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Concentrations of and application protocols for hydrogen peroxide bleaching gels: Effects on pulp cell viability and whitening efficacy

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ARTICLE INFO

Article history:
Received 9 July 2013
Received in revised form
30 October 2013
Accepted 31 October 2013

Keywords:
Tooth bleaching
Dental pulp
Odontoblasts
Cytotoxicity

ABSTRACT

Objectives: To assess the whitening effectiveness and the trans-enamel/trans-dentinal toxicity of experimental tooth-bleaching protocols on pulp cells.

Methods: Enamel/dentine discs individually adapted to trans-well devices were placed on cultured odontoblast-like cells (MDPC-23) or human dental pulp cells (HDPCs). The following groups were formed: G1 – no treatment (control); G2 to G4 – 35% H_2O_2, 3 × 15, 1 × 15, and 1 × 5 min, respectively; and G5 to G7 – 17.5% H_2O_2, 3 × 15, 1 × 15, and 1 × 5 min, respectively. Cell viability and morphology were evaluated immediately after bleaching (T1) and 72 h thereafter (T2). Oxidative stress and cell membrane damage were also assessed (T1). The amount of H_2O_2 in culture medium was quantified (Mann–Whitney; $\alpha = 5\%$) and colour change (ΔE) of enamel was analysed after 3 sessions (Tukey's test; $\alpha = 5\%$).

Results: Cell viability reduction, H_2O_2 diffusion, cell morphology alteration, oxidative stress, and cell membrane damage occurred in a concentration-/time-dependent fashion. The cell viability reduction was significant in all groups for HDPCs and only for G2, G3, and G5 in MDPC-23 cells compared with G1. Significant cell viability and morphology recovery were observed in all groups at T2, except for G2 in HDPCs. The highest ΔE value was found in G2. However, all groups presented significant ΔE increases compared with G1.

Conclusion: Shortening the contact time of a 35%-H_2O_2 gel for 5 min, or reducing its concentration to 17.5% and applying it for 45, 15, or 5 min produce gradual tooth colour change associated with reduced trans-enamel and trans-dentinal cytotoxicity to pulp cells.

Clinical significance: The experimental protocols tested in the present study provided significant tooth-bleaching improvement associated with decreased toxicity to pulp cells, which may be an interesting alternative to be tested in clinical situations intended to reduce tooth sensitivity and pulp damage.

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0300-5712/$ – see front matter
http://dx.doi.org/10.1016/j.jdent.2013.10.021

1. Introduction

Hydrogen peroxide (H_2O_2) has been widely used by clinicians to whiten darkened/coloured teeth.[1–4] However, the high incidence of post-operative sensitivity claimed by patients subjected to tooth-bleaching therapies[1–4] indicates that this kind of aesthetic procedure can damage pulp tissue.[5–7] Acute inflammation or even partial necrosis of the coronal pulp tissue has previously been shown in teeth subjected to highly concentrated in-office bleaching gels.[5,6] It has been reported that the inward diffusion of H_2O_2 into the pulp chamber is crucial for post-operative tooth sensitivity.[1,2] This phenomenon is directly related to the concentration of H_2O_2 in the bleaching gel as well as the period of application of this dental product to enamel.[8,9]

The low molecular weight of H_2O_2 and its subproducts favours the rapid diffusion of these reactive oxygen species (ROS) through mineralised dental tissues[10] to cause oxidative stress in pulp cells.[7] Intense cell death[11,12] or stimulus for odontoblastic marker over-expression and cell differentiation[13–15] may occur, depending on the amount of ROS that reaches the pulp cells. In this way, reducing the trans-enamel and trans-dentinal H_2O_2 diffusion during tooth-bleaching procedures may be an interesting alternative to minimise the oxidative damage caused in pulp cells by this aesthetic procedure, whilst also stimulating pulpal healing. Therefore, the aim of this study was to assess the effects of H_2O_2 concentration in bleaching gels and their application periods on the trans-enamel and trans-dentinal diffusion of H_2O_2, and its influence on pulp cell viability, correlating these data with tooth-whitening effectiveness.

2. Materials and methods

2.1. Cell culture

Two cell linage from pulp tissue were evaluated in this study. The odontoblast-like MDPC-23 cell is an immortalised cell linage from rat dental papilla, which exhibits an odontoblast phenotype. This cell linage has been extensively used to test the toxic effects of bleaching gels.[11,12,16] This is because in clinical situation the odontoblasts are the first cells that receive any component released from bleaching gels capable of diffusing through enamel and dentine to reach the pulp chamber. A primary culture of human dental pulp tissue was also used in this study since it represents a mixed culture from pulp cells containing a subpopulation of mesenchymal stem cells (MSC). The MSCs from pulp tissue, which have the ability to differentiate into odontoblast-like cells, play an important hole in the pulp-dentine complex healing and homeostasis.

The primary culture of human dental pulp cells (HDPCs) were obtained from freshly impacted third molars from donors who signed a corresponding form approved by the Ethics Committee of Araraquara School of Dentistry, SP, Brazil (Proc. no. 13/11). The pulp tissue was aseptically removed and minced into small fragments, which were subjected to enzymatic digestion by collagenase (Worthington Biochemical Corporation, Lakewood, NJ, USA). The cells released from digested tissue were then subcultured in complete DMEM. The identification of stem cells on HDPC culture was performed by immunofluorescence assay. After fixation and permeabilisation, the cells were incubated with 1:50 Vimentin, Nestin, Nanog, and Oct3/4 (Santa Cruz Biotechnology, Santa Cruz, CA, USA) primary antibodies (12 h at 4 °C), followed by incubation with 1:100 secondary antibody conjugated with fluorescein isothiocyanate (FITC). Nuclear fluorescence was performed with 4′,6′-diamino-2-phenylindole (DAPI). Positive staining for the primary antibodies tested (Fig. 1) indicated that the HDPC culture used in the present investigation contains a subpopulation of MSCs derived from the neural crest.[17,18] Cells from the 4th to 6th passages were used. The MDPC-23 cells were cultured and subcultured into cell culture flasks with complete DMEM at 37 °C and 5% CO_2 until adequate numbers of cells were obtained.

2.2. Experimental procedure

Enamel/dentine discs (5.6 mm diameter × 3.5 mm thickness)[16] obtained from 24- to 30-month-old bullocks were adapted to acrylic trans-wells (Corning Inc., Corning, NY, USA) by means of a fluid light-cured resin (TopDam, FGM, Joinville, SC, Brazil). Each disc/trans-well set was individually sterilised by ethylene oxide.[19] The HDPCS and MDPC-23 cells were seeded into 24-well plates (6×10^4 cells/cm^2) and incubated at 37 °C and 5% CO_2 for 24 h before the experiment (80% confluence). Thereafter, the culture medium was replaced by 300 μL DMEM without SFB, and the disc/trans-well set was placed on the cultured cells in such way that only the dentine surface was maintained in direct contact with DMEM. The DMEM without SFB was placed in contact with the cells prior the bleaching procedure in order to avoid interaction between SFB components and the H_2O_2 that diffused through the disc/trans-well set. Two concentrations of H_2O_2 were tested in this study, a 35% H_2O_2 gel (Whiteness HP–FGM, Joinville, SC, Brazil); and a 17.5% H_2O_2 gel, which was obtained by dilution of the 35% H_2O_2 gel in distilled water immediately before the bleaching procedure. The bleaching procedure was performed on the central surface of enamel, according to the different protocols established, giving rise to the following groups: G1 – no treatment (negative control); G2 – three 15-min applications of a 35%-H_2O_2 gel (traditional protocol – positive control); G3 – one 15-min application of a 35%-H_2O_2 gel; G4 – one 5-min application of a 35%-H_2O_2 gel; G5 – three 15-min applications of a 17.5%-H_2O_2 gel; G6 – one 15-min application of a 17.5%-H_2O_2 gel; and G7 – one 5-min application of a 17.5%-H_2O_2 gel. Immediately after the end of the bleaching procedure, the disc/trans-well set was removed, and cell analysis was performed at two experimental time-points: T1 – immediately after bleaching; and T2–72 h after bleaching (DMEM plus 10% SFB was changed daily).

2.3. Cell viability assay

Cell viability was assessed by the MTT assay as previously described.[16] The cells were incubated for 4 h with MTT solution (Sigma–Aldrich Corp.) at 37 °C and 5% CO_2. After that, the absorbance of formazan crystals in the viable cells was assessed in an ELISA microplate reader (570 nm) (Tp Reader, Thermoplate, Nanshan District, Shenzhen, China).