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Special Issue Invited Review

Could Light-Based Technologies Improve Stem Cell Therapy for Skin Wounds? A Systematic Review and Meta-Analysis of Preclinical Studies†

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ABSTRACT

Several diseases or conditions cause dermatological disorders that hinder the process of skin repair. The search for novel technologies has inspired the combination of stem cell (SC) and light-based therapies to ameliorate skin wound repair. Herein, we systematically revised the impact of photobiomodulation therapy (PBM) combined with SCs in animal models of skin wounds and quantitatively evaluated this effect through a meta-analysis. For inclusion, SCs should be irradiated *in vitro* or *in vivo*, before or after being implanted in animals, respectively. The search resulted in nine eligible articles, which were assessed for risk of bias. For the meta-analysis, studies were included only when PBM was applied *in vivo*, five regarding wound closure, and three to wound strength. Overall, a positive influence of SC + PBM on wound closure (mean difference: 9.69; 95% CI: 5.78–13.61, $P < 0.00001$) and strength (standardized mean difference: 1.7, 95% CI: 0.68–2.72, $P = 0.001$) was detected, although studies have shown moderate to high heterogeneity and a lack of information regarding some bias domains. Altogether, PBM seems to be an enabling technology able to be applied postimplantation of SCs for cutaneous regeneration. Our findings may guide future laboratory and clinical studies in hopes of offering wound care patients a better quality of life.

INTRODUCTION

The skin is the largest organ in the human body playing a key role in the maintenance of the body's functions. Complications in skin healing still challenge healthcare leading to annual costs for wound care exceeding $15 billion worldwide (1). Besides, several diseases or conditions cause dermatological disorders that hinder the process of skin repair and healing.

Regenerative medicine is the branch of medicine focused on the development of methods and efficient strategies to properly achieve the regrowth, repair, or replacement of injured cells, organs and tissues (2). It stands out as one of the most promising fields in healthcare, which mostly includes the therapeutic use of mesenchymal stem cells (SCs) alone or in combination with biocompatible materials and biologically active molecules to assemble functional constructs that will further substitute tissues and/or promote endogenous regeneration. SCs are defined by their self-renewal and differentiation capacity into one or multiple specialized cell types and can be obtained from embryonic or somatic tissues (3). These cells also secrete growth factors and cytokines and have immunomodulatory properties that enhance tissue regeneration and prevent scar formation (4). In this context, regenerative medicine has been proposed to improve cutaneous regeneration and create artificial skin tissues (5,6).

Light-based therapies are enabling technologies that have fueled the interest in regenerative medicine due to the growing demand for less invasive approaches (7). The first scientific evidence about the use of light to treat diseases dates back more than a century when Niels Finsen was awarded the Nobel Prize for Medicine or Physiology in 1903 for his contribution to skin disease treatment with concentrated light radiation (8). However, it was just with the development of the laser in the 1960s that light-based technologies gained notoriety. Currently, lasers, light-emitting diodes (LEDs) and lamps in proper wavelengths have been used to treat different conditions and improve the patient's quality of life, mainly through photobiomodulation therapy (PBM).

PBM is a promising light-based technology that has been used to treat skin diseases with encouraging outcomes since it can accelerate tissue repair by interrelated mechanisms (9). PBM has also been demonstrated to influence SC viability, differentiation, proliferation or migration, leading to an improvement in their recoverability (10). Thus, PBM combined with SCs could be useful for the development of new approaches to repair skin wounds in regenerative medicine, as well as restore the function of compromised cells.

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A considerable number of animal models addressing the association of SCs and PBM at pre or postexposure have been published over the last decades. In the wake of the 50 years since the founding of the American Society for Photobiology, much of our knowledge about the biological effects of red and near-infrared light has evolved. Thus, it seems quite appropriate to verify if PBM may be an adjuvant to SC therapy to treat skin wounds. In this systematic review, we gathered efforts to join evidence from animal studies to evaluate whether light-based technologies can enhance SC therapy for cutaneous repair. We carefully examined the validity of the preclinical evidence to identify gaps, guide future research and provide insights for a possible translation from the laboratory bench to clinical practice.

MATERIALS AND METHODS

This systematic review was conducted and reported following SYRCLE (Systematic Review Center for Laboratory animal Experimentation) guidelines (11). We included and compared preclinical studies involving animal models that received SCs *in vitro* pre-exposed or *in vivo* postexposed to light radiation. SCs could be inoculated alone or seeded on scaffolds depending on the intervention model. The protocol was registered in the PROSPERO database under the number CRD42022329856.

The search strategy included articles from the electronic databases of PubMed, Scopus, Web of Science and EMBASE. The search period comprised published articles from entry until April 30, 2022. Keywords related to cell therapy, tissue engineering and light therapy were used with Boolean operators AND/OR for the search. Keywords were established according to the analysis of the criteria of the PICO question (P: population, animal models; I: intervention, light + cell therapy; C: comparison, cell therapy; O: outcome, skin repair), with the following question: Could light-based therapies be an adjuvant to cell therapy in pre or postexposure of SCs for ameliorating cutaneous wound healing? We used the following keywords: Animal* OR "*in vivo*" OR "preclinical" AND "Phototherapy" OR "Photobiomodulation" OR "Light therapy" OR "LED therapy" OR "Light-emitting diode" OR "Laser therapy" AND "stem cells" OR "tissue engineering" OR "cell therapy" OR "cellular therapy" OR "regenerative medicine."

The study selection included original articles with intervention in any animal model concerning the use of SCs combined with light in pre or postexposure for the healing of skin wounds. Studies in the clinical practice of Veterinary Medicine were excluded. Two reviewers (TMY and MSR) performed the search independently and duplicates (repeated articles) were removed before the screening. Subsequently, we screened titles and/or abstracts, and those not aligned with the purpose of the review were excluded. Articles published in other languages than English, reviews, letters, comments, abstracts/conference proceedings, *in vitro* studies and clinical trials were also excluded. We later excluded articles reporting the use of light or cell therapy as the only intervention as well as light combined with drugs and SCs.

Articles retrieved by the search were tabulated into a Microsoft Excel spreadsheet, which included author information, article title, type of SC, animal model, light parameters, irradiation protocol and outcome. For eligibility, studies should present the number of animals/groups, surface markers or differentiation assays to confirm the use of SCs, reliable light parameters and irradiation protocol (radiant exposure, irradiance and exposure time) and nonirradiated SC group as a control group. All reviewers assessed the studies for eligibility and disagreements were solved after discussion.

The quality of included articles was achieved by SYRCLE's risk of bias (RoB) tool (12) and judged by two reviewers (FPS and MSR). The degree of bias was categorized as low, high or unclear related to the (1) selection bias (sequence generation, baseline characteristics and allocation concealment); (2) performance bias (random housing and blinding); (3) detection bias (random outcome assessment and blinding); (4) attrition bias (incomplete outcome data); (5) reporting bias (selective outcome reporting) and (6) other sources of bias. We used the Kappa coefficient to appraise the agreement between reviewers and disagreements were resolved *via* discussion among all reviewers.

We performed a meta-analysis with data extracted regarding the percentage of wound closure and the maximum force (N), which is related to the wound tensile strength, for included studies where PBM was applied *in vivo*. Mean values and standard deviations from plots were obtained using the *WebPlotDigitizer* (13) if not reported by the authors. We extracted data for SC and SC + PBM groups from 14 to 16 days after injury to standardize the follow-up period. We performed a random effect meta-analysis in which the effect for wound closure was measured as the mean difference (MD). For the force, the fixed effect and standardized mean difference (SMD) were used because the animals were similarly handled but data were differently scaled. Comparisons between SC and SC + PBM groups were obtained with 95% confidence intervals (95% CI). We used the Chi-square test and I^2 to evaluate heterogeneity in the effect size of the primary outcomes. Statistical analysis was carried out through Cochrane RevMan software (Review Manager 5.4).

RESULTS

Our research identified 1736 articles through database searching, of which 779 duplicates were removed. Subsequently, 957 publications were screened and then 943 were excluded after reading the title and/or abstract. As a result, 14 articles were assessed for eligibility, resulting in nine articles that were included in this review. Five studies were disqualified because they did not fit our inclusion criteria, that is, three showed absent and/or unreliable light protocols (14–16), one did not report surface markers or differentiation assays for SCs (17) and one did not present nonirradiated SC group as a control group (18) (Fig. 1).

We noticed that out of the nine selected studies, all of them were conducted using adipose-derived SCs (ADSCs) (19–27). We also found that monolayer was the predominant type of cell culture, used in seven articles (19–23,26,27). However, some studies also covered other types of cell culture, including spheroids (20,25) and clusters (24). Yet, we observed that in all articles SCs were directly applied to the site of action. Indeed, in eight studies cells were intradermally delivered (19–24,26,27), whereas only one reported that cells were intramuscularly inoculated (25) (Table 1).

All animal models involved in the experimental research were rodents, including five studies with Wistar rats (19,21–23,27) and four comprising mice [two with BALB/c (20,25) and two with BALB/c nude mouse strain (24,26)]. The male gender was preferred among all of them, except for one study that used female mice (26). Age was more heterogeneous ranging from 5 weeks (24) to 3 months (22,27). Three studies did not report animal age (19,21,23), two used 7-week-old mice (20,25) and one used 6-week-old mice (26).

In terms of the cell graft, we found that in five works the research was conducted using human ADSCs (20,22–25) and one applied canine ADSCs (26). Therefore, those were considered xenotransplants since all experimental animal models were rodents. In the other studies, allotransplants were employed, all from rats (19,21,27).

The skin flap was one of the disease models observed, which was present in two out of the nine selected articles (20,25). Although four works reported skin-infected wounds in diabetic Wistar rats, three of them induced diabetes mellitus type 1 (DM1) (19,21,22), whereas the other induced diabetes mellitus type 2 (DM2) (27). In those works, a full-thickness wound was created in the middle of a bipedicle flap, which was further infected with methicillin-resistant *Staphylococcus aureus* (19,21,22,27). Two studies promoted full-thickness skin wounds

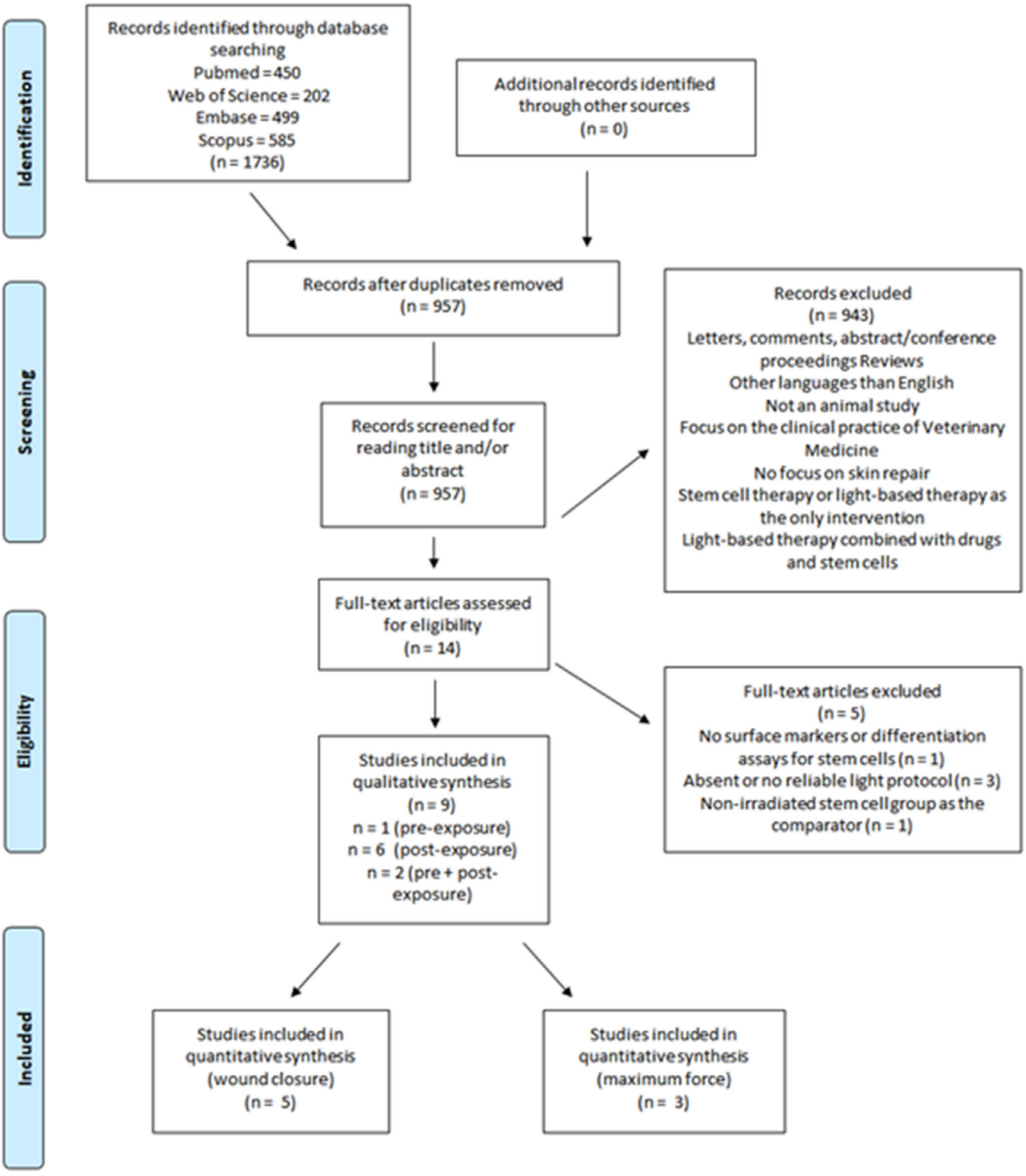

Figure 1. PRISMA flow chart of the literature search and study selection. [Colour figure can be viewed at wileyonlinelibrary.com]

in athymic mice (24,26) and one induced a third-degree burn in rats (23) (Table 1).

Regarding PBM therapy, we found that in one article, SCs were irradiated *in vitro* before transplantation into the animal's wounds (20). In six studies, PBM was applied after SC transplantation into the skin wound (22–27). In the other two studies, SCs were pre and postirradiated (19,21). Even though all nine studies reported the use of red and/or near-infrared wavelengths, PBM protocols were rather diversified (Tables 2 and 3). Moreover, all of them reported that PBM combined with SCs at

Table 1. Stem cell and animal characteristics of the selected studies.

Cell characteristics					Animal characteristics				
Authors	Cell type and culture	Type of graft	Delivery route	SC confirmation	Animal model	Gender	Age	n/group	Disease model
Ahmadi *et al.* (19)	Diabetic rat ADSC, monolayer	Allograft	Intradermal	Flow cytometry	Wistar rat	Male	NR	6	DM1 infected wound
Park *et al.* (20)	hADSC, spheroid	Xenograft	Intradermal	Flow cytometry	BALB/c mice	Male	7 weeks	8	Skin flap
Ahmadi *et al.* (21)	Diabetic rat ADSC, monolayer	Allograft	Intradermal	Flow cytometry	Wistar rat	Male	NR	6 (2)	DM1 infected wound
Ebrahimpour-Malekshah *et al.* (22)	hADSC, monolayer	Xenograft	Intradermal	Flow cytometry	Wistar rat	Male	3 months	6	DM1 infected wound
Andrade *et al.* (23)	hADSC, monolayer	Xenograft	Intradermal	Flow cytometry	Wistar rat	Male	NR	24 (6)	Third-degree skin burn
Park *et al.* (24)	hADSC, cluster	Xenograft	Intradermal	Flow cytometry	BALB/c nude mice	Male	5 weeks	9*	Full-thickness skin wound
Park *et al.* (25)	hADSC, spheroid	Xenograft	Intramuscular	Flow cytometry	BALB/c mice	Male	7 weeks	8	Skin flap
Kim *et al.* (26)	Canine ADSC, monolayer	Xenograft	Intradermal	Flow cytometry	BALB/c nude mice	Female	6 weeks	18 (6)*	Full-thickness skin wound
Moradi *et al.* (27)	Rat ADSC, monolayer	Allograft	Intradermal	Flow cytometry	Wistar rat	Male	3 months	6	DM2 infected wound

ADSC, adipocyte stem cells; hADSC, human ADSC; DM1, diabetes mellitus type 1; DM2, diabetes mellitus type 2; NR, not reported. The number in parentheses refers to animals euthanized per subgroup during the experimental period. *Denotes wounds instead of animals per group.

pre or postexposure ameliorates cutaneous repair by different mechanisms depending on the skin wound model (19–27) (Tables 2 and 3).

For PBM in pre-exposure, in two articles the authors used two different light sources, a red and infrared laser at 630 and 810 nm applied consecutively (19,21). For both light sources, the same light parameters were applied in both studies. However, in one of them, cells were exposed to light irradiation every other day, for 3 days (21), whereas in the other, cells were exposed to light just once (19). Besides, both studies were conducted in DM1 rats that were monitored for 16 days (19,21) (Table 2).

In the third eligible work with SCs irradiated *in vitro*, the authors investigated the role of PBM-formed spheroid and monolayer SCs before transplantation into skin flaps induced in BALB/c mice (20). For this, the authors used a red light-emitting diode (LED) that was applied within 3 consecutive days. Mice were observed for 14 days (Table 2).

Regarding the selected articles involving PBM applied after SCs transplantation, we noticed that in all of them the experiments were undertaken in the wavelength range between 630 and 890 nm. Four studies comprised infrared pulsed laser at 890 nm (19,21,22,27), two used red LEDs at 660 ± 20 nm (24,25) and two included red laser, one at 660 nm (23) and the other at 632.8 nm (26) (Table 3).

Interestingly, all the studies using the infrared laser at 890 nm were carried out in infected wounds of diabetic animals by the same group (19,21,22,27). Animals received PBM 6 days week^{-1} and were monitored for 16 days (Table 3). However, in three of them, the authors developed DM1 in Wistar rats (19,21,22), while in the other, DM2 was induced (27).

Regarding the other study undertaken in the skin flap model, mice were daily irradiated by a red LED at 660 nm after SC graft with a 14-day follow-up (25). Two other articles reported full-thickness skin wounds in athymic BALB/c mice induced by biopsy punch (24,26). In one of them, cluster cells were transplanted to mice and skin wounds were exposed to a red LED at 660 nm, daily treated for 13 days and monitored for 14 days (24). The other one used a He–Ne laser at 632.8 nm applied within 20 days into the skin wound (26). Animals were euthanized on day 21 (Table 3).

In terms of skin burns, we found just one study describing the use of SCs and PBM after cell graft (23). In this case, third-degree burns were exposed to a red laser at 660 nm, three times a week for 21 days, totaling 10 sessions, when animals were euthanized (Table 3).

Fig. 2 displays the overall risk of bias in the nine studies included in this review. We noticed that all of them showed that SC and SC + PBM groups were similar at baseline, even though one study did not report that the experiment was randomized (25). On the other hand, all studies were unclear regarding allocation concealment, random and blinding housing and random outcome assessment once authors did not address these items. We assigned a low risk of bias to all studies concerning the blinding of outcome assessment and incomplete outcome data. Indeed, all of them provided quantitative data for the outcome, which was adequately addressed in their analysis. In contrast, three studies were scored as high risk of bias for selective reporting, while we observed a lack of information comparing methodology and results (20,24,26).

We also identified another point that could cause bias in study results and deserves to be highlighted. As the exposure of SCs to PBM *in vivo* involves further animal handling, animals should be sham-irradiated. Only one study considered this issue (23). An almost perfect agreement of 88.89% was achieved between reviewers (Kappa index = 0.78).

Table 2. PBM parameters *in vitro* before SC implantation.

Authors	Light source	Wavelength (nm)	Mode	Radiant exposure (J cm^{-2})	Irradiance (mW cm^{-2})	Exposure time (s)	Sessions	Animal follow-up (days)	Outcome (PBM + SC *vs* SC)
Ahmadi *et al.* (19)	Laser	630; 810	Continuous	1.2	26; 1	46; 92	1	16	Reduced bacterial CFU in the wound. Increased bending stiffness and stress high load of the wound. A higher number of neutrophils and fibroblasts
Park *et al.* (20)	LED	660 ± 20 nm	Continuous	6	10	600*	3 daily	14	Enhanced secretion of angiogenic growth factors from SCs in skin flaps. Reduced percentage of skin necrosis. A higher score on blood flow
Ahmadi *et al.* (21)	Laser	630; 810	Continuous	1.2	26; 1	46; 92	3 in every other day	16	Increased maximum force and energy absorption of the wound. Decreased number of mast cells

CFU, colony-forming units. *Calculated by reviewers.

Fig. 3 presents the results of the meta-analysis comparison for wound closure (Fig. 3A). Five studies compared the percentage of wound contraction (in %) for SC ($n = 45$) and SC + PBM groups ($n = 45$). There was evidence of an increase in wound closure rate among animals receiving PBM compared to SCs alone (MD: 9.69; 95% CI: 5.78–13.61, $P < 0.00001$). As expected based on methodological heterogeneity, we noticed a high heterogeneity among studies ($I^2 = 93\%$).

Worth noting that in two studies (24,26), the authors used the wounds instead animals as sampling units. Thus, we conducted a sensitivity analysis removing these studies to assess their influence on wound closure (Fig. 3B). Once again, we observed a significant wound closure favoring SC + PBM groups (SMD: 6.16; 95% CI: 0.82–11.49, $P = 0.02$), but heterogeneity changed substantially ($I^2 = 68\%$).

Additionally, three studies from the same group were compared concerning the force (in N), totalizing 17 animals per group (Fig. 4). In this case, there was also evidence of an enhanced maximum force in skin wounds for the SC + PBM group (SMD: 1.7, 95% CI: 0.68–2.72, $P = 0.001$), with moderate heterogeneity ($I^2 = 65\%$).

DISCUSSION

The skin is the largest organ in the human body playing a key role in the maintenance of the body's functions. It is also the most exposed tissue prone to being easily injured. Full-thickness skin injuries that reach into the dermis and are larger than 1 cm in diameter do not heal on their own and should receive specialized care to prevent infections, cosmetic deformities, extensive scarring and impaired joint mobility (28,29). Autologous skin grafts are the gold standard treatment for serious cutaneous wounds, but their use is limited by commonly insufficient remaining healthy donor sites to harvest from and difficulties supporting the skin graft when the dermis' function is impaired (29). Therefore, novel technologies that stimulate skin repair to overcome these limitations are of great interest.

Currently, the commercially available tissue-engineered therapies for wound healing comprise acellular biological and synthetic/biosynthetic matrices, cellular products (fibroblasts/keratinocytes with or without biological/synthetic substrates) and platelet-derived growth factor products (30). Unfortunately, these technologies still present modest benefits, mainly because some systems take a long time to vascularize and integrate within host tissue, and important skin structures and cell variety are usually absent. The use of SCs emerges as another approach to break some of these barriers since these cells are natural skin residents and actively participate in the process of wound healing (30). Thus, administering these kinds of cells, preferably obtained from the same patient to the wound bed, would be of great help.

For cell therapy purposes, adult SCs are usually preferred because they do not encounter ethical and legal barriers as embryonic SCs. Additionally, they still preserve multipotent and high proliferative capabilities and can be retrieved from different tissues and organs, being adipose tissue-derived SCs one of the most advantageous cell types for autografts since they can be harvested in high quantities (31). All the studies selected for this review used ADSCs as SCs sources and they are of particular benefit for wound healing considering they express important growth factors and interleukins involved in skin regeneration, such as vascular endothelial growth factor (32), hepatocyte growth factor (33), insulin-like growth factor-1 (34) and interleukin-6 (35).

The delivery route of SCs was another homogeneous characteristic among the selected studies. All of them administered the SCs in the vicinity of the injuries, intradermally or intramuscularly. A recent meta-analysis investigating the efficacy of SC therapy for skin flaps showed that the intra-arterial injection promoted more effects on flap survival, although the other routes (intradermal and intravenous) were also positive (36). The authors alerted that intravascular administration can cause

Table 3. PBM parameters *in vivo* after SC implantation.

Authors	Light source	Wavelength (nm)	Mode	Radiant exposure (J cm^{-2})	Irradiance (mW cm^{-2})	Exposure time (s)	Sessions	Follow-up (days)	Outcome (SC + PBM *vs* SC)
Ahmadi *et al.* (19)	Laser	890 ± 10	Pulsed 80 Hz, 180 ns	0.2/point (9 points)	1.0	1800*	6 days/ week	16	Reduced bacterial CFU in the wound. A higher number of neutrophils, macrophages and fibroblasts
Ahmadi *et al.* (21)	Laser	890 ± 10	Pulsed80 Hz, 180 ns	0.2/point (9 points)	1.0	1800*	6 days/ week	16	Increased energy absorption of the wound. Decreased number of mast cells
Ebrahimpour-Malekshah *et al.* (22)	Laser	890 ± 10	Pulsed 80 Hz, 180 ns	0.2/point (9 points)	1.0	1800*	6 days/ week	16	Reduced bacterial CFU in the wound. Increased wound strength, wound closure rate, and angiogenesis. Reduced number of neutrophils, macrophages and inflammatory cells. Increased number of fibroblasts and vascular length
Andrade *et al.* (23)	Laser	660	Continuous	70	1.43*	49*	3/week (Total 10)	21	Increased percentage of wound closure by modulating the inflammatory process. Cutaneous tissue with better quality
Park *et al.* (24)	LED	660	Continuous	30	50	600	Daily, 13 days	14	Enhanced wound healing including neovascularization and regeneration of skin appendages. Increased secretion of growth factors in the wound bed
Park *et al.* (25)	LED	660	Continuous	30	50	600	Daily, 14 days	14	Enhanced survival of SCs in the skin flap with higher blood reperfusion and stimulated secretion of growth factors. Enhanced skin flap healing with smaller necrotic areas
Kim *et al.* (26)	Laser	632.8	Continuous	1.2	60*	20	Daily, 20 days	21	Accelerated wound closure, a higher number of vessels and skin appendages. Enhanced re-epithelization, dermal regeneration and granulation tissue formation. Increased survival of transplanted SCs in the wound bed
Moradi *et al.* (27)	Laser	890	Pulsed, 80 Hz, 180 ns	0.324/point (9 points)	1.0	2700*	6 days/ week	16	Reduced bacterial CFU in the wound. Reduced wound area. Increased bending stiffness, maximum force, stress maximum load and energy absorption

CFU, colony-forming units. *Calculated by reviewers.

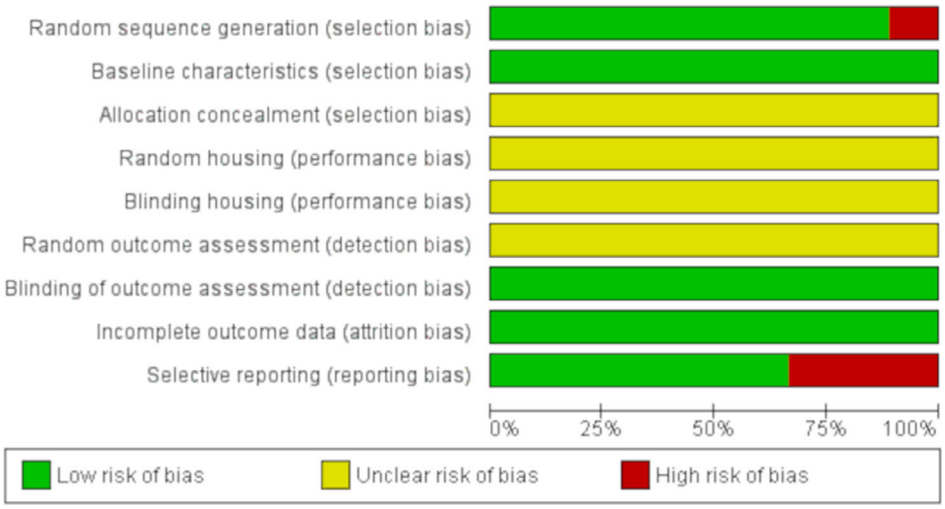

Figure 2. Scores for the risk of bias in the eligible studies.

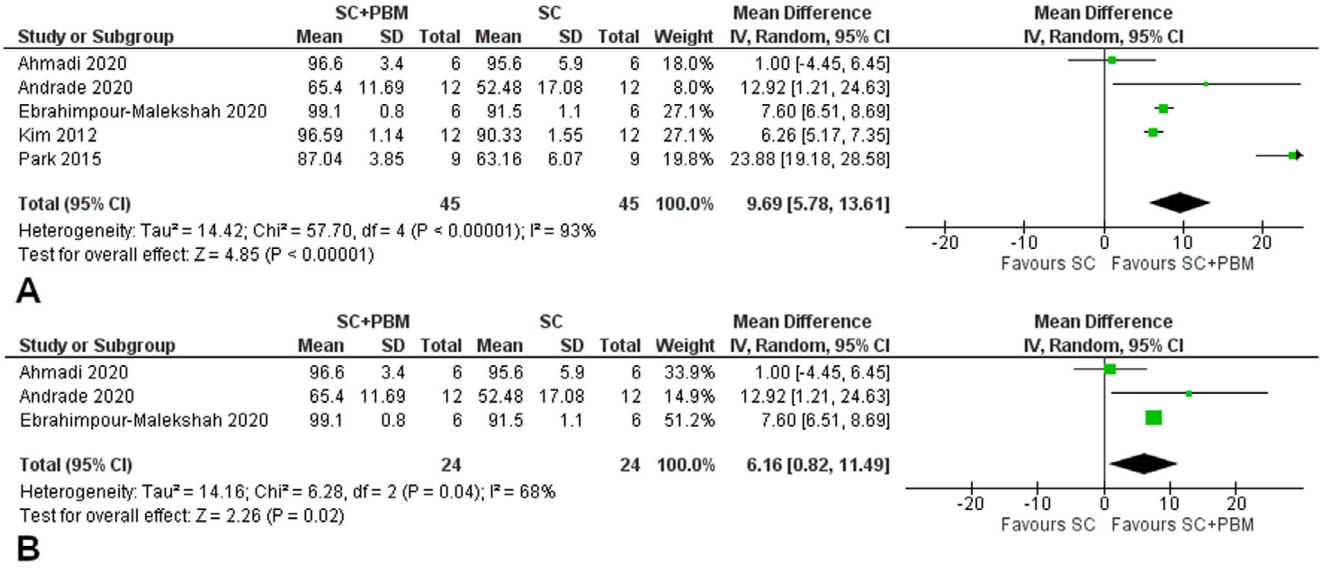

Figure 3. Forest plots for wound closure rate (A) and sensitivity analysis excluding two studies (B). PBM was applied *in vivo* after SC implantation.

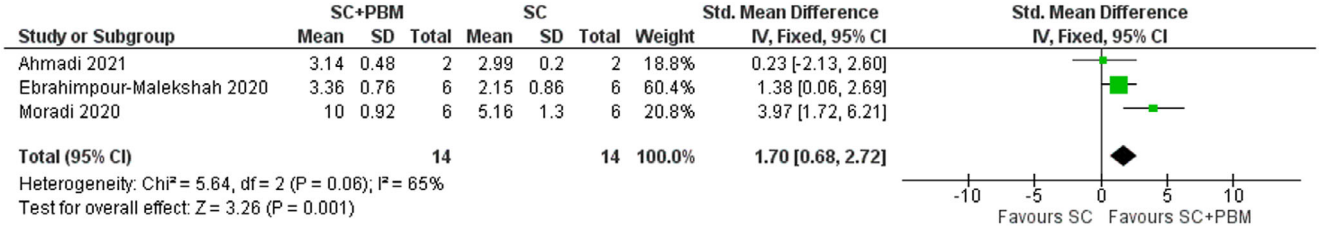

Figure 4. Forest plot for the maximum force related to the wound tensile strength. PBM was applied *in vivo* after SC implantation.

vascular damage. In virtue of the small number of studies, the best delivery route remains uncertain.

Rat and mouse models of wound healing are the most abundant among preclinical *in vivo* studies (37), which explains rodents being the prevalent animal model among the selected studies in this review. Although rodents present important differences in the mechanisms of wound healing impairing a straightforward translation to clinical research, rodents and humans are rather similar in the phases of tissue repair (homeostasis, inflammation, proliferation and remodeling) (38,39). Indeed, rodents heal by wound contraction whereas humans heal by granulation tissue formation. All studies with full-thickness punch wounds encompassed in this review applied strategies to prevent skin contraction using the splinted model, when a

silicone ring is sutured around the wound to favor granulation tissue deposition (19–22,24–26).

Photobiomodulation is a well-recognized therapy for wound healing with approved clinical use (9,40). Its benefits lie mainly in recruiting cells to the wound site, modulating growth factors and inflammatory secretion within the injury milieu, and stimulating vascularization. Red and near-infrared wavelengths are preferred for therapeutic purposes due to higher depth light penetration, and all the studies comprised in this review used light sources emitting between this range. However, we noticed high variability among irradiation protocols regarding radiant exposure (1.2–70 J cm^{-2} per session), irradiance (1–60 mW cm^{-2}), exposure time (20–2700 s), wave mode (continuous or pulsed), light source (laser or LED) and frequency of treatment sessions (daily to 3 times a week and only one exposure). These treatment protocols were rather heterogeneous to enable the extraction and recommendation of an ideal dosage.

In this review, we aimed to evaluate whether PBM could promote additional benefits on wound healing when combined with SCs in animal models. Due to the small number of eligible publications administering *in vitro* preirradiated SCs, our meta-analysis only included the works that irradiated SCs *in vivo*, that is, after transplantation of SCs to the injury site. Thus, a comparison between the treatment order was not possible.

Our results indicated an overall positive effect of the PBM on wound healing and tensile strength when used in combination with SCs *in vivo*, even though moderate to high heterogeneity was observed among the included studies. Methodological variability within the research may explain this heterogeneity, encompassing animal species, wound type, donor/recipient health status, SC type and the treatment protocols mentioned above.

Noteworthy, two studies in this review adopted similar experimental designs although observing different outcomes (19,22). One of them was the only work where *in vivo* PBM presented a neutral effect on wound closure and maximum force (19). Both induced ischemic infected wounds in diabetic rats, which were treated using the same irradiation protocol. The only difference between them was the SCs source: Ahmadi *et al*. (19) harvested ADSCs from DM1 rats, whereas Ebrahimpour-Malekshah *et al*. (22) administered ADSCs from healthy human donors. It is well-known that SCs obtained from diabetic individuals present altered biological characteristics such as migratory and proliferative potential as well as growth factors and cytokine secretion that are associated with compromised wound healing (41,42). These metabolic particularities could help explain the contrasting results observed in both studies.

Indeed, it is an emerging concern in the field of regenerative medicine to reverse the impaired functions of diabetic-derived ADSCs (d-ADSCs) (43) since diabetics are prone to develop foot ulcers, which are the leading cause of member amputations (44). Therefore, these patients are great candidates to be benefited from SC therapy to facilitate their compromised wound healing process. Ahmadi *et al*. (19) achieved interesting results rescuing d-ADSCs function by preconditioning them *in vitro* with PBM before transplantation, which seems to be a promising strategy that should be considered when using autologous SCs to treat diabetic wounds.

Besides, infection is commonly found in skin wounds, mainly in diabetic patients. We identified four articles that used infected wounds in diabetic mice (19,21,22,27). Although authors have reported reduced bacterial load in the wounds for PBM groups at pre or postexposure (19,22,27), we assume that PBM was able to activate the immune system by recruiting neutrophils and macrophages to fight infection. PBM using red or near-infrared light is not recommended to treat infections. In this case, the use of photodynamic therapy (PDT) could promote a better outcome by combining light with a photosensitizing drug (e.g. methylene blue, porphyrins) to kill microorganisms by oxidative stress (45).

It is important to highlight that our study aimed to assess whether light alone would enhance SC therapy for wound repair. In this sense, including additional interventions such as the use of drugs would fall far from our initial objective and was one of our exclusion criteria. However, Huang and collaborators used light and a photoactive drug [5-aminolevulinic acid (ALA), a photosensitizer precursor] to evaluate the effects of PDT combined with human umbilical cord SCs on infected wounds (46). Although authors have concluded that combining ALA-PDT with SCs significantly enhanced the therapeutic effect compared to single therapies, it would be rather difficult to imply whether this application would work better than using only PBM. There is still a scarcity of studies regarding PDT combined with SCs to make proper analysis and comparisons.

Low survival rates of grafted SCs are one of the major obstacles hindering advances in the regenerative medicine field (47). Albeit not being able to outline the mechanisms underlying the positive effects of PBM associated with SC to treat wound healing, a few among the selected studies described increased SC survival after transplantation, a response that was observed when the cells were irradiated *in vitro* (20) or *in vivo* (24–26). Also, the use of aggregated cells in the form of clusters or spheroids seems to boost SC survival, once they grow in hypoxic conditions and are metabolically prepared to endure ischemic environments such as the wound site (20,24,25).

The lack of vascularization in the injured tissue affects not only SC survival but can also lead to tissue-engineered rejection or skin-graft necrosis (48). Regarding this subject, several studies were designed to evaluate ischemic conditions using a skin flap model with (19,21,22,27) or without (20,25) a wound inside the flap. The common ground shared by these works was better vascularization indicators when PBM was applied, such as higher vessel-like structures, a higher number of phenotypic endothelial cells and increased blood flow, with more pronounced effects when PBM-preconditioned SCs were also irradiated *in vivo*. A possible mechanism that could explain this impact on vascular properties is the increased expression of angiogenic growth factors in PBM-treated wounds.

When dealing with preclinical reports and especially when using a treatment that involves visible light, the overall quality of the research is hindered by the difficulties of double-blinding the experimental design, which directly impacts the risk of bias. Indeed, we notice that all studies appear to neglect the ARRIVE guidelines (49) as the authors did not report important methodological details. Due to this poor reporting, all studies were judged as unclear risk of bias for four domains (see Fig. 2). Moreover, we observed some inconsistencies in the comparison of the methodology and results of three studies, which were assessed with a high risk of bias (20,24,26). Surely, future laboratory research would be favored if studies provided a more standardized methodology and meticulous reporting. Additionally, it is important to highlight that animals should be equally handled in intervention and control groups to prevent bias. Thus, animals should be sham-irradiated in any study using light-based technologies.

In conclusion, this review provides *in vivo* evidence of the potential clinical benefits of the combination of SCs with PBM on skin wound repair, even though there is no confirmed dosage due to the heterogeneity of the included studies. Besides, it is worth mentioning that two studies investigated PBM effects on SCs irradiated *in vitro* (before) and *in vivo* (after transplantation) (19,21). Interestingly, both studies reported a better outcome for wound healing in these conditions. Thus, further studies are welcome to pursue the best protocol of PBM to improve the quality of life of wound care patients in regenerative medicine.

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