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Effects of polarized and non-polarized red-light irradiation on proliferation of human Wharton's jelly-derived mesenchymal cells

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ABSTRACT

Light emitting diode (LED) irradiation has recently been introduced as an encouraging strategy for promotion of cell proliferation. Human umbilical cord Wharton's jelly-derived mesenchymal (hUCM) cells are among the most available mesenchymal cells with a promising application in regenerative medicine. The aim of the present study was to examine the effect of polarized (PL) and non-polarized (NPL) red-light emitted by LED on various proliferation properties of hUCM cells. Cell proliferation was assessed 48 h after irradiation of hUCM cells by different energy densities. Cell density increased to a significant level both in PL and NPL irradiation at 0.954 J/cm^2 following WST-1 assay. Staining of irradiated and non-irradiated cells with Hoechst after 3 and 6 days revealed an increased proliferation rate in irradiated cells, but the non-irradiated cells proliferated more than irradiated cells at day 9 of cultivation. Similar results were obtained in trypan blue assay. Scratch repair test for 18 h with an interval of 6 h did not reveal a significant difference between irradiated and non-irradiated cells. In addition, CFU-F assay in PL irradiated cells was higher than control when 500 cells/plate was cultivated. Totally, this study revealed that hUCM cells could be induced to achieve higher number of cells by PL and NPL red-light irradiation after 48 h.

1. Introduction

Physical factors such as electromagnetic fields, laser and light emitting diode (LED) irradiation have recently been introduced as a new method for cell proliferation [1]. Ferraresi et al. reported that red LED irradiation improves cell proliferation in exercise-trained mice [2]. Also, Li et al. demonstrated that red LED irradiation enhances proliferation of bone marrow mesenchymal cells [3]. Nowadays, polarized (PL) and non-polarized (NPL) LED lights are widely used in treatment of chronic wound healing [4]. Among the different light sources and devices of phototherapy, LEDs are cost-effective and can easily be used in various procedures [5]. From the technical and ethical aspects, human umbilical cord Wharton's jelly derived mesenchymal (hUCM) cells are easily harvested and propagated in the cell culture units as one of the most available mesenchymal cell sources [6]. These cells, while having self-renewal capacity are multipotent [7], show low immunogenicity and are very suitable for clinical usages [8]. Cell proliferation enhancement is very important for research works as well as regenerative medicine [9]. Whether PL light irradiation could have different impacts on mesenchymal cells compared with NPL light has not yet been investigated. Thus, the aim of the present study was to determine the effect of PL and NPL red light emitted from LED on proliferation of human Wharton's jelly derived mesenchymal cells.

2. Materials and methods

2.1. Isolation of hUCM cells

All the materials of this study were obtained from Sigma Company (Sigma-Aldrich, Mo, USA) unless stated otherwise. Ethical review committee at Kerman University of Medical Sciences, Kerman, Iran, approved the study. We used a previously described

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method to isolate hUCM cells [10]. Briefly, fresh neonatal human umbilical cords (N = 3) were obtained from full-term infants delivered by Cesarean section after a written consent was taken from the parents. Umbilical cords were immersed in Hanks' solution and transferred to the cell culture laboratory. Following removal of amniotic membrane and blood vessels, Wharton's jelly was apportioned to approximately 2–3 mm^3 pieces and seeded onto Petri dishes. Dulbecco's modified Eagle's medium-F12 (DMEM-F12), supplemented with streptomycin (100 μg/mL), penicillin (100 IU/mL), amphotericin B (2.5 μg/mL), and 10% fetal bovine serum (FBS) was then added for three weeks. Half of the medium was refreshed every 2–3 days until the cells peered at the fragment's borders and reached 80–90% confluence. Third to fifth-passage hUCM cells were used for experiments.

2.2. Evaluation of osteogenic and adipogenic differentiation of hUCM cells

hUCM cells at a density of 2×10^3 cells/cm^2 were cultured on sterile glass slides. Adipogenic differentiation medium consisted of DMEM-F12, dexamethasone (100 nM), indomethacin (50 μg/ml) and 10% FBS. Osteogenic differentiation was carried out using DMEM-F12 supplemented with β-glycerophosphate (10 mM), dexamethasone (10 nM), ascorbic acid (50 μg/ml) and 10% FBS [11]. The medium was refreshed every 3–4 days. Twenty one days after induction of osteogenic differentiation and 14 days after induction of adipogenic differentiation, the cells were evaluated with Alizarin Red and Oil Red O, respectively.

2.3. Flow cytometry analysis

Fourth-passage hUCM cells at concentration of 3×10^5 were used for flow cytometric analysis. The cells were fixed with 4% formaldehyde and incubated for 25 min at 4 °C with 10% goat serum to block the nonspecific primary antigen binding sites. After washing by PBS, the cells were conjugated with phyco-erythrin (PE) against CD_{105}, CD_{90}, CD_{73}, CD_{45} and CD_{34} (Chemicon, Temecula, USA) for 1.5 h at 4 °C [10,11]. The cells were washed and analyzed by a flow cytometer machine (FACS Caliber, BD, USA) and WinMDI software (USA, West Lafayette, IN).

2.4. Light irradiation

A handmade LED device (625 nm wavelength, 10-nm bandwidth; SE. Electronics, China) was used as light sources. LED power density was measured by appropriate meter (Melles-Griot, USA) and adjusted to 2.65 mW/cm^2. In addition, the LED spectrum was tested by the spectrometer (Avantes, Netherlands). The hUCM cells at a density of 1×10^4 cells were seeded onto 96-well culture plates and they were randomly allocated as control or the irradiated groups (polarized or non-polarized lights). After 24 h incubation period, the cells were irradiated once for 2, 4 or 6 min to achieve radiation energies of 0.318 J/cm^2, 0.636 J/cm^2 and 0.954 J/cm^2, respectively. The cells were kept inside the incubator during the LED irradiation under the similar conditions (37 °C, CO_2 5%) as the control group (non-irradiated cells). 48 h after irradiation, the cell proliferation was evaluated using WST-1.

2.5. Evaluation of cell proliferation by WST-1

The hUCM cells (passages 3–4) were trypsinized, and at a density of 1×10^4 were loaded into each well of standard 96-well culture plates and incubated for 24 h in a humidified CO2 incubator at 37 °C. Irradiated groups were irradiated by LED (see supra). After 48 h incubation, 10 μl Wst-1 reagent (Roche Co, Germany) was added to each well and following 1 min shaking, incubated again for 1.5 h. The optical density (OD) was measured at 450 nm (with a reference value of 630 nm) by an ELISA reader (Biotek Co, USA) instrument. The rate of cell survival and activity was measured by the following equation: (OD of irradiated groups/OD of control group) x 100. The experiments were repeated four times independently.

2.6. Evaluation of hUCM cells proliferation by Hoechst staining

The hUCM cells from the third passage were plated on sterile glass cover slips (1×10^4 cells) and incubated for 24 h. The cells were irradiated for 6 min (radiation energies of 0.954 J/cm^2) by PL or NPL according to the study groups. Afterward, the cells were cultured for 3, 6 and 9 days. The cells were stained with Hoechst for the assessment of cell proliferation. The cells in the control group were stained with Hoechst as well. Eventually, cell counting was done using a fluorescent microscope (x100, 5 field), the cells nuclei were counted in the midpoint (highest cell density area) of glass coverslip and the stage was then moved 2 mm forward, backward, right and left according to the center of glass coverslip [11].

2.7. Viability assay by trypan blue

The hUCM cells were cultured in a 35 mm plates (5×10^4 cells). LED irradiation was carried out similar to the Hoechst method; 3, 6 and 9 days later the cells were collected in 5 ml sterile tubes and centrifuged at 1500 rpm for 4 min 10 μl sample of tube was mixed with the same volume of 0.4% trypan blue. Finally, the cells were moved to an improved Neubauer slide and were counted by an optical microscope (Nikon, Japan) the experiments were repeated 3–5 times.

2.8. In vitro scratch assay

The hUCM cells at a density of 1×10^5 cells/dish were seeded onto 35 mm culture dishes. After reaching to more than 90% confluency to create a standard scratch, the cells were separated in a straight line by a 200-μl pipette tip [12]. In order to remove the cell debris, culture plates were washed by 1.5 ml PBS, and 2.5 ml culture medium was replaced. The scratch margins were marked using an ultrafine tip marker. The light irradiation (6 min, 0.954 J/cm^2) was performed and all culture dishes were kept in an incubator for 18 h, while scratch healing was assessed every 6 h.

2.9. CFU-F assay

The hUCM cells were plated at 100, 250 and 500 cells/dish in 35 mm culture dishes and incubated overnight. Light exposure (6 min, 0.954 J/cm^2) was carried out. 14 days after light irradiation the cells were washed twice with PBS and subsequently fixed with 4% paraformaldehyde for 35 min. The cultures were washed again with PBS and stained with methylene blue (0.5%, for 20 min). The dishes were rinsed three times with deionized water and dried at room temperature. The colonies (collection of at least 10 cells) were counted at 100X magnification with a light microscope. Colony forming efficiency (CFE) was calculated by the following equation: CFE = (Number of colony/number of initial plated cells) x 100. Also, colony diameters were assessed by appropriate software (Analysis. Olympus, Japan) and were compared between the groups.

2.10. Statistical analysis

Data were presented as the mean ± SD. We used one-way ANOVA and Tukey post hoc test for data analyzing. A P value less than 0.05 was considered statistically significant.

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