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Phototherapy up-regulates dentin matrix proteins expression and synthesis by stem cells from human-exfoliated deciduous teeth

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ABSTRACT

Objectives: The aim of this study was to evaluate the effects of infrared LED (850 nm) irradiation on dentin matrix proteins expression and synthesis by cultured stem cells from human exfoliated deciduous teeth (SHED).

Methods: Near-exfoliation primary teeth were extracted ($n = 3$), and SHED cultures were characterized by immunofluorescence using STRO-1, CD44, CD146, Nanog and OCT3/4 antibodies, before experimental protocol. The SHEDs were seeded (3×10^4 cells/cm^2) with DMEM containing 10% FBS. After 24-h incubation, the culture medium was replaced by osteogenic differentiation medium, and the cells were irradiated with LED light at energy densities (EDs) of 0 (control), 2, or 4 J/cm^2 ($n = 8$). The irradiated SHEDs were then evaluated for alkaline phosphatase (ALP) activity, total protein (TP) production, and collagen synthesis (SIRCOL™ Assay), as well as ALP, collagen type I (Col I), dentin sialophosphoprotein (DSPP), and dentin matrix acidic phosphoprotein (DMP-1) gene expression (qPCR). Data were analyzed by Kruskal–Wallis and Mann–Whitney tests ($\alpha = 0.05$).

Results: Increased ALP activity and collagen synthesis, as well as gene expression of DSPP and ALP, were observed for both EDs compared with non-irradiated cells. The ED of 4 J/cm^2 also increased gene expression of COL I and DMP-1.

Conclusions: In conclusion, infrared LED irradiation was capable of biostimulating SHEDs by increasing the expression and synthesis of proteins related with mineralized tissue formation, with overall better results for the energy dose of 4 J/cm^2.

Clinical significance: Phototherapy is an additional approach for the clinical application of LED in Restorative Dentistry. Infrared LED irradiation of the cavity's floor could biostimulate subjacent pulp cells, improving local tissue healing.

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1. Introduction

Pulp tissue inflammation may be exacerbated during cavity preparation and following cavity restoration with non-biocompatible dental materials.[1] Therefore, specific procedures and biomaterials should be clinically applied to enhance pulpal healing by up-regulating collagen-rich dentin matrix production and its mineralization by pulp cells.[1] It has been shown that light-emitting diode (LED) irradiation may improve the repair of different tissues, including pulp.[2–4] Furthermore, previous studies have demonstrated that LED irradiation can biostimulate cultured odontoblast-like MDPC-23 cells[5] and human dental pulp cells (HDPCs).[6]

It has been reported that LED irradiation in the infrared range of the spectrum (from 700 nm to 1 mm wavelength) is able to pass through the dentin barrier[7] and photobiostimulate underlying cells,[8] increasing the cell viability and alkaline phosphatase activity of odontoblast-like cells as well as up-regulating collagen type I (Col I) expression.[5,9,10] Additionally, infrared light can improve healing of bone defects mechanically created in rats[11] and reduce pain in temporomandibular disorders.[12]

Based on these data, it is reasonable to consider that phototherapy technology with LED represents an interesting alternative for clinical application in restorative dentistry, particularly for stimulation of pulp cells subjacent to the cavity floor. Therefore, the authors hypothesized that infrared LED irradiation, at specific parameters, could enhance dentin matrix and dentin mineralization protein expression/synthesis by pulp cells. The aim of this study was to evaluate the effects of infrared LED (850 nm) irradiation on dentin matrix protein gene expression and synthesis by cultured SHEDs.

2. Materials and methods

2.1. Primary culture obtained from deciduous teeth

The present study was approved by the Ethical Committee of UNESP—Univ. Estadual Paulista (protocol 63/11). Three near-exfoliation sound primary teeth were donated by the child's legal guardian, who provided a signed consent form. Pulp cells were isolated by enzymatic digestion with collagenase type I and dispase (Worthington Biochemical Corp., Lakewood, NJ, USA).[13] Cells were cultured in 75 cm^2 flasks in monolayer on DMEM supplemented with 10% fetal bovine serum (GIBCO, Grand Island, NY, USA), 100 IU/mL and 100 μg/mL, respectively, of penicillin and streptomycin, and 2 mmol/L of glutamine (GIBCO, Grand Island, NY, USA). The medium was refreshed every 2 days, and the culture was maintained semiconfluent. All cultures were incubated at 37 °C in a 5% CO_2 high-humidity environment. Cells at passages 3 and 4 were used in this study.

2.2. Irradiation device and experimental protocol

For cell irradiation, a standardized device (LEDtable) containing 24 infrared diodes (850 ± 10 nm) was used.[6] Each diode was accompanied by a collimator lens that limited the area of irradiation to the area of one single well of a 24-well culture plate (2 cm^2). Therefore, each diode was able to irradiate the cells adhering to the bottom of each well individually, with no interference from the adjacent well. The irradiance used was 40 mW/cm^2, and the energy densities (EDs) investigated were 0 (control group), 2 J/cm^2 (time of irradiation, 50 s), and 4 J/cm^2 (time of irradiation, 1 min and 40 s).

The irradiation protocols used in this study were validated by preliminary tests that monitored temperature change from the moment the diodes were activated up to 2 min without interruption, and the possible interference of the culture medium colour (phenol-red DMEM) with absorbance. The results of these preliminary studies showed no temperature increase and no interference of medium colour with infrared light absorbance (data not shown).

SHEDs in DMEM with 10% FBS were seeded (3×10^4 cells/cm^2) in wells of sterilized 24-well plates and maintained for 24 h in a humidified incubator with 5% CO_2 and 95% air at 37 °C (Isotemp, Fisher Scientific, Bellefonte, PA, USA). Then, DMEM was replaced by an odontoblastic/osteogenic differentiation medium (DMEM with 5 mM β-glycerophosphate and 100 μM ascorbic acid; Sigma/Aldrich, St. Louis MO, USA) containing 0.5% FSB (nutritional stress induction).[9,10,14] After 12-h incubation, the irradiations were delivered in a dark room, and protein gene expression and synthesis by SHEDs cells were assessed after 72 h. The time-point of 72 h after irradiation was selected based on previous studies.[5,9,10]

2.3. Cell characterization by immunofluorescence

An immunofluorescence assay for stem cell markers was performed before the differentiation and irradiation experiments. Cells from three individuals (P1, P2, and P3) were seeded (8×10^4cells/mL) in wells of a 96 well-plate (TPP Techno Plastic Products AG, Trasadingen, Switzerland) and incubated for 24 h. Then, cells were fixed in 4% buffered formalin at 4 °C overnight, washed 3 times with PBS, and subjected to immunolabelling. Samples seeded for Nanog and OCT3/4 identification (cytoplasm markers; Santa Cruz Biotechnology, Dallas, TX, USA) were permeabilized with Triton 0.1% for 10 min. For CD44, CD146, and STRO-1 identification (membrane markers; Santa Cruz Biotechnology), no permeabilization was performed, and samples were in contact with PBS during this period. After primary antibody incubation, 3 more washing cycles were performed, and samples were placed in contact with 30 μL of TBST solution (Tris-buffered saline and Tween 20) with 5% BSA (bovine serum albumin) for 30 min. The samples were then incubated with 30 μL of a solution containing primary antibodies (CD44—dilution 1:100; CD146—dilution 5:1,000; STRO-1—dilution 1:10; Nanog—dilution 1:10; and OCT3/4—dilution 1:10) for 1 h. After being washed in PBS, samples were in contact with secondary antibodies conjugated with FITC (IgM—dilution 1:400, and IgG—dilution 1:400; Santa Cruz Biotechnology) for an additional 1 h. Negative controls treated only with secondary antibodies were evaluated. After new washing, sample nuclei were labelled with Hoechst (dilution 1:5,000; Invitrogen, Carlsbad, CA, USA) for 15 min. Cells were then assessed on IN Cell Analyzer 2000 (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA), and 9 selected areas were considered for quantitative data analysis with IN Cell Investigator Software v1.6.