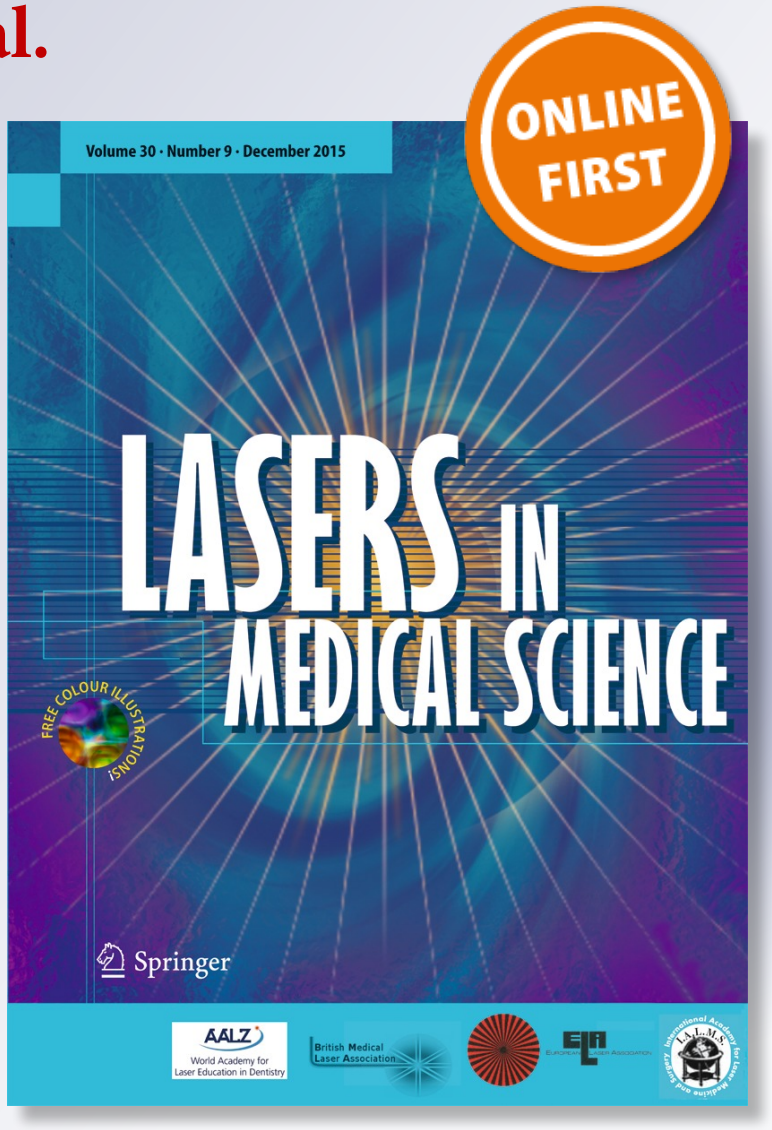
Different effects of energy dependent irradiation of red and green lights on proliferation of human umbilical cord matrix-derived mesenchymal cells

Samereh Dehghani Soltani, Abdolreza Babaee, Mohammad Shojaei, Parvin Salehinejad, Fatemeh Seyedi, Mahshid JalalKamali, et al.

Lasers in Medical Science

ISSN 0268-8921

Lasers Med Sci
DOI 10.1007/s10103-015-1846-y



Springer

Lasers Med Sci
DOI 10.1007/s10103-015-1846-y

ORIGINAL ARTICLE

Different effects of energy dependent irradiation of red and green lights on proliferation of human umbilical cord matrix-derived mesenchymal cells

Samereh Dehghani Soltani[1] · Abdolreza Babaee[1] · Mohammad Shojaei[2] · Parvin Salehinejad[1] · Fatemeh Seyedi[3] · Mahshid JalalKamali[4] · Seyed Noureddin Nematollahi-Mahani[1,5]

Received: 18 March 2015 / Accepted: 30 November 2015

Abstract Light-emitting diodes (LED) have recently been introduced as a potential factor for proliferation of various cell types in vitro. Nowadays, stem cells are widely used in regenerative medicine. Human umbilical cord matrix-derived mesenchymal (hUCM) cells can be more easily isolated and cultured than adult mesenchymal stem cells. The aim of this study was to evaluate the effect of red and green lights produced by LED on the proliferation of hUCM cells. hUCM cells were isolated from the umbilical cord, and light irradiation was applied at radiation energies of 0.318, 0.636, 0.954, 1.59, 3.18, 6.36, 9.54, and 12.72 J/cm^2. Irradiation of the hUCM cells shows a significant ($p<0.05$) increase in cell number as compared to controls after 40 h. In addition, cell proliferation on days 7, 14, and 21 in irradiated groups were significantly ($p<0.001$) higher than that in the non-irradiated groups. The present study clearly demonstrates the ability of red and green lights irradiation to promote proliferation of hUCM cells in vitro. The energy applied to the cells through LED irradiation is an effective factor with paradoxical alterations. Green light inserted a much profound effect at special dosages than red light.

✉ Seyed Noureddin Nematollahi-Mahani
nnematollahi@kmu.ac.ir

[1] Department of Anatomy, Afzalipour School of Medicine, Kerman University of Medical Sciences, Kerman, Iran

[2] Afzal Research Institute, Kerman, Iran

[3] Department of Anatomy, Jiroft University of Medical Sciences, Kerman, Iran

[4] Semiconductors Group, Photonics Research Center, Graduate University of Advanced Technology, Kerman, Iran

[5] Neuroscience Research Center, Institute of Neuropharmacology, Kerman University of Medical Sciences, Kerman, Iran

Keywords Light-emitting diode · Umbilical cord matrix-derived cells · Cell proliferation · Red and green lights

Introduction

Human umbilical cord matrix-derived mesenchymal (hUCM) cells have self-renewal potential and competence of multilineage differentiation. They can more easily be isolated and cultured than adult mesenchymal stem cell [1, 2]. Previous studies have reported that chemical agents including growth factors and hormones regulate cell proliferation [3, 4]. Physical factors such as low-level light irradiation provided by light-emitting diodes (LED) and low-energy lasers have recently been introduced as a potential factor for proliferation of various cell types including fibroblasts, osteoblast, keratinocytes, etc. [5–7]. Li et al. reported that LED irradiation promotes proliferation of human bone marrow mesenchymal stem cells [8]. Proliferation of these cells was shown to be associated with photobio-modulation effects that increase cytoplasmic calcium ion content, activate protein synthesis, and promote cell growth and cell division [9, 10]. Nowadays, low-level light therapy is widely used in treatment of dermatitis, veterinary medicine, sports medicine, and wound healing [11–13]. Compared to lasers, LEDs are cost-effective and light-weight. Also, according to the experiments, LEDs are an effective alternative to lasers for photo-stimulation and photodynamic therapy [8, 14, 15]. In the recent years, the red and green light have been found to affect various biological processes [16–18]. On the other hand, improvements of stem cells proliferation capacity are vital for the further advance of cell culture and tissue engineering. Thus, we aimed at this study to evaluate the effect of red and green lights produced by LED on the

Published online: 29 December 2015

proliferation of human umbilical cord matrix-derived mesenchymal cells.

Materials and methods

Isolation and culture of hUCM cells

All the materials were purchased from Sigma company (Sigma-alderich, Mo, USA) unless stated otherwise. Institutional ethical review board committee at Kerman University of Medical Sciences, Kerman, Iran, approved the study. A written consent was taken from parents for the use of umbilical cord. hUCM cells were harvested from normal and fresh neonatal human umbilical cords (UCs) as described elsewhere [19] with minor modifications. Briefly, UCs were transferred to the laboratory in Hanks' balanced salt solution (HBSS) supplemented with 50 mg/mL streptomycin sulfate and 100 IU/mL penicillin.

Wharton's jelly was then divided into 2- to 3-mm pieces and loaded onto Petri dishes (Falcon BD, Franklin Lakes, NJ, USA). Dulbecco's modified Eagle's medium (DMEM/F12), supplemented with 10 % FBS (PA Biologicals, Sydney, Australia), 100 μg/ml streptomycin, and 100 μg/ml penicillin, was added for 2 weeks. Half of the medium was replaced every 3 to 4 days until the cells migrated from the fragment margins and reached 80 % confluence. Passage 2 to 4 hUCM cells were used for the experiments.

Evaluation of the differentiation potential of hUCM cells

Differential capability of hUCM cells were assessed with osteogenic and adipogenic differentiation of these cells. hUCM cells (passages 3–4) at a density of 2×10^4 cells/cm^2 were seeded onto sterile glass slides with DMEM/F12 supplemented with either adipogenic (100 nM dexamethasone and 50 μg/ml indomethacin) or osteogenic (50 μg/ml ascorbic acid, 10 nM dexamethasone, and 10 mM β-glycerophosphate) differentiation medium for 21 days. The medium was refreshed every 3 days. Cells induced with the adipogenic and osteogenic formulas were stained with Oil Red O and Alizarin Red S, respectively [3, 20].

Flow cytometry analysis

To assess surface antigen expression, third-passage hUCM cells were detached and approximately 2×10^5 viable cells were loaded into 5-ml tubes. The cells were then washed, fixed with 10 % formaldehide, and incubated for 30 min at 4 °C. After centrifugation and washing, the cells were suspended in 10 % normal goat serum in PBS to block nonspecific binding sites. The cells were labeled with the following antibodies: phyco-erythrin (PE)-conjugated-CD73, PE-conjugated-CD34, PE-conjugated-CD105 (Chemicon, Temecula, CA), and PE-conjugated-CD90 (Dako, Glostrup, Denmark) for 1 h at 4 °C [21, 22]. The control sample was stained with matched isotype antibody (PE-conjugated mouse IgG monoclonal isotype standard). At least 10,000 events were recorded for each sample by a flow cytometer machine (BD Biosciences), and data were analyzed using Win MDI software (West Lafayette, IN).

Light irradiation

In this study, a handmade LED device was used. This device consisted of green (532 nm with 20-nm bandwidth) and red (630 nm with 10-nm bandwidth from SE Electronics, China) lights. The power density and distribution was measured by appropriate meters (Melles-Griot, US) and adjusted to 5.3 mW/cm2. The spectrum of the LEDs was checked by the spectrometer (Avantes, The Netherlands). The LED array was designed to fit into standard 96-well culture plates. These plates were randomly assigned as control or the treated groups (green or red LEDs). The cells were irradiated once for 1, 2, 3, 5, 10, 20, 30, or 40 min at radiation energies of 0.318, 0.636, 0.954, 1.59, 3.18, 6.36, 9.54, and 12.72 J/cm^2, respectively. All the exposed cells were maintained inside the incubator during the irradiation under the same conditions as the non-exposed cells (control). Forty-eight hours after irradiation, the cells were examined for cell viability and proliferation.

Determination of cell proliferation by Wst-1

hUCM cells were trypsinized, and 1×10^4 cells were loaded into each well of 96-well culture plates. The cells were incubated at 37 °C with 5 % CO_2 in the humidified air for 24 h. The treatment groups received various LED irradiations, and a control group received no irradiation. After 48-h incubation, 10 μl WST-1 (Roche, Germany) reagents were added to each well and incubated for 1.5 h in humid incubator at 37 °C. The optical density (OD) was measured at 450-nm wavelength with a reference value of 630 nm using an ELISA reader instrument (BIOTEK, USA). Experiments were repeated at least three times in triplicate. The rate of cell activity and survival was calculated by the following equation: (OD of treatment/OD of control) × 100.

Characterization of hUCM cells proliferation by Hoechst staining

hUCM cells were cultured at a density of 1×10^4 cells/cm^2 on clean, sterile glass coverslips and incubated for 24 h at 37 °C with 5 % CO2 in the air. These cells were irradiated for 1 min and 5 min (radiation energies of 0.318 and 1.59 J/cm^2) by red

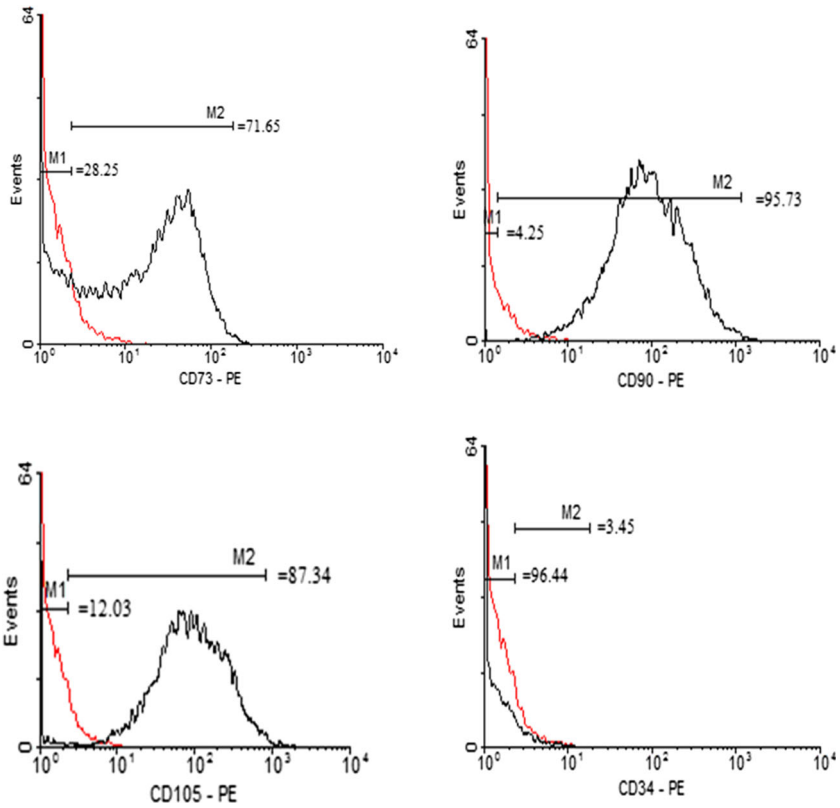


Fig. 1 hUCM cells flow cytometric analysis. The *black histograms* demonstrate the antibody stained cells, and the *red histograms* demonstrate the isotype control-stained cells

and green LEDs, respectively. Afterward, the cells were cultured in DMEM/F12 supplemented with 10 % FBS, 100 U/ml penicillin, and 100 μg/ml streptomycin for 7, 14, and 21 days. The slides were stained with Hoechst for the evaluation of cell proliferation. The slides in the control group (non-exposed cells) were stained with Hoechst as well. Cell counting was performed by a fluorescent microscope at ×200, with the following method: Cells nuclei were counted in the center of glass coverslip (highest cell density area) and the stage was then moved 2 mm forward, backward, left, and right according to the center of slide.

Viability assay by trypan blue

A total of 1.5×10^5 viable cells were seeded into 3-cm plates containing DMEM/F12 supplemented with 10 % FBS. Light irradiation was similar to Hoechst method (see supra). Forty-eight hours later, the cells (floating and trypsin-detached cells) were collected in 15-mL tubes and centrifuged at 2700 rpm for 3 min, and a sample was mixed with equal volume of trypan blue. The cells were then transferred to a Neubauer hemocytometer slide and were counted using an optical microscope (Nikon, TS100, Japan). Viable cells do not take up the dye and

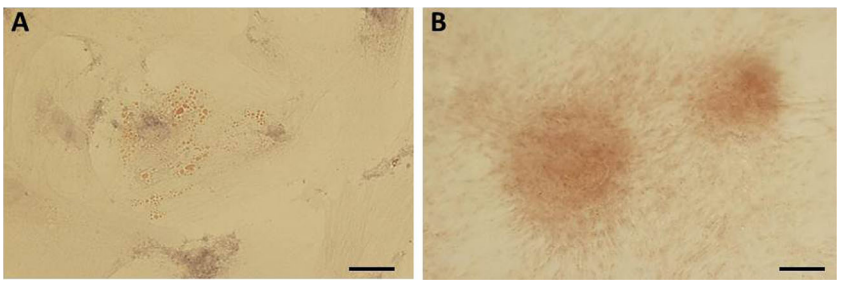


Fig. 2 Adipogenic and osteogenic differentiation of hUCM cells. Oil Red O staining (**a**) showed a collection of lipid-containing vacuoles. Subsequent to osteogenic induction, formation of calcium deposits by means of Alizarin Red S staining (**b**) was visible. (Scale bars: **a** 20 μm; **b** 40 μm)

appear bright, while dead cells uptake the dye and appear blue. Each plate had three repeats of counting. Experiments were repeated at least three times, and the mean was used for analysis.

Statistical analysis

Data were presented as the mean ± SD. One-way ANOVA followed by Tukey post hoc test was used for data processing. A difference of $p < 0.05$ was considered statistically significant.

Results

Features of hUCM cells

Twelve days after the umbilical cord Wharton's jelly pieces were cultured, fusiform, fibroblast-like cells were detected at the boundary of the explants. The isolated cells expressed mesenchymal stem cells markers CD_{73}, CD_{90}, and CD_{105} (71.6, 95.7, and 87.3 %, respectively) but did not express hematopoietic cell surface marker CD_{34} (3.4 %) (Fig. 1).

Adipogenic differentiation of hUCM cells was assessed after 18 days of adipogenic treatment. A collection of lipid-containing vacuoles was discovered with Oil Red O staining (Fig. 2a). Osteogenic activity was determined by the formation of calcium deposits in extracellular matrix detected by means of Alizarin Red S staining (Fig. 2b). No Alizarin Red S- or Oil Red O-positive cells were observed in the control cultures.

Determination of cell proliferation by Wst-1

The results, presented in Fig. 3, show that cell proliferation measured by means of Wst-1 assay revealed a significant ($p < 0.05$) increase in hUCM proliferation and activity in comparison to non-irradiated controls. The red probe at all radiation energies, except 9.54 and 12.72 J/cm^2, provided higher result than the control non-irradiated culture. However, no statistical significant differences were detected among the groups. In addition, the green probe induced a non-significant increase of cells at all radiation energies except 9.54 J/cm^2. Furthermore, the green probe provided a higher increase in the number of cells (0.636 and 3.18 J/cm^2, $p < 0.05$; 0.954 and 1.59 J/cm^2, $p < 0.01$) than the red probe.

Characterization of hUCM cells proliferation by Hoechst staining

Hoechst staining was used to determine whether green and red LED's treatment could enhance hUCM proliferation over a long period of cultivation. The results showed that the cell proliferation on days 7, 14, and 21 in irradiated groups was significantly ($p < 0.001$) higher than that in the non-irradiated groups (Fig. 4). Moreover, the cell number at 7 and 14 days post-irradiation in green light group was significantly (day 7 $p < 0.001$; day 14 $p < 0.01$) higher than that in red light irradiated group. However, on day 21, the red probe induced a significant ($p < 0.01$) increase of cells compared with green probe (Fig. 5a).

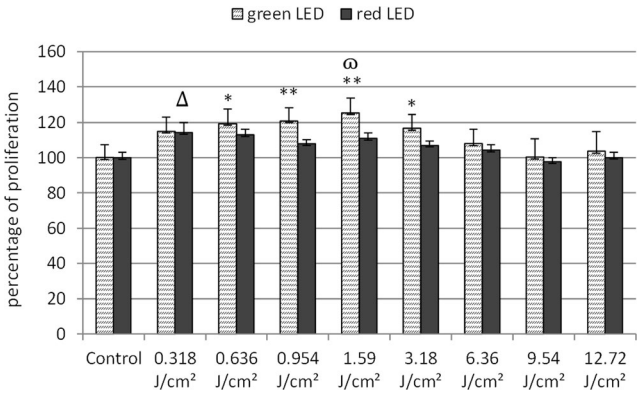


Fig. 3 Significantly higher proliferation of hUCMs was observed after 48 h exposure to green and red LED irradiation compared with the control groups (, different from the control group in green light at $p < 0.05$; Δ, different from the control group in red light at $p < 0.05$). Comparison of the results in green and red light irradiation show that the green probe resulted in a significant increase in the number of hUCM cells (0.636 and 3.18 J/cm^2, $p < 0.05$; 0.954 and 1.59 J/cm^2, $p < 0.01$)

Viability assay by trypan blue

The number of viable cells in LED's treatment groups was higher in comparison to control group, but there was no significant difference between the groups (Fig. 6).

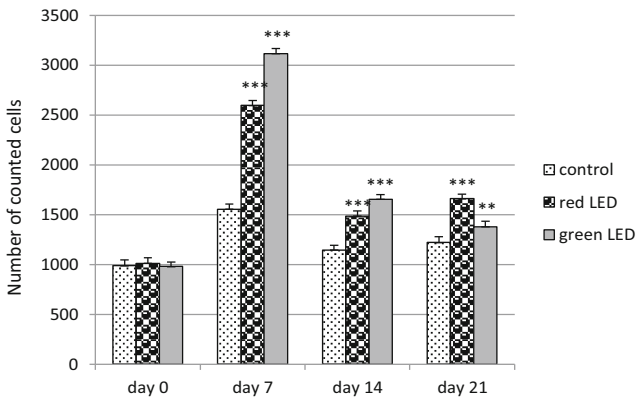


Fig. 4 Results of hUCM cells proliferation by Hoechst staining. The data showed that the cell proliferation on days 7, 14, and 21 in irradiated groups were significantly higher than that in the control groups ($p < 0.001$)

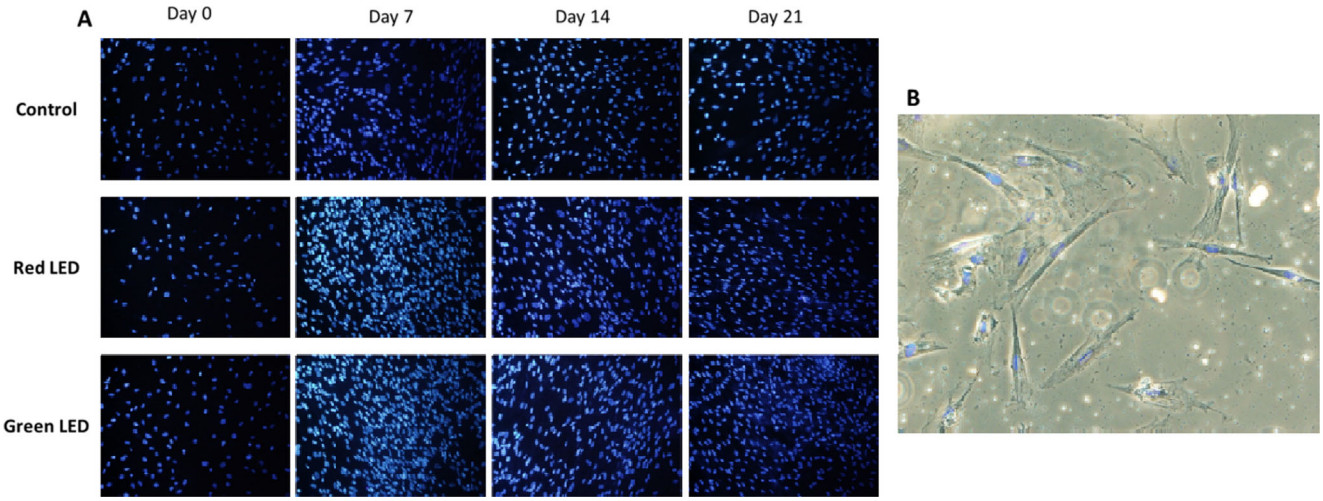


Fig. 5 Hoechst staining was used to assess the cell proliferation on days 0, 7, 14, and 21 (**a**). hUCM cells at passage 3 are a monolayer of large, flat cells under phase contrast; nuclei of these cells are labeled with Hoechst (**b**)

Discussion

In the present study, the effects of red and green lights at different energy dosages on hUCM cells were investigated. The previous photobiostimulation studies using LEDs or low-power lasers have shown enhanced cellular proliferation, activity, and maturation [16, 23, 24]. These effects have been observed for osteoblast and fibroblast cultures consistently [7, 25]. The mechanism of cell proliferation following light stimulation is not fully understood, but it has been attributed to an increase in oxidative metabolism of mitochondria [26, 27]. The absorption of LED photons by the cytochrome c oxidase (a respiratory chain enzyme) increases production of ATP which may accelerate mitoses [28]. The way light interacts with the cells will depend on the parameters and characteristics of light devices, mainly the exposure time and wavelength, and/or the cell types used [29, 30].

In a recent study, Peng et al. have shown that proliferation of bone marrow mesenchymal stem cells can be promoted by LED irradiation [31]. In our study, the results of the WST-1 demonstrated that LED irradiation is also capable of stimulating proliferation of hUCM cells cultured in conventional environment. Based on the results of WST-1 test, the LED irradiation (at the lower doses) increases cell proliferation while later (higher doses), it reverses the trend. Whether higher doses of green and red LED would alleviate, a stressful condition on living cells needs to be investigated. Reactive oxygen species (ROS) and NO measurements may elucidate the mechanisms underlying dual function of red and green LED irradiation. Mesenchymal stem cells are hopeful for use in regenerative medicine [21]; hence, appropriate in vitro conditions such as chemical or physical inducers are a necessary factor for improved proliferation of these cells. Ong et al. reported that green light-emitting diode can activate the motility of stem cells isolated from human orbital fat tissue [32]. In agreement with Ong study, we showed that green LED (570 nm) irradiation improves hUCM cells proliferation at different radiation energies especially 1.59 J. Motility and proliferation of stem cells result in an effective tissue repair [33]. To achieve more population of stem cells for clinical applications, numerous approaches have been introduced to maintain and accelerate the proliferation potentialities [34, 35]. Whether, other types of LED wavelength and the consequence of irradiation would alter the proliferation capacity of mesenchymal stem cells needs to be investigated.

Conclusions

Our results demonstrated that human umbilical cord matrix-derived mesenchymal cells could successfully be induced for

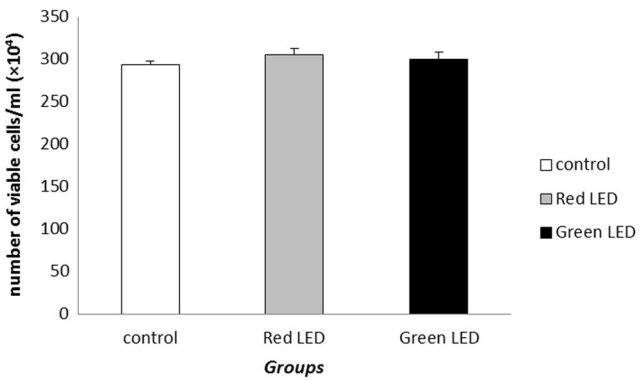


Fig. 6 Number of viable cells/ml ($\times 10^4$) detected by the trypan blue assay

higher proliferate rate by green and red LEDs. The energy applied to the cells through LED irradiation is an effective factor with paradoxical alterations. Green light inserted a much profound effect at special dosages than red light.

Acknowledgments S. Dehghani-soltani was a MSc student at Department of Anatomy, Afzalipour School of Medicine, Kerman, Iran.

Compliance with ethical standards Institutional ethical review board committee at Kerman University of Medical Sciences, Kerman, Iran, approved the study. A written consent was taken from parents for the use of umbilical cord.

Funding Kerman University of Medical Sciences Research affair supported this research by a grant numbered 92–58.

Conflict of interest The authors declare that they have no conflict of interest

Informed consent The mothers donated umbilical cords after a written informed consent was obtained

References

1. Karahuseyinoglu S, Cinar O, Kilic E, Kara F, Akay GG, Demiralp DO et al (2007) Biology of stem cells in human umbilical cord stroma: in situ and in vitro surveys. Stem Cells 25(2):319–331
2. Ma L, Feng XY, Cui BL, Law F, Jiang XW, Yang LY et al (2005) Human umbilical cord Wharton's Jelly-derived mesenchymal stem cells differentiation into nerve-like cells. Chin Med J (Engl) 118(23):1987–1993
3. Salehinejad P, Alitheen NB, Mandegary A, Nematollahi-Mahani SN, Janzamin E (2013) Effect of EGF and FGF on the expansion properties of human umbilical cord mesenchymal cells. In Vitro Cell Dev Biol Anim 49(7):515–523
4. Pountos I, Georgouli T, Henshaw K, Bird H, Jones E, Giannoudis PV (2010) The effect of bone morphogenetic protein-2, bone morphogenetic protein-7, parathyroid hormone, and platelet-derived growth factor on the proliferation and osteogenic differentiation of mesenchymal stem cells derived from osteoporotic bone. J Orthop Trauma 24(9):552–556
5. Agha R, Beeson W, Beeson WH (2012) In vitro comparison of light-emitting diodes and carnosic acid effects on keratinocyte proliferation and wound healing. Am J Cosmet Surg 29(1):30–37
6. Aleksic V, Aoki A, Iwasaki K, Takasaki AA, Wang C-Y, Abiko Y et al (2010) Low-level Er: YAG laser irradiation enhances osteoblast proliferation through activation of MAPK/ERK. Lasers Med Sci 25(4):559–569
7. Vinck EM, Cagnie BJ, Cornelissen MJ, Declercq HA, Cambier DC (2003) Increased fibroblast proliferation induced by light emitting diode and low power laser irradiation. Lasers Med Sci 18(2):95–99
8. Li W, Chen H, Wang C (2006) Effect of light emitting diode irradiation on proliferation of human bone marrow mesenchymal stem cells. J Med Biol Eng 26(1):35
9. Passarella S, Karu T (2014) Absorption of monochromatic and narrow band radiation in the visible and near IR by both mitochondrial and non-mitochondrial photoacceptors results in photobiomodulation. J Photochem Photobiol B Biol 140:344–358
10. Kreslavskia VD, Fominaa IR, Losc DA, Carpentierd R, Kuznetsovc VV, Allakhverdieva SI (2012) Photochemistry reviews. J Photochem Photobiol C: Photochem Rev 13:190–203
11. Fekrazad R, Seraj B, Ghadimi S, Tamiz P, Mottahary P, Dehghan M-M (2014) The effect of low-level laser therapy (810 nm) on root development of immature permanent teeth in dogs. Lasers Med Sci 1–7
12. Min PK, Goo BL (2013) 830 nm light-emitting diode low level light therapy (LED-LLLT) enhances wound healing: a preliminary study. Laser Ther 22(1):43
13. Leal ECP, Lopes-Martins RB, Frigo L, De Marchi T, Rossi RP, De Godoi V et al (2010) Effects of low-level laser therapy (LLLT) in the development of exercise-induced skeletal muscle fatigue and changes in biochemical markers related to postexercise recovery. J Orthop Sports Phys Ther 40(8):524–532
14. Sampaio SCPO, de C Monteiro JS, Cangussu MCT, Santos GMP, dos Santos MAV, dos Santos JN et al (2013) Effect of laser and LED phototherapies on the healing of cutaneous wound on healthy and iron-deficient Wistar rats and their impact on fibroblastic activity during wound healing. Lasers Med Sci 28(3):799–806
15. de Sousa APC, Paraguassu GM, Silveira NTT, de Souza J, Cangussu MCT, dos Santos JN et al (2013) Laser and LED phototherapies on angiogenesis. Lasers Med Sci 28(3):981–987
16. Holder MJ, Milward MR, Palin WM, Hadis MA, Cooper PR (2012) Effects of red light-emitting diode irradiation on dental pulp cells. J Dent Res 91(10):961–966
17. Fushimi T, Inui S, Nakajima T, Ogasawara M, Hosokawa K, Itami S (2012) Green light emitting diodes accelerate wound healing: characterization of the effect and its molecular basis in vitro and in vivo. Wound Repair Regen 20(2):226–235
18. Vinck EM, Cagnie BJ, Cornelissen MJ, Declercq HA, Cambier DC (2005) Green light emitting diode irradiation enhances fibroblast growth impaired by high glucose level. Photomed Laser Surg 23(2):167–171
19. Salehinejad P, Alitheen NB, Nematollahi-Mahani SN, Ali AM, Omar AR, Janzamin E et al (2012) Effect of culture media on expansion properties of human umbilical cord matrix-derived mesenchymal cells. Cytotherapy 14(8):948–953
20. Kaviani M, Ezzatabadipour M, Nematollahi-Mahani SN, Salehinejad P, Mohammadi M, Kalantar SM et al (2014) Evaluation of gametogenic potential of vitrified human umbilical cord Wharton's jelly-derived mesenchymal cells. Cytotherapy 16(2):203–212
21. Wang L, Ott L, Seshareddy K, Weiss ML, Detamore MS (2011) Musculoskeletal tissue engineering with human umbilical cord mesenchymal stromal cells. Regen Med 6(1):95–109
22. Wang HS, Hung SC, Peng ST, Huang CC, Wei HM, Guo YJ et al (2004) Mesenchymal stem cells in the Wharton's jelly of the human umbilical cord. Stem Cells 22(7):1330–1337
23. AlGhamdi KM, Kumar A, Moussa NA, Hassan A (2012) Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells. Lasers Med Sci 27(1):237–249
24. Li W-T, Leu Y-C, Wu J-L (2010) Red-light light-emitting diode irradiation increases the proliferation and osteogenic differentiation of rat bone marrow mesenchymal stem cells. Photomed Laser Surg 28(S-157):S-157–S-165
25. Khadra M, Lyngstadaas SP, Haanaes HR, Mustafa K (2005) Effect of laser therapy on attachment, proliferation and differentiation of human osteoblast-like cells cultured on titanium implant material. Biomaterials 26(17):3503–3509
26. Li S, Wang Q-L, Chen X, Mi X-q (2014) Photobiomodulation for cobalt chloride-induced hypoxic damage of RF/6A cells by 670 nm light-emitting diode irradiation. Int J Photoenergy 2014, 971491
27. Wong-Riley MTT, Bai X, Buchmann E, Whelan HT (2001) Light-emitting diode treatment reverses the effect of TTX on cytochrome oxidase in neurons. Neuroreport 12(14):3033–3037

28. Ekizer A, Uysal T, Guray E, Yuksel Y (2013) Light-emitting diode photobiomodulation: effect on bone formation in orthopedically expanded suture in rats—early bone changes. Lasers Med Sci 28(5):1263–1270
29. Asai T, Suzuki H, Kitayama M, Matsumoto K, Kimoto A, Shigeoka M et al (2014) The long-term effects of red light-emitting diode irradiation on the proliferation and differentiation of osteoblast-like MC3T3-E1 cells. Kobe J Med Sci 60(1): E12–E18
30. Wu HP, Persinger MA (2011) Increased mobility and stem-cell proliferation rate in Dugesia tigrina induced by 880nm light emitting diode. J Photochem Photobiol B 102(2):156–160
31. Peng F, Wu H, Zheng Y, Xu X, Yu J (2012) The effect of noncoherent red light irradiation on proliferation and osteogenic differentiation of bone marrow mesenchymal stem cells. Lasers Med Sci 27(3):645–653
32. Ong W-K, Chen H-F, Tsai C-T, Fu Y-J, Wong Y-S, Yen D-J et al (2013) The activation of directional stem cell motility by green light-emitting diode irradiation. Biomaterials 34(8):1911–1920
33. Seo Y-K, Park J-K, Song C, Kwon S-Y (2014) Comparison of light-emitting diode wavelength on activity and migration of rabbit ACL cells. Lasers Med Sci 29(1):245–255
34. Sensebé L, Bourin P (2009) Mesenchymal stem cells for therapeutic purposes. Transplantation 87(9S):S49–S53
35. Indrawattana N, Chen G, Tadokoro M, Shann LH, Ohgushi H, Tateishi T et al (2004) Growth factor combination for chondrogenic induction from human mesenchymal stem cell. Biochem Biophys Res Commun 320(3):914–919