Effect of low-level laser-treated mesenchymal stem cells on myocardial infarction

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Abstract Cardiovascular disease is the leading cause of death worldwide. Although cardiac transplantation is considered the most effective therapy for end-stage cardiac diseases, it is limited by the availability of matching donors and the complications of the immune suppressive regimen used to prevent graft rejection. Application of stem cell therapy in experimental animal models was shown to reverse cardiac remodeling, attenuate cardiac fibrosis, improve heart functions, and stimulate angiogenesis. The efficacy of stem cell therapy can be amplified by low-level laser radiation. It is well established that the bio-stimulatory effect of low-level laser is influenced by the following parameters: wavelength, power density, duration, energy density, delivery time, and the type of irradiated target. In this review, we evaluate the available experimental data on treatment of myocardial infarction using low-level laser. Eligible papers were characterized as in vivo experimental studies that evaluated the use of low-level laser therapy on stem cells in order to attenuate myocardial infarction. The following descriptors were used separately and in combination: laser therapy, low-level laser, low-power laser, stem cell, and myocardial infarction. The assessed low-level laser parameters were wavelength (635–804 nm), power density (6–50 mW/cm²), duration (20–150 s), energy density (0.96–1 J/cm²), delivery time (20 min–3 weeks after myocardial infarction), and the type of irradiated target (bone marrow or in vitro-cultured bone marrow mesenchymal stem cells). The analysis focused on the cardioprotective effect of this form of therapy, the attenuation of scar tissue, and the enhancement of angiogenesis as primary targets. Other effects such as cell survival, cell differentiation, and homing are also included. Among the evaluated protocols using different parameters, the best outcome for treating myocardial infarction was achieved by treating the bone marrow by one dose of low-level laser with 804 nm wavelength and 1 J/cm² energy density within 4 h of the infarction. This approach increased stem cell survival, proliferation, and homing. It has also decreased the infarct size and cell apoptosis, leading to enhanced heart functions. These effects were stable for 6 weeks. However, more studies are still required to assess the effects of low-level laser on the genetic makeup of the cell, the nuclei, and the mitochondria of mesenchymal stromal cells (MSCs).

Keywords Cardiovascular diseases · Low-level laser · Low-power laser · Laser parameters · Laser effects · Stem cell

Introduction

Cardiovascular disease (CVD) is the leading cause of death worldwide [1]. Conventional therapeutic approaches aim to restore blood flow by dissolving the thrombus or decreasing myocardial oxygen consumption. However, new therapeutic approaches aim to limit the scar size after myocardial infarction (MI). For end-stage cardiac diseases, cardiac transplantation is still the most effective therapy. However, organ transplantation faces the limitations of the availability of donors and the possibility of organ rejection, and thus, new approaches for treatment are required. Among the most promising approaches is the administration of exogenous stem cells into the heart to overcome the insufficiency of cardiac resident stem cells to repair the damage after acute injury [2].
Mesenchymal stromal cells (MSCs) are multipotent cells with the ability to differentiate into osteoblasts, adipocytes, chondrocytes, and myocytes. MSCs could be obtained from the bone marrow, adipose tissue, lung, umbilical cord, teeth, placenta, and other organs. They are identified by their fibroblast-like morphology and ability to form colonies in vitro. They adhere to plastic, and proliferate in vitro into cells of different lineages.

Following acute MI, administration of MSCs into the infarcted area was shown to reverse cardiac remodeling [2], attenuate cardiac fibrosis, and improve heart functions [3]. While many of the experimental data on stem cell therapy in MI were promising, clinical trials using MSCs therapy for cardiac diseases provide conflicting results. Some studies [4, 5] reported no significant improvement in the left ventricular ejection fraction (LVEF) in patients receiving bone marrow stem cell (BM-SCs) therapy, where stem cells were administered through intracoronary injection (IC) 1, 6, or 7 days after percutaneous coronary intervention (PCI). Other clinical trials on the other hand have been promising. Significant improvement in LVEF was observed after 3, 4, and 6 months in patients who received BM-SCs after 1, 4, 5, 7, 12, or 18 days after PCI [6, 7]. Nevertheless, more studies showed that the long-term improvement in heart functions was minor [8]. This was reported in three double-blind clinical trials on ischemic heart failure patients receiving autologous MSCs and followed up for 6 months after the transplant [8]. More optimization of several factors is still required [9]. One of the limiting factors for stem cell therapy is the significant cell loss after the injection into the heart. This loss may be due to mechanical damage (caused by the injection itself) or due to the harsh microenvironment of the damaged heart (hypoxic insult, reperfusion injury, and the inflammatory process) [10]. Novel approaches thus aim to increase stem cell proliferation without causing damage in the cellular DNA, or due to chromosomal deletion or translocation [11]. One of the most promising approaches to overcome this cell loss is the application of light therapy (phototherapy) to the transplanted cells.

Light amplification by stimulated emission of radiation (laser) therapy is considered a novel and promising approach in wound healing, as it affects mostly injured tissues, and has minimal deleterious effects on healthy, uninjured cells [12]. Among all the types of laser, low-level laser (LLL) and red- or near-infrared laser with wavelength range (600–1100 nm) have beneficial effects on cells including cell proliferation, migration, improved adhesion, and prevention of cell apoptosis [13]. LLL increases the viability of adipose-derived MSCs, their proliferation [14], angiogenic potential [15] and anti-inflammatory effects [16], and their differentiation ability into smooth muscle cells [17] and osteocytes [18]. Furthermore, LLL increases the proliferation rate of BM-SCs [19, 20] and their osteogenic differentiation capacity [21]. In the heart, it was demonstrated that LLL-treated MSCs have cardioprotective effect in myocardial infarction (MI) animal models [22–24]. Laser therapy was administered either by irradiation of cultured stem cells in vitro, followed by their re-implantation in vivo, or by direct in vivo irradiation of the bone marrow or the affected myocardium. Many hypotheses have been proposed for the cardioprotective effect of LLL-treated MSCs including increasing the mitochondrial respiration and ATP synthesis, stimulating angiogenesis, increasing the stem cell survival, reducing the inflammatory responses, decreasing the number of injured cardiomyocytes, and reducing scarring. The biostimulatory effect of LLL seems to be influenced by several parameters including the wavelength of the laser, its power density, dose, delivery time, energy density, frequency, and the type of irradiated cells [25].

In this review, we identify the optimum LLL parameters used for treating MI by reviewing all LLL parameters used in pre-clinical trials, and comparing their effect focusing on the attenuation of the scar tissue and the promotion of angiogenesis as primary targets for MI therapy.

Our hypothesis is that for improving heart functions and treating MI, LLL with wavelength in the range of 780 to 950 nm and energy density in the range of 10 to 50 J/cm² given every day or every other day will be adequate to treat either BM-MSCs, myocardium, or the bone marrow itself. This effect is supposed to last at least for 2 weeks in animal models.

**Irradiating with laser**

Upon irradiating the tissue with laser, the beam is transmitted to the surface, reflected, scattered, or absorbed at each layer (Fig. 1). As a photon, it can only be absorbed by its chromophore to transfer energy, producing an effect on the tissues. *Transmission* indicates the passage of all the photons through the tissue without producing any effect, and this occurs in the absence of chromophores. *Reflection* occurs at all interfaces of media through which laser beam is travelling. *Scattering* of energy can happen due to lack of homogeneity in the tissue structures, such as molecules, organelles, cells, or larger tissue.
structures. Greater scattering means less penetration. Therefore, it is necessary to increase the photon absorption by reducing laser reflectance and scattering [26]. Adjustments in duration and energy density can thus overcome the challenge of delivering the maximum number of photons to the target chromophore and avoid unneeded absorption by surrounding tissues [26].

In order for LLL to exert an effect, LLL photons must be absorbed by chromophores. This depends mainly on LLL wavelength since both absorption and scattering of light by tissue are wavelength dependent [27]. Thus, blue, green, and yellow light are effective for cells growing in transparent culture medium, while red and near infrared light are effective for treating animals and patients [27].

Absorbed by the tissue, LLL induces physical and chemical changes in the chromophore molecules of the respiratory chains yielding biological changes [28]. These changes include redox properties changes, electron transfer acceleration, nitric oxide (NO) release from cytochrome c oxidase, and superoxide generation [29].

**LLL therapeutic mechanism**

The exact therapeutic mechanism of LLL is still debatable. LLL irradiation increases oxygen consumption and mitochondrial products such as ATP, NADH, protein, and RNA. It was suggested that LLL is absorbed by cellular respiratory chain component. Since the absorption spectrum of cytochrome c oxidase in different oxidation states was very similar to the action spectra for cellular responses to LLL, it is considered as the primary chromophore for the red-near infrared range in mammalian cells [30]. Cytochrome c oxidase is a transmembrane protein complex, consisting of two nuclear copper centers and two nuclear heme centers, that facilitate the transfer of electrons from water-soluble cytochrome c oxidase to oxygen (final electron acceptor) in the respiratory electron transport chain involved in producing ATP [31].

Different mechanisms had been proposed for the therapeutic effect of LLL: First, LLL reverses the inhibitory effect of nitric oxide on cytochrome c oxidase by photodissociation of nitric oxide from the binuclear center CuB/a3 of cytochrome c oxidase. Hence, LLL results in increased respiration rate and ATP production [32] (Fig. 2). This is known as “NO hypothesis.”

Second, LLL promotes the metabolism of oxygen in the electron transport chain producing superoxide anions O_2^- (considered as reactive oxygen species, ROS) as a natural by-product. Also, other redox chain compounds such as NADPH oxidase can be activated by LLL. Thus, LLL induces high amounts of ROS shifting the cell redox state to greater oxidation. This is known as “redox properties alteration hypothesis.” Changes in redox state activate transcription factors related to cell proliferation, migration, nucleic acid synthesis, protein synthesis, cytokine production, and cell cycle progression [25] (Fig. 3). Among these transcription factors are redox factor-1 (Ref-1)-dependent activator protein-1 (AP-1) (Fos and Jun) and nuclear factor κβ. Thus, LLL effects vary according to cells redox states: at reduced state (low intracellular pH), cells will significantly respond to LLL; while at optimal redox state, cells will weakly respond to LLL or will not respond at all. Another mechanism by which LLL regulates cell functions is via increasing ATP level that regulates adenyl cyclase (cAMP), and increases proton gradient increasing the activity of the Na^+/H^+ and Ca^{2+}/Na^+ antiporters and ATP-driven carriers for ions: Na^+/K^+ ATPase and Ca^{2+} pumps. Both Ca^{2+} and cAMP are very important second messengers regulating gene expression [27]. This may explain how LLL stimulates cell growth by suppressing cell apoptosis and regulating the expression of cell proliferation genes [27].

On the cellular level, LLL triggers mast cell degranulation releasing the pro-inflammatory cytokine TNF-α from the cells [33]. This leads to increased infiltration.
by leukocytes, further enhancing the cellular proliferation [33].

Laser parameters

In vitro biostimulation by LLL depends on many parameters, such as wavelength, output power, energy density, duration, delivery time, type of irradiated cells, and frequency of irradiation (as shown in Table 1). Some of these parameters may be useful for certain targets and not others. For instance, the parameters used for increasing cell proliferation may influence protein synthesis negatively [36]. For example, using LLL of 904 nm wavelength and 3 J/cm² energy density increased the cell proliferation rate significantly, but it also changed the ultrastructure of the cultured cells. The mitochondria became edematous and the rough endoplasmic reticulum became dilated [36], and the excretion of proteins into the extracellular environment was impaired. Therefore, the optimization of the combination of parameters to reach a certain target is necessary.

Wavelength

Wavelength affects both the absorption by chromophores and the depth of laser penetration into tissues. It was found that for the purpose of treating animals and patients by LLL, the maximum effect “optical window” is from 650 to 930 nm. Wavelength of 600 to 660 nm is considered the best for superficial therapies, while a wavelength of 801 to 980 nm is better for deep tissue therapy [37]. Zhang et al. used LLL of 635 nm wavelength and 0.96 J/cm² energy density to irradiate cultured BM-MSCs 3 weeks after induction of MI in rats. The irradiation did not improve heart functions although it enhanced the cell survival, cytoprotection, and angiogenesis [34]. Furthermore, these effects were unstable after 1 week as shown by cell apoptosis, vascular density, left ventricular end diastolic volume, left ventricular end systolic volume,
| Wavelength (nm) | Power density (mW/cm²) | Energy density (mW/cm²) | Duration (s) | Target | Frequency | Delivery time (after MI) | Effects |
|-----------------|------------------------|-------------------------|--------------|--------|-----------|-------------------------|---------|
| 635             | 6.37                   | 0.96                    | 150          | In vitro-cultured BM-MSCs: 2 × 10⁶ cells, 80-90% confluent | 1        | 3 weeks | The combination of these parameters:  
  • Did not improve heart function compared to the control group as shown by the  
    ○ Insignificant difference in left ventricular end diastolic volume 6.89 ± 0.73 compared to 7.10 ± 0.7 in the control group  
    ○ Insignificant difference in left ventricular end systolic volume 5.53 ± 0.82 vs 5.77 ± 0.81  
    ○ Insignificant difference in left ventricular ejection fraction 48.64 ± 7.57 vs 46.49 ± 6.62  
    ○ Insignificant difference in left ventricular fractional shortening 20.08 ± 3.95 vs 18.92 ± 3.21  
  • Increased angiogenesis  
  • Increased cytoprotection as shown by the increase of GRP78  
  • Increased vascular densities 24 ± 3.22 vs 19 ± 2.28%  
  • Increased cell survival 1.07 ± 0.28 vs 0.68 ± 0.22%  
  • Decreased cell apoptosis 1.81 ± 0.63 vs 3.55 ± 0.89% |
| 804             | Not mentioned          | Not mentioned           | Not mentioned | In vitro-cultured BM-MSCs | 1        | 30 min | The combination of these parameters:  
  • Reduced infarct size to 76% compared to positive control, and to 53% compared to treating by MSCs alone  
  • Increased the density of c-kit⁺ cells 6.3-fold compared to treating by MSCs alone  |
| 804             | 50                     | 1                       | 20           | In vitro-cultured BM-MSCs: 1.5 × 10⁶ cells, 90% confluent | 1        | 30 min | The combination of these parameters:  
  • Reduced the infarct size to: 50% compared to positive control, and to 53% compared to treating by MSCs alone  
  • Increased the density of c-kit⁺ cells 6.3-fold compared to control positive  
  • Increased angiogenesis 2-fold compared to treating by MSCs alone  
  • Increased the vascular densities 2-fold compared to the positive control, and 1.4-fold compared to treating by MSCs alone  
  • Increased cytoprotection as shown by the reduction of p38 MAPK by 12.5% |
| 804             | 10                     | 1                       | 100          | Bone marrow | 1        | 20 min | The combination of these parameters:  
  • Reduced the infarct size to 76% compared to positive control  
  • Increased the density of c-kit⁺ cells 25-fold compared to control positive  
  • Reduced the ventricular dilatation to 75% compared to positive control |
| 804             | 10                     | 1                       | 100          | Bone marrow | 1        | 4 h after MI, sacrifice after 3 weeks | The combination of these parameters:  
  • Reduced the infarct size to 54% compared to positive control |
and left ventricular fractional shortening. This may be due to the poor capacity of LLL of 635 nm wavelength to penetrate tissues [34].

On the other hand, Tuby et al. applied LLL of 804 nm wavelength and 1 J/cm² energy density to irradiate cultured BM-MSCs 30 min after induction of MI in rat model; the irradiation enhanced cardiac functions, attenuated the infarct size, increased the survival of transplanted cells, increased cell proliferation, and enhanced angiogenesis [23, 24]. Cardiac functions were assessed by p38 mitogen-activated protein kinases, which are involved in the cardiac dysfunction and reactive collagen deposition. Cell survival and proliferation were assessed by the increased density of c-kit+ cells and BrdU-labeled cells [23, 24]. Angiogenesis was assessed by the increased expression of vascular endothelial growth factor (VEGF), the higher density of blood vessels of LLL-treated MSCs, and the improved vascular density in the infarct and peri-infarct area [23, 24].

Another study by the same group used LLL of 804 nm wavelength and 1 J/cm² energy density to irradiate the bone marrow directly for 20 min or 4 h after MI induction in rat model. The treatment increased the proliferation of MSCs, their homing, and the formation of new cardiomyocytes; and decreased the infarct size, the ventricular dilatation, and the concentration of infiltrating macrophages. These effects lasted for 6 weeks [22, 35]. However, using an infrared viewer (guiding beam) in order to determine the irradiation area may have confounded the results at least partially.

**Power density and energy density**

Power density is defined as the rate of delivery of photons per unit area. The optimal power density depends on the used wavelength and tissue type. For instance, for bio-stimulation purposes, using 2–12 mW/cm² maximized the output benefit [38].

Whereas the energy density is the amount of energy stored by the tissue per unit volume, it determines the magnitude of laser interaction. The optimum energy density depends largely on the required effect (cell proliferation was maximized using 0.5 or 1 J/cm² while the secretion of angiogenic factors and the myogenic differentiation induced by 5-azacytidine were maximized by 5 J/cm²) [20].

Both power density and energy density are considered key biological parameters for the effectiveness of laser therapy. Within the lower (5 mW) and higher thresholds (10 J/cm²), laser therapy is effective, as strong light penetrates the tissues deeper than weak light. However, outside this range, laser therapy is either too weak to have any effect or so intense that the tissue interaction is inhibited [39].

The optimum energy density of LLL for the cell proliferation is debatable. One group reported that using LLL of 635 nm wavelength and different energy densities of 0.5, 1,
2, and 5 J/cm² to irradiate cultured BM-MSCs, 0.5 J/cm² produced the highest proliferation rate [20]. Another group used LLL of 660 nm wavelength and either 1 or 0.5 J/cm² energy density twice at 0 and 48 h to irradiate cultured BM- and adipose-MSCs. An energy density of 1 J/cm² enhanced BM- and adipose-MSC proliferation more effectively than 0.5 J/cm² [11]. This discrepancy may be related to other different parameters such as the wavelength (635 vs 660 nm), the duration of irradiation (75 vs 33 and 16 s), and the frequency of irradiation.

In studies on LLL-MSC therapy in MI rat model, researchers used only three different power densities: 6.37 mW/cm² (635 nm) [34], 10 mW/cm² (804 nm) [22, 35], and 50 mW/cm² (804 nm) [24]. However, since the researchers used also different exposure times, the amount of energy stored by the tissue per unit volume (energy density) was kept constant [0.96 J/cm² 39, and 1 J/cm² [22, 24, 35]]. For example, Tuby et al. used 50 mW/cm² for 20 s, which had yielded 1000 mW/cm² = 1 J/cm², while in another study, Tuby et al. used 10 mW/cm² for 100 s which also had yielded 1000 mW/cm² = 1 J/cm². Noticeably, 10 mW/cm² reduced the infarct size and increased the density of c-kit⁺ cells more than 50 mW/cm² [24, 35]. However, the effects of energy densities could not be compared in such system, and more studies are still required to determine their effect on the therapeutic behavior of MSCs in cardiac damage.

**Frequency of treatment**

Interestingly, LLL may lose some of its efficacy if given too frequently “biphasic dose response” [40]. In vivo showed that using only two different treatment frequencies: 3 [22] and 1 have effective therapeutic outcome on MI [23, 24, 34, 35]. Tuby et al. had shown that using three doses of LLL of 804 nm wavelength and 1 J/cm² energy density to treat the BM 4 h after MI induction in rat model increased stem cell density but to a dramatically lower extent compared to using one dose while its potential to reduce the infarct size remains unaffected [22, 35].

**Different parameters yield different effects**

Collectively, different parameters of LLL treatment may yield very different, even opposite effects. For instance, using LLL of 804 nm wavelength and 3 J/cm² energy density for 60 s to treat BM-MSCs increased cell proliferation [19] while using LLL of 808 nm wavelength and 4 J/cm² energy density for 91 s failed to stimulate proliferation [41]. This may be due to the combined effect of using a slightly greater wavelength, a higher energy density, and frequent administration. Thus, more studies are still required for the standardization of the parameters required for generating each effect.

**Delivery time**

LLL treatment delivery time is a pivotal factor in determining the clinical applicability of LLL. In a series of studies on the effect of LLL-treated MSCs on MI in rat model, Tuby et al. reported that LLL reduced the infarct size and increased cell proliferation effectively if given 20 min [35], 30 min [23, 24], or 4 h after MI [22, 35]. These effects lasted for 6 weeks. This provides evidence that LLL irradiation can be applied in the clinical setting [35]. However, Zhang et al. showed that LLL treatment after 3 weeks failed to improve heart functions [34].

**Target of the treatment**

Direct treatment of the BM by LLL irradiation increased the density of c-kit⁺ cells significantly and reduced the scar size and ventricular dilatation more effectively than treating the myocardium [35]. Also, LLL direct treatment to BM increased the density of c-kit⁺ cells more efficiently than LLL treatment of in vitro-cultured BM-MSCs (25-fold vs rarely found) while both of them reduced the scar size by 76% [24, 35].

**Measurement of the effects of LLL treatment on MI**

In order to assess the effect of LLL-treated BM-MSCs in repairing cardiac injury, clinical efficacy would be assessed by measuring left ventricular end diastolic fraction, end systolic fraction, ejection fraction, fractional shortening, and ventricular dilatation. Histological evaluation is usually determined by the size and extent of scarring reduction. Other contributing therapeutic benefits include improved angiogenesis, increase of ATP synthesis, cell survival, cell proliferation and differentiation, and homing of MSCs to the site of injury (Table 1).

**Scarring reduction**

Recent studies provided evidence that treatment by either LLL or administration of MSCs reduced the scar tissue significantly after MI, while treatment by both of them (LLL-treated MSCs) may reduce it even more. Tuby et al. showed that using LLL of 804 nm wavelength and 1 J/cm² energy density to treat either BM-MSCs or the bone marrow itself in rat model of MI reduced the infarct size and increased the formation of new myogenic cells with abundant mitochondria and ribosomes [22–24, 35].
Angiogenesis

LLL may enhance the formation of new blood vessels. Tuby et al. reported that using LLL of 804 nm wavelength and 1 J/cm² energy density for 20 s increased the expression of vascular endothelial growth factor (VEGF) and the density of blood vessels [24]. Furthermore, Zhang et al. showed that using LLL of 635 nm wavelength and 0.96 J/cm² energy density for 150 s increased the vascular densities in the infarct and the peri-infarct area [34].

Cardioprotective effects

Tuby et al. showed that using LLL of 804 nm wavelength and 1 J/cm² energy density 20 or 30 min or 4 h after MI induction in rat model enhanced heart functions. This was shown by the reduction of ventricular dilatation [35] and p38 mitogen-activated protein kinases (involved in cardiac dysfunction and reactive collagen deposition) [24]. However, Zhang et al. reported that using LLL of 635 nm wavelength and 0.96 J/cm² energy density for 150 s did not improve heart functions. However, it had a cytoprotective effect as it protected cells against MI injury by decreasing the malondialdehyde (lipid peroxidation) and increasing VEGF and GRP78 expression, and protected against oxidative stress by increasing the superoxide dismutase.

Cell survival and proliferation

LLL seems to enhance the proliferation and/or survival of BM-MSCs. Tuby et al. showed that using LLL of 804 nm wavelength and 1 J/cm² energy density for 20 or 30 min or 4 h after MI induction to treat BM-MSCs increased the density of stem cells for 3 weeks, but faded after 6 weeks [22–24, 35]. Also, LLL increased BM-MSCs proliferation rate [24]. The latter effect may be due to absorption of laser energy by intracellular chromophores, which increases ATP production, and consequently upregulates DNA, RNA, and protein synthesis [32]. Moreover, Zhang et al. showed that using LLL of 635 nm and 0.96 J/cm² energy density improved BM-MSC survival and reduced BM-MSC apoptosis [34].

Assessing the possibility of malignant transformation, Hou et al. determined the cellular DNA growth pattern using LLL of 635 nm wavelength and 0.96 J/cm² energy density. They showed that cells irradiated with LLL showed increase in cell number and cellular DNA synthesis rates comparable to the growth of non-irradiated groups. They concluded that the natural characteristics of BM-MSCs remain unaffected by LLL-induced proliferation and that LLL did not cause malignant proliferation [20].

A study by Smith et al. may have shed some light on the conditions necessary for LLL-induced cell proliferation [42]. When the cultured cells were already growing robustly in vitro culture, LLL-induced proliferation was limited; while if cells were poorly growing, LLL irradiation stimulated better cell proliferation [42]. Thus, in order to maximize LLL yield, cells should be 20% confluent and placed in phosphate-buffered saline (PBS) in the dark at the time of the irradiation [19].

Cell differentiation

Tuby et al. showed that using LLL of 804 nm wavelength and 1 J/cm² energy density increased desmin (marker of muscle cells)-immunostained cells and enhanced BM-MSC ability to differentiate into cardiomyocytes [22]. These data suggest that the observed differentiation effect is not caused by BM-MSC treatment alone, since BM-MSCs poorly differentiated into cardiomyocytes upon transplantation into the heart [43]. On the other hand, Zhang et al. reported that using LLL of 635 nm wavelength and 1 J/cm² energy density failed to induce BM-MSC differentiation into cardiomyocytes 1 week after the irradiation. This may be due to the longer time (up to several weeks) required for BM-MSCs to differentiate into myogenic cells [34].

Homing of MSCs

LLL therapy was shown to promote MSC survival into the damaged organs. Tuby et al. reported that using LLL of 804 nm wavelength and 1 J/cm² energy density increased MSC concentration in the circulating blood [22]. Interestingly, LLL was found to enhance MSC-specific homing to the injured site rather than its random deposition on the entire LV [23, 24, 35].

Moreover, the same study showed also that LLL increased the concentration of macrophages in the circulating blood [22]. Macrophages were stimulated to migrate from the BM and home onto the infarcted area. This effect may prove valuable as a possible mechanism for reducing the infarct size.

Missing effects

In order to have a comprehensive assessment of the effect of treating MSCs with LLL, other factors should be taken into consideration, in addition to cell proliferation, migration, homing, apoptosis, and differentiation. For example, the effect of laser treatment on the genetic makeup of the nuclear or mitochondrial DNA must be assessed, especially when concerns of mutation, malignancy, and tumor formation are considered. A study by Oron et al. used LLL, of 803 nm wavelength and 1.08 J/cm² energy density for 3 min to treat a dog model of MI. The therapy increased ATP levels in the infarcted area, and stimulated new myogenic protein synthesis. It also reduced the number of damaged mitochondria, slowed down the destruction of the cardiomyocytes, and
diminished the infarct size [44]. This effect was also shown in a rat model [44]. In another study, Barboza et al. showed that using LLL of 660 nm wavelength and 1 J/cm² energy density for 33 s caused no damage to MSCs nuclei either by fragmentation or by pyknosis [11]. However, these data cannot negate the possibility of causing chromosomal translocations or deletions that must be assessed by means of karyotyping [11]. Another important factor is the status of the cells to be treated by LLL since this will affect the secondary cellular signaling cascades that clearly determine the cells’ final photobiological response. For instance, pathological cells significantly respond to LLL due to the reduced cellular responses, while nonpathological cells minimally or do not respond [45].

Conclusion and future perspective

The combination of LLL and stem cell therapy can be implemented by targeting cultured BM-MSCs, the myocardium, or the bone marrow itself by the irradiation. This treatment has shown to significantly reduce the scar tissue and promote angiogenesis; enhance cell survival, cell proliferation, and differentiation; and improve homing of the stem cells to the site of injury, leading to enhanced cardiac functions [22–24, 34, 35]. The extent of these effects varies according to the status of cells (healthy or pathological), their redox status (reduced or optimum), and LLL parameters (wavelength, power density, energy density, delivery time, frequency, and irradiated target, Table 1).

In this review, we show that the best outcome for treating MI using LLL was achieved by direct irradiating of the bone marrow by only one dose of LLL. The most effective parameters were the use of 804 nm wavelength and 1 J/cm² energy density within 4 h post-MI. This treatment enhanced heart functions, decreased the infarct size, reduced cell apoptosis, and increased stem cell survival, proliferation, and homing. These effects were stable for 6 weeks [22–24, 34, 35]. The mechanisms underlying these effects are not completely understood. However, one of the mechanisms of action seems to be via the effect of LLL on exciting the photoreceptors of mitochondrial and cell membranes and converting light energy into chemical energy (ATP) within the cell. Conclusions about either the efficacy of LLL treatment of MI or the mechanism of action are, however, based on very few number of studies. A large number of preclinical and clinical studies are required. Moreover, important effects of LLL on the genetic makeup of the cell, the nuclei, and the mitochondria of MSCs must be investigated.

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Compliance with ethical standards

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

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