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Transenamel and transdentinal cytotoxicity of carbamide peroxide bleaching gels on odontoblast-like MDPC-23 cells

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Abstract

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Aim To evaluate the transenamel and transdentinal cytotoxicity of bleaching gels based on carbamide peroxide (CP) on odontoblast-like cells after different contact times of the products with enamel.

Methodology Enamel/dentine discs were obtained from bovine incisors and placed in artificial pulp chambers. Bleaching gels containing 10% or 16% CP were applied for 8 h day^{-1} on the enamel side of the discs during periods of 1, 7 or 14 days. Deionized water and artificial saliva served as controls. The extracts (culture medium plus bleaching gel products that diffused through the discs) were collected and applied on previously cultured MDPC-23 cells for 1 h. Cell metabolism was evaluated by the MTT assay, and the data were analysed statistically by one-way ANOVA and Tukey's test ($\alpha = 0.05$). Cell morphology was analysed by SEM.

Results There was no significant difference ($P > 0.05$) between the controls and the groups bleached with 10% CP gel. In the groups bleached with 16% CP gel, however, cell metabolism decreased significantly ($P < 0.05$) by 40.32%, 30.16% and 26.61% at 1, 7 and 14 days, respectively. There was no significant difference ($P > 0.05$) between 1, 7 or 14 applications of the gels for either of the CP concentrations.

Conclusion Regardless of the number of applications on an enamel surface, the 10% CP bleaching gel did not cause transenamel and transdentinal cytotoxicity to the MDPC-23 cell cultures. However, diffusion of products from the 16% CP gel through enamel and dentine and cytopathic effects to the pulp cells occurred even after a single application of this product on enamel.

Keywords: carbamide peroxide, cytotoxicity, odontoblasts, tooth bleaching.

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Introduction

Discoloured teeth can be restored by tooth bleaching, which is a relatively simple and noninvasive technique (Dahl & Pallesen 2003, Joiner 2006). Owing to its reactive properties, H_2O_2 is the main active chemical component of many agents used in tooth bleaching therapies. H_2O_2 can be used in its pure form or as the final product of the degradation of other bleaching substances, such as carbamide peroxide (CP). Traditionally, two techniques are used for vital tooth bleaching therapies. In the technique applied professionally, a gel based on high concentrations of H_2O_2 or CP is applied by the dentist during a clinical treatment session. Under the dentist's supervision, home tooth

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bleaching involves the patient wearing custom-made trays filled with the CP-based bleaching gels at lower concentrations for up to 8 h day^{-1} usually for a period of two weeks (Wetter *et al.* 2009). It is considered as a safe, low-cost and effective technique, especially in teeth without severe discolorations or if rapid treatment is not a priority.

During the bleaching period, the CP in contact with water is decomposed into urea and H_2O_2. The urea produces ammonia and carbon dioxide, contributing to the maintenance of an alkaline pH, which potentiates the action of the bleaching agent (Sun 2000). In addition, carbopol is an important component of bleaching gels used in home bleaching therapies. Carbopol acts as a thickening agent and retards CP degradation, permitting a more gradual release of H_2O_2 onto tooth tissues, which avoids pulpal and periodontal damage and maintains the efficacy of the bleaching gel for a longer period (Haywood & Heymann 1989).

However, as H_2O_2 is a product of the reaction of CP with water, the adverse effects of this molecule may also be seen in home bleaching therapies. It is known that H_2O_2 is a thermally instable chemical agent with a high oxidative power, which dissociates into free radicals and other reactive oxygen species (ROS), such as hydroxyl radicals (OH^-). ROS are chemically reactive molecules containing oxygen. Examples include oxygen ions and hydrogen peroxide (H_2O_2). These reactive molecules are capable of diffusing through enamel and rapidly cross the dentinoenamel junction reaching the subjacent dentine. In this substrate, these molecules attack the long-chained, dark-colour chromophore molecules and split them into smaller, less coloured and more diffusible molecules, which produce the bleaching effect (Dahl & Pallesen 2003). However, it has been demonstrated that the H_2O_2 is able to diffuse through tooth tissues and reach the pulp chamber (Benetti *et al.* 2004, Gökay *et al.* 2005, Camargo *et al.* 2007). Free radicals are ROS that have a single unpaired electron in an outer shell, which is highly reactive (Cecarini *et al.* 2007). In contact with the pulp cells, free radicals and other ROS may cause several pathological effects, such as degradation of lipids, proteins and nucleic acids, causing tissue ageing and other degenerative processes (Dahl & Pallesen 2003, Kawamoto & Tsujimoto 2004, Tredwin *et al.* 2006).

Cell damage caused by peroxides has been extensively investigated (Coldebella *et al.* 2009, De Lima *et al.* 2009, Dias Ribeiro *et al.* 2009, Trindade *et al.* 2009, Lima *et al.* 2010). However, little is known about the cytotoxic effects of bleaching gel components that are capable of diffusing through enamel and dentine and reaching the pulp cells, especially the odontoblasts. In mammalian teeth, odontoblasts are organized in a monolayer that underlies the coronal and root dentine, and thus, any chemical agent or toxic product that diffuses through the enamel and dentinal tubules will first interact with these peripheral pulp cells, which play an important role in pulp healing (Goldberg & Smith 2004).

Recent laboratory studies (Coldebella *et al.* 2009, Dias Ribeiro *et al.* 2009, Trindade *et al.* 2009) have evaluated the cytotoxicity of bleaching gels used in professionally applied bleaching therapies on odontoblast-like MDPC-23 cells. Briefly, these studies have found that products released from 35% H_2O_2 bleaching gels were capable of diffusing through enamel and dentine and cause significant cell damage. In a study with human premolar teeth, the application of a 38% H_2O_2 bleaching gel did not cause histological alterations in the pulp tissue (Kina *et al.* 2010). However, the application of the same bleaching gel in human mandibular incisors caused coagulation necrosis in part of the coronal pulp, probably due to the thickness of enamel and dentine in these teeth (De Souza Costa *et al.* 2010). A recent study has found that the application of a gel containing CP at 16% on dentine discs for 6 h resulted in the diffusion of products with cytotoxic effects to odontoblast-like cells, whilst the 10% CP gel did not produce toxic effects (Lima *et al.* 2010). However, whether products of the degradation of these gels cross both enamel and dentine to cause damage to pulp cells after long periods of application requires further investigation. Therefore, this study evaluated the transenamel and transdentinal cytotoxicity of 10 and 16% CP-based bleaching gels on odontoblast-like MDPC-23 cells after different contact times of the products with enamel, simulating home tooth bleaching.

Materials and methods

Preparation of discs

One hundred and twenty sound bovine permanent central incisors were collected and scaled for the removal of periodontal tissue remnants and other debris. Teeth with enamel cracks, hypoplasia, calculus in the middle third of the root or other morphological alterations were excluded. Enamel/dentine blocks were cut transversally from the middle third of the buccal

surface of each tooth with a water-cooled, double-faced diamond disc (KG Sorensen, Barueri, SP, Brazil). Next, the specimens were rounded with a high-speed, water-cooled cylindrical diamond bur (1095; KG Sorensen) to obtain specimens having a diameter of 5.2 mm containing enamel and dentine. Dentine surfaces were polished with wet 400- and 600-grit silicon carbide paper (T469-SF- Norton; Saint-Gobain Abrasivos Ltda., Jundiaí, SP, Brazil). The final thickness of the enamel/dentine discs was 3.5 mm, as measured with a digital caliper (Model 500-144B; Mitutoyo Sul América Ltda., São Paulo, SP, Brazil). A 0.5 mol L^{-1} ethylenediaminetetraacetic acid (EDTA) solution, pH 7.2, was applied on dentine surface for 30 s to remove the smear layer, and the discs were then rinsed thoroughly with sterile deionized water (Jacques & Hebling 2005).

Artificial pulp chambers

Each enamel/dentine disc was adapted individually to artificial pulp chambers with two compartments, which have been described previously (Trindade *et al.* 2009). The discs were positioned in the upper compartment of the artificial pulp chamber with their enamel side facing upwards to receive the bleaching treatments and were secured between two silicone rings (Rodimar Rolamentos Ltda, Araraquara, SP, Brazil) that promoted a tight seal between the upper and lower compartments of the device. In the lower compartment, circular perforations permitted free contact of the culture medium with the dentine surface of the disc. A 5-mm stainless steel matrix (Injecta Products Odontológicos, Diadema, SP, Brasil) was adapted between the silicone ring and the lateral wall of the upper compartment of the ACP. The ACPs with the discs in position were autoclaved at 120 °C for 15 min and received an additional seal with autoclaved number 7 wax (Wilson®; Polidental, Cotia, SP, Brazil) in the region between the enamel/dentine disc and the stainless steel matrix in a laminar flow chamber.

Culture of MDPC-23 cells

The MDPC-23 cell line used in this study was kindly provided by Dr. Carl T. Hanks and Dr. Jacques E. Nör, from the University of Michigan, USA. The cells were cultured in Dulbecco's modified Eagle's medium (DMEM; Sigma Chemical Co., St. Louis, MO, USA) supplemented with 10% foetal bovine serum (FBS, Gibco, Grand Island, NY, USA), with 100 IU mL^{-1} penicillin, 100 μg mL^{-1} streptomycin and 2 mmol L^{-1} glutamine (Gibco) in an humidified incubator with 5% CO_2 and 95% air at 37 °C (Isotemp Fisher Scientific, Pittsburgh, PA, USA). The MDPC-23 cells were subcultured at every 3 days until an adequate number of cells were obtained. The cells were then seeded (30 000 cells cm^{-2}) in sterile 24-well plates (Costar Corp., Cambridge, MA, USA), which were maintained in the humidified incubator with 5% CO_2 and 95% air at 37 °C for 72 h.

Bleaching procedure and analysis of cell metabolism (MTT assay)

Bleaching gels containing 10% and 16% CP (Whiteness HP; FGM, Joinville, Brazil) were evaluated. For the bleaching procedure, the artificial pulp chambers with the dentine/enamel disc in position were placed individually in the wells of sterile 24-well plates, containing 1 mL of DMEM culture medium without FBS. The enamel surface of the discs (faced upwards) was washed with 1 mL of sterile deionized water, dried with absorbent paper and then 30 mg of either one of the bleaching gels was left in contact with enamel for 8 h day^{-1} during periods of 1, 7 and 14 days, giving origin to two controls and six experimental groups, as shown in Table 1. After each 8-h bleaching time in a humidified incubator with 5% CO_2 and 95% air at 37 °C, the gel was aspirated and the enamel surface was thoroughly rinsed with 1 mL of sterile deionized water with concomitant aspiration, and 100 μL of artificial saliva (3.9% monobasic potassium phosphate; 3.6% potassium chloride; 2% sodium chloride; 2% potassium chloride; 3.7% magnesium chloride; 0.2% phenochem; 10% natrosol gel; distilled water qsp) was applied. The saliva remained in contact with the enamel for 16 h in the incubator, which corresponds to the time needed for a possible remineralization of the tissue to occur (Attin *et al.* 1997). In the control groups

Table 1 Experimental and control groups

Groups	*n*[a]	Treatment
G1	15	Deionized water
G2	15	Artificial saliva
G3	15	10% carbamide peroxide (1 day)
G4	15	10% carbamide peroxide (7 days)
G5	15	10% carbamide peroxide (14 days)
G6	15	16% carbamide peroxide (1 day)
G7	15	16% carbamide peroxide (7 days)
G8	15	16% carbamide peroxide (14 days)

[a]The experiment was undertaken in three independents times.

G1 and G2, the enamel surface remained in contact with deionized water and artificial saliva, respectively.

In the 7- and 14-day treatment periods, the culture medium in contact with the dentine side of the disc was replaced by fresh culture medium without FBS and then the last gel application (7th or 14th) was undertaken to obtain the extracts. Thus, the extracts applied to the cells were obtained only from the last gel application, in such a way that the time allowed for CP gel decomposition in the 1-, 7- and 14-day groups was the same. The extracts, namely culture medium containing products released from last application of the bleaching gel that crossed the enamel/dentine disc, were collected at the end of the 1-, 7- and 14-day treatment periods. Aliquots of 500 μL of extract were collected and applied on the wells of 24-well plates containing previously cultured MDPC-23 cells, which were maintained in the humidified incubator with 5% CO_2 and 95% air at 37 °C for 1 h.

In each group, 12 wells were used for the analysis of cell metabolism by the cytochemical demonstration of succinic dehydrogenase (SDH) activity, which is a measure of the mitochondrial respiration of the cells employing the methyl tetrazolium (MTT) assay (Mosmann 1983). The extracts were aspirated and replaced by 900 μL of DMEM plus 100 μL of MTT solution (5 mg mL^{-1} sterile PBS; Sigma Chemical Co., St. Louis, MO, USA), remaining in the incubator for an additional period of 4 h. Thereafter, the culture medium with the MTT solution was aspirated and replaced by 600 μL of acidified isopropanol solution (0.04 N HCl) in each well to dissolve the formazan crystals resulting from the cleavage of the MTT salt ring by the SDH enzyme present in the mitochondria of viable cells. Three 100-μL aliquots of each well were transferred to 96-well plates (Costar Corp., Cambridge, MA, USA). Cell metabolism was 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). The values obtained from the three aliquots were averaged to provide a single value for each well. The means were calculated for the groups and transformed into percentages, which represented the percentage of cell viability. The negative control (G1) was defined as having 100% of cell metabolism. Three independent experiments were undertaken at different times to demonstrate reproducibility. The data obtained from the MTT assay were analysed statistically by one-way ANOVA and Tukey's test at 5% significance level.

Analysis of cell morphology by scanning electron microscopy (SEM)

Three wells in each group were used for the analysis of cell morphology by SEM. For this purpose, sterile 13-mm-diameter cover glasses (Fisher Scientific, Pittsburgh, PA, USA) were placed on the bottom of the wells of 24-well plates immediately before seeding of the MDPC-23 cells (30 000 cells cm^{-2}). The extracts were applied on the cells and incubated for 1 h. Following this period, the extracts were aspirated and the viable cells that remained adhered to the glass substrate were fixed in 1 mL of buffered 2.5% glutaraldehyde for 120 min. Next, the cells were subjected to three 5-min rinses with 1 mL PBS, post-fixed in 1% osmium tetroxide for 60 min and processed for examination with a scanning electron microscope (DSM 960; Carl Zeiss Inc., Oberkochen, Germany).

Results

Cell metabolism (MTT assay)

The results of cell metabolism obtained from the MTT assay after exposure of the MDPC-23 cells to the extracts are presented in Fig. 1. There was no significant difference ($P > 0.05$) between the control groups G1 and G2. No significant difference ($P > 0.05$) was found either between the control group G1 and the groups bleached with 10% CP gel (G3, G4 and G5). Considering the control group G1 as having 100% of cell metabolism, the metabolic activity of the MDPC-23 cells that remained adhered to the glass substrate after bleaching decreased by 12.47%, 6.38% and 5.81% at 1, 7 and 14 days, respectively. No significant difference ($P > 0.05$) was found between G3, G4 and G5 at any of the evaluation periods.

The control group (G1) differed significantly from the groups bleached with 16% CP gel (G6, G7 and G8) ($P < 0.05$). The cell metabolism decreased by 40.32%, 30.16% and 26.61% at 1, 7 and 14 days, respectively. No significant difference ($P > 0.05$) was found between G6, G7 and G8 at any of the evaluation periods.

In the 1- and 7-day periods, the 16% CP gel caused a significantly more accentuated decrease ($P < 0.05$) in cell metabolism than the 10% CP gel. At 14 days, however, no statistically significant difference ($P > 0.05$) was found between the 10% (G5) and 16% (G8) CP gels regarding the metabolic activity of the odontoblast-like MDPC-23 cells.

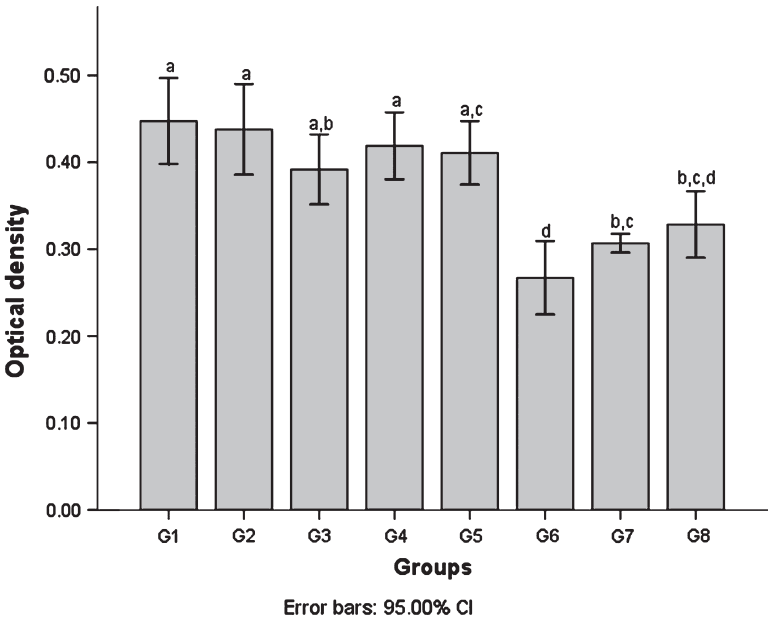

Figure 1 Bar graphs illustrating the mean and standard deviation of optical density (MTT assay) of the three independent experiments. Different letters indicate significant difference between groups (Tukey's test, $P < 0.05$).

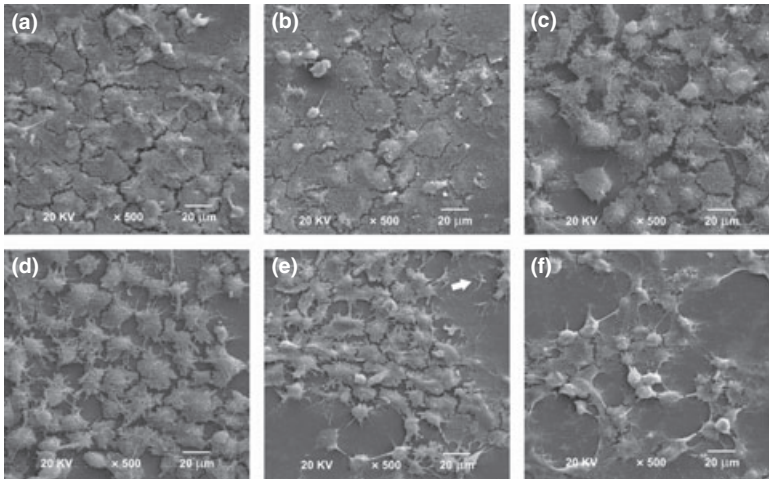

Figure 2 Panel of SEM micrographs (×500) representative of the experimental and control groups. (a,b) Deionized water (G1) and saliva (G2) controls: A large number of MDPC-23 cells near confluence can be observed adhered to the cover glass. The glass substrate was almost completely covered by MDPC-23 cells with a large cytoplasm. Note the numerous cytoplasmic processes originating from the cell membrane. (c,d) 10% carbamide peroxide (CP) (1 and 7 days, respectively): Note a large number of cells adhered to the cover glass, with noticeable reduction in the size of the cytoplasm. (e,f) 16% CP (1 and 7 days, respectively): Note a decrease in the number of cells adhered to the cover glass. Note that the rests of the cytoplasmic membrane of lethally damaged cells are clearly observed on the glass substrate (arrow).

Cell morphology (SEM)

In the water and saliva control groups (G1 and G2), a large number of MDPC-23 cells remained adhered to the glass substrate. The cells were near confluent and had a wide cytoplasm covering the entire surface of the cover glass. The cells had normal morphology, exhibiting several cytoplasmic processes originating from their membrane, which were apparently keeping them adhered to the glass substrate (Fig. 2a,b).

In the groups bleached with the 10% CP gel (G3, G4 and G5), there was a discrete decrease in cell number. However, the large number of cells that remained adhered to the glass substrate showed morphological alterations, especially reduction in cell size (Fig. 2c,d), which created intercellular spaces. In the groups in which the extracts were obtained after bleaching of enamel with the 16% CP gel, there was a more accentuated reduction in the number of cells that remained adhered to the cover glass, forming large cell-free areas, in which rests of structures of dead cells could be seen. These characteristics determined by the analysis of cell morphology and organization on the glass substrate indicate the occurrence of cell damage and death after bleaching with the gel containing CP at 16% (Fig. 2e,f).

Discussion

Tooth bleaching is an aesthetic clinical procedure that has potential adverse effects on tooth and pulp tissues that are not yet fully understood. Clearly, the indiscriminate use of tooth bleaching is a cause of concern. Several studies have demonstrated that the application of bleaching gels to enamel surface results in the diffusion of H_2O_2 to the pulp chamber and that this diffusion is proportional to the bleaching agent concentration (Benetti *et al.* 2004, Gökay *et al.* 2005, Camargo *et al.* 2007). Because of concern over the possible cytotoxic effects of products released from bleaching gels on the dental pulp, recent studies have evaluated the cytotoxicity of a bleaching gel with high peroxide concentrations on pulp cell cultures (Coldebella *et al.* 2009, Dias Ribeiro *et al.* 2009, Trindade *et al.* 2009). Those authors applied a 35% H_2O_2 gel on the enamel surface of bovine tooth discs and observed that products of the bleaching gel degradation were capable of diffusing through the tooth tissues to cause intense toxic effects to pulp cell cultures, characterized by an accentuated decrease in the cell metabolism associated with morphological alterations and even cell death. The death of odontoblasts, which are specialized cells responsible for maintaining the integrity of the pulpodentinal complex, causes an early ageing of the pulp tissue. This is because of the need of recruitment of undifferentiated mesenchymal cells to differentiate into odontoblasts, thus reducing the tissue healing capacity associated with the limited number of cells in the remaining pulp (Goldberg & Smith 2004).

In view of the confirmed cytotoxicity of bleaching agents with high H_2O_2 concentrations, home tooth bleaching may be a better alternative to minimize such deleterious effects. This technique uses CP at concentrations between 10% and 22% (Wetter *et al.* 2009), which results in the release of H_2O_2 at a low concentrations (Haywood 1992). In addition, the release of H_2O_2 is slow and gradual because of the presence of carbopol in the bleaching gel (Haywood & Heymann 1989). Therefore, the aim of this study was to evaluate the transenamel and transdentinal cytotoxicity of home bleaching gels on odontoblast-like cells.

Following a methodology described in the literature for the evaluation of the cytotoxic effects of bleaching agents (Coldebella *et al.* 2009, Dias Ribeiro *et al.* 2009, Trindade *et al.* 2009, Lima *et al.* 2010), artificial pulp chambers were used in the present study to simulate a clinical condition in the laboratory. The application of the bleaching gel to the enamel surface of the specimens allowed the products of gel degradation to diffuse into the culture medium in contact with dentine. Bovine teeth were used because their structural characteristics, such as number and diameter of dentinal tubules, are similar to those of human teeth (Schmalz *et al.* 2001, Fonseca *et al.* 2004, Krifka *et al.* 2008). The thickness of the bovine enamel/dentine discs (3.5 mm; 1.3-mm enamel and 2.2-mm dentine) was comparable to that described for human teeth (0.9- to 1.05-mm enamel and 2.33-mm dentine) (Harris & Hicks 1998, Sulieman *et al.* 2005). The mouse dental papillae cells (MDPC-23 cells) used in the present study characterize an immortalized odontoblast-like cell line with odontoblast phenotype, which present a high alkaline phosphatase activity and dentine sialoprotein and phosphoprotein expression (Hanks *et al.* 1998). This cell line has been used to evaluate the biological effects of different dental materials and/or their components for over a decade (Costa *et al.* 1999, 2003a,b, He *et al.* 2004, Aranha *et al.* 2006, Souza *et al.* 2006, De Souza Costa *et al.* 2007, Nishida *et al.* 2010).

The use of a 10% CP gel over different bleaching periods (1, 7 and 14 days) did not cause a significant decrease in cell metabolism when compared to the control. Benetti *et al.* (2004) has shown *ex vivo* that the application of a 10% CP gel to bovine enamel for 1 h resulted in the penetration of approximately 0.0712 μg mL^{-1} of H_2O_2 in the pulp chamber. However, they did not demonstrate whether this concentration could cause deleterious effects on pulp cells. In a recent study (De Lima *et al.* 2009), the direct application of 0.2547 μg mL^{-1} of H_2O_2 on MDPC-23 cells did not cause a significant decrease in cell metabolism compared to the control group (culture medium). In

addition, the application of a bleaching gel containing CP at 10% on 0.5-mm-thick dentine discs over a period of 6 h did not result in the diffusion of toxic products to cultures of the same cells (Lima *et al.* 2010). Likewise, in the present study, the application of 10% CP gel to enamel for an even longer time (8 h day^{-1}) did not result in the diffusion of bleaching gel products at toxic levels to cause significant damage to the odontoblast-like cells. Therefore, the application of a 10% CP gel for 14 consecutive days simulating home tooth bleaching did not cause toxic cytopathic effects on pulp cell cultures. Hanks *et al.* (1993) observed that a 15-min application of 10% CP bleaching gels on 0.5-mm-thick dentine discs resulted in intense transdentinal diffusion of H_2O_2 after 15 min that was capable of causing toxic effects in Blab/c 3T3 fibroblast culture, with 60% decrease in cell viability. The present study and the study by Lima *et al.* (2010) used a different cell line (MDPC-23 cells), which could explain, at least in part, these differing results. A previous *in vivo* study (Fugaro *et al.* 2004) has also found that tooth bleaching with 10% CP may be considered a safe technique. In this study, the bleaching gel was applied in human premolars for 6 h day^{-1} over 14 consecutive days and only one-third of the bleached teeth had small histological pulpal alterations, which were reversible with time.

In the present study, the specimens subjected to bleaching with 16% CP gel had a significant decrease in cell metabolism compared to the control group for all evaluation periods (1, 7 or 14 days). It has been shown that the diffusion of products of bleaching gel degradation is proportional to the gel concentration and its contact time with enamel (Benetti *et al.* 2004, Gökay *et al.* 2005, Camargo *et al.* 2007). Therefore, the increase in the CP gel concentration from 10 to 16% probably resulted in a greater diffusion of by-products through the enamel/dentine discs, making the gel with higher CP concentration more toxic to odontoblast-like cells, even when a single dose was applied. This fact has also been observed in a recent study (Lima *et al.* 2010) in which the application of a gel containing CP at 16% on dentine discs over 6 h resulted in significant decrease in metabolic activity of MDPC-23 cells, whilst the application of a gel containing the same substance at 10% did not produce toxic effects to these cell lines. According to Hanks *et al.* (1993), H_2O_2 diffusion through tooth tissues is directly proportional to the bleaching agent concentration and the contact time with the tooth surface.

The higher cytotoxicity of the 16% CP gel observed in the present study was confirmed by the organization of the MDPC-23 cells on the glass substrate as revealed by SEM. A large number of cells were damaged lethality and detached from the glass substrate after death, leaving cell-free areas on the cover glass surface to which rests of cell structures remained adhered. The groups bleached with the 10% CP gel did not show a reduction in the number of cells, but only a discrete reduction in the cell size, which seems to be related to a minor decrease in the metabolic activity of the MDPC-23 cells.

H_2O_2 is a ROS and its degradation produces other types of ROS and free radicals. The presence of these molecules at a high exogenous concentration in contact with cells, such as occurs in tooth bleaching procedures, may cause severe adverse effects to several cell components, including damage to cellular DNA (mutagenesis and carcinogenesis), lipid peroxidation, protein oxidation and fragmentation, reduction in cell proliferation and even induction of cell apoptosis and necrosis (Sies 1993, Slater *et al.* 1995, Shackelford *et al.* 2000, Martindale & Holbrook 2002). It has been shown that odontoblast-like MDPC-23 cells exposed to H_2O_2 resulted in a significant increase in intracellular ROS production, producing an oxidative stress (Min *et al.* 2008). Increase in alkaline phosphatase activity and extracellular mineralization matrix has also been observed, which are cell markers for dentine production and are also related to the H_2O_2-derived oxidative stress (Lee *et al.* 2006).

In the present study, cell viability tended to increase as the number of bleaching gel applications increased, though without significant difference between the evaluation periods. This direct relationship between bleaching gel concentration and cell metabolism seems contradictory. However, these results may be explained by the possible remineralizing capacity of the artificial saliva, which was left in contact with enamel for 16 h in the intervals between the bleaching treatments. The saliva has specific ions that have an effective participation in the remineralization of the enamel altered by tooth bleaching procedures (Delvin *et al.* 2006). The remineralizing action of saliva on the bleached enamel has been observed by Spalding *et al.* (2003) in a study in which tooth samples were treated with 35% hydrogen peroxide for 20 min and stored in saliva for 1 week. SEM analysis revealed the formation of a granular area on the enamel surface, similar to that observed after application of fluoride solutions, which suggests a possible remineralization phenomenon by the action of saliva. In the present study, saliva was used to replicate the clinical situation. For all experi-

mental groups, the most severe cytotoxic effects were observed in the earlier period (1 day), in which the analysis of cell metabolism (MTT assay) was performed immediately after having the bleaching gel in contact with enamel for 8 h. In the following periods of 7 and 14 days, milder cytotoxic effects were observed for both CP gel concentrations, which indicates that saliva somehow protected the tooth structures against the diffusion of toxic products released from the gels. Further studies are needed to elucidate the mechanism of action of saliva in preventing the transenamel and transdentinal diffusion of products bleaching agents.

In the present laboratory study, the application of both bleaching gels followed the clinical protocol usually employed in home bleaching therapies. According to Meireles *et al.* (2008), similar aesthetic outcomes are obtained with either 10% or 16% CP bleaching agents. Therefore, based on the recent clinical data and on the results of the present study, it may be suggested that 10% CP gels have a whitening potential comparable to that of the 16% CP gel, though with lower risks of causing pulpal alterations in the bleached teeth. Teeth with vital pulp have a dentinal fluid flow produced by the osmotic intrapulpal pressure, cytoplasmatic processes of odontoblasts and other intratubular components, which may prevent the diffusion of bleaching gel components through the dentinal tubules (Sauro *et al.* 2007). The pulp also has a lymphatic system that participates in the elimination of external products that reach this specialized connective tissue by transdentinal diffusion. Furthermore, because of the oxidative stress generated by the presence of free radicals, the defence system of the pulp cells may be activated, releasing several endogenous antioxidant agents, such as the enzymes superoxide dismutase, catalase and peroxidases, vitamins A and E and ascorbic acid, which act on the degradation of H_2O_2 (Bowels & Burns 1992, Esposito *et al.* 2003). Therefore, considering that all physiological factors mentioned earlier may interfere in the pulp tissue damage-healing process, clinical studies are needed to evaluate the safety of home bleaching therapies with 16% CP gels.

Conclusions

Regardless of the number of applications on enamel surfaces, the 10% CP bleaching gel did not cause transenamel and transdentinal cytotoxicity to MDPC-23 cell cultures. However, diffusion of products from the 16% CP gel through enamel and dentine and cytopathic effects to the pulp cells occurred even after a single application of this product on enamel.

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