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Responses of human dental pulp cells after application of a low-concentration bleaching gel to enamel

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ABSTRACT

Objective: To evaluate the effect of a 17.5% H_2O_2 gel on the odontoblastic differentiation capability of human dental pulp cells (HDPCs).

Design: The bleaching gel was applied for 45, 15 or 5 min to enamel/dentine discs adapted to transwells, positioned over previously cultured HDPCs. In the control group, no treatment was performed on the discs. Immediately after samples were bleached, the cell viability (MTT assay) and death (Live/Dead assay) as well as the mRNA gene expression of inflammatory mediators (TNFα, IL-1β, IL-6, and COX-2; real-time PCR) were evaluated. The mRNA gene expression of odontoblastic markers (DMP-1, DSPP, and ALP) and mineralized nodule deposition (alizarin red) were assessed at 7, 14 and 21 days post-bleaching. The amount of H_2O_2 in contact with cells was quantified. Data were evaluated by Kruskal–Wallis and Mann–Whitney tests ($\alpha = 5\%$).

Results: Significant cell viability reduction and cell death were observed for bleached groups relative to control in a time-dependent fashion. Also, significant overexpression of all inflammatory mediators tested occurred in the 45- and 15-min groups. In the bleached groups, the expression of ALP, DMP-1, and DSPP and the deposition of mineralized nodules were reduced in comparison with those in the control group, at the initial periods (7 and 14 days). However, the 15- and 5-min groups reached values similar to those in the control group at the 21-day period.

Conclusions: The 17.5% H_2O_2 gel was cytotoxic to pulp cells; however, cells subjected to short-term bleaching are capable of expressing the odontoblastic phenotype over time.

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1. Introduction

It is well known that tooth-bleaching therapy can promote odontoblast death due to oxidative damage mediated by hydrogen peroxide and its by-products, capable of diffusing through enamel and dentine at toxic concentrations.[1,2] These cells are essential for the homeostasis of the dentine-pulp complex, since they are responsible for the deposition and mineralization of dentine matrix throughout the life of the tooth, as well as for the orchestration of the inflammatory and immune responses of pulp tissue, since the noxious signal acts on the tooth surface.[3,4]

Odontoblasts are highly differentiated terminal cells. Therefore, when they are lost, reparative dentinogenesis takes place, which involves mesenchymal stem cell (MSC) recruitment, proliferation, and differentiation into odontoblast-like cells, resulting in the deposition of mineralized matrix and restoration of the homeostasis of pulp tissue.[5–7] It was previously demonstrated that very low concentrations of H_2O_2 induce odontoblastic differentiation and mineralized matrix deposition[8,9]; conversely, toxic concentrations of H_2O_2 enhance inflammatory mediator expression associated with the impairment of odontoblastic differentiation capability.[10–14]

Recently, it was demonstrated that application of a low-concentration H_2O_2 gel (17.5%) from 5 to 45 min onto dental structure was able to reduce in about 11.3–4.5 times, respectively, the indirect toxicity of in-office tooth-bleaching to human dental pulp cells (HDPCs), when compared with traditional therapy (35% H_2O_2; 3 × 15 min). The cells were able to proliferate significantly 3 days after samples were bleached, demonstrating that pulp cells were able to overcome the initial oxidative stress.[2] However, the effect of this initial H_2O_2 toxicity on the odontoblastic differentiation capability of HDPCs as well as mineralized matrix deposition was not addressed. Therefore, the present study investigated the effect of a 17.5% H_2O_2 gel, applied to enamel surfaces for different periods, on the immediate viability of HDPCs and late odontoblastic differentiation and mineralized matrix deposition capability.

2. Materials and methods

2.1. Cell culture

The human dental pulp cell (HDPC) primary culture was obtained by enzymatic digestion of pulp tissue from one freshly extracted third molar, obtained from one donor (24 years of age) (Proc. n° 13/11; Ethics Committee of Araraquara School of Dentistry, SP, Brazil) after sign the informed consent according to the code of ethics of the world medical association (Declaration of Helsinki). The pulp tissue was removed aseptically, cut into small pieces (1 mm), and then incubated for 24 h in complete Dulbecco's Modified Eagle Medium (DMEM; supplemented with 100 IU/mL penicillin, 100 μg/mL streptomycin, 2 mmol/L glutamine; Gibco, Grand Island, NY, USA) containing 10% foetal bovine serum (FBS; Gibco) and 200 units/mL of type II collagenase (Worthington Biochemical Corporation, Lakewood, NJ, USA). After this period, the released cells were subcultured in complete DMEM with 10% FBS. Cells from the 4th to 6th passages were used.

2.2. Enamel/dentine specimens

Bovine incisors, from 24- to 30-month-old bullocks, were cross-sectioned in its buccal surface with a diamond trephine bur (Dinser brocas diamantadas LTDA, São Paulo, SP, Brazil) coupled to bench drilling machine (FSB 16 Pratika; Schultz, Joinville, SC, Brazil) to obtain 5.6 mm-diameter discs containing both enamel and dentine. To obtain the standardized thicknesses similar to those of central incisors (3.5 mm),[15] we polished the dentine surfaces with 400- and 600-grit abrasive papers, followed by EDTA 0.5 N treatment for 30 s for smear layer removal. The enamel surfaces of the discs were cleaned with a solution of pumice stone and water by means of a low-speed handpiece. The discs were adapted to acrylic transwells (polycarbonate membrane/8 μm pore size; Corning Inc., Corning, NY, USA) by means of a fluid light-cured resin (TopDam, FGM, Joinville, SC, Brazil), which promoted a lateral seal, and the set was then sterilized with ethylene oxide.

2.3. Experimental design

The HDPCs were seeded into wells of 24-well plates (6×10^4 cells/well) for 24 h in DMEM supplemented with 10% FBS, reaching 80% confluence. The culture medium was then replaced by 300 μL of DMEM with no FBS, and the disc/transwell set was positioned onto the previously cultured cells, in such a way that only the dentine was in direct contact with the culture medium, and the enamel surface remained exposed to receive the bleaching procedure. The bleaching gel containing 17.5% of H_2O_2 was obtained by dilution of a 35% H_2O_2 gel (Whiteness HP; FGM) in distilled water immediately before the bleaching procedure (1:1).[2] The gel (30 mg) was applied to the enamel surface for different periods according to the experimental groups: *45-min group* – 3 applications of 15 min each; *15-min group* – one application of 15 min; *5-min group* – one application of 5 min; and *control group* – no treatment. Immediately after the bleaching treatment, the transwell/disc set was removed, the culture medium in contact with the cells was collected, and the cells were washed with 1 mL of phosphate-buffered saline (PBS) solution.

2.4. Trans-enamel and trans-dentinal cytotoxicity

2.4.1. Cell viability analysis (MTT assay)

The cells were incubated for 4 h at 37 °C and 5% CO_2 with the MTT solution (Sigma–Aldrich Corp., St. Louis, MO, USA) diluted in DMEM (1:10). The formazan crystals formed in viable cells were then dissolved in acidified isopropanol, and the absorbance of the resulting solution was read at 570 nm (ELISA microplate reader, Tp Reader, Thermoplate, Nanshan District, Shenzhen, China). The mean absorbance of the control group was considered as 100% of cell viability, and the percentage values for experimental groups were calculated based on this parameter ($n = 6$).