 655-nm wavelength in an ELISA microplate reader. Total protein expression was calculated according to a standard curve. Data were analyzed statistically by ANOVA and Tukey's test for pairwise comparisons ( $\alpha=0.05$ ). Two independent experiments were undertaken at different times to demonstrate the reproducibility.

### Microhardness Analysis

Thirty-two enamel/dentin blocks (6x6 mm) were obtained from bovine incisors and embedded in self-curing

acrylic resin. The enamel surface was ground flat with silicon carbide paper (320-, 600-, 1200- and 1500-grit; T469-SF-Norton, Saint-Gobain Abrasivos Ltda., Jundiaí, SP, Brazil) and polished with diamond pastes (6, 3 and 1  $\mu$ m, Arotec, Arotec S.A., Cotia, SP, Brazil). Initial surface microhardness was determined (baseline) for selection of the blocks that were randomly assigned to the following groups ( $n=8$ ): G1 - without treatment (control group); GII - bleaching with 16% CP gel; GIII - bleaching with 16% CP gel + 0.05% fluoride solution; and GIV - bleaching with 16% CP gel + 0.2% fluoride solution. The bleaching procedure was performed according to previously described procedures, during 14 days. The surface microhardness was measured after 1, 7 or 14 treatment days. Five indentations were made on the enamel surface with a microhardness tester and a Knoop indenter (Buehler, Lake Bluff, IL, USA) at each evaluation period with a 50 g load for 15 s. Means of the 5 indentations of each evaluation period were calculated and used for statistical analysis.

Data were subjected to two-way ANOVA (*group* and *period*) and Tukey's test for multiple comparisons ( $\alpha=0.05$ ). Two independent experiments were undertaken at different times to demonstrate the reproducibility.

## Results

### Cell Metabolism and ALP Activity

The results of cell metabolism (SDH quantification) and ALP activity are presented in Table 2. Considering the control group G1 as having 100% of cell metabolism, the metabolic activity of the MDPC-23 cells decreased by

Table 2. Succinic dehydrogenase (SDH) quantification and alkaline phosphatase (ALP) activity for the experimental and control groups

Group	SDH quantification (O.D. 570 nm) (n=10)	ALP activity (U/mg of protein) (n=6)
G1	0.424* (0.399-0.481) <sup>***</sup>	1.288** (0.151) <sup>a</sup>
G2	0.225 (0.176-0.253) <sup>b</sup>	0.420 (0.160) <sup>c</sup>
G3	0.249 (0.230-0.267) <sup>b</sup>	0.556 (0.205) <sup>bc</sup>
G4	0.226 (0.219-0.255) <sup>b</sup>	0.647 (0.300) <sup>bc</sup>
G5	0.267 (0.215-0.276) <sup>b</sup>	0.830 (0.142) <sup>b</sup>
G6	0.276 (0.232-0.304) <sup>ab</sup>	0.823 (0.226) <sup>b</sup>
G7	0.271 (0.251-0.287) <sup>ab</sup>	0.698 (0.054) <sup>bc</sup>
G8	0.255 (0.241-0.289) <sup>b</sup>	0.634 (0.143) <sup>bc</sup>

\*Values represents median (percentile 25 - percentile 75) for SDH quantification (absorbance, 570 nm) of two independent experiments.

\*\*Values represents mean (standard deviation) for ALP activity (U/mg of protein) of two independent experiments. \*\*\*Different letters indicate significant difference among groups (vertical) (Mann-Whitney or Tukey's test;  $p<0.05$ ).

47%, 41.3%, 46.7%, 37%, 34.9%, 36.1% and 39.9% for G2, G3, G4, G5, G6, G7 and G8, respectively. Significant cell metabolism reduction was observed in G2, G3, G4 and G5, when compared to the control group (G1) ( $p < 0.05$ ). The groups G6, G7 and G8 did not differ statistically from the negative control group ( $p > 0.05$ ).

Nevertheless, no significant difference in cell metabolism was observed when the bleached and non-fluoridated groups (G2, G3 and G4) were compared with the bleached and fluoridated groups (G5, G6, G7 and G8), at any of the evaluation periods ( $p > 0.05$ ). A significant reduction in ALP activity was also observed for all experimental groups (G2-G8) compared with the control group (G1) ( $p < 0.05$ ).

Table 3. Knoop microhardness values for control and experimental groups according to treatment days

Group	Treatment days			
	Baseline	1 day	7 days	14 days
G1	284.86 (14.73) <sup>aA</sup>	284.61 (15.12) <sup>aA</sup>	286.82 (15.23) <sup>aA</sup>	284.98 (16.72) <sup>aA</sup>
GII	285.55 (27.04) <sup>aA</sup>	254.29 (14.63) <sup>bB</sup>	239.60 (18.21) <sup>bB</sup>	228.32 (16.27) <sup>bC</sup>
GIII	292.11 (21.44) <sup>aA</sup>	270.99 (30.15) <sup>abA</sup>	253.19 (18.48) <sup>bB</sup>	253.19 (18.51) <sup>bB</sup>
GIV	288.77 (14.58) <sup>aA</sup>	262.34 (26.04) <sup>abA</sup>	249.00 (19.59) <sup>bB</sup>	251.64 (19.53) <sup>bB</sup>

Values represent mean (standard deviation), n=8, of two independent experiments. Different lowercase letters within rows (horizontal) and uppercase letters within columns (vertical) indicate statistically significant difference among groups (Tukey's test,  $p < 0.05$ ).

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### PI Staining

A significant difference was observed between all experimental groups and the control group G1 ( $p < 0.05$ ). The percentage of PI-positive cells increased by 13.3% in G1 (control group). In groups G2, G3, G4, G5, G6, G7 and G8 the percentage of PI-positive cells increased by 32.4%, 32.2%, 26.8%, 25.5%, 26.8%, 29.5% and 29.6%, respectively (Fig. 1). No significant difference was observed among the experimental groups (G2-G8), independent of fluoride concentration (0.05% or 0.2%) and evaluated periods (1, 7 or 14 days) ( $p > 0.05$ ).

### Knoop Microhardness

Knoop microhardness data are presented in Table 3.

Significant microhardness decreases were observed at 1- and 7-day treatment periods for GII, GIII and GIV compared with the control group and baseline ( $p < 0.05$ ). However, at 14-day treatment period, maintenance of microhardness values occurred in the fluoridated groups with solutions at 0.05% (GIII) and 0.2% (GIV), and a significant difference between these groups and GII (bleached-only group) was observed ( $p < 0.05$ ). No significant difference was found between GIII and GIV at any evaluation period ( $p > 0.05$ ).

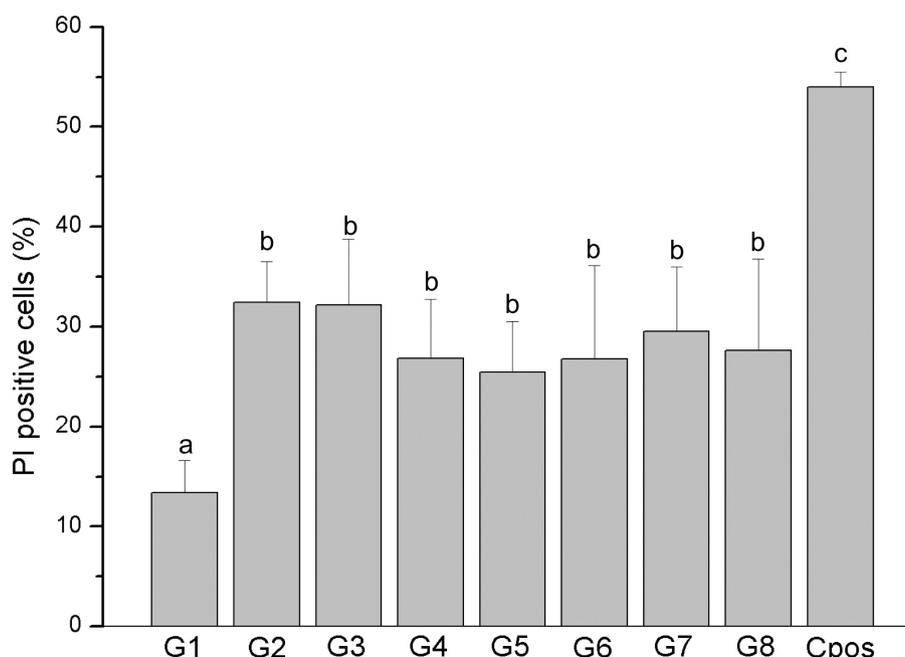


Figure 1. Percentage of I-stained MDPC-23 cells. Different letters indicate statistically significant difference among groups (Tukey's test,  $p < 0.05$ ). The 0.06% HP was applied in the positive control (Cpos).

## Discussion

The data obtained in the present study, which are in line with previous investigations (7,8), showed similar reduction in cell metabolism for all experimental groups in which 16% CP gel was used, even after a single application of the product to the enamel surface. Min et al. (21) reported that odontoblast-like MDPC-23 cells exposed to high concentrations of HP exhibit a significant increase in intracellular ROS production, which cause intense oxidative stress in this cell line. Moreover, notable cytotoxicity occurred *in vitro* when the same immortalized odontoblast-like cell line was exposed to components released from a bleaching gel capable of diffusing across enamel/dentin discs (4,5). In the present study, PI-positive cells increased about 30% in all experimental groups compared to control group (G1). Thus, it may be speculated that the cell metabolism reduction was probably caused by cell membrane damage and cell death by necrosis. In addition, in the present study, a significant decrease in ALP activity, an important enzyme expressed by odontoblasts that plays a key role in pulp repair and regeneration, particularly in dentin matrix mineralization, was observed in all bleached groups, even in those with fluoridation. This ALP activity reduction presented the same pattern as observed in the MTT analysis, determining that the toxic components released from the 16% CP bleaching gel that diffused through hard tooth structures definitively decrease the cultured pulp cell viability.

Several studies have proposed fluoride application combined with at-home bleaching gels in order to: 1) prevent mineral loss during tooth bleaching procedures (13-16), and 2) minimize the intensity and duration of post-operative tooth sensitivity (17-19). The hypothesis is that the fluorapatite precipitation can reduce tooth permeability to HP without affecting the oxidizing potential of the active bleaching agent (17-19). However, the scientific data obtained in the present study demonstrated that the topical application of fluoride to bleached enamel did not prevent the toxic effects of the 16% bleaching gel.

Armênio et al. (17) evaluated tooth sensitivity experience in patients subjected to a 16% CP gel with or without subsequent application of a 1.23% sodium fluoride. The authors observed no difference between the groups in which the patients were subjected to placebo or fluoride treatment. Kose et al. (18) used a desensitizer gel containing 5% potassium nitrate and 2% sodium fluoride before application of a 16% CP bleaching gel *in vivo*. Those authors observed that the prevalence of tooth sensitivity was similar to the placebo group; however, patients of the placebo group presented tooth sensitivity in 33.6% of the bleaching days, which was significantly higher than the percentage observed in the desensitizer

group (20.1%). These data are in line with those of Reis et al. (19), who did not observe differences in tooth sensitivity when patients treated or not with a desensitizer gel (5% potassium nitrate and 2% sodium fluoride) immediately before in-office tooth bleaching were evaluated; however, lower sensitivity was reported in the experimental group 24 h after bleaching procedures.

Tooth sensitivity after bleaching procedures has been associated with inward HP diffusion through enamel and dentin to reach the pulp chamber (1-9,11). In this situation, this reactive oxygen specie (ROS) causes inflammatory pulp response (2,3) that induces cell-release derived factors capable to excite and sensitize pulp nociceptors (22). It has been suggested that potassium ions can block the synapse between nerve cells, reducing nerve excitation associated with pain (23). Therefore, it may be suggested that the reduced tooth sensitivity duration reported in those studies above (18,19) was caused, at least in part, by the potassium nitrate present in the desensitizers. It was previously demonstrated that home-bleaching gels application on enamel causes superficial porosity in this hard tissue, allowing inward HP diffusion to the pulp chamber (1,10). It was reported that HP can diffuse along the enamel proteins affecting the organic phase of enamel (10). Then, the product can cause local disruption of the crystalline structure, which can result in mineral loss. Addition of fluoride to bleaching gel compositions or the topical application of fluoride solutions after a bleaching procedure has been proposed to minimize HP diffusion into the pulp chamber and, consequently, tooth sensitivity (17).

However, in the present study, the use of 0.2% or 0.05% fluoride solutions after each application of a 16% CP bleaching gel did not prevent its toxic effects to pulp cells. It was also analyzed whether fluoride solutions had the ability to increase enamel microhardness after application of 16% CP gel. This protocol was performed in order to correlate the hardness of enamel subjected to fluoride treatment followed by an at-home bleaching therapy with the trans-enamel and transdentinal cytotoxicity of this esthetic procedure. It was demonstrated a significant reduction in enamel microhardness at 1, 7 or 14-day periods after application of non-fluoridated 16% CP gel on enamel. On the other hand, maintenance of enamel microhardness was observed in the fluoridated groups between 7 and 14 post-operative days compared to control group, regardless of fluoride concentration.

Analysis of these data confirms the potential of fluoridated solutions remineralizing bleached enamel after treatment with a 16% CP gel. However, this remineralized enamel surface was unable to prevent the trans-enamel and transdentinal diffusion of toxic products released from the 16% CP gel, as observed 14 days after treatment. Therefore,

it seems that the de-/remineralization process that occurs during tooth bleaching in combination or not with fluoride therapy does not avoid the inward HP movement through enamel and dentin and its consequent cytotoxicity to pulp cells. Based on data previously demonstrated in the literature, which were corroborated by this *in vitro* study, Armenio et al. (17) asserted that the benefits of daily use of fluoride gel after home-bleaching procedures are not completely successful. In this way, it may be suggested that the fluoride gel-associated bleaching therapy with carbamide peroxide may be useful and an interesting alternative for those patients who have a prior history of tooth sensitivity.

Detailed evaluation of the results provided in previous investigation and their association with the data obtained in the current study, indicate that the use of 10% CP gel for at-home bleaching gel remains as the safest current tooth bleaching therapy regarding the maintenance of health pulp cells (7,8). A recent *in vivo* study also demonstrated that nightguard vital bleaching with 10% CP gel provides patient satisfaction with minimal side effects up to 17 years post-treatment (24). However, data obtained from *in vitro* studies cannot be directly extrapolated to clinical situations. This is because several different procedures including tooth bleaching are carried out in teeth that present outward dentinal fluid movement as well as cytoplasmic processes of odontoblasts, collagen and other structures inside the dentinal tubules. All these components and structures may interfere with the transdentinal diffusion of bleaching gel products *in vivo* (25).

It was demonstrated in this study that the bleaching gel containing 16% carbamide peroxide causes trans-enamel and transdentinal toxic effects to cultured MDPC-23 cells and also reduces enamel microhardness after 1, 7 and 14 days of bleaching gel application. Although the 0.05% and 0.2% fluoride solutions have shown potential for remineralization of bleached enamel, both of them are not able to prevent the toxic effects of a 16% CP bleaching applied on enamel. However, further clinical studies are needed to assess the safety of at-home bleaching therapies involving diverse techniques and products.

## Resumo

O objetivo do presente estudo foi avaliar o possível efeito protetor de soluções fluoretadas aplicadas sobre o esmalte dentário frente à citotoxicidade trans-amelodentinária de um gel clareador com 16% de peróxido de carbamida (PC). O gel de PC foi aplicado sobre discos de esmalte/dentina adaptados a câmaras pulparem artificiais (8 h/dia) durante períodos de 1, 7 ou 14 dias, seguido de aplicação de soluções fluoretadas (0,05% ou 0,2%) durante 1 min. Os extratos (meio de cultura em contato com a dentina) foram aplicados sobre células MDPC-23 durante 1 h, seguido de análise do metabolismo celular (teste do MTT), atividade de fosfatase alcalina (ALP) e danos à membrana celular (citometria de fluxo). A microdureza Knoop do esmalte dental foi avaliada. Os dados

foram analisados pelos testes de ANOVA e Kruskal-Wallis. Para o teste do MTT e atividade de ALP, redução significativa entre os grupos controle e clareados foram observados ( $p < 0,05$ ). Nenhuma diferença entre os grupos clareados foi observada ( $p > 0,05$ ), independente da aplicação das soluções fluoretadas ou tempo de tratamento. A análise por citometria de fluxo demonstrou lesão à membrana celular em torno de 30% para todos os grupos clareados. Após 14 dias de tratamento, os espécimes clareados e fluoretados apresentaram aumento significante na microdureza do esmalte ( $p < 0,05$ ). Pôde-se concluir que apesar do aumento na dureza do esmalte decorrente da aplicação das soluções fluoretadas, este tratamento não preveniu os efeitos tóxicos causados pelo gel com 16% de PC sobre as células odontoblastóides.

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