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REVIEW ARTICLE

Low-level laser therapy: a useful technique for enhancing the proliferation of various cultured cells

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Abstract The aim of this work is to review the available literature on the details of low-level laser therapy (LLLT) use for the enhancement of the proliferation of various cultured cell lines including stem cells. A cell culture is one of the most useful techniques in science, particularly in the production of viral vaccines and hybrid cell lines. However, the growth rate of some of the much-needed mammalian cells is slow. LLLT can enhance the proliferation rate of various cell lines. Literature review from 1923 to 2010. By investigating the outcome of LLLT on cell cultures, many articles report that it produces higher rates of ATP, RNA, and DNA synthesis in stem cells and other cell lines. Thus, LLLT improves the proliferation of the cells without causing any cytotoxic effects. Mainly, helium neon and gallium-aluminum-arsenide (Ga-Al-As) lasers are used for LLLT on cultured cells. The results of LLLT also vary according to the applied energy density and wavelengths to which the target cells are subjected. This review suggests that an energy density value of 0.5 to 4.0 J/cm^2 and a visible spectrum ranging from 600 to 700 nm of LLLT are very helpful in enhancing the proliferation rate of various cell lines. With the appropriate use of LLLT, the proliferation rate of cultured cells, including stem cells, can be increased, which would be very useful in tissue engineering and regenerative medicine.

Keywords Low-level laser therapy · Cell culture · Stem cells · Proliferation · Tissue engineering · Regenerative medicine

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Introduction

Biotechnology has provided various tools and techniques to generate treatments for previously incurable diseases. Cell culture was one of the most useful techniques produced at the turn of the 20th century [1]. It played a role in the production of viral vaccines and hybrid cells as well as the development of recombinant DNA technology [1]. Numerous mammalian and non-mammalian cell lines can be cultured [2]. The growth rate of mammalian cells is relatively slow compared to bacteria. Bacterial cells can double every 30 min, while mammalian cells require about 18 to 24 h to double [3]. This makes the mammalian culture vulnerable to contamination, as a small number of bacteria would soon outgrow a larger population of animal cells [3]. Therefore, the development of various techniques involved with the consistency and reproducibility of the cells have been the focus of biotechnologists [4].

Low-level laser therapy (LLLT) has been used in wound healing for the last 30 years [5]. It is also widely applied in different branches of regenerative medicine (e.g., tissue regeneration) [6] and dentistry, where it is used to enhance the healing process [7]. Earlier, LLLT was shown to have beneficial effects on a variety of pathological conditions

including pain relief [8] and inflammation [9]. Recently, it has been reported that LLLT enhances the proliferation of mesenchymal and cardiac stem cells [10]. Although the biological mechanisms underlying the biostimulatory effects of LLLT are not fully understood, it has been reported by several investigators that LLLT modulates cellular metabolic processes, leading to an enhanced regenerative potential for biological tissues [5, 11]. The effects of LLLT on tissue metabolism are known as "laser biostimulation". The positive biostimulatory effects of LLLT on tissues are well known [12, 13].

In this review, we aimed to systematically review the published scientific literature between 1923 and 2010 regarding the use of LLLT to enhance the proliferation of different lines of cultured cells without compromising the characteristics and native properties of the cells. Moreover, we aimed to shed light on additional points like getting maximum cell yield after LLLT treatment. We searched databases including MEDLINE/PubMed, Embase, and Google Scholar for LLLT, cell culture, stem cells, proliferation, tissue engineering, and regenerative medicine. We found no controlled studies that compared different parameters of LLLT to get the maximum cell yield.

Low-level laser therapy

LLLT irradiation refers to the use of red-beam or near-infrared lasers with a wavelength of 600–1,100 nm and an output power of 1-500 mW. This type of radiation is a continuous wave or pulsed light that consists of a constant beam of relatively low energy density (0.04–50 J/cm^2), and the laser is directed at the target tissue or monolayer of cells using powers measured in milliwatts (mW). LLLT can prevent cell apoptosis and improve cell proliferation, migration, and adhesion at low-levels of red/near-infrared light illumination [14, 15]. At low doses (2 J/cm^2), LLLT stimulates proliferation, while at high doses (16 J/cm^2) LLLT is suppressive, pointing to the dose dependence of biological responses after light exposure [16]. However, stimulation in cell proliferation has been shown outside these ranges [17, 18]. A number of different laser light sources, including helium-neon, ruby, and gallium-aluminum-arsenide, have been used to deliver LLLT in different treatments and on different schedules.

LLLT transmits energy at low levels and therefore does not emit heat, sound, or vibrations. Its reactions are non-thermal because there is no immediate increase in the temperature of the laser-irradiated tissue. Experiments following LLLT exposure have shown that the immediate increase in heat of the target tissue is negligible (±1°C) [19]. It has been confirmed by many investigators that the temperature remained unchanged in fibroblast suspensions during LLLT irradiation [20, 21]. Schneede et al. also reported a temperature increase of less than 0.065°C, with laser irradiation of 40 mW/cm^2, using a microthermo probe in a monolayer of cells [22]. In contrast, high-energy lasers (e.g., carbon dioxide lasers and neodymium-YAG lasers) are able to raise the tissue temperature high enough to cut and vaporize them [23].

Molecular mechanisms of cell proliferation induced by LLLT

LLLT can stimulate a number of biological processes, including cell growth, proliferation [24], and differentiation [25, 26]. In vitro, the effects on cell proliferation by LLLT have been studied in various cell types including fibroblasts, endothelial cells, skeletal cells, keratinocytes, myoblasts, and other cell types [24–30]. However, the molecular mechanism associated with the stimulatory effects of LLLT has not been fully clarified [31]. One classic mechanism involved is that the laser energy is absorbed by intracellular chromophores and converted to metabolic energy, since cellular ATP levels increase almost twofold after He–Ne laser irradiation [32]. ATP acts via multiple P2 nucleotide receptor subtypes to increase intracellular calcium concentration (Ca^{2+}) [33–35]. Simultaneously, ATP regulates protein synthesis, DNA synthesis, and expression of immediate-early and delayed-early genes [35, 36]. In addition, Wilden et al. demonstrated that ATP stimulates activation of ERK1/ERK2 in a phosphatidylinositol 3-kinase (PI3K)-independent manner, and cell proliferation requires both ERK1/ERK2 and PI3K activity (pathways) [37]. In many cells, the extracellular signal-regulated kinase (ERK) cascade plays an important role in cellular proliferation. ATP-induced activation of ERK1/ERK2 is dependent on the dual-specificity kinase mitogen-activated protein kinase/ERK kinase (i.e., MEK) but independent of phosphatidylinositol 3-kinase (PI3K) activity [38]. PI3K is a lipid kinase that promotes diverse biological functions including cellular proliferation, survival, and motility. The PI3K pathway is an important driver of cell proliferation and cell survival, most notably in cells that are responding to growth-factor–receptor engagement [39], whereas the ERK pathway is a major regulator of cell proliferation [40].

To further understand the mechanism, it is necessary to identify the signal transduction pathways of cell proliferation stimulated by LLLT. It has been reported that LLLT specifically activates MAPK/ERK pathway and consequently induces satellite cell proliferation [41, 42]. In another study, it was revealed that LLLT specifically activates RTK/PKCs signaling pathway to promote cell proliferation [43] and triggers a significant activation of

ROS/Src pathway [44]. Furthermore, Akt can be activated by either Src or PKCs protein kinase [45–48]. Therefore, it is likely that Akt is involved in LLLT-induced cell proliferation. On the other hand, LLLT treatment can increase the level of intracellular ROS generation [44, 49–51]. The increased intracellular oxidants can mediate the activation of Akt [52, 53]. These reports suggest the existence of ROS/Akt signaling pathway during LLLT-induced proliferation.

Cell signaling after LLLT

Cell proliferation is a very important physiological effect of LLLT that is used in basic experimental cell culture procedures and clinical practice [54]. LLLT promotes proliferation of multiple cells, which (especially red and near-infrared light) is mainly through the activation of mitochondrial respiratory chain and the initiation of cellular signaling. The alterations in photoacceptor function are the primary reactions, and the subsequent alterations in cellular signaling and cellular functions are secondary reactions [55]. The primary reactions after light absorption are singlet-oxygen hypothesis, redox properties alteration hypothesis, nitric oxide hypothesis, transient local heating hypothesis, and superoxide anion hypothesis [56]. The secondary reactions after light absorption are cellular signaling pathways, mitochondrial retrograde signaling included [57].

However, the mechanisms of cell proliferation induced by LLLT are poorly understood [54]. Various mechanisms for the mitogenic effects of low-power laser irradiation have been proposed, including ligand-free dimerization and activation of specific receptors that are in the "right energetic state" to accept the laser energy, leading to their autophosphorylation and down-stream effects [49], activation of calcium channels resulting in increased intracellular calcium concentration and cell proliferation [58–62]. Irradiation of red and near-infrared light is absorbed by mitochondrial respiratory chain components, resulting in the increase of reactive oxygen species (ROS), and adenosine triphosphate (ATP)/or cyclic AMP, and initiating a signaling cascade that promotes cellular proliferation and cytoprotection [57, 62–67]. Following increased ATP and protein synthesis after LLLT, the expressions of growth factors and cytokines increase and ultimately lead to cell proliferation [68, 69].

Biophysical mechanism of action of LLLT

Phototherapy is based on the effects of light energy on cell metabolism of living systems. Biological responses of cells to visible and near-IR (laser) radiation occur due to physical and/or chemical changes in photoacceptor molecules, components of respiratory chains [70, 71]. Some of the major changes induced by laser in the irradiated cell are changes in redox properties and acceleration of electron transfer, nitric oxide (NO) release from catalytic centre of cytochrome c oxidase, superoxide generation, photodynamic action, and changes in biochemical activity induced by local transient heating of chromophores [72].

Biological effects of LLLT

Stimulation of cells with LLLT has been examined in numerous contexts. LLLT stimulates wound healing, collagen synthesis, nerve regeneration, enhanced remodeling and repair of bone, restoration of normal neural function following injury, normalization of abnormal hormonal function, pain attenuation, stimulation of endorphin release, and modulation of the immune system [73–77]. The photonic energy is converted to chemical energy within the cell, in the form of ATP, which leads to normalization of cell function, pain relief, and healing. Cell membrane permeability is altered, followed by physiological changes in the target cells [78]. The effects of LLLT on wound healing are often attributed to increased cell proliferation [79].

It has been reported that irradiation of cells at certain wavelengths can activate specific biochemical reactions as well as alter the whole cellular metabolism [49]. LLLT has shown a variety of effects including increased cell numbers [80], increased DNA synthesis [81], and increased collagen production [73] in several in vitro studies on cultured human fibroblasts. Another interesting finding is that laser irradiation also stimulates cell attachment to a plastic substrate [20]. Boulton and Marshall observed that laser-irradiated cultures exhibit a significant increase in the number of human skin fibroblasts that grow on the plastic substrates compared to their respective non-irradiated controls after 24 and 48 h [20]. The effects of LLLT on cell cultures have been studied most extensively by Karu [82]. It was also reported that the stimulation of cellular proliferation is dependent on the dose of laser irradiation, as lower doses increase the cell proliferation rate and other cellular functions while higher doses of LLLT have negative effects, as described above [80, 83].

Mechanism of action of LLLT

One theory regarding the mechanism of action of LLLT purports that the laser is capable of influencing photoreceptors in the cells. This mechanism is referred to as

photobiology or biostimulation. It has been reported that photobiostimulation occurs via the electron transport chain enzymes in mitochondria, inducing high cell respiration rates by either the endogenous porphyrins in the cell or by cytochrome c [84], which increases cellular metabolism and function [80, 85]. The biostimulating effect of LLLT results in an increase in microcirculation, higher production rates for ATP, RNA, and DNA synthesis, thus improving cellular oxygenation, nutrition, and regeneration [86] and an enhanced mitochondrial electron transport system [85]. Photons enter the cell and are readily absorbed by biological chromophores located either in the mitochondria or in the cell membrane. These chromophores strongly interact with the laser irradiation. The photonic energy is converted to chemical energy within the cell, in the form of ATP, which enhances cellular functions and cell proliferation rates. Cell membrane permeability is altered, followed by physiological changes in the target cells. The magnitude of the laser biostimulation effect depends on the wavelength used as well as the physiological state of the cell at the moment of irradiation [87].

To explain the biostimulation effect of LLLT, Karu proposed a chain of molecular events starting with the absorption of light by a photoreceptor and leading to the photoactivation of enzymes in the mitochondria, including the signal transduction and amplification events, and ending with the photoresponse [70, 88]. Light is absorbed by components of the respiratory chain, which leads to changes in both the mitochondria and the cytoplasm. At low-laser doses, additional Ca^{2+} is transported into the cytoplasm by an antiport process that triggers or stimulates various biological processes such as DNA and RNA synthesis, cell mitosis, and cell proliferation. At higher doses, too much Ca^{2+} is released, which results in hyperactivity for the calcium-adenosine triphosphatase (ATPase) calcium pumps and exhausts the ATP pool of the cell, thereby inhibiting cell metabolism [88, 89].

Effects of different LLLT wavelengths on cellular functions

LLLT has been used with visible, infrared, and ultraviolet (UV) light, but the most effective results have come from using the visible spectrum, ranging from 600–700 nm [90]. In various in vitro studies it was observed that 860-nm laser light stimulates cellular proliferation [16], 812-nm laser light increases DNA synthesis [81], 660-nm laser light upregulates the production of basic fibroblast growth factor [91], and 632.8-nm laser light transforms fibroblasts into myofibroblasts in cultured fibroblasts [92]. It was also observed that a 632.8-nm laser light used in cultured keratinocytes was found to increase cellular proliferation [93], stimulate the release of IL-1 and IL-8 [24], and increase the motility rate [94], while a 780-nm laser light was shown to stimulate cellular proliferation [63] (Table 1). Macrophages are activated by 632.8-nm laser light, and various laser wavelengths reportedly increase growth factor secretion from cultured macrophages [95, 96]. There are relatively less data available on the effects of low-intensity laser irradiation on vascular endothelial cells. However, increased vascular endothelial cell proliferation has been described in vitro [97].

It has also been reported that the proliferation rate is at a maximum in the presence of 665-nm and 675-nm light, while 810-nm light inhibits the proliferation of cultured fibroblasts [98]. Therefore, not every report concerning LLLT supports its efficacy. Low-intensity laser irradiation from a gallium-aluminum-arsenide laser failed to increase the proliferation, migration, or adhesion of cultured keratinocytes or fibroblasts [99]. In addition, it has been reported by many investigators that in vitro biostimulation is dependent on many factors, including laser irradiation parameters such as wavelength, fluence, laser output power [100], and energy density [101, 102], as well as the type of cell being irradiated [98]. Parameters that are helpful for increasing proliferation rates can sometimes have adverse effects on protein synthesis [101, 103]. Therefore, it is crucial to know the correct combination of parameters (e.g., wavelength, power density, and energy density) to arrive at the maximum proliferation rate of cells.

Karu also stated that the laser effect depends on the radiation, wavelength, dose, and intensity as well as on other cell culture conditions [70, 104]. Therefore, it is possible that cells in tissues or cellular cultures may not respond to LLLT in exactly the same way and that similar parameters can have different effects on different cultured cells.

Biphasic dose response of LLLT

LLLT delivered at low doses may produce a better result when compared to the same wavelength delivered at high doses; this phenomenon is called "biphasic dose response" or "hormesis" [105]. The modern term "hormesis" was first used by Stebbing in 1982 [106] and has been thoroughly reviewed by Calabrese [105, 107]. A biphasic dose response has been demonstrated many times in LLLT research by several investigators [108, 109] and the "Arndt-Schulz Law" is frequently quoted as a suitable model for describing the dose-dependent effects of LLLT [68, 110–112]. According to the 'Arndt-Schulz law', weak stimuli slightly accelerate vital activity and stronger stimuli raise it further; but when a peak is reached, then stronger stimuli suppress it, until a negative response is finally achieved [113].

Table 1 Effects of low-level laser therapy on different cell lines

S. No.	Type of cells	Wavelength (nm)	Power (mW)	Total energy (J)#	Energy density (J/cm^2)	Beam diameter (cm)	Application techniques	Results	Study
1	Mesenchymal and Cardiac Stem Cells (MSCs and CSCs)	804	400	8 and 24	1 and 3	1.0	Pulsed laser	LLLT promoted proliferation of MSCs and CSCs in vitro	Tuby et al. [10]
2	Olfactory ensheathing cells	810	127	0.5 and 71.25	0.2, or 68	1.12*	Continuous wave laser	OEC proliferation was also found to be significantly increased in light-treated groups in comparison to the control group	Byrnes et al. [17]
3	MSCs	405	1.57#	0.07, 0.141, 0.211, and 0.282	9, 18, 27, 36	0.635**	Continuous wave laser	Irradiation was able to promote extracellular calcification of MSCs	Kushibiki and Awazu [18]
4	Whole blood	660	282#	3.5, 7, 21.1, and 35.25	5.0	3.0	Continuous wave laser	Lymphocyte proliferation was significantly higher in samples irradiated in the presence of whole blood compared with lymphocytes irradiated after isolation from whole blood	Stadler et al. [27]
5	Normal human keratinocytes (NHK)	780	6.5	1.17	0 to 3.6 (0–180 s)	0.64**	Continuous wave laser	Proliferation of NHK exposed to 0.45–0.95 J/cm^2 was significantly enhanced by 1.3–1.9-fold relative to sham-irradiated controls	Grossman et al. [63]
6	Human skin fibroblasts (HSF)	632.8	33	27.22, 54.45, and 174.24	2.5, 5.0, or 16.0	3.4	Continuous wave laser	Cumulative effect of lower doses (2.5 or 5 J/cm^2) determined the stimulatory effect, while multiple exposures at higher doses (16 J/cm^2) resulted in an inhibitory effect	Hawkins and Abrahamse [68]
7	Human umbilical vein endothelial cell	670	250	25, 50, and 100	2–8	2.07***	Continuous wave laser	Doses of between 2 and 8 J/cm^2 induced statistically significant cell proliferation	Schindl et al. [97]
8	Endothelial and fibroblasts cells	665, 675, and 810	1.58#	3.17	10	0.635**	Continuous wave laser	Fibroblasts proliferated faster than endothelial cells in response to laser irradiation. Maximum cell proliferation occurred with 665- and 675-nm light, whereas 810-nm light was inhibitory to fibroblasts	Moore et al. [98]
9	Human lung fibroblasts and human foreskin keratinocytes	830	5-100	0.05 and 12	0.12 to 4.24	0.425	Continuous wave laser	Fibroblasts and keratinocytes failed to produce an increase in the number of cells in comparison with their respective non-irradiated controls	Pogrel et al. [99]
10	Human gingival fibroblasts	670 (L1),780 (L2), 692 (L3), and 786 (L4).	10 (L1), 50 (L2) and 30 (L3 and L4)	0.02	2	0.112*	Continuous wave laser	The infrared laser (780 nm) induced significantly higher cell growth in cells grown in nutritional deficit than the visible laser (670 nm). Lasers of equal power output (red 692-nm and infrared 786-nm) have similar effects on the fibroblasts	Almeida-Lopes et al. [100]

Table 1 (continued)

S. No.	Type of cells	Wavelength (nm)	Power (mW)	Total energy (J)#	Energy density (J/cm^2)	Beam diameter (cm)	Application techniques	Results	Study
11	NIH 3T3 fibroblasts cells	904	120	0.96 and 1.92	3 to 5	0.3*	Pulsed laser	3 and 4 J/cm^2 increased the cell numbers about threefold to six-fold compared to control cultures. 5 J/cm^2 had no effect on cell growth	Pereira et al. [101]
12	Bone marrow-derived mesenchymal stem cells (BMSCs)	635	60	4.5, 9, 18, and 45	0, 0.5, 1.0, 2.0, or 5.0	0.34	Continuous wave laser	LLLT significantly stimulated BMSCs proliferation and 0.5 J/cm^2 was found to be an optimal energy density	Hou et al. [122]
13	BMSCs	632.8	10	6	0.3	5	Continuous wave laser	Higher ossification levels were observed in the irradiated samples when compared to the control group	Abramovitch-Gottlib et al. [131]
14	MSCs	647	8.98-9.89	0.093, 0.279, and 0.836	0.01, 0.03, and 0.11#	3.2*	Continuous wave laser	Irradiation significantly increased osteoblast mineralization in irradiated cells	Kim et al. [132]
15	Human dental pulp stem Cells (hDPScs)	660	40	0.12	3	0.214*	Continuous wave laser	hDPSC responds positively to LLLT	Eduardo et al. [133]
16	Adult human adipose-derived stem cells (ADSC)	635	50	45	5	3.3	Continuous wave laser	LLLT increased cellular viability, proliferation, and expression of beta 1-integrin	Mvula et al. [134]
17	HeLa cells	580-860, 632.8	0.05	0.0005	0.01	0.7*	Continuous wave laser	Cell attachment to the glass surface increased after irradiation of samples of HeLa cells in suspension	Karu et al. [137]
18	Human endothelial cells and Human saphenous vein smooth muscle cells (HECs and HSVSMCs)	632	5	0.24 and 14.4	0.10 to 6.3	1.6	Continuous wave laser	LLLT stimulated growth of HECs and HSVSMCs in culture	Kipshidze et al. [138]
19	Human gingival fibroblasts (HGF)	809	10	0.75, 1.5, and 3	1.96–7.84	0.635**	Continuous wave laser	The irradiated HGF cells revealed a considerably higher proliferation activity	Kreisler et al. [139]
20	Rat calvaria osteoblast-like cells	780	10	0.12	3	0.226*	Continuous wave laser	LLLT increased the proliferation of cells	Fujihara et al. [140]
21	Porcine aortic smooth muscle cells (SMC)	780	0-450	243 and 486	1 and 2	11.28*	Continuous wave laser	LLLT increased SMC proliferation by 16 and 22% (1 and 2 J/cm^2, respectively) compared to non-irradiated cells	Gavish et al. [141]
22	Peripheral blood progenitor cell (PBPC)	685	25	0.95, 4.8, 9.6, 14.4, and 19.25	0.1–2.0	0.35	Continuous wave laser	LLLT increased (1.0 J/cm^2) or decreased (2.0 J/cm^2) the potential of long-term cryopreserved PBPC for growth of colony forming unit	Nascimento and Callera [142]
23	Epithelial cell cultures (Vero cell line)	660 and 780	40 and 70	0.112 and 0.175	3 or 5	0.214*	Continuous wave laser	The number of viable cells for cultures irradiated three times was higher than that of non-irradiated control cultures	Eduardo et al. [143]

24	Wounded human skin fibroblasts	632.8 in the dark or 830 in the light	18.8 and 54	47, 41.52, and 394	5	3.4	Continuous wave laser	632.8 nm (5 J/cm^2) in the dark or 830 nm in the light was the most effective dose to stimulate cell proliferation, which may ultimately accelerate or improve the rate of wound healing	Hawkins and Abrahamse [144]
25	Normal human neural progenitor cells	808	600	0.6	0.05	2.5	Pulsed laser	The quantity of ATP in laser-treated cells was significantly higher than that in the non-treated cells	Oron et al. [145]
26	Cultured lymphocytes	904	10	36	45.86#	1.0	Pulsed laser	The amount of ATP in irradiated cell cultures was significantly higher than that in the control	Benedicenti et al. [146]
27	Murine mesenchymal stem cells (mMSCs)	660	60	1.5- 4.5	1.9-11.7	1.0	Continuous wave laser	Low energy density of LLLT increased and high energy density of LLLT decreased the proliferation of mMSCs	Horvát-Karajz et al. [147]
28	HIG-82 rabbit synovial fibroblasts cells	660	100	12	4.8	3.0	Continuous wave laser	LLLT promoted HIG-82 synovial fibroblast proliferation	Taniguchi et al. [148]
29	ADSCs	636	110	45.43	5	2.14*	Continuous wave laser	LLLT increased the viability and proliferation of ADSCs	Mvula et al. [149]
30	Human gingival fibroblasts (HGF)	685	25	3.5	2	1.12*	Continuous wave laser	LLLT increased the proliferation of HGF cells	Saygun et al. [150]
31	HGF	660	10-29	0.14	2	0.3*	Continuous wave laser	LLLT influenced cell growth	Azevedo et al. [151]
32	Human periodontal ligament fibroblasts (PDLF)	809	10	0.75- 3	1.96-7.84	0.635**	Continuous wave laser	The irradiated cells revealed a considerably higher proliferation activity than the controls	Kreisler et al. [152]

These parameters were not mentioned in the references, they were calculated using the following formulas:

- Power = power density × irradiated area
- Total energy (J) = Watt × seconds
- Energy density = J / irradiated area

*Beam diameters were not mentioned in the references; they were calculated from irradiated area using the formula (area of circle = πr^2)

**Cells were irradiated in 96-well tissue culture plate; this area (standard area of 96-well plate) was used for calculation of beam diameter

***Cells were irradiated in a 12-well tissue culture plate; this area (standard area of 12-well plate) was used for calculation of beam diameter

A "biphasic" curve can be used to explain the expected dose response to light at a subcellular, cellular, tissue, or clinical level. The biphasic curve will be helpful to identify the sufficient energy level that will be applied to get maximum biostimulation. If insufficient energy is applied then there will be no response (because the minimum threshold has not been met). If more energy is applied, then a threshold is crossed and biostimulation disappears and is replaced by bioinhibition instead [15, 112].

Effects of reciprocity of exposure time and irradiance of LLLT on cellular proliferation

It has been reported that the outcome of LLLT can be influenced by varying the irradiance and exposure time, despite keeping the energy density constant. A unique dose–frequency regime may exist for tissues and cell lines and that the determination of that treatment paradigm is necessary in order to achieve maximal stimulation of cellular metabolism and proliferation [108]. Lanzafame et al. also explained that identification of the proper treatment parameters for the particular cell lines or tissue is crucial for achieving maximum photobiostimulation [108].

Contradictory reports of LLLT on cell proliferation

Cell proliferation of various cultured cell lines induced by LLLT has been reported by various investigators, as is described above. However, few negative effects of LLLT regarding cell proliferation have been reported. In one report, 830-nm Ga-Al-As lasers could not enhance the proliferation of cultured fibroblasts and keratinocytes as presented in Table 1 [99]. In another study, it was observed that fibroblasts were proliferated faster than endothelial cells in response to laser irradiation. These observations suggest that both wavelength and cell type influence cell proliferation response to low-level laser irradiation [98].

It is also noticeable that maximum cell proliferation occurred at 665–675 nm, whereas irradiation at 810-nm (or higher) wavelengths inhibited cell division. The magnitude of the LLLT effect on cell proliferation depends on the physiological state of the cell at the moment of irradiation. It has been reported that LLLT can stimulate cell proliferation if the cells are growing poorly at the time of irradiation. However, if the cells are fully functional, or growing in a serum-rich environment (10% FBS) at the moment of irradiation, then there is nothing for LLLT to stimulate and no therapeutic benefit will be observed [88].

Types of LLLT used in cell proliferation

Although many types of LLLT have been used to deliver irradiation to different cell lines in order to achieve the maximum proliferation rate (Table 1), mainly two types of LLLT are used for in vitro studies. One uses a helium neon (He-Ne) laser at a wavelength of 632 nm that transmits a red light, while the second type uses gallium-aluminum-arsenide (Ga-Al-As) with a wavelength of 830 nm, which is in the infrared portion of the spectrum. Most in vitro studies have been carried out with the He-Ne laser [99].

Effects of LLLT on bone marrow mesenchymal stem cells

Bone marrow-derived mesenchymal stem cells (BMSCs) have been used to treat many diseases like osteogenesis imperfecta, mucopolysaccharidoses, graft-versus-host disease, and myocardial infarction [114–117]. Due to their availability and expansion capacity, BMSCs have become a promising source of adult stem cells for regenerative medicine [118]. Usually, adequate numbers of BMSCs are required for clinical applications because the efficacy of grafted BMSCs is limited in host pathological microenvironments, even when transplanted at very high cell dosages [119–121]. Therefore, Hou at al. used LLLT to improve the proliferation rate of BMSCs (Table 1) using an indium-gallium-arsenate-phosphate (In-Ga-As-P) diode laser with a wavelength of 635 nm to irradiate the BMSCs [122]. During the experiments, BMSCs were irradiated for 75, 150, 300, and 750 s with energy density values of 0.5, 1.0, 2.0, and 5.0 J/cm^2, respectively (a control group was similarly treated but the cells were not irradiated). Hou et al. did not find any significant cytotoxicity differences between the non-irradiated and irradiated groups [122]. On the other hand, the proliferation rate of BMSCs was significantly higher in the irradiated group when compared to the non-irradiated groups.

Effects of LLLT on mesenchymal stem cells in stimulating an osteogenic phenotype

BMSCs, being pluripotent, can differentiate into several cell lineages when a suitable immediate environment (including extracellular matrix, spatial and temporal signals, growth factors, and cell–cell interactions) is provided [123–125]. Recently it was reported that BMSCs can be expanded and terminally differentiated into osteoblasts, chondriocytes, adipocytes, myoblasts, neural cells, or hematopoietic-supporting stroma in the presence of a suitable microenvironment and/or stimuli [123, 126, 127].

It has also been reported that LLLT on osteoblasts stimulates matrix production, DNA synthesis in cultured cells, and bone nodule formation [128]. These consequences have been validated by in vivo studies, proving the biostimulatory effect of LLLT on bone regeneration [129, 130]. Abramovitch-Gottlib et al. seeded BMSCs on three-dimensional (3-D) coralline (*Porites lutea*) biomatrices and irradiated them with a polarized He-Ne red laser (wavelength of 632.8 nm) to stimulate the osteogenic phenotype of BMSCs [131]. The irradiated samples showed enhanced tissue formation with the appearance of phosphorous peaks along with calcium and phosphate incorporation into the newly formed tissue. Higher ossification levels were observed in the irradiated samples when compared to the control group. Therefore, Abramovitch-Gottlib et al. suggested that 3-D crystalline biomatrices in conjunction with LLLT have biostimulatory effects on the conversion of BMSCs into bone-forming cells and on the induction of ex-vivo ossification [131].

LLLT promotes proliferation of mesenchymal and cardiac stem cells in culture

Tuby et al. used a diode (Ga-As) laser at 804 nm with an energy density of 1 to 3 J/cm^2 to irradiate mesenchymal and cardiac stem cells (Table 1) [10]. Control cells were also treated in the same way as the irradiated cells but the control cells were not irradiated. The number of mesenchymal and cardiac stem cells increased significantly after the LLLT when compared to the control cells. That study also demonstrated that LLLT promotes the proliferation of mesenchymal and cardiac stem cells in vitro. These results may have an important impact on regenerative medicine. The power and energy densities used during this LLLT did not induce any adverse effects on cells in culture and did not cause any histopathological changes in myogenic satellite cells in culture. Therefore, it can be concluded that the power and energy densities applied during this LLLT can be safely employed for the irradiation of cells in vitro.

LLLT enhances osteogenic differentiation in mesenchymal stem cells

Recently, red light at 647 nm was used to enhance osteogenic differentiation in mesenchymal stem cells [132]. Mesenchymal stem cells were irradiated with low-energy red light at 647 nm for different time periods and energy densities. Non-irradiated (control) cells were maintained under the same conditions as the irradiated cells. The red light at 647 nm was observed to significantly increase osteoblast mineralization in irradiated cells, after 4 to 5 days, when compared to non-irradiated cells. This result indicates that red light promotes osteoblast differentiation.

In another study, mesenchymal stem cells (MSCs) were irradiated with a blue laser (wavelength 405 nm) for 180 s via a fiber attached to the bottom of the culture dish. This study showed that blue laser irradiation was able to promote extracellular calcification of MSCs and induced the translocation of circadian rhythm protein cryptochrome 1 (CRY1 protein) from the cytoplasm to the nucleus. CRY1 is a master regulator of circadian clock that regulates the extracellular calcification of MSCs and this clock controls the bone mass [133].

LLLT enhances the proliferation of human dental pulp stem cells

Human dental pulp stem cells (hDPSCs) were treated with LLLT to increase the proliferation rate [134]. These stem cells were irradiated with indium-gallium-aluminum-phosphate (InGaAlp) lasers at a wavelength of 660 nm and an energy density of 3 J/cm^2, along with a control group (non-irradiated cells). This experiment showed that hDPSCs respond positively to LLLT, with improvements in cell growth when compared to the control group (Table 1). These results open the possibility of using LLLT for improving the growth rate of other types of stem cells.

Effects of LLLT on adult human adipose-derived stem cells

Adult human adipose-derived stem cells were irradiated using a diode laser at a wavelength of 635 nm and an energy density of 5 J/cm^2 [135]. The results of this experiment revealed that the proliferation rate of irradiated cells, measured by optical density, increased significantly when compared to non-irradiated cells. Moreover, Western-blot analysis and immunocytochemical labeling proved that the stem cell marker β 1-integrin was present at increased levels in irradiated cells compared to non-irradiated cells.

How to use LLLT to obtain the maximum proliferation of cells

Cell cultures should be 20% confluent at the time of irradiation to obtain the maximum yield from LLLT. The medium should be replaced with phosphate buffered saline (PBS) at the time of irradiation. Otherwise, serum will interfere with the reaction during irradiation. This procedure should be performed in the dark [10]. Most studies suggest that laser biostimulation occurs at fluences between

0.05 and 10 J/cm^2, whereas fluences above 10 J/cm^2 have bioinhibitory effects [5, 136, 137].

Conclusion and summary

In the past, LLLT has been used for pain relief, inflammation reduction, and wound healing. Recently, LLLT has been used to enhance the proliferation of stem cells and several other cell lines, which is essential for performing several different experiments related to disease control in humans. Effects of LLLT on proliferation of cell cultures depend on consumed energy density, total energy, number of irradiated points, and diameter of beam or irradiated area, type of laser, and sometimes on the type of cells as well (Table 1), as some cell lines give effective results with one type of laser and negative results with other laser types (Table 1). Most of the reports [81, 101–104, 144] showed that infrared wavelengths caused positive effects in fibroblast cell cultures, whereas other contradictory effects were reported [99]. These parameters are given and compared in Table 1. This review suggests that an energy density value from 0.5 to 4.0 J/cm^2 and a visible spectrum ranging from 600 to 700 nm for LLLT are very helpful in enhancing the proliferation of various cell lines. LLLT is able to increase cell numbers, DNA, and RNA synthesis and collagen production, and in addition is able to initiate mitosis in cultured cells. LLLT stimulates the photoreceptors present on the mitochondrial and cell membranes to convert light energy into chemical energy in the form of ATP within the cell, which enhances cellular functions and cell proliferation rate. Certain rules must be followed to obtain the optimal benefits from LLLT, as described in this review.

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