Chapter

Combined Administration of Stem Cells and Photobiomodulation on Wound Healing in Diabetes

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Abstract

Wound healing is an active and compound biological course which can be divided into four steps: hemostasis, inflammation, proliferation, and remodeling. Diabetes mellitus induces weakened wound healing by disturbing one or more of the biological functions of these steps. Diabetic foot ulcers result from the simultaneous action of multiple disturbing causes. Mesenchymal stem cells, especially autologous ones, are easily accessible with noninvasive methods and have been shown to provide a regenerative microenvironment at wound sites. Despite current knowledge, major hurdles remain to be overcome in order to achieve effective therapeutic effects. Photobiomodulation is the use of light to reduce pain and inflammation and stimulate healing and the proliferation of stem cells, which would be very useful in increasing stem cell function and in regenerative medicine. The current study analyzes the results of studies using separate and combined administrations of stem cells and photobiomodulation on diabetic wound healing in patients and animal models. We hypothesize that the combined application of photobiomodulation and stem cells will accelerate the repair process and assist the healing of foot ulcers in diabetes mellitus patients.

Keywords: wound healing, diabetes mellitus, diabetic foot ulcers, mesenchymal stem cells, adipose tissue-derived stem cells, photobiomodulation

1. Introduction

Diabetes mellitus (DM) is the most important cause of illness and death, affecting 422 million adults worldwide [1]. Epidemiological studies of DM in the U.S. have shown that almost one out of every three people in the U.S. is prone to preDM or suffering from DM. The Centers for Disease Control and Prevention (CDC) recently reported that more than 100 million adults in the U.S. have DM or pre-DM. In 2015, a total of 30.3 million people of all ages, or 9.4% of the U.S. populace, were reported to have DM. Moreover, a total of 33.9% of the U.S. adults aged 18 years or older (84.1 million people) had pre-DM in 2015. Almost half (48.3%) of adults aged 65 years or older have pre-DM which, if left untreated, will develop into Type 2 DM within 5 years [2]. Of the entire population of the U.S., 33% are predicted to be afflicted by DM by the year 2050 [3]. Diabetic foot ulcer (DFU) is still the predominant cause of hospitalization for patients with DM,

and DM is the chief reason for more than 50% of nontraumatic leg amputations. Obviously, these operations increase the death ratio [3].

In this chapter notes are provided about the following subjects: 2, acute wound healing in healthy subjects; 3, a mechanistic approach to wound healing in DM; 4, DFU;5, administration of stem cells in DFUs; 6, adipose tissue-derived stem cells (ADSC); 7, regenerative potential of ADSC; 8, PBM and its effects on cells and stem cells; 9, how the combined application of photobiomodulation (PBM) and ADSCs accelerates healing in DFU; and 10, finally we will deliver our conclusions in section 10.

2. Acute normal skin injury repair course in healthy subjects

The acute normal skin injury repair course can be separated into four overlying steps: 1. coagulation; 2. inflammation; 3. proliferation; and 4. remodeling. During the first step, blood-clotting actions preclude extreme hemorrhage and deliver temporary protection to the injured area. The development of inflammation directs the use of leukocytes, neutrophils, and macrophages; the creation of growth factors; and the stimulation of fibroblasts, keratinocytes, and angiogenesis. Achievement of the proliferation step in wound repair directs the creation of extracellular matrix (ECM), i.e. rich, vascularized granulation tissue. Lastly, ECM maturation and cell apoptosis direct the creation of scar tissue with physical features that are similar to unwounded skin [4]. The repair of an acute skin injury comprises synchronized cellular and molecular responses. First, immune cells migrate to the injury site, then they initiate pathogen clearance, while also participating in the repair course. Cut epidermal borders upregulate wound-related genes, thereby allowing mutual cell migration. Local and blood-borne fibroblasts increase and migrate to produce wound granulation tissue, provide organization and signaling clues, and deliver new ECM. Some fibroblasts differentiate into myofibroblasts to help wound closure. The wound bed is perfused with oxygen and nutrients through new blood vessels derived by angiogenesis [5].

3. A mechanistic approach to wound healing in DM

DM causes the repair course directed to a non-healing wound (chronic wound or ulcer) to lag, resulting in practical restrictions, gait trouble, and contamination. The weakening of repair in DM patients is well-known, but the connection between pathophysiology and weakened skin injury repair in DM is still an unidentified etiology. The repair course requires cooperation between inflammatory cells and biochemical mediators encouraged by many elements. Nevertheless, alterations in the cellular and biochemical elements and accomplishments are concerns associated with wound healing failure in DM patients. Neutrophils, monocytes, macrophages, keratinocytes, fibroblasts, T and B cells, mast cells, and endothelial cells all contribute to wound repair and dynamically to the creation and regulation of various cytokines and growth factors. Monocytes, which later transform into macrophages, are the principal manufacturers of pro-inflammatory cytokines, including interleukin-1 (IL) -1β, tumor necrosis factor (TNF)-α, IL-6 and cytokines, and growth factors such as vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF)-1, and transforming growth factor (TGF) - β in both healthy and diabetic subjects. Neutrophils, such as T and B cells, are also important producers of TNF-α and IL-10 cells among others, keratinocytes, fibroblasts, mast cells, and endothelial cells which participate in the production of VEGF, IGF-1, and TGF-β.

Macrophages are fundamental providers in healing. Hyperglycemia and oxidative stress alter the epigenetic code that results in alterations to the polarization and plastination of macrophages. Dysregulated macrophage polarization is one of the key hindrances to wound repair. Investigations have revealed that in DM, a compound function is included at the molecular level which is accountable for hindered wound repair. Actions like the continued production of pro-inflammatory cytokines, weakened angiogenic response and microvascular difficulties, weakened macrophage and neutrophils function, weakened keratinocytes and fibroblast migration, and increased and weakened creation of healing-associated elements like decreased growth factor creation have been reported in animal simulations of DM. The steps of the remedial course in diabetic sufferers are also delayed (in the inflammatory stage) by other elements as well as specific metabolic insufficiencies, weakened functional responses like hypoxia due to glycation of hemoglobin, and changes in red blood cell membranes and the tapering of blood vessels. Hypoxia reduces the oxygen stream to wounds because of tapering blood vessels. Hemoglobin glycation causes a lack of nutrients and oxygen to tissue, which further interrupts the repair course. Diabetic wounds continuously stimulate the unfolded protein response (UPR) and increase expression of pro-inflammatory chemokine in comparison with normal wounds. Native ischemia because of microvascular problems in DM significantly delays the repair course [6].

Reduced IGF-1 and TGF-β values at sites of tissue injury have been described in both diabetic animals and human (h) s with DM and are accountable for delayed repair to skin injuries. TGF-β employs and encourages the motivation of inflammatory cells, including neutrophils, macrophages, and lymphocytes, as well as keratinocytes, fibroblasts, and the creation of growth factors, which hasten neovascular formation, and the creation and delayed deterioration of ECMs. The decreased attentiveness of TGF-β has been described in skin injury repair in diabetic subjects. Many studies have proven that matrix metalloproteinase (MMP)-encoding genes have a TGF-β1-dependent preventative component in the promoter region, which down-regulates expression of the gene. Reduced TGF-β values and improved expression of MMPs induce the extreme deterioration of growth factors. Accompanied by MMP-encoding genes, transcription factors like Smad-2, Smad-3, and Smad-4 also trigger and suppress TGF-β target genes. TGF-β1 triggers Smad-2 and 3 for the creation of collagen. Reductions in TGF-β1 values augment the use of triggered inflammatory cells to hinder progression from the inflammatory step to the proliferation step in the repair course of diabetic wounds. Elevated TGF-β3 values are supposed to reduce TGF-β1 values in diabetic subjects, which leads to augmented macrophage action and reduced collagen creation. In DM, elevated glucose levels increase macrophage action, directing more reactive oxygen species (ROS) to extend the inflammatory step. Reduced values and expression of these growth factors weaken and extend the skin injury repair course in DM [6].

4. DFU

Disturbances in the coordination of glucose homeostasis induce hyperglycemic prominence and result in the initiation of certain metabolic pathways that, in their unusual situation, lead to the progression of vascular deficiency, nerve damage caused by ulcerations in inferior limbs because of changed patterns of plantar pressure, and consequently foot abnormalities. Abuse to the foot produced by trauma to the affected area remains hidden to the patient because of damage to afferent sensory nerves [7]. Diabetic neuropathy results in foot muscular inequality, inadequate

feeling in the skin, and ultimately foot irregularities that lead to augmented force applied to the skin when walking. Collectively, the above-mentioned occurrences are accompanied by foot ischemia [8] and DFU formation.

When a foot ulcer develops, the foot is at increased risk for aggressive infection, and as soon as it is combined with a peripheral artery occlusive disease, the sufferer will have dangerous foot ischemia [8]. Thus, the etiology for DFU is composite. Disruption of harmony in glucose homeostasis causes hyperglycemic status, results in activation of certain metabolic pathways which in their abnormal state subsequently leads to development of vascular insufficiency, nerve damages headed by ulceration in lower extremity due to plantar pressures and foot deformity. Staphylococcus is the most common infectious bacterium [9]. A diabetic foot infection may be a warning limb complaint. Infection is identified by the occurrence or augmented ratio of inflammation markers. Frequently, these markers are less noticeable than anticipated. Imaging investigations can identify or better define profound, soft tissue-infected areas and are regularly required to detect pathological results in bone. The primary bactericidal cure as well as the length of cure are observational. There is a considerable delay in DFU injury repair that has been correlated to many irregularities [9]. Today, DM is the chief origin of non-traumatic amputations in the U.S. Generally, around 5% of DM patients develop DFUs, and 1% of them wind up with an amputation. Around 60% of diabetic patients will develop neuropathy, ultimately leading to a DFU. The danger of a DFU is augmented in people with flatfoot, as they apply uneven pressure across the foot, leading to local inflammation in risky areas of the foot. The yearly occurrence of DFU ranges from 9.1 to 26.1 million cases globally, and about 15% to 25% of DM patients will develop a DFU sometime during their lifespan.

As the number of newly identified DM cases rises annually, the occurrence of DFU is also destined to rise. DFUs are accountable for higher medical charges than any other diabetic difficulty. The usual cost of curing one DFU is $8,000, that of an infected DFU is $17,000, and that of a chief amputation is $45,000. Over 80,000 amputations are done yearly on diabetic patients in the U.S., and approximately 50% of patients with amputations will develop ulcers and infections in the other foot within 1.5 years. Sadly, 58% of people with DM will experience a second amputation 3–5 years after the first one. Furthermore, the prevalence of death occurring 3 years after a first amputation has been estimated to be as high as 20%–50%, and these statistics have not altered considerably in the past 30 years despite major developments in the medicinal and surgical management of DM patients [10]. Management of DFUs is mainly based on severity (score), blood vessel status, and the existence of contamination. Inhibiting the reappearance of DFUs remains a chief medical objective [11]. Numerous novel cures correlated to these aberrations have been discovered in wound repair with differing achievements [9].

5. Administration of mesenchymal stem cells (MSC) in DFUs

As previously described, DFUs are one of the more frequent and severe difficulties of DM, as wound repair is weakened in the diabetic foot. Investigations concentrated on comprehensively understanding these functions could allow for a precisely directed cure for DFUs. The main treatments for DFUs are currently wound debridement, weight off-loading, neovascularization, and contamination treatment. Nevertheless, some DFUs are extremely impervious to routine cures, and the development of wound repair remains to be the goal of numerous cure policies. Novel cure choices such as bioengineered skin substitutes, ECM proteins, cytokines,

and negative pressure wound therapy, have been developed as supplementary remedies for DFUs [12]. Stem cell therapies have appeared as top-notch cure methods with the possibility of returning tissue to its pre-injury state.

The use of cellular therapy in the treatment of skin injuries is presently a dynamic field of research. Multi-potent adult stem cells are an attractive option for cell therapy, as they have a high possibility of proliferation and the capability of differentiating into diverse cell types and creating a range of cytokines and growth factors essential to wound repair. This study concentrated on the involvement of three types of adult stem cell populations through a skin injury repair course and their beneficial possibilities for use in cell therapy.

Endothelial progenitor cells (EPCs) are endothelial precursors involved in the revascularization of injured tissue and tissue repair. Their vascular repairing potentials have been described in a range of translational and human investigations into ischemic illnesses, together with myocardial infarction, stroke, and peripheral arterial illness. Furthermore, numerous articles have stated that EPC engraftment can enhance wound repair by improving new blood vessel formation in granulation tissue. It has been reported that the administrated EPCs released a variety of wound repair-related growth factors and cytokines, thus encouraging the implementation of monocyte/macrophage and exciting endogenous new blood vessel formation during the course of skin injury repair. Another study showed that the transplantation of human cluster of differentiation (CD) 133^+ progenitor cells into streptozotocin-induced diabetic mice amplified the wound closure rate and capillary density in granulation tissues. These results suggest that EPC engraftment would be favorable for the cure of skin wounds, specifically chronic wounds which are often connected with reduced peripheral blood flow and continue to be tough to heal using existing beneficial tactics [13].

Bone marrow-derived mesenchymal stem cells (BM-MSCs), comprise another talented nominee for the reparation or substitution of injured tissue. BM-MSCs have the ability to differentiate into numerous lineages, such as endothelial cells, neural cells, and hepatocytes, among others. Furthermore, research has shown that BM-MSCs participate in wound repair by differentiating into numerous cutaneous cell types. It has further been reported that BM-MSCs differentiate into keratinocytes, endothelial cells, pericytes, and monocytes. One study reported that BM-MSCs significantly improved wound repair in both diabetic and nondiabetic mice; BM-MSC-treated wounds displayed augmented wound contraction by discharging proangiogenic elements including VEGF and angiopoietin-1. Analysis of paracrine elements released from BM-MSCs with real-time polymerase chain reaction (PCR) and of BM-MSC-CM by enzyme-linked immunosorbent assay (ELISA) showed that BM-MSCs secreted VEGF, IGF-1, epidermal growth factor (EGF), keratinocyte growth factor (KGF), angiopoietin-1, and stromal derived factor (SDF)-1. These paracrine elements from MSC-condition media (CM) displayed a pronounced influence in utilizing $CD14^+$ monocytes, keratinocytes, and endothelial cells in injured tissue, thus encouraging the skin injury repair course [13].

6. Adipose tissue-derived stem cells (ADSCs)

ADSCs are placed inside the stromal vascular fraction of adipose tissue. They have the ability to differentiate into adipogenic, osteogenic, chondrogenic, and myogenic cells when they are cultivated in particular culture circumstances. New information has shown the possible effects of ADSCs on new blood vessel formation in ischemic illness animal simulation. ADSCs discharge numerous powerful antigenic elements and were also shown to collaborate in angiogenesis by differentiating into endothelial cells in an *in vivo* study. The engraftment of ADSCs is reported to

encourage wound contraction and enhance blood perfusion in injured skin. When ADSCs were cultivated in hypoxic circumstances, they released VEGF 5-time more than in normoxic circumstances [13].

In regenerative medicine, adult stem cells are the greatest encouraging cell types for cell-based therapies. Human adipose tissue has been presented as a novel origin for multipotent stem cells. These so-named ADSCs are considered perfect for use in regenerative therapies. Their chief benefit over MSC extracted from other origins, e.g., from bone marrow, is that they can be simply and repeatable collected using negligibly aggressive methods with little injury. ADSCs are multipotent and can differentiate into numerous cell types. Interestingly, ADSCs are categorized by immunosuppressive properties and have little immunogenicity. Their discharge of trophic elements make compulsory the healing and regenerative results in an extensive variety of administrations. Generally, these specific characteristics of ADSCs make them very much applicable for medical uses. Therefore, the beneficial probability of ADSCs is huge [13].

7. Regenerative potential of adipose tissue-derived stem cells

The beneficial impacts of ADSCs have been determined to be valuable in regenerative therapies for many illnesses. Specifically, ADSCs can be collected, handled, and cultured in a nominally aggressive, yet calm and persuasive method, and they have the great probability of differentiating into mature cells along the mesodermal, ectodermal, and endodermal lineages. Throughout recent years, crucial advancements have been made concerning the separation, morphological features, molecular biology, and in vitro differentiation potential of stem cells, and it has become clear that ADSCs might facilitate beneficial effects. Not only do they act as tissue-specific progenitor cells, but they also participate in a number of chief functions, e.g., paracrine-mediated signaling of angiogenesis, inflammation, cell homing, and cell survival. The above-mentioned essential results have assisted us in gradually closing the hole between basic knowledge and clinical application; meanwhile, ADSCs have been used in clinical trials all over the globe, presenting as harmless and realistic options in a range of simulations.

Nevertheless, before ADSCs can be used in conventional medical administrations, numerous obvious queries associated with ADSCs must be resolved. With the intention of fully appreciating the fundamental functions which control ADSCs, future experimentations should, for example, concentrate on additional accurate markers for the improved and source-precise classification of ADSCs. Moreover, the genetic alteration of ex vivo-cultivated cells should not be ignored, and the controllers concerning differentiation, migration, and cell viability after in vivo engraftment must be clarified. Furthermore, as the scientific comprehension of the regenerative capabilities and, therefore, the potential uses of ADSCs increases, the possible dangerous threats must be addressed, and the supervisory outline that directs their medical usage must be established. Presently, precise supervisory instructions are set by the country in which treatment occurs. Clearly, the worldwide standardization of rules of use is crucial. In conclusion, scientific advancements, supervisory rules, and a commercial substructure are all vital factors in the development and conversion of this talented MSC origin [14].

Conversely, there are some methodological questions for the application of MSCs and ADSCs for skin regeneration in DM patients, as discussed below.

1. The elevated extracellular glucose density in diabetic wounds leads to the collection of advanced glycosylation end products (AGEs). The creation

of AGEs prevents proliferation, leads to hADSCs apoptosis, prevents the differentiation, proliferation, and homeostasis of ADSCs into endothelial cells, and also prevents the production of collagen protein, ultimately hindering wound repair [15].

2. Despite the rising usage of MSCs in medicinal human research, the curative benefit continues to be insignificant [16]. This is partially related to the normal, limited disease-modifying ability of MSCs [16]. At the same time, tissue destruction and the remedial response lead to the excretion of interior danger signals [17], comprising Toll-like receptors (TLRs) and interleukin-1 receptors, type 1 (IL-1R1) ligands, that alter the immune microenvironment [18]. TLRs and IL-1R1 unfavorably impress the cure of numerous injured organs [19]. IL-1R1/myeloid differentiation primary response 88 (MYD88) signaling unfavorably regulates bone repair in mice by injuring the regenerative abilities of murine MSCs. Furthermore, IL-1b that is released at bone injury areas inhibits the regenerative abilities of MSCs [20]. Thus, new approaches to increasing the strength of MSCs is an active area of life science research with medical importance [16]. MSCs have been one of the profoundly investigated options for cell therapy. As the homing ability of MSCs is a key influential element of effective MSC-based cures, the progress of homing effectiveness is important for creating faultless, positive outcomes. Therefore, tactics to stimulate and reinforce the function, mobilization, and homing of MSCs have advanced a key option in regenerative medicine [21].

3. Stem cell numbers have been reduced in some animal simulations of skin damage. Wu et al. observed a significant rise in the survival of stem cells 7 days after inducing skin damage and a quick reduction in cell viability 14 days after damage [22]. Muhammad et al. presented that the engraftment of ADSCs hastens the course of acid burn skin injury repair [23].

8. PBM and its effect on cells and stem cells

The term "LASER" originated as an acronym for "light amplification by stimulated emission of radiation." Laser radiation could encourage a photobiomodulatory impact on cells and tissues, participating in a concentrating inflection of cell behaviors, increasing the courses of tissue repair. PBM, also recognized by its former term low-level laser therapy (LLLT), is a safe technique that participates in pain reduction and decreases inflammation, along with improving cure and tissue healing. It also encourages cell propagation and increases stem cell differentiation [24]. PBM is a fast-developing technology applied many medical situations where stimulus of repair, decrease in pain and inflammation, and renovation of action are needed. While skin is obviously exposed to light more than any other organ, it still reacts fine to red and near-infrared wavelengths. The photons are absorbed by mitochondrial chromophores in skin cells. Therefore, electron transport, adenosine triphosphate nitric oxide release, blood flow, ROS increase, and various signaling paths are triggered. Stem cells can be activated, permitting augmented tissue repair and healing [25]. PBM, with its above-mentioned properties, can mediate numerous illnesses and circumstances, such as DM, brain damage, spinal cord injury, dermatological circumstances, oral annoyance, and diverse fields in dentistry. Most studies have reported a rise in the propagation ratio of radiated cells [24]. PBM definitely controlled the in vitro propagation of the ADSC examined, and new in

vitro documents have been presented where PBM meaningfully augmented hADSCs cell survival in comparison to control and PBM-treated hBM-MSC groups [26].

9. How the combined application of PBM and ADSCs can accelerate DFU healing

Concerning the low viability ratio of ADSCs transplanted onto a wound, the application of some superior pretreatment agents not only makes available a good biological circumstance for the transplanted ADSC, but also encourages their propagation, differentiation, and paracrine capabilities and causes them to discharge more cytokines and growth factors [27]. Because PBM can augment the proliferation ratio of cultivated ADSCs [26], it can be considered as an effective approach for the preconditioning of ADSCs in in vitro situations preceding ADSC transplantation. In Zare et al. study [26] both in vitro human bone marrow-derived mesenchymal stem cells (hBM-MSCs) and h adipose-derived stem cells (hADSCs) were irradiated with 36 protocols using two different laser types (helium-neon [He-Ne] and diodes), four different laser wavelengths (HeNe laser, 630 nm, 810 nm, 630 + 810 nm); three different energy densities (0.6 J/cm^2, 1.2 J/cm^2, 2.4 J/cm^2); and three different PBM times (1, 2, and 3). A total of 1×10^4 MSCs were seeded in each well of a 24 well-plate. Next, the He–Ne laser at 632.8 nm, (IR-2000; IAEA, Tehran, Iran), red laser at 630 nm, NIR laser at 810 nm, and 630 nm +810 nm (NILTVIR202 Noura Instruments, Tehran, Iran) were applied. It should be mentioned that immediately after switched on the laser machine, it was ready for PBM therapy. In order to ensure exposure of the entire well (15.6 mm well) to PBM, the He–Ne laser emission was expanded by an optic culminator and the spot size of the red and NIR lasers were increased by a cone shaped pine hole culminator. Control MSCs did not receive PBM. **Table 1** lists the PBM protocol specifications.

Zare et al. study [26] demonstrated that PBM with the combined 630 + 810 nm lasers significantly stimulated 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay which was measured the effects of PBM on MSC viability, and significantly decreased population doubling time (PDT) and apoptosis rate of hBM-MSCs and hASDCs in vitro. There were no pharmacological side effects of PBM on MSC as evidenced by measuring apoptosis rate of MSCs. Zare et al. reported new in vitro evidence where PBM administered at 630 nm (one and two times, 0.6 and 1.2 J/cm^2) and 630 + 810 nm (three times, 2.4 J/cm^2) significantly increased hADSC cell viability compared to its control and the PBM-treated hBM-MSC groups. PBM-based medical trials and experiments will display new uses for PBM and MSC remedies [28].

Laser type	Wavelength (nm)	Power (W)	Duration of each session (s)	Energy density (J/cm^2)	Laser beam diameter (cm)	Laser beam area (cm^2)	Power density (W/cm^2)
He-Ne	632.8	0.005	229, 458, 917	0.6, 1.2, 2.4	1.56	1.91	0.00261
Red	630	0.05	23, 46, 92	0.6, 1.2, 2.4	1.56	1.91	0.0261
Near infrared (NIR)	810	0.05	23, 46, 92	0.6, 1.2, 2.4	1.56	1.91	0.0261

Table 1.
Specifications of the photobiomodulation (PBM) protocol.

Accordingly, Ahmadi et al. examined the efficiency of several preconditioned ADSCs and PBM regimes on healing an infected ischemic delayed-healing wound in type 1 diabetic rats. Their study included five groups of rats: (1) control, (2) control ADSCs [diabetic ADSCs were engrafted into the wound bed], (3) ADSCs + PBM in vivo (diabetic ADSCs were transplanted into the wound, followed by in vivo PBM therapy), (4) ADSCs + PBM in vitro, and (5) ADSCs + PBM in vitro + in vivo.

Ahmadi et al. for in vitro study seeded a total of 1×10^4 passage-4 ADSCs in each well of a 24-well plate for each of three groups: healthy control ADSC, diabetic control ADSC, and experimental diabetic ADSC. Here, red laser alone plus infrared laser alone (NILTVIR202 Noura Instruments, Tehran, Iran) at two energy densities (1.2 J/cm^2 and 2.4 J/cm^2) were used to irradiate the ADSC every other day for three sessions according to a previously published protocol. Ahmadi et al. found that diabetic ADSCs preconditioned with PBM had significantly increased the MSC viability, and significantly decreased PDT, and apoptotic rate of ADSCs in comparison with diabetic ADSCs. **Table 2** lists the in vitro, and in vivo PBM parameters. The control ADS did not receive PBM. The wounds of the rats in groups 3 and 5 were subjected to PBM in vivo (**Figure 1**).

Specifications of in vitro photobiomodulation							
Laser type	**Wavelength (nm)**	**Power (W)**	**Time of each session (s)**	**Energy density (J/cm^2)**	**Laser beam diameter (cm)**	**Laser beam area (cm^2)**	**Power density (W/cm^2)**
Red	630	0.05	46	1.2	1.56	1.91	0.0261
Near infrared	810	0.05	46	1.2	1.56	1.91	0.0261
Specifications of in vivo photobiomodulation							
Parameters				**Dose and unit**			
Peak power output				75 W			
Average power				0.001 W			
Power density				0.001 W/cm^2			
Wavelength				890 nm			
Wavelength range of the device				890 ± 10 nm			
Pulse frequency				80 Hz			
Spot size				1 cm^2			
Diameter				1.12 cm			
Pulsed duration				180 ns			
Duration of exposure for each point				200 s			
Energy density				0.2 J/cm^2			
Number of laser shootings in each session				9			
Energy densities for one session and for the total sessions				1.8 and 25.2 J/cm^2			
PBM radiation scheduling				Immediately after surgery, 6 days per week, for 16 consecutive days			
Probe				L07			
Company				MUSTANG 2000, Technica Co., Russia			

Table 2.
Specifications of in vitro and in vivo photobiomodulation parameters.

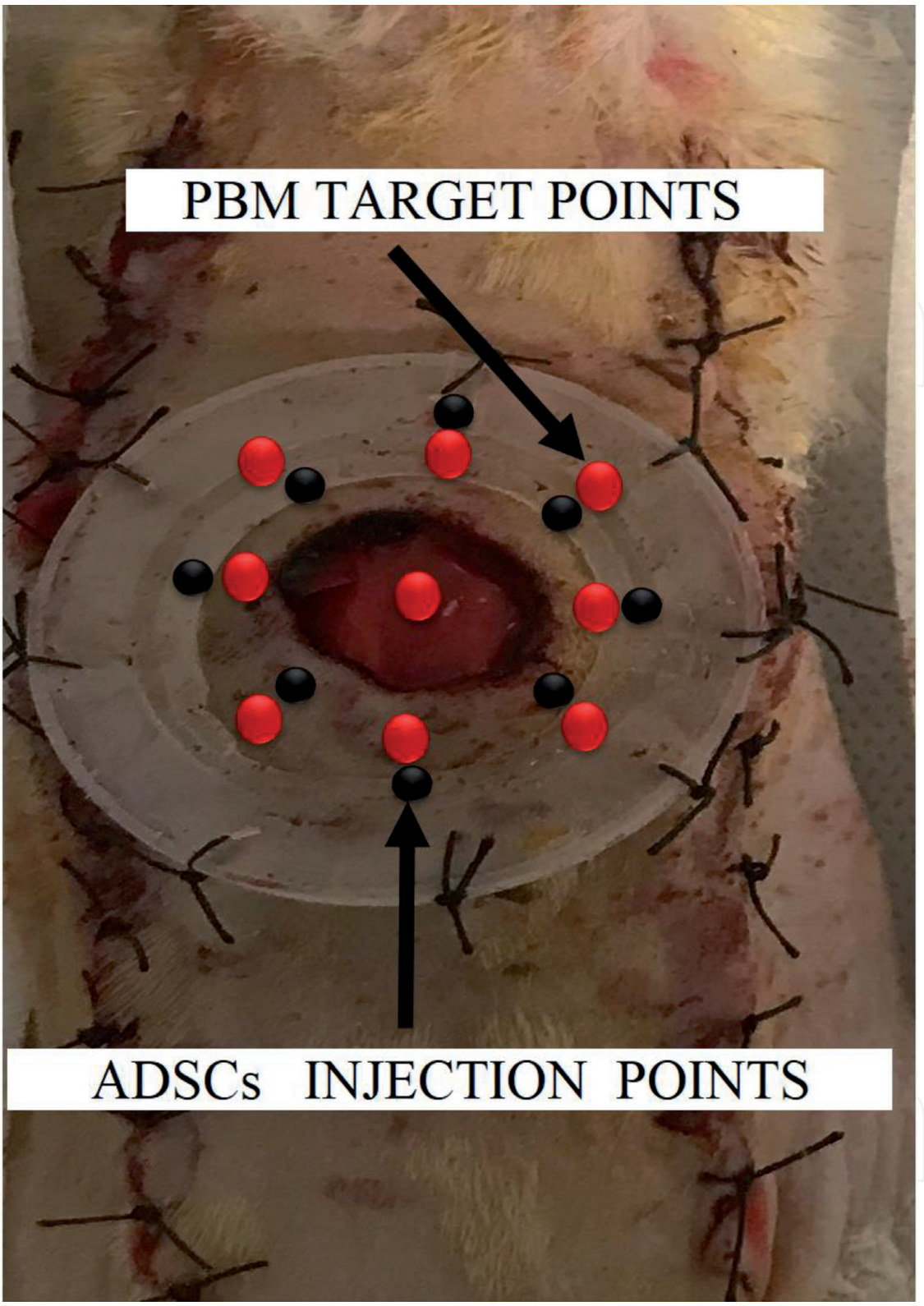

Figure 1.
A photo of the wound, photobiomodulation (PBM) target points, and adipose tissue -derived stem cell (ADSCs) injection points.

Table 2 lists the complete specifications of the PBM protocols for invitro and in vivo studies. There were no pharmacological side effects of PBM on MSC in Ahmadi et al. study as evidenced by histological examination of wounds.

Groups 3 and 5 showed significant reductions in bacterial contamination compared to groups 1 and 2. Groups 2, 3, 4, and 5 showed significantly enhanced wound contraction ratios in comparison with group 1. Groups 2–5 displayed

significantly increased wound strength compared to group 1.In most cases, group 5 had significantly better results than groups 2, 3, and 4. Ahmadi et al. concluded that preconditioning diabetic ADSCs with PBM in vitro plus PBM in vivo significantly accelerated healing in the diabetic rat model of an ischemic infected delayed-healing wound [29]. In other related studies, the same results were reported. Khosravi et al. reported that the in vitro preconditioning of hADSCs with PBM significantly amplified bone repair in a rat model of critical size femoral defect in vivo [30]. Liao et al. explored the therapeutic potential of hADSCs preconditioned with PBM. Cultured ADSCs were treated with PBM. In addition, a mouse photoaged skin simulation was proven by UVB radiation. Liao et al. concluded that PBM is a persuasive bioenhancer of ADSCs and may improve the healing possibility of ADSCs for medical use [31]. While few studies give some evidence for the positive effects of PBM alone for wounds in diabetic patients [32], or PBM plus skin grafts for burn ulcers in diabetic patients [33, 34], there have been no clinical trials using human models to show stem cells plus PBM as an effective agent in wound care regimes to date. Further well designed clinical trials are necessary to determine the true value of ADSCs plus PBM in routine wound care regimes for patients with DM.

10. Conclusions

Present knowledge dictates that when an organ is healthy, the inflammatory phase of wound healing is well orchestrated, lasting only a few days, and the steps of tissue repair proceed normally. However, when an organ is hyperglycemic, as with DM, the inflammatory process is extended, the integrity of the skin is not restored, and DFU occurs. DFUs are a serious clinical problem and affect millions of people around the world. They need repetitious cures, impose extensive medical expenses, and create a major economic burden on healthcare systems worldwide. Thus, much work has been concentrated on evolving new healing approaches for wound treatment. Preclinical studies have shown that preconditioning diabetic ADSCs with PBM in vitro significantly increases ADSC function over that of diabetic ADSCs. Preconditioning diabetic ADSC with PBM significantly hastened healing in ischemic MRSA–infected, delayed-healing wounds in rats with type one DM compared to the control, ADSC alone, and ADSC plus PBM-in vivo rats. The combined administration of preconditioned diabetic-ADSC with PBM plus PBM therapy in vivo demonstrated a significantly superior effect compared to other treatment protocols [29].

Whereas our hypothesis (combined application of PBM and stem cells can accelerate repairing process and assist healing DFU in animal models and patients) was confirmed through preclinical studies [29, 30, 31], we suggest further animal and clinical trial investigations be conducted in order to provide more documentation. Hopefully these outcomes would help the use of ADSCs plus PBM as a routine treatment protocol for the healing of severe DFU in patients with DM.

We confirm there were no conflicts of interest.

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