



# Modulatory effect of photobiomodulation on stem cell epigenetic memory: a highlight on differentiation capacity

Arezoo Rezaie Nezhad Zamani<sup>1</sup> · Shirin Saberianpour<sup>1</sup> · Mohammad Hossein Geranmayeh<sup>2</sup> · Farhad Bani<sup>3</sup> · Leila Haghighi<sup>4</sup> · Reza Rahbarghazi<sup>1,5</sup>

Received: 16 May 2019 / Accepted: 3 September 2019 / Published online: 7 September 2019  
© Springer-Verlag London Ltd., part of Springer Nature 2019

## Abstract

Differentiation potential of stem cells into various lineages makes these cells as promising sources to treat multiple diseases. In this regard, the use of different strategies and protocols to increase differentiation capacity is highly demanded. Low-level laser therapy, a relatively noninvasive technique, has the capacity to accelerate the healing of numerous injuries and a portion of restorative capacity could be correlated with the stem cell activation and differentiation. Several mechanisms have been diagnosed to participate in orientation of stem cells to functional mature cells. Among them, the status of DNA methylation orchestrates the maintenance of tissue-specific gene expression during the differentiation procedure. DNA methylation is a momentous event in embryogenesis and functional maturation. This review article highlighted the potency of laser irradiation (low-level intensities) in the differentiation of stem cells by modulation of methylation. The analysis of these modalities could help us to understand the underlying mechanisms participating in the therapeutic effects of photobiomodulation.

**Keywords** Stem cells · Low-level laser irradiation · DNA methylation · Differentiation

## Introduction

Stem cells (SCs) are touted as a unique cell source to alleviate various diseases. These cells are in null phenotype and have the potential to proliferate, preserve self-renewal activity, and differentiate into different multiple cell phenotypes. It seems that numerous stimuli and key signals could hamper/harness these bioactivities [1, 2]. Along with these descriptions, SC therapy paves a way for a promising treatment approach in the cases that conventional modalities are ineffective [3, 4]. Based

on the previous data, SCs are classified into embryonic stem cells (ESCs), adult stem cells (ASCs), and fetal stem cells (FSCs). It has been demonstrated that SCs encompass heterogeneous populations not only given to their origins but also in the case of trans-differentiation capacity [5, 6].

ESCs are defined as primordial cells to be used in modern biology and human medicine [7]. ESCs are originated from the inner cell mass of human blastocysts during embryogenesis. In the term of differentiation, ESCs display dramatic potential of self-renewal and differentiation with potency to generate all cell types in vivo [8]. Despite a high potential differentiation capacity, the application of ESCs is limited according to immune cells response [9].

In contrast to ESCs, ASCs are present in various tissues especially adult bone marrow, etc. Compared to the ESCs, these cells possess only limited differentiation capacity with an orientation to specific cell lineages. Two main types of bone marrow-derived SCs include hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs) which have been used extensively by authorities in the field of tissue engineering and SC biology [10, 11]. In most of these experiments, the underlying mechanisms participating in cell orientation promote differentiation by genetic elements and protein effectors.

✉ Reza Rahbarghazi  
Rezarahbardvm@gmail.com; rahbarghazi@tbzmed.ac.ir

<sup>1</sup> Stem Cell Research Center, Tabriz University of Medical Sciences, Tabriz, Iran  
<sup>2</sup> Neuroscience Research Center, Imam Reza Medical Center, Tabriz University of Medical Sciences, Tabriz, Iran  
<sup>3</sup> Department of Medical Nanotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran  
<sup>4</sup> Department of Parasitology and Mycology, School of Medicine, Iran University of Medical Sciences, Tehran, Iran  
<sup>5</sup> Department of Applied Cell Sciences, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran

## Effect of methylation/demethylation in SC differentiation

The gradual conversion of a single fertilized egg into the formation of a complete organism is an intricate process procedure. In addition to the production of numerous cell number, it requires the acquisition of functional activity and morphologies in a well-designed 3D niche [12]. Cell differentiation is defined as a crucial process in which distinct cell phenotype acquires specific function and profound alterations in the gene profile [13] (Table 1). In this regard, trans-differentiation of SCs is the most common changes seen inside the body [23]. Numerous experiments showed that differentiation of embryonal cells happens in cells at the G<sub>1</sub> phase. In this phase, cells are sensitive to external signals and the accumulation of distinct transcription factors that promote cell differentiation [24, 25]. In addition to numerous de novo molecular adaptations, chromatin modification involved a pattern termed bivalent domains. The region Lys27 methylation related to pluripotency consisted of smaller regions of Lys4 methylation participate in chromatin modification. Therefore, methylation of these regions is commonly seen during differentiation of ESCs [26]. During ESC differentiation, most of the bivalent

regions become monovalent and devoid of activating and inhibiting factors [26, 27]. The change of transcriptional and methylation pattern has a fundamental role in cell conversion to the specific cell type [28]. In addition to the complete gene suppression and/or expression during SC differentiation toward various lineages, some reversible modification could affect gene activity. Of these changes, epigenetic programming commonly happens inside the SCs committed to specific lineage [13].

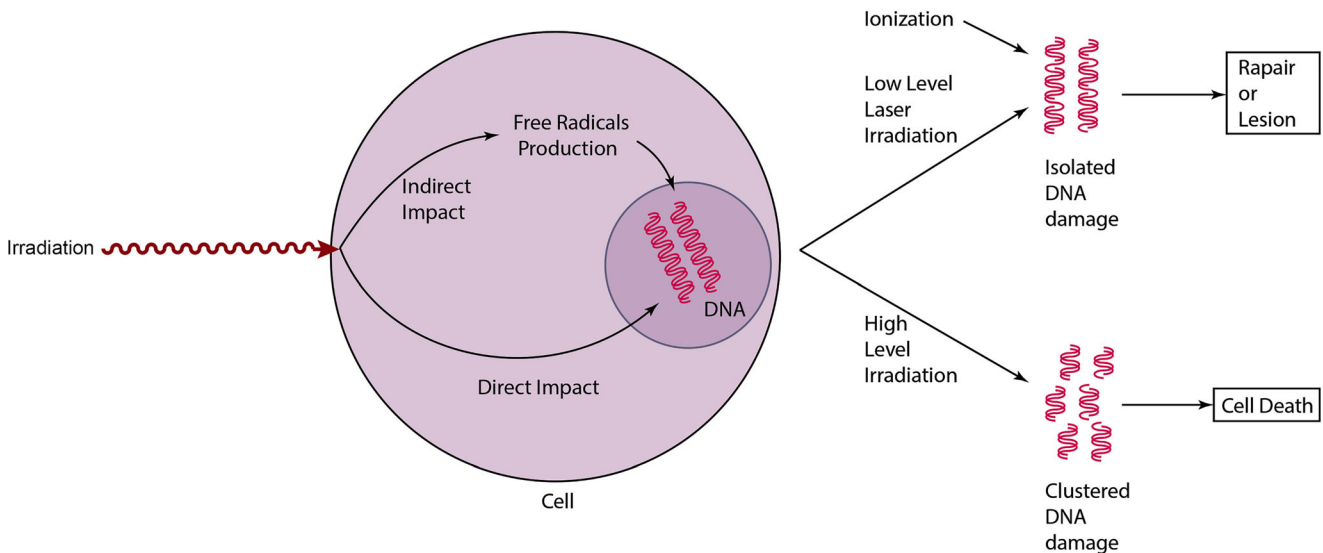
The epigenetic modification includes changes in gene bio-activity without any alterations on structure and sequence. These modifications are viewed as the reversible, heritable events and mostly comprise of methylation, acetylation, and histone modification [29] (Fig. 1). DNA methylation is conceived as the common investigated epigenetically event with a highly significant role in controlling gene activity during SC differentiation [30, 31]. This phenomenon has critical effects on the stemness maintenance and the preservation of lineage orientation toward various cell types, and in some circumstances, it is described as an epigenetic memory of SCs [23].

To methylate gene, the methyl group is added by DNA methyltransferases (DNMTs) on carbon 5 of the cytosine into CpG sequences during SC development and differentiation

**Table 1** Effect of laser on cell differentiation

Study	Laser type	Exposure duration	Irradiation impact	Ref.
Kim et al.	He–Ne	20 s/day from day 0–20 (1.2 J/cm <sup>2</sup> )	There is no obvious difference in the number of cytokeratin-positive cells between the irradiated and non-irradiated ASCs	[14]
Leonida et al.	Nd:YAG	Three irradiation cycles (30 s each cycle) separated by 30-s intervals with two intensities (18.75 and 28.1 W/cm <sup>2</sup> ) were used	Promotes osteoblastic differentiation of MSCs	[15]
Abramovitch-Gottlib et al.	He–Ne	10 min per day from days 1 to 28 (~0.5 mW/cm <sup>2</sup> )	Increased differentiation of MSCs into the osteoblastic lineage	[16]
Bernard Mvula and Heidi Abrahamse	Diode laser	9 min and 10 s (5 J/cm <sup>2</sup> )	Increased differentiation of ADSCs into smooth muscle cells	[17]
Soleimani et al.	GaAlAs	Osteoblastic differentiation: 12 s (2 J/cm <sup>2</sup> ) and 24 s (4 J/cm <sup>2</sup> ); Neuron differentiation: 18 s (3 J/cm <sup>2</sup> ) or 36 s (6 J/cm <sup>2</sup> ) at days 1, 3, and 5	Enhanced BMSCs differentiates into neuron and osteoblast-like cells	[18]
Hou et al.	InGaAsP	Exposure duration for 75, 150, 300, and 750 s with energy density of 0.5, 1.0, 2.0, and 5.0 J/cm <sup>2</sup> , respectively.	Increased myogenic differentiation of BMSCs	[19]
Wang et al.	The LED array, Filtered lamp, Diode laser	188 s (3 J/cm <sup>2</sup> ) in different wavelength five times (every 2 days) on hASCs cultured in the osteogenic medium over 3 weeks	Promoted osteoblastic differentiation of hASCs	[20]
Zhang et al.	LLL	ND*	Increased differentiation of BMSC into osteoblast	[21]
Stein et al.	He–Ne	On days 2 and 3 after seeding for 3 s or different durations (1, 3, and 10 s) (at a power density of 180 mW/cm <sup>2</sup> ) that corresponds to 0.14, 0.43, and 1.43 J/cm <sup>2</sup>	Increase in the differentiation of human osteoblast cells	[22]

ND none determined



**Fig. 1** The direct and indirect effects of low-level laser irradiation on target DNA and genome pool. A prolonged and or high irradiation doses could contribute to DNA damage and irreversible outcomes

[32]. There are two types of DNMTs. Dnmt1 is a basic enzyme and targets nucleotide cytosines at hemimethylated CpG sites [33]. This enzyme plays a key role in SC differentiation and self-renewal pre and postnatal periods [34]. In addition to type 1 Dnmt, Dnmt3a and 3b also take part in the methylation of DNA by the converting unmethylated sites into methylated form [35]. Of note, epigenetic procedures can regulate gene expressions which accompanied with modification of chromatin structure in specific phases of SCs entered to development and differentiation by two pathways that include the prohibition binding specific transcription factors and collaboration of proteins containing methyl-CpG-binding domain (MBD) that eventually suppress the target genes [32, 36]. Experiments show that DNA methylation could be also termed epigenetic programming because of developmental genes silencing and activation of tissue-specific genes. In contrast, the epigenetic pattern can be modulated in phenomena called reprogramming and anaplastic changes. Under these conditions, epigenetic factors become silent, contributing to the acquisition of developmental potential [13]. Therefore, one could hypothesize that there is a reverse relationship between methylation and multipotentiality in SCs. Commensurate with this claim, various experiments have verified the inherent role of epigenetic modifications on SC differentiation by using chromatin-modifying drugs in order to investigate the differentiation capacity of SCs to several lineages [29]. Demethylation of the promoter is accompanied by activation of specific genes in various stage of the development process. It should mention that while differentiating, promoters become more methylated [34]. Epigenetic modifications on gene promoters may lead to dual influences, having said that it facilitates (or avoid) the recruitment of additional chromatin modifying enzymes or transcriptional regulators that would

drive SC differentiation [29]. In addition to modulation of epigenetic indices in distinct cell types, experiments show that remarkable quantity of epigenetic information inside somatic cells is transferred to daughter cells. In ESCs, the balance between hypo/hypermethylation of genetic pool could predetermine cell fate and differentiation into specific lineages. The decrease of methylation rate, for example in ESCs, could trigger cell orientation to specific lineage and expression of differentiation-associated markers [37]. SC renewal and differentiation requires selective transcriptome profile and is promoted by an inevitable dialog between transcription factors and epigenetic modulators, changing the structure of chromatin and packaged eukaryotic genome. Epigenetic changes cause to the regulation of the memory of active and silent gene states and determine SC fates [38]. Therefore, the impact of epigenetic modifications in the maintenance/loss of the multipotentiality and differentiation process is an area of intense care in SC biology [35]. In addition to adult SCs, DNA methylation is commonly seen in the embryonic stage. The blastocyst stage is characterized by a high rate of epigenetic activities such as DNA methylation, inactivation of X chromosomes, and remodeling of chromatin.

Conducted researches related to in vitro fertilization evaluation have surprisingly indicated the existence of multiple epigenetic aberrations in human embryos at the early-stage development, inspiring the fact that expanded ESCs differ in the light of epigenetic status; thereby, these unique characteristics contribute to diverse differentiation capacity. However, a vivid consensus can be inspired regarding distinct epigenetic features in various hESC cell lines. Of note, the conditions which these cells are expanded possibly alter epigenetic status. Despite these comments, consolidate data exist neither about the stability of ESCs epigenetic profile after prolonged in vitro

culture nor how it may change as the cells differentiate along different developmental pathways [39].

## Natural effects of photobiomodulation

Low-level laser therapy (LLLT) or photobiomodulation (PBM) is defined as a photochemical process relies on production of low intensity of light by lasers [40]. By using LLLT, low power irradiation is used on the target biological sites without heat generation, so it is also touted as “cold laser” therapy in the field of cancer and regeneration [41]. Regarding different side effects of conventional treatments, scientists and researchers try to find novel treatment modalities in order to limit unwanted consequences in candidate patients. In this regard, LLLT is one of the promising procedures which accompanied with trivial side effects on body surface such as cutaneous irritation, itching, and redness [42]. In contrast to other modalities, the advantages of LLLT hugely outweigh the existing negligible drawbacks and disadvantages [43]. To evaluate the efficacy of LLLT, various parameters related to laser type and target tissue consistency or cell response should be noted. As a matter of fact, the wavelength of the laser, power density, exposure time, and energy density is critical [44]. The suitable wavelengths for LLLT are restricted to specific ranges at red and near infrared at the wavelength of 600–1070 nm. It should be mentioned that shorter wavelengths (< 600 nm) are efficiently absorbed by tissue chromophores. Wavelengths in the range of 600–700 nm are prominently effective for the treatment of target sites located near to cutaneous tissue while wavelengths between 780 and 950 nm have the potential to reach beneath tissues. Of note, the ranges between 700 and 770 nm contain less biochemical activity. In wavelengths more than 1200 nm, water molecules have significant absorption. Therefore, roughly 810 nm is conceived as the optimum wavelength in this treatment technique [41, 45].

After the absorbance of LLLT by chromophores, it stimulates the movement of ground state electrons to higher energy orbits. In this case, stimulated electron is transported through biological transmembrane carriers such as cytochrome C oxidase and delivered to the ultimate electron acceptors. This process creates a proton gradient which assists in ATP production. Furthermore, LLLT contributes to an enhanced reactive oxygen species (ROS) production and the activation of multiple transcription factors [46] (Fig. 1). LLLT endeavors to preserve SCs cellular DNA from unwanted modifications included chromosomal deletion or translocation. It exerts splendid impacts on SCs functions and bioactivity such as survival rate, migration, and adhesion. In line with these comments, LLLT could direct SCs to distinct activity and phenotype. In the next step, we aim to debate more about the potency of LLLT on SC biology, trans-differentiation into specific

lineages, or preserving stemness feature by the modulation methylation rate.

One of the well-accepted and substantial mechanisms of LLLT is a photochemical process in mitochondria following receiving of photons which cause to increase the activity of cytochrome C oxidase, nitric oxide (NO) release and production of ATP, and changes in the intracellular content of signaling molecules such as calcium ions and ROS [47]. ROS are classified as Janus face mediators that are useful in low contents while detrimental effects are evident at high concentrations and these effects depend on the feature and protocol LLLT. In a better word, low levels of ROS are generated via the normal metabolism rate in mitochondria. When the basal mitochondrial membrane potential is changed, the cellular content of ROS can be modulated.

In cells under normal condition, the direct action of photons by Cox promotes changes in mitochondrial membrane potential and ROS accumulation. After a decrease of mitochondrial membrane potential due to current oxidative stress, excitotoxicity, and suppression of electron transport, light captivation increases mitochondrial membrane potential toward normal levels while ROS generation is halted [48]. Of note, ROS levels have a pivotal impact on regulating cellular bioactivities such as self-renewal capacity, cellular division, and differentiation. For example, at trivial levels, ROS perform as secondary messengers along with various signaling pathways regulate proliferation, survival, and differentiation of SCs [49]. Furthermore, proper levels of ROS within SCs assist to keep their potency. In contrast, the uncontrolled levels of ROS could hamper cellular hemostasis and thereby inhibit physiological biochemical reactions correlated with stemness and normal bioactivities [50].

## Laser for cell differentiation

SC trans-differentiation into different cell types is requisite to reconstitute the injured tissues [17]. LLLT can modulate SC bioactivity by the increase of migration, cellular division, and survival rate, as well as promotion of proteins related to differentiation [51]. Therefore, SC differentiation attitude can be affected by specific stimulators such as LLLT [52]. Through recent decades, different investigations have been published related to LLLT efficacy in induction of differentiation. Despite a few numbers of researches on cell differentiation induced by laser irradiation, positive effects of LLLT on differentiation have approximately been illustrated. In some cases, there are no evident differences in differentiation properties pre- and post-irradiation [53].

In an experiment done by Kim and co-workers, they did not find a noticeable difference between differentiation rate of laser-treated adipose-derived mesenchymal stem cells by He–Ne cold laser (632.8 nm) and non-irradiated cells [14]. It is

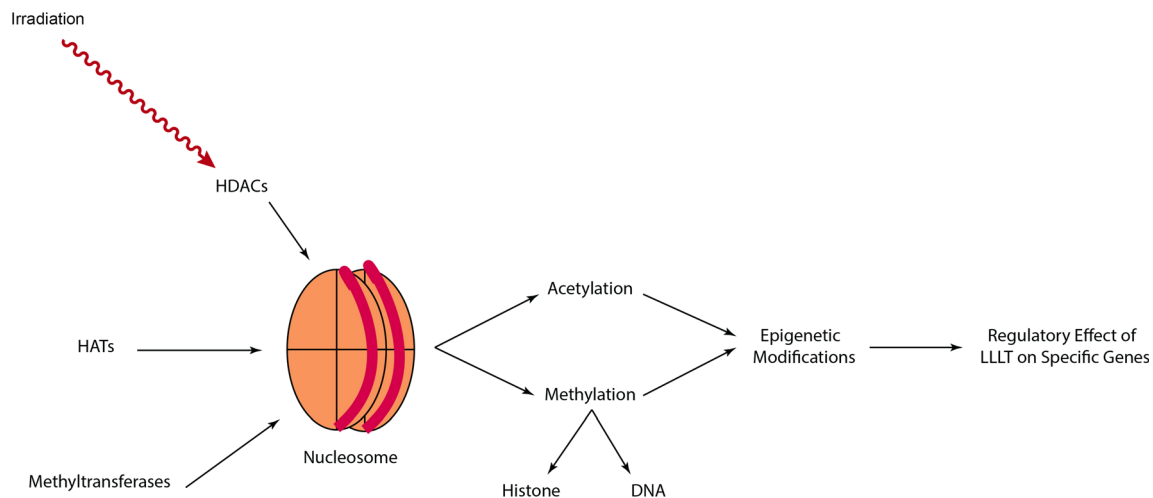
proposed that differentiation of MSCs is based on light source quality and feature, laser intensity, and light exposure treatment protocol. Based on the released data, the minimum exposure time point was near to 4 weeks [18]. In one study, MSCs were seeded on lyophilized collagen sponge Gingistat and subjected to 1064-nm irradiation provided by Nd:YAG laser and, 14 days after irradiation, differentiation was dramatically [15].

LLLT has potential to trans-differentiate into various cell lineages. LLLT (He–Ne laser functions as an osteogenesis-inducing factor with a continuous mode (at the wavelength of 632.8 nm) and evaluated previously on osteoblast differentiation of MSCs. In irradiated MSCs, alkaline phosphatase (ALP) activity, as an osteoblast differentiation indicator, was promoted in the early stages following laser treatment. The profound decrease in ALP activity was seen in the later stages, suggesting cellular maturation and bone formation [16]. In addition to cell differentiation, the proliferation of human osteoblast cells has positively affected by LLLT (He–Ne laser, at 632.8 nm) [22]. The application of growth factors is the main method to induce cell differentiation into various lineages. Growth factors (GF) are polypeptides which play an important role in differentiation. Low-intensity laser irradiation and GF can lead to differentiation of ADSCs into smooth muscle cells [17].

Although there is a positive relation in the majority of studies, it is believed that light source and laser irradiation does not eventuate to significant differentiation in MSCs. So auxiliary agents such as non-coherent red LED of BMSC specimens with osteogenic medium improved differentiation rate and GF [17, 53].

Human bone marrow–derived MSCs (BMSCs) irradiated by 810 nm gallium aluminum arsenide (GaAlA) laser at energy densities of 3 or 6 J/cm<sup>2</sup> acquired neuron-like phenotype at

days 1, 3, and 5. The use of GaAlAs at energy densities of 2 or 4 J/cm<sup>2</sup> promoted osteoblast-like phenotype. In fact, low-level laser irradiation (LLLI) acts in a dose-dependent manner to promote BMSC-to-osteoblasts differentiation. In support of this claim, ALP activity was significantly promoted in the 4 J/cm<sup>2</sup> treated LLLI compared to the 2 J/cm<sup>2</sup> counterpart [18]. It also described that LLLI provided by indium–gallium–arsenate–phosphate (InGaAsP) diode laser at 635 nm and 5 J/cm<sup>2</sup> could dictate myogenic differentiation of BMSCs [19]. These data confirm the potential role of LLLI energy density on phenotype acquisition and trans-differentiation quality. In addition to energy density, the entity and wavelength of LLLI could direct cell polarization toward various lineages. In an empirical study, the impact of four multiple wavelengths (420, 540, 660, and 810 nm) at an energy density of 3 J/cm<sup>2</sup> was examined on human ASCs. LLLI at the wavelength of prompt osteoblast differentiation compared to 660 and 810 nm. Biochemical analyses confirmed an increase in intracellular calcium level at 420 and 540 nm [20]. Regarding the wavelength value in inducing cell differentiation, it is better to mention that near infrared light enhances cytochrome C oxidase activity while abrogates the bioactivity of nitric oxide synthetase. The activation of cytochrome C oxidase, in turn, increases mitochondrial membrane integrity and ATP production. Concomitant with these changes, the profound shift in metabolic profile from glycolytic to oxidative status is induced by light exposure, conceiving as an important factor in osteogenic differentiation [20]. LLLI not only promotes BMSC differentiation toward osteoblast-like phenotype but also decreases the expression of adipocyte-related markers in BMSCs. Molecular analysis revealed the critical role of  $\beta$ -catenin in cells exposed to the LLLI compared to the non-treated control. LLLI has the ability to increase the levels of  $\beta$ -catenin which further promotes Wnt signaling pathway by engaging APN receptors located inside the nucleus. Totally,



**Fig. 2** The potency of low-level laser irradiation on the epigenetic pattern of target genes to change acetylation and methylation. These changes participate in the differentiation of stem cells toward specific lineages

LLLI increases the  $\beta$ -catenin transport to the nucleus. It seems that the activation of  $\beta$ -catenin/Wnt axis induces the osteogenic capacity of BMSCs [21].

### Effect of LLLI on SC differentiation by modulating methylation

Similar to numerous modulators, it is proved that the epigenetic mechanisms are apparently regulated via external clues such as laser irradiation. During LLLT, cellular mechanisms including DNA synthesis and gene expression, cell proliferation, and differentiation are prominently changed. Therefore, the critical role of epigenetic mechanisms in the molecular responses through LLLT is supported [54]. In addition to numerous LLLI effects, some adverse outcomes were also evident. The ionizing radiation of the body was found to result in the induction of acute myeloid leukemia (r-AML); meanwhile, LLLI could also promote DNA damage in each cell inside the body [55–57]. Therefore, one could hypothesize that both somatic and progenitor cells are efficiently affected by LLLI. The potency of laser irradiation was extensively investigated on the global DNA methylation and the expression of *Dnmt1* and *Dnmt3a* genes in a rat model of skin wound healing. Laser-emitted diodes were used in animals at a wavelength of 604 nm in two different doses 0.8 and 1.6 J/cm<sup>2</sup>. Total DNA methylation reduction was evident in the experimental condition, confirming DNA methylation during the healing process. Therefore, epigenetic mechanisms participate in the regeneration of epithelial tissues. In support of these changes, the expression of *Dnmt3a* was induced at a density of 1.6 J/cm<sup>2</sup> compared to the control [54]. De Farias and colleagues explored the impact of photobiomodulation (InGaAlP laser, 660 nm, 4 J/cm<sup>2</sup>, 4 s) on the site of histone 3 acetylation (acH3) and NF- $\kappa$ B expression on ulcer healing in the oral cavity. In situ irradiation not only modulated the levels of acH3 and NF- $\kappa$ B but also increased migration and differentiation of keratinocytes [58] (Fig. 2).

### Conclusion

Manipulation of DNA methylation by several modalities such as LLLT is considered to yield various therapeutic outcomes. In light of accessibility, ability to adapt to different therapeutic conditions and potential therapeutic properties according to wavelength, energy intensity, and LLLT could be applied for the modulation of various pathologies and injuries. Given the small number of studies related to the impact of LLLT on SC methylation, further investigations are needed to address precisely the restorative effects. For further insurance, it can be fruitful to examine the expression of differentiation-related genes and methylation rate in the development of SCs.

**Acknowledgments** We would thank the personnel of Stem Cell Research Center for collaboration.

**Authors' contributions** A.R.N.Z., S.S., M.H.G., F.B., and L.H. collected the data and wrote manuscript. R. R. conducted the study.

**Funding information** This manuscript is supported by a grant from Tabriz University of Medical Sciences.

### Compliance with ethical standards

**Conflict of interest** The authors declare that there is no conflict of interest.

### References

1. Biehl JK, Russell B (2009) Introduction to stem cell therapy. *J Cardiovasc Nurs* 24(2):98
2. Masoud Maleki, (2015) Stem cell therapy of cataract. *BioImpacts* 5(4):165–167
3. Alexander MS, Casar JC, Motohashi N (2015) Stem cell differentiation and therapeutic use. *Stem Cells Int* 2015
4. Saeed Azandeh, Anneh Mohammad Gharravi, Mahmoud Orazizadeh, Ali Khodadi, Mahmoud Hashemi Tabar, (2016) Improvement of mesenchymal stem cell differentiation into the endoderm lineage by four step sequential method in biocompatible biomaterial. *BioImpacts* 6(1):9–13
5. Nadig RR (2009) Stem cell therapy—hype or hope? A review. *J Conserv Dent: JCD* 12(4):131
6. Mohammadian M, Shamsasenjan K, Lotfi nezhad P, Talebi M, Jahedi M, Nickkhal H, Minayi N, Movassagh pour A (2013) Mesenchymal Stem Cells: New Aspect in Cell-Based Regenerative Therapy. *Adv Pharm Bull* 3(2):433–437. <https://doi.org/10.5681/apb.2013.070>
7. Keller G (2005) Embryonic stem cell differentiation: emergence of a new era in biology and medicine. *Genes Dev* 19(10):1129–1155
8. Elbuluk A, Einhorn TA, Iorio R (2017) A comprehensive review of stem-cell therapy. *JBJS Rev* 5(8):e15
9. Bradley A, Evans M, Kaufman MH, Robertson E (1984) Formation of germ-line chimaeras from embryo-derived teratocarcinoma cell lines. *Nature* 309(5965):255
10. Alison M, Islam S (2009) Attributes of adult stem cells. *The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland* 217(2):144–160
11. Hwang NS, Zhang C, Hwang YS, Varghese S (2009) Mesenchymal stem cell differentiation and roles in regenerative medicine. *Wiley Interdiscip Rev Syst Biol Med* 1(1):97–106
12. Kandath C, McLellan MD, Vandin F, Ye K, Niu B, Lu C, Xie M, Zhang Q, McMichael JF, Wyczalkowski MA (2013) Mutational landscape and significance across 12 major cancer types. *Nature* 502(7471):333
13. Huang K, Fan G (2010) DNA methylation in cell differentiation and reprogramming: an emerging systematic view. *Regen Med* 5(4):531–544
14. Kim H, Choi K, Kweon O-K, Kim WH (2012) Enhanced wound healing effect of canine adipose-derived mesenchymal stem cells with low-level laser therapy in athymic mice. *J Dermatol Sci* 68(3):149–156
15. Leonida A, Paiusco A, Rossi G, Carini F, Baldoni M, Caccianiga G (2013) Effects of low-level laser irradiation on proliferation and osteoblastic differentiation of human mesenchymal stem cells seeded on a three-dimensional biomatrix: in vitro pilot study. *Lasers Med Sci* 28(1):125–132

16. Abramovitch-Gottlieb L, Gross T, Naveh D, Geresh S, Rosenwaks S, Bar I, Vago R (2005) Low level laser irradiation stimulates osteogenic phenotype of mesenchymal stem cells seeded on a three-dimensional biomatrix. *Lasers Med Sci* 20(3–4):138–146
17. Mvula B, Abrahamse H (2014) Low intensity laser irradiation and growth factors influence differentiation of adipose derived stem cells into smooth muscle cells in a coculture environment over a period of 72 hours. *Int J Photoenergy* 2014
18. Soleimani M, Abbasnia E, Fathi M, Sahraei H, Fathi Y, Kaka G (2012) The effects of low-level laser irradiation on differentiation and proliferation of human bone marrow mesenchymal stem cells into neurons and osteoblasts—an in vitro study. *Lasers Med Sci* 27(2):423–430
19. Jf H, Zhang H, Yuan X, Li J, Yj W, Hu S (2008) In vitro effects of low-level laser irradiation for bone marrow mesenchymal stem cells: proliferation, growth factors secretion and myogenic differentiation. *Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery* 40(10):726–733
20. Wang Y, Huang Y-Y, Wang Y, Lyu P, Hamblin MR (2016) Photobiomodulation (blue and green light) encourages osteoblastic-differentiation of human adipose-derived stem cells: role of intracellular calcium and light-gated ion channels. *Sci Rep* 6:33719
21. Zhang R, Wang Q, Zhang A, Xu J, Zhai L, Yang X, Liu X (2018) Low-level laser irradiation promotes the differentiation of bone marrow stromal cells into osteoblasts through the APN/Wnt/ $\beta$ -catenin pathway. *Eur Rev Med Pharmacol Sci* 22(9):2860–2868
22. Stein A, Benayahu D, Maltz L, Oron U (2005) Low-level laser irradiation promotes proliferation and differentiation of human osteoblasts in vitro. *Photomed Laser Ther* 23(2):161–166
23. Kim M, Kang T-W, Lee H-C, Han Y-M, Kim H, Shin HD, Cheong HS, Lee D, Kim S-Y, Kim YS (2011) Identification of DNA methylation markers for lineage commitment of in vitro hepatogenesis. *Hum Mol Genet* 20(14):2722–2733
24. Mummery C, Van den Brink C, De Laat S (1987) Commitment to differentiation induced by retinoic acid in P19 embryonal carcinoma cells is cell cycle dependent. *Dev Biol* 121(1):10–19
25. Lange C, Calegari F (2010) Cdks and cyclins link G1 length and differentiation of embryonic, neural and hematopoietic stem cells. *Cell Cycle* 9(10):1893–1900
26. Bernstein BE, Mikkelsen TS, Xie X, Kamal M, Huebert DJ, Cuff J, Fry B, Meissner A, Wernig M, Plath K (2006) A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* 125(2):315–326
27. Voigt P, Tee W-W, Reinberg D (2013) A double take on bivalent promoters. *Genes Dev* 27(12):1318–1338
28. Jaenisch R, Young R (2008) Stem cells, the molecular circuitry of pluripotency and nuclear reprogramming. *Cell* 132(4):567–582
29. M Perez-Campo F, A Riancho J (2015) Epigenetic mechanisms regulating mesenchymal stem cell differentiation. *Curr Genomics* 16 (6):368–383
30. Sen GL, Reuter JA, Webster DE, Zhu L, Khavari PA (2010) DNMT1 maintains progenitor function in self-renewing somatic tissue. *Nature* 463(7280):563
31. Smith ZD, Meissner A (2013) DNA methylation: roles in mammalian development. *Nat Rev Genet* 14(3):204
32. Berdasco M, Esteller M (2011) DNA methylation in stem cell renewal and multipotency. *Stem Cell Res Ther* 2(5):42
33. Boland MJ, Nazor KL, Loring JF (2014) Epigenetic regulation of pluripotency and differentiation. *Circ Res* 115(2):311–324
34. Sheaffer KL, Kim R, Aoki R, Elliott EN, Schug J, Burger L, Schübeler D, Kaestner KH (2014) DNA methylation is required for the control of stem cell differentiation in the small intestine. *Genes Dev* 28(6):652–664
35. Altun G, Loring JF, Laurent LC (2010) DNA methylation in embryonic stem cells. *J Cell Biochem* 109(1):1–6
36. Podobinska M, Szablowska-Gadomska I, Augustyniak J, Sandvig I, Sandvig A, Buzanska L (2017) Epigenetic modulation of stem cells in neurodevelopment: the role of methylation and acetylation. *Front Cell Neurosci* 11:23
37. Kim M, Costello J (2017) DNA methylation: an epigenetic mark of cellular memory. *Exp Mol Med* 49(4):e322
38. Zhou Y, Kim J, Yuan X, Braun T (2011) Epigenetic modifications of stem cells: a paradigm for the control of cardiac progenitor cells. *Circ Res* 109(9):1067–1081
39. Bibikova M, Chudin E, Wu B, Zhou L, Garcia EW, Liu Y, Shin S, Plaia TW, Auerbach JM, Arking DE (2006) Human embryonic stem cells have a unique epigenetic signature. *Genome Res* 16(9):1075–1083
40. Cotler HB, Chow RT, Hamblin MR, Carroll J (2015) The use of low level laser therapy (LLLT) for musculoskeletal pain. *MOJ Orthop Rheumatol* 2(5)
41. Chung H, Dai T, Sharma SK, Huang Y-Y, Carroll JD, Hamblin MR (2012) The nuts and bolts of low-level laser (light) therapy. *Ann Biomed Eng* 40(2):516–533
42. Costa MM, Silva SB, Quinto ALP, Pasquinelli PFS, dos Santos VQ, de Cássia Santos G, Veiga DF (2014) Phototherapy 660 nm for the prevention of radiodermatitis in breast cancer patients receiving radiation therapy: study protocol for a randomized controlled trial. *Trials* 15(1):330
43. Amid R, Kadkhodazadeh M, Ahsaie MG, Hakakzadeh A (2014) Effect of low level laser therapy on proliferation and differentiation of the cells contributing in bone regeneration. *J Lasers Med Sci* 5(4):163
44. Moore P, Ridgway TD, Higbee RG, Howard EW, Lucroy MD (2005) Effect of wavelength on low-intensity laser irradiation-stimulated cell proliferation in vitro. *Lasers in Surgery and Medicine: The Official Journal of the American Society for Laser Medicine and Surgery* 36(1):8–12
45. Sibata C, Colussi V, Oleinick N, Kinsella T (2000) Photodynamic therapy: a new concept in medical treatment. *Braz J Med Biol Res* 33(8):869–880
46. Hashmi JT, Huang Y-Y, Osmani BZ, Sharma SK, Naeser MA, Hamblin MR (2010) Role of low-level laser therapy in neurorehabilitation. *Pm&r* 2(12):S292–S305
47. Huang YY, Nagata K, Tedford CE, McCarthy T, Hamblin MR (2013) Low-level laser therapy (LLLT) reduces oxidative stress in primary cortical neurons in vitro. *J Biophotonics* 6(10):829–838
48. de Freitas LF, Hamblin MR (2016) Proposed mechanisms of photobiomodulation or low-level light therapy. *IEEE Journal of selected topics in quantum electronics* 22(3):348–364
49. Maraldi T, Angeloni C, Giannoni E, Sell C (2015) Reactive oxygen species in stem cells. *Oxidative Med Cell Longev* 2015
50. Nugud A, Sandeep D, El-Serafi AT (2018) Two faces of the coin: minireview for dissecting the role of reactive oxygen species in stem cell potency and lineage commitment. *J Adv Res*
51. Abrahamse H (2012) Regenerative medicine, stem cells, and low-level laser therapy: future directives. Mary Ann Liebert, Inc. 140 Huguenot Street, 3rd Floor New Rochelle, NY 10801 USA,
52. Kushibiki T, Hirasawa T, Okawa S, Ishihara M (2015) Low reactive level laser therapy for mesenchymal stromal cells therapies. *Stem Cells Int* 2015
53. Fekrazad R, Asefi S, Allahdadi M, Kalhori KA (2016) Effect of photobiomodulation on mesenchymal stem cells. *Photomed Laser Surg* 34(11):533–542
54. de Matos Gomes MV, Manfredo MH, Toffoli LV, Castro-Alves DC, do Nascimento LM, da Silva WR, Kashimoto RK, Rodrigues-Jr GM, Estrada VB, Andraus RA (2016) Effects of the led therapy on the global DNA methylation and the expression of Dnmt1 and Dnmt3a genes in a rat model of skin wound healing. *Lasers Med Sci* 31(7):1521–1526

55. Pierce DA, Shimizu Y, Preston DL, Vaeth M, Mabuchi K (1996) Studies of the mortality of atomic bomb survivors. Report 12, part I. Cancer: 1950-1990. *Radiat Res* 146(1):1–27
56. Preston DL, Shimizu Y, Pierce DA, Suyama A, Mabuchi K (2003) Studies of mortality of atomic bomb survivors. Report 13: solid cancer and noncancer disease mortality: 1950–1997. *Radiat Res* 160(4):381–407
57. Warner JK, Wang JC, Hope KJ, Jin L, Dick JE (2004) Concepts of human leukemic development. *Oncogene* 23(43):7164
58. de Farias GA, Wagner V, Correa C, Webber L, Pilar E, Curra M, Carrard V, Martins M, Martins M (2019) Photobiomodulation therapy modulates epigenetic events and NF- $\kappa$ B expression in oral epithelial wound healing. *Lasers Med Sci*

**Publisher's note** Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.