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Review Article

Allogeneic mesenchymal stem cells therapy for burn wound rat model: A systematic review and meta-analysis

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Abstract

Burn wounds are a burdensome problem affecting many people and cause high morbidity and mortality. Therefore, a more effective burn wound treatment is urgently needed**.** Here the objective of the study is to evaluate the allogeneic mesenchymal stem cells' efficacy in treating burn wounds in rats. English language articles were collected from the following databases: ProQuest, PubMed, EBSCOhost, SAGE, JSTOR, GARUDA, and Open Gray (January 1st, 2011, to March 29th, 2023). The inclusion criteria are original articles, rat burns experimental models, stem cells therapy using MSCs for experimental groups, non-functional solution, excipient or no treatment for control group, and healing rate of the burn wounds as the main outcome. We found 9 articles in English. Hot plates or radiation were used to create burn model in 170 rats. The burn wounds degree ranged from partial (n=2) to full-thickness (n=4). Around 0.1–3.2 million stem cells were administered through various routes. The wound re-epithelialization rate was analyzed statistically and is shown more significant in the treatment group. Allogeneic MSCs may have benefits for treating burn wounds, although more experiments are still needed. RCTs are recommended to evaluate the efficacy and adverse effects of the MSCs treatment.

Keywords: allogeneic; mesenchymal; stem cells; burn; rat

Key words

Allogeneic; Mesenchymal; Stem cells; Vurn; Rat model.

Introduction

Burn wound can be defined as a defect in skin and other organic tissues due to exposure to high temperatures, radiation, friction, electricity, or chemicals. As one of the severe trauma that is impacting millions people around the world, burn wounds are known to become public health burden with great number of mortality and morbidity.[1,2,3] WHO estimates that approximately 11 million people suffered from burns. Of these, 265,000 people died every year from fire, and many more died from scalds, electrical burns, and other forms of burns.[4] About 96% of fire-related fatal burns occurred in developing countries with low- to middle-income. In Indonesia, as a middle-income country, burns are recorded as the second most common form of trauma with an average of 1,200 cases per year. In addition, millions of people are disabled and disfigured throughout their lives, often leading to stigma and exclusion, because of burns.[5] People with comorbidities, old age, and multi-drug therapy

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are more likely to have burn complications.[6,7]

The management of burn wound patients has improved significantly due to modern medical technology achievements. The primary goals of burn management are practical wound management, rapid wound closure, and functional aesthetically scars, which largely determine the prognosis and survival of those burn wound patients.[8] There are some strategies to promote burn wound healing, such as debridement, therapeutic agents, and skin transplantation. Skin transplantation leads to a significant improvement in the treatment result. Mesh-skin graft can be expanded to achieve better coverage in severe burn patients with limitation of the donor skin. Unfortunately, maturation processes of the mesh grafts might take several weeks and patients are often unsatisfied aesthetically with the result. Complications such as skin contracture or infection can occur, leading to the need for further treatment and prolonged healing process.[9,10]

Therefore, there is an urgent need for more effective and efficient treatment strategies to manage burn wounds. One of these strategies is cell therapy. Stem cell, which is used as cell therapy, is able to proliferate and differentiate into a variety of cell types. It is essential for renewing physiological tissues and promoting regeneration after trauma. It has been used in different types of wounds.[8,11,12] Various stem cells have been used for regeneration of skin and also burn healing, such as embryonic, adult, induced pluripotent, mesenchymal, adipose, and melanocyte stem cells. Among them, allogeneic mesenchymal stem cells or MSCs tend to be used in various studies, but the results were varied. MSCs are favored for their unique abilities, such as (1) differentiation into multiple cell types of different lineages, (2) migration to damaged tissues, and (3) production of soluble substances that can induce healing process.[13] Due to their proliferative effects on keratinocytes and blood vessels, application of stem cells can more effectively promote re-epithelialization and angiogenesis of wounds.[14–18]

As a model of human diseases, compared to mice and other organisms, rats have many advantages and more commonly used in medical research. The successfulness of rat embryonic stem cell isolation has expanded its usefulness further. There are various genetic strains of rats that are suitable for many research criteria. Using rats as a model is cheap and easy to care for. The anatomical structure of rats is well-known. They are relatively resistant to surgical infection and their functional analysis techniques have been well-developed.[19,20] The physiological functions of rats are easy to be monitored, and in many cases are similar to the corresponding human conditions.[19] In addition, the murine genome has been extensively sequenced and showed similarities to the human genome. For these reasons, rats are preferred as models of human pathology.[18]

Although clinical trials have been run and reported, many research on stem cells in mediating burn wound healing are carried out in small animal models, especially rats. The rat experiment has a special method that can increase understanding of the physiology and pathology of wound healing process and become the basis for further clinical trials. There have been many reviews discussing the potential of stem cells to treat burn wounds, but the re-epithelialization rate of allogeneic stem cells treated burn wounds in rats has not been strictly evaluated. Thus, we conducted a systematic review and meta-analysis in purpose to make evaluation of the experimental results obtained so far.

Methods

This review followed the Preferred Reporting Items guidelines for Systematic Reviews and also Meta-analysis. Supplementary **Table 1** shows the PRISMA 2020 checklist.

Literature Search We conducted a comprehensive literature search to assess the association of allogeneic stem cell therapy and the burn wounds of rat model. ProQuest, PubMed, EBSCOhost, SAGE, JSTOR, GARUDA, Open Gray were retrieved from January 1st, 2011 to March 29th, 2023. The search terms applied in PubMed are the following: ((((("mesenchymal stem cells"[All fields]) OR" msc "[all fields]) AND" skin "[all fields]) OR" skin "[MeSH term] OR" derm * "[all fields] OR" derm * "[MeSH term]) AND "wound" [all fields] AND" record "[all fields]) OR" record "[MeSH term]) AND "rat" [all fields]. Literature search was limited to those published in English. In addition, we manually searched additional possible studies for references.

Study Selection This study inclusion criteria were: (1) Published as an original article; (2) Burn experimental rat models were used; (3) The experimental groups were treated with stem cells (various kind of mesenchymal stem cells, bone marrow stem cells, adipose stem cells, etc.); (4) The control groups only got non-functional solution, excipient or no treatment at all; (5) The main result was reduced burn wound area. The secondary result was the re-epithelialization rate of burn wound healing. The research exclusion criteria were: (1) There was no control group or just comparing stem cells and other therapies in the study; (2) Reviews, clinical trials and case reports; (3) Repeated publications; (4) Lack of data availability.

Data Extraction Two authors extracted further information independently about the research that has been included, and the third author resolved their differences. The extracted data were as follows: (1) The name of the first author and publication year; (2) Rat characteristics (including weight, number, degree of burn, area); (3) Administration method of treatment group and control group; (4) Stem cells profile (type of cell, the source, the dose, and the transplant type); (5) Primary and also secondary results. If the included results were shown in graphical form (i.e. linear chart), we used digital ruler software (ImageMeter application) to get numerical measurement based on the scaled image of each chart.

Quality Assessment Two independent reviewers assessed the bias risk by applying the 10-item animal research scale (SYRCLE's risk of bias in animal research tool). Sequence generation, characteristics of baseline, concealment of allocation, random housing, blind investigators, then assessment for animals randomly, blind outcome assessor, incomplete data outcome, then reporting of selective outcome, and others could be the aspects for risk of bias.[21]

Type of Outcome Measures Two indicators were applied to assess the potential and efficacy of allogeneic MSCs in treating the burn wounds: wound area measurement and wound re-epithelialization rate. Wound area measurement was the actual area (mm^2) in the original article and in this systematic review. Wound re-epithelialization rate was counted by [(initial wound existing - wound area at cleared time point)/initial wound region] x 100.[22]

Results

As many as 157 literatures were obtained in the initial search. They were filtered then by titles, keywords, and abstracts, and finally 32 qualified articles were found. All full text of those

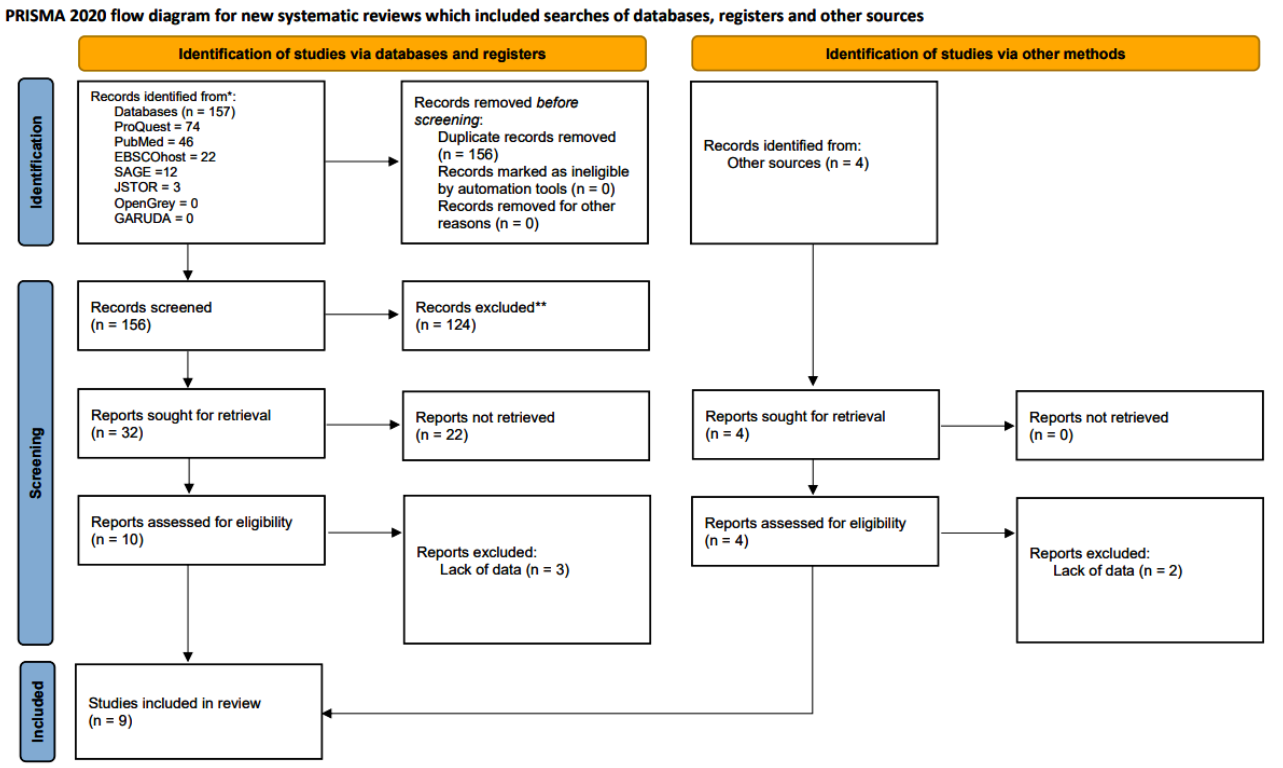

Figure 1 PRISMA flowchart.

32 articles were read and 20 articles were excluded. The PRISMA P 2020 flow chart was used for the evaluation of the literatures as shown in **Figure 1**. The remaining nine literatures reported experimental studies. Because of the little amount of research and also the endpoints heterogeneity, a formal statistical analysis cannot be performed. Instead, a detailed review of the system was carried out.

Characteristic of eligible studies All studies used in this review were published in English between 2013 and 2020. Rats were used in all studies, although the species in each study was different. Sprague Dawley was used in four studies,[26] Wistar in three studies,[27–29] and Lewis inbreeding in one study.[30] Male rats were used in seven studies, both sexes in one study,[30] and one study[25] did not mention the gender of the rats. The weight of the rats ranged from 200 to 400 grams. Two kinds of methods were used to create burn wounds, i.e hot plate contact in seven studies[14,15,23,24,26,27,31] and radiation in the remaining studies.[28,30] The length of process to burn the rats' skin varied from 2 to 312 seconds and the resulting wound area varied from 1 to 8.5 cm^2. Six studies reported the depth of the wound, ranging from deep second degree or partial burn (n=2)[23,26] to third degree or full thickness burn (n=4),[24,27,30,31] while the other three studies did not report it. The location of the wounds were on the back (n=7),[23–26,28,29,31] the spine (n=1)[30] or on the left abdomen (n=1)[27] **(Table 1)**.

All of the eight experimental studies[15,23,24,26–28,30,31] assessed the potency of exogenous stem cells on burn wound healing, and one study[25] assessed the effect of stem cells through a burn model in comb shape. In this study, a 150-gram brass comb was used to produce several spaced apart burn wounds. The burn constitutes the

Table 1 Summary of the rat studies.

Author & Year	*Rat's Characteristic*				*Burn Models*			
	Species	*Weight (grams)*	*Sex*	*Age*	*Method*	*Wound Size*	*Area*	*Burn Degree*
Feng et. al. (2019) [23]	Sprague-Dawley	250 to 300	Male	8 weeks old	Type: Contact burns Tools: Copper plate placed in boiling water Temperature: 90℃ Duration: 30 seconds	1 cm x 1 cm	Dorsum	Second degree (deep-partial thickness)
Zhou et. al. (2019) [24]	Sprague-Dawley	No data	Male	1 year old	Type: Contact burns Tools: The head of the iron Temperature: 100℃ Duration: 10 seconds	2 cm2	Dorsum	Third degree
Kakabadze et. al. (2019) [30]	Lewis inbreed	200 to 250	Both sexes	8 to 10 weeks	Type: Radiation burns Tools: A radiation device (RUM-17, Russia; 250 kV; 10 mA; 60 Gy). The distance from target to the point of measurement was 25 cm.	1 to 1.3 cm in diameter	Spinal	Third degree
Imam *et al*., (2019) [31]	Albino	200 to 250	Male	Adult	Type: Contact burns Tools: Stainless steel tunning fork in boiling water Temperature: 100°C Duration: 15 seconds	2 cm2	Dorsum	Third degree (full-thickness)
Temnov et. al. (2018) [28]	Wistar	Experimental group 235 to 315 (M±m, 283.1±12.0 g) Control group 283±8.6	Male	Adult	Type: Radiation burns Dose: 110 Gy X-rays; 21.4 Gy/min; 30 kV; 6.1 mA; filter 0.1 mm AI Duration: 312 seconds	8.2 and 8.5 cm2	Pre-iliac-lumbar region of the back	No data
Franck *et al*., (2018) [27]	Wistar	250 to 280	Male	Twenty-three ninety days old	Type: Contact burns Tools: Welding station with ceramic surface Temperature: 100°C Duration: 30 seconds	484 mm2	Left abdominal (80 mm from the lower border of the last costal arch with one side parallel to the midline of the abdomen)	Total skin thickness burns
Chen et. al. (2017) [29]	Wistar	250 to 300	Male	Adult	Type: Contact burns Tools: Hot steam directed toward the skin through an electrovalve and a round-shaped rubber funnel Duration: 2 seconds	3 cm in diameter	Dorsum	No data
Guo et. al. (2016) [26]	Sprague-Dawley	200 to 250	Male	2 months old	Type: Contact burns. Tools: Boiled water in the vitreous pipe. Temperature: 100 ℃ Duration: 10 seconds	3 cm in diameter	Dorsum	Second degree (deep-partial thickness)
Singer et. al. (2013) [25]	Sprague-Dawley	300 to 400	No data	No data	Type: Contact burns. Tools: 150-gram brass comb Temperature: 100°C Duration: 30 seconds	Four rectangles that are each 10 x 25 mm, which separated by three grooves (5 x 25 mm each)	Dorsum	No data

Table 2 Summary of the results.

Author & Year	*C (n)*	*T (n)*	*Healing Control*	*Healing Treated*	*Wound Re-epithelialization Rate (%)*		*Significance*
					C	**T**	
Feng et. al. (2019)[23]	6	6	Wound area measurement (mm^2): Week 1 → 100 Week 2 → 60 Week 3 → 32.1 Week 4 → 31.5	Wound area measurement (mm^2): Week 1 → 58.8 Week 2 → 29.3 Week 3 → 25.5 Week 4 → 14.2	Day 0-7 → 60.00 Day 0-14 → 32.10 Day 0-21 → 31.50 Day 7-14 → 53.50 Day 14-21 → 98.13	Day 0-7 → 49,83 Day 0-14 → 43.38 Day 0-21 → 24.15 Day 7-14 → 87.03 Day 14-21 → 55.69	Wound healing was improved in the ASCs-treated group at all given time points. This result was significant especially in the first two weeks ($p < 0.05$). Between the 3rd and 4th week, the percentage of living follicles in the ASCs treatment group increased gradually compared to the control group ($p < 0.05$).
Zhou *et al*. (2019)[24]	9	9	Wound measurement (mean pixel number): Day 3 → 7.6×10^5 Day 12 → 5.6×10^5 Day 21 → 2.2×10^5 Day 27 → 0.7×10^5	Wound measurement (mean pixel number): Single dose: Day 3 → 7.2×10^5 Day 12 → 4.8×10^5 Day 21 → 1.8×10^5 Day 27 → 0.2×10^5 Multiple dose: Day 3 → 7.1×10^5 Day 12 → 4.8×10^5 Day 21 → 0.9×10^5 Day 27 → 0.1×10^5	Day 3-12 → 73.68 Day 3-21 → 28.95 Day 12-21 → 39.29	Day 3-12 → 66.60 Day 3-21 → 25.00 Day 12-21 → 37.50	On day 21, the wound size of the treatment group was significantly smaller compared to the PBS group ($P<0.05$).
Kakabadze et. al. (2019)[30]	15	15	Wound area measurement (cm^2): Day 0 → 7 Day 3 → 5.83 Day 7 → 3.62 Day 14 → 2.0 Day 30 → 0.62 Day 45 → 0.24	Wound area measurement (cm^2): Day 0 → 7.0 Day 3 → 2.92 Day 7 → 0.83 Day 14 → 0.14	Day 0-7 → 51.71 Day 0-14 → 28.57 Day 7-14 → 55.25	Day 0-7 → 11.86 Day 0-14 → 2.00 Day 7-14 → 16.88	No statistical analysis
Imam *et al*., (2019)[31]	10	10	Wound area measurement (cm^2): Day 0 → 2.0 Day 7 → 1.7 Day 21 →0.4	Wound area measurement (cm^2): Day 0 →2.0 Day 7 → 1.6 Day 21 → 0.3	Day 0-7 → 85.00 Day 0-21 → 20.00 Day 7-21 → 23.53	Day 0-7 → 80.00 Day 0-21 → 15.00 Day 7-21 → 18.75	The treated burn wound area on day 21 after burn induction was significantly reduced. On day 7, there was a statistically insignificant reduction. Rats that received MSCs and EPOa / MSCs only on day 21 showed better epithelialization with significant difference, compared to those that did not receive on day 7.
Temnov et. al. (2018)[28]	8	8	Wound measurement (cm^2): Day 15 → the wounded area gradually reduced by 5.9±0.6 cm^2 Day 29 → the wounded area gradually reduced up to 2.2±0.3 cm^2 Day 50 to 71 → the area of the lesion ranged from 1.4±0.6 cm2 on the 50th day to 1.9±0.8	Wound measurement (cm^2): Day 15 → the wounded area gradually reduced by 6.1±0.4 cm^2 Day 29 → the wounded area gradually reduced by up to 2.2±0.3 cm^2 Day 36 to 71 → there was a constant reduction in the burn area up to 0.2±0.1 cm^2	N/A	N/A	On day 36 to the end of radiotherapy, there was a significant difference in clinical manifestations between the treated and control group ($P<0.05$).

Author	*C*	*T*	*Healing Control*	*Healing Treated*	*Wound Re-epithelialization Rate (%)*		*Significance*
			cm2 on the 71st day.				
Franck *et al*. (2018)[27]	12	11	Day 0 → N/A Day 4 → N/A Day 14 → 343.7 ± 65.70 mm^2	Day 0 →N/A Day 4 →N/A Day 14 → 275.3 ± 61.01 mm^2	N/A	N/A	The burned area of the experimental group was significantly reduced on day 14 after the first ASC transplant compared to the control group (p = 0.027).
Chen et. al. (2017)[29]	3	3	In the control group, the result from the wound measurement (cm^2): Day 0 → 6.8 Day 7 → 6.7 Day 12 → 5.8 Day 14 → 4.6 Day 16 → 3.5 Day 18 → 3.2 Day 21 → 2.4	In the intervention group, the result from the wound measurement (cm^2): Day 0 → 6.9 Day 7 → 6.8 Day 12 → 3.4 Day 14 → 2.2 Day 16 → 1.4 Day 18 → 1.1 Day 21 → 1.0	Day 0-7 → 98.53 Day 0-14 → 67.65 Day 0-21 → 35.29 Day 7-14 → 68.66 Day 14-21 → 52.17	Day 0-7 → 98.55 Day 0-14 → 31.88 Day 0-21 → 14.49 Day 7-14 → 32.35 Day 14-21 → 45.45	Data were expressed as mean ± SD (n = 3) (P < .001).
Guo et. al. (2016)[26]	16	16	Wound measurement (%): Day 0 → 0 Day 3 → 9 Day 7 → 20.5 Day 14 → 61.7 Day 21 → 77.6	Wound measurement (%): SIS Day 0 → 0 Day 3 → 14.8 Day 7 → 37.5 Day 14 → 82.4 Day 21 → 97.4 SIS + MSC Day 0 → 0 Day 3 → 19.1 Day 7 → 57.4 Day 14 → 89.4 Day 21 → 99.0	Day 0-7 → 79.50 Day 0-14 → 38.30 Day 0-21 → 22.40 Day 7-14 → 48.18 Day 14-21 → 58.49	Day 0-7 → 62.50 Day 0-14 → 17.60 Day 0-21 → 2.60 Day 7-14 → 28.16 Day 14-21 → 14.77	On day 7, the wound closure rate of MSCs-seeded SIS was higher than that of treatment with SIS alone (P <0.01). After 21 days of treatment with SIS, the burn wounds were closed, but the burn wounds of the untreated group did not fully heal (P <0.05).
Singer et. al. (2013)[25]	10	10	After 24 hours → all interspaces became completely necrotic (100%) After 7 days → The mean percentage of combined area of the interspaces that underwent necrosis was 100% (95% CI = 93.4% to 100%).	After 24 hours → 29 of the 48 interspaces (60%) experienced some degree of survival After 7 days → approximately 80% (95% confidence interval [CI] = 65.7% to 88.3%) of the combined area of the burn interspaces underwent necrosis.	N/A	N/A	On day 7, there was a significant difference between the treated and control group (p < 0.001).

Abbr. : C= control group, T=Treatment group

Author & Year	*Stem Cell Type*	*Phenotyping*	*Dosage*	*Administration*	*Days (After Induction)*
Feng et. al. 2019)[23]	ASCs	No	5×10^5/0.2mL	Local injection (intradermal)	Week 1, 2, 3, and 4
Zhou et. al. (2019)[24]	ASCs were isolated from the inguinal fat pad	No	2×10^6/500 µl	Local injection (subcutaneous)	Day 3, 12, 21 and 27
Kakabadze et. al.(2019) [30]	Bone marrow stem cells	No	2.5 x 10^6 mononuclear cells	MSCs-seeded decellularized amniotic membrane were placed to the burn wound area	Day 0, 7, 14, 18
Imam *et al*., (2019)[31]	Bone marrow stem cells	Yes	1 x 10^6 cells/mL/cm^2	Local injection	Day 0, 7, 21
Temnov et. al. (2018) [28]	Bone marrow stem cells paracrine factors	No	General protein 8 mg/mL	Local injection (subcutaneously 0.5 mL)	Every week starting 15 days after radiation
Franck *et al*., (2018)[27]	ASCs	Yes	3.2 x 10^6 cells/mL/rat	Local injection (intradermal)	Day 0, 4,14
Chen et. al. (2017) [29]	ASCs from inguinal fat pads	No	1 x 10^6 cells/mL/rat	Local injection (subcutaneous)	Day 0, 7, 12, 14, 16, 18, and 21
Guo et. al. (2016) [26]	Bone marrow stem cells	No	MSCs were seeded on the SIS patches (5×10^5 cells/cm2)	MSCs-seeded SIS and SIS alone were sutured to the burn wound area	Day 3, 7 , 14, 21
Singer et. al. (2013) [25]	Bone marrow stem cells	No	1 x 10^6 cells/mL/rat	Systemic injection (intravenous (tail)/tail vein)	24 hours until 7 days (each day)

necrotic zone and the gap constitutes the ischemic zone. Phenotyping analysis of MSC was performed in two of nine studies. Bone marrow mesenchymal stem cells or called BMSC has been applied in four studies,[14,26,30,31] bone marrow stem cells paracrine factors in one study,[28] and adipose stem cells (ASC) in four studies,[23,24,27,29] As much as 0.1 to 3.2 million stem cells were given by local (n=6)[15,23,24,27,28,31] or systemic (n=1)[14] injections. Meanwhile, in the study by Temnov *et al*. (2018),[28] they injected 8 mg/ml of paracrine factor proteins from BMSC. In two studies,[26,30] stem cells were applied topically as a patch. Wound area was measured from the day the burn wound was created until the fourth week. It is also worth to be mentioned that all of the studies reviewed here used phosphate buffered saline (PBS) or normal saline (NS) as a placebo for the control groups **(Table 1)**.

Risk of Bias We used the Laboratory Animal Experiment System Review Center (SYRCLE) risk of bias tool to evaluate the study quality. In all of these included studies,[14,15,23,24,26–28,30,31] they only reported selective results. On the other hand, allocation concealment was not reported by most of the study. Allocation of experimental settings should be used to avoid statistical bias and overestimation or underestimation. However, these two measures are rarely reported in animal studies. Of all the included studies, only 20% reported the use of housing randomization, 10% of the randomized outcome assessments reported the use of blinding of results, and 40% used detection blinding. Furthermore, none of the studies reported any statement of conflict of interest. Although some authors mentioned the use of randomization or blinding in their experiments, few authors fully explained their methods. Therefore, most studies are assessed as unclear for most types of risk of bias. It is worth to be noted that not all of the 10 items from SYRCLE risk of bias tool were mentioned clearly in the studies we gathered. Several of them only partially mentioned the information we need to determine the degree of deviation.

Macroscopic Assessment This review studied

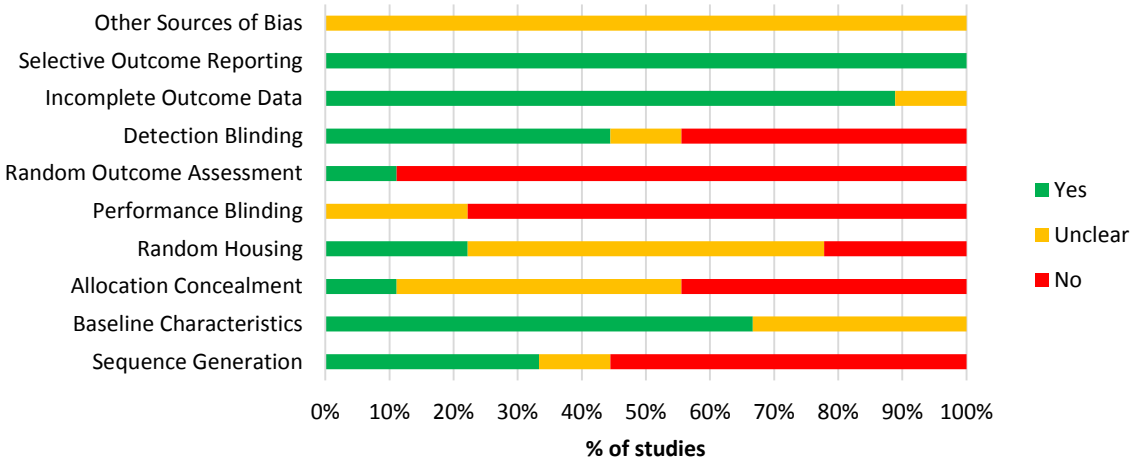

Figure 2 SYRCLE's Risk of Bias.

177 rats, divided into treatment and control groups. Of the nine studies,[14,15,23,24,26–28,30,31] macroscopic findings of the wounds were assessed either through direct inspection, digital and manual measurement, or software analysis. The results were presented as wound area measurement and wound re-epithelialization rate. All studies had reported the positive influence of allogeneic stem cells on these parameters. Eight[15,23–28,31] of the nine studies[14,15,23,24,26–28,30,31] showed that stem cell therapy has a significant effect on reducing wounds, and one study[30] did not perform statistical analysis **(Table 2)**.

Wound Re-epithelialization Rate (Day 0-7) Based on the results of the analysis (table 3 and table 4), there was high heterogeneity between experiments (I^2= 73.230%; p= <0.001), so that the Random Effects Model (REM) was used. Total effect size between the studies was 76.58 (55.28-97.87) with 95% confidence interval (figure 3). There was a significantly different wound healing rate between using allogeneic stem cells and placebo. The funnel plot showed **(Figure 3)** that four publications have low risk of bias and one publication has moderate risk of bias. We concluded that these publications' bias is low.

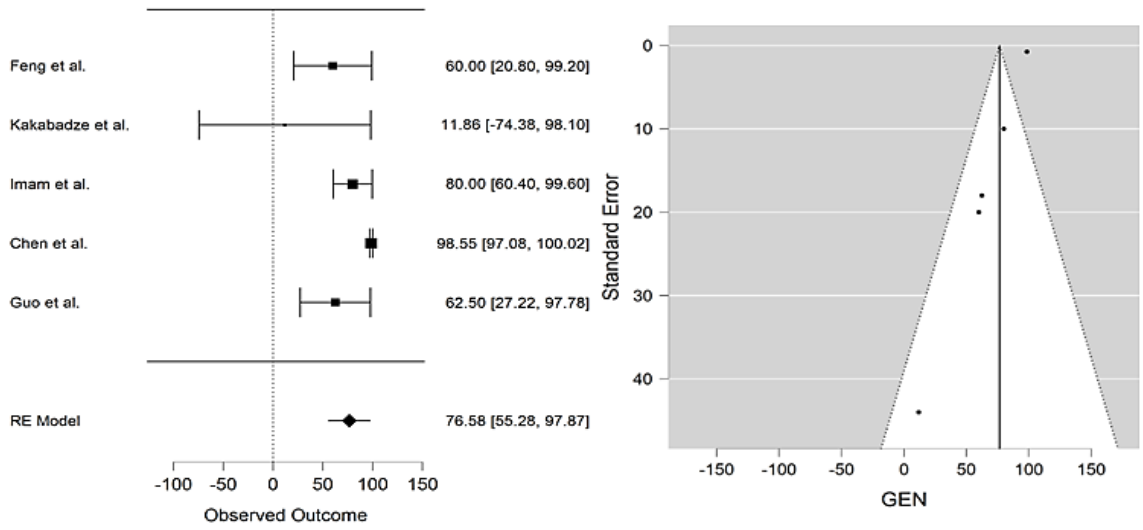

Figure 3 Forest Plot and Funnel Plot (re-epithelialization rate day 0-7)

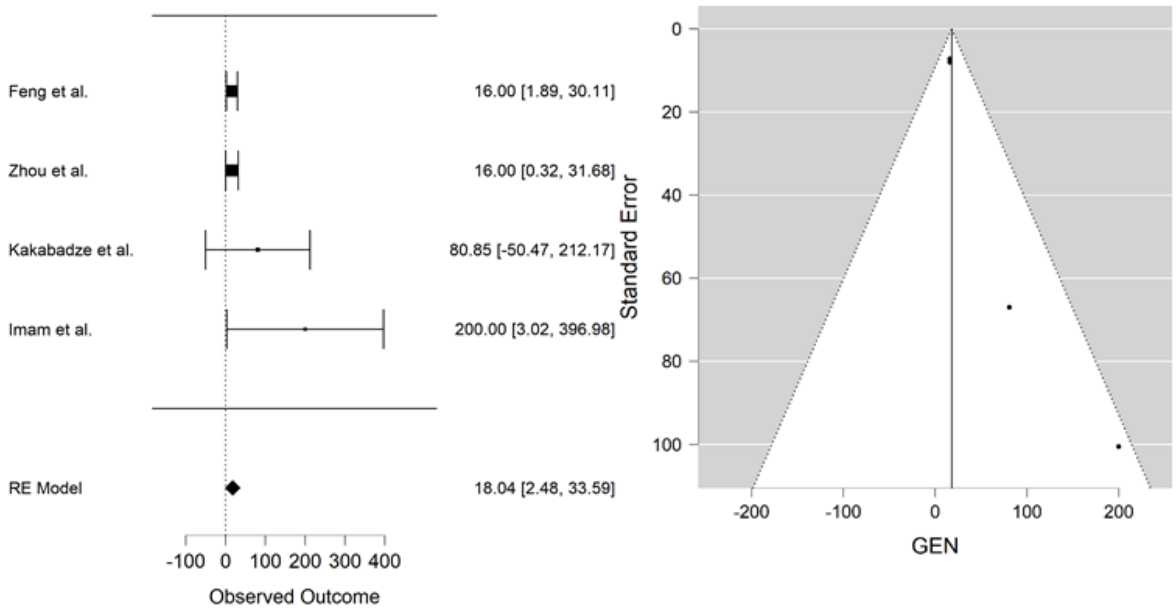

Figure 4 Forest Plot and Funnel Plot (re-epithelialization rate day 7-14).

Table 3 Coefficients (re-epithelialization rate day 0-7 and 7-14).

Duration		Estimate	Standard Error	z	p
0 – 7 d	intercept	76.577	10.866	7.048	< .001
7 – 14 d	intercept	18.035	7.937	2.272	0.023
0 – 14 d	intercept	31.863	0.749	42.557	< .001

Note. Wald test

Table 4 Residual Heterogeneity Estimates (re-epithelialization rate day 0-7 and 7-14).

Duration		*Estimate*
0 – 7 days	τ^2	341.982
	τ	18.493
	I^2	73.230
	H^2	3.736
7 – 14 days	τ^2	70.919
	τ	8.421
	I^2	29.554
	H^2	1.420
0 – 14 days	τ^2	0.000
	τ	0.000
	I^2	0.000
	H^2	1.000

Wound Re-epithelialization Rate (Day 7-14)

Based on the results of the analysis, there was no heterogeneity between experiments (I^2= 29,554%; p= <.023), so that the Fixed Effects Model (FEM) was used. Total effect size between the studies was 18.04 with 95% confidence interval. There was a significant effect of stem cell for re-epithelialization of the burn wounds (day 7-14) with total effect size 18.04.

Based on the funnel plot **(Figure 4)**, all publications have low risk of bias. However, due to the asymmetry shown in the funnel plot, we could conclude that there was risk for publication bias.

Wound Re-epithelialization Rate (Day 0-14)

Based on the results of the analysis **(Table 3, 4)**, there was no heterogeneity between experiments (I^2= 0.000%; p= <.001), so that the Fixed Effects Model (FEM) was used. Total effect size between the studies was 45.65 with 95% confidence interval **(Figure 5)**. There was a

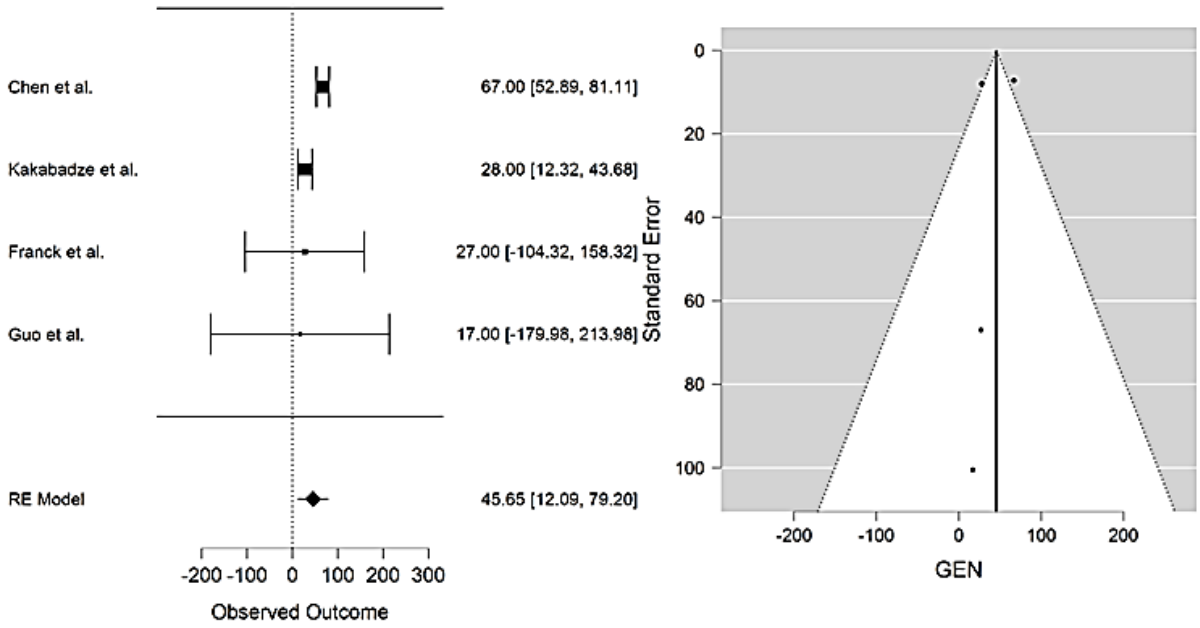

Figure 5 Forest Plot and Funnel Plot (re-epithelialization rate day 0-14).

significant potency of allogeneic mesenchymal stem cells for wound re-epithelialization of the burn wounds (day 0-14) with total effect size 45.65

Based on the funnel plot **(Figure 5)** due to the asymmetry shown in the funnel plot, we could conclude that there was risk for publication bias.

Discussion

The combination between systematic review and meta-analysis process enables more objective and systematic assessment for the results. We have assessed the MSCs therapeutic activity on healing of various degree burn wounds. A total of nine in vivo experimental studies[14,15,23,24,26–28,30,31] were included in this systematic review, and just five out of nine studies by Feng *et al*. (2019),[23] Kakabadze *et al*. (2019),[30] Imam *et al*. (2019),[31] Chen *et al*. (2017),[29] and Guo *et al*. (2016)[26] have been analysed in this meta-analysis due to the homogeneity of the wound assessment time point. These studies investigated and analyzed the macro-processes of different wound healing components of the treated group and control group, which are wound area measurement and wound re-epithelialization rates. Based on our systematic review, all of them found positive influence of MSCs on the overall healing, and all of the studies agreed that there was a decrease in burn area size before and after the therapy was given. In our meta-analysis, it was shown that there was a significant difference of re-epithelialization rate between the group treated with MSCs and the control group.

The reduction effect of MSCs in burn wound area was investigated, especially at 7, 14, and 21 days after burn induction. Two studies that were included[28,30] also observed the influence of MSCs after 36–71 days. Burn wound healing process usually occurs within 14 days. Faster wound healing will reduce the infection risk due to pathogen colonization on the wound. Although the burn wounds in the beginning are sterile from pathogen colonization, if wound healing is delayed, there is an increasing risk of infection and septicemia, especially in immunocompromised patients.[33,34] In our study, the wound healing activity was most pronounced

between 7 to 21 days at which time most wounds had closed. A rapid wound closure is important to decrease the complication risk of the burn wound.[32]

Wound healing consisted of continuous process, that includes inflammation response, cell proliferation, neovascularization, granulation, re-epithelialization, and lastly remodeling or scarring.[27,35] In this study, we assessed the re-epithelialization rate from the MSCs in burn wounds. The highest re-epithelialization rate was 98.55% in a study by Chen *et al*. (2017)[15] at day 7 with 1 x 10^6 cells/mL stem cells by local injection, followed by 80.00% in a study by Imam *et al*. (2019),[31] also at day 7 with 2 x 10^6 cells/mL stem cells by local injection. The re-epithelialization rates in the majority of the studies reached their peak in 7 to 14 days after the burn infliction and stem cells treatment. Although wound healing is consisted of complex and multifactorial pathways, the main thing of a healed wound is the restoration of intact epidermis barrier. Thus, it does not matter how the underlying epidermis structures might perfectly restored, a wound that has not been epithelialized can not be claimed as "healed". Therefore, wound epithelialization is important to define the feature of wound repair.[36]

MSCs derived products, for example exosomes, have a therapeutic effect in burn wound healing similar to MSCs. These derivative products show positive activity in reducing inflammation, modifying the activation of fibroblast, and promoting the production of collagen. Furthermore, they promoted neovascularization and re-epithelialization.[37] These activities could explain the significant difference ($P<0.05$) in clinical manifestations between control group and the treated group that received bone marrow stem cells paracrine factors.[28]

Histological assessment was frequently used as an approach to evaluate the MSCs mechanism in burn wounds healing. Experimental studies by Feng *et al*. (2019),[23] Zhou *et al*. (2019),[24] Temnov *et al*. (2018), Kakabadze *et al*. (2018),[30] Guo *et al*. (2016),[26] and Chen *et al*. (2017)[15] also conducted histological analysis to assess and observe the re-epithelialization activity of MSCs-treated burn wounds when being compared to control. The stem cells enhanced the burn wound healing through promoting granulation process for the tissue formation, maturity of the wound, and also revascularization of the tissue. Stem cells could also decrease the level of inflammation with less scar progression and fibrosis formation. They stimulated faster and greater burn wound healing. However, there was an obstacle to conclude evidence-based suggestions due to the qualitative method of this procedure. Some studies enabled a sort of comparison by trying to convert the discoveries into semi-quantitative scoring systems. An unified scoring system of histological wound healing would be a great value if it was developed and adopted across the researchers in this field. It seems that the most significant progress to help understanding the MSCs utility in clinical settings may come out if the outcome measurement included biopsy samples analysis quantitatively to investigate specific markers in each wound healing phase. The infiltrating inflammatory cells, the proliferating endothelial cells, fibroblasts, or collagen quantification could be included in histological analysis to assess progression through the burn wound healing phases. These approaches could reflect the burn wound pathogenesis more comprehensively.[38,39]

The differences in rat's characteristic, source of the stem cell, burn wound induction methods, and burn wound degree in these studies were the limitation of our study. These limitations made direct comparison between their findings is not possible. Most of the studies (n=7)[14,15,23,24,26,27,31]

in this review used a hot plate to create burn wounds that resulted in partial-thickness (n=2)[23,26] to full-thickness burns (n=3),[24,27,31] and others (n=2)[14,15] did not specify the degree of the burn wound. Partial to full-thickness burns are the most frequent burnings that occur following contact with hot metal, liquid, or steam, flames, and other sources such as electrical and chemical. These burn injuries influence the structure of epidermis and also beneath it (for example: the blood vessels, follicles of hair, and peripheral nervous system.[40]

The other limitation in using stem cells therapy in burn wounds is identifying the right stem cells pool, delivery system, and the dosage for these cells. Autologous cell therapy might not available rapidly and sufficiently after burning and requires cell culture to obtain appropriate number of cells. Thus, this process takes time and can be a specific problem in the burn wound treatment which requires rapid healing. To overcome this problem, it is necessary to use allogeneic stem cells.[41,42]

Future experimental animal studies should apply the risk of bias tools like SYRCLE of the study design phase and has to be clearly written out in the published works of researchers. More clinical studies should be done to identify and recognize the gaps of knowledge and discontinuity of pre-clinical to clinical research. Better future clinical study should be designed as confirmatory research, whether it is regarding proper delivery system or the dose of stem cells therapy.[39]

In summary, our systematic review and meta-analysis provide a comprehensive overview of the MSCs delivery system and dosage, as well as the wound re-epithelialization rate assessment in rat models. Although the animal trials in rat models have shown significant positive effects, the randomized controlled trials still need to be done in purpose to assess the clinical potency of stem cell treatment in burn wounds.

Conclusion

Allogeneic mesenchymal stem cells have potential in the treatment of burns. Our study showed significant differences between treated group and control group in wound area measurement and re-epithelialization rate. In view of the differences in treatment methods and techniques, more research is needed to define standardized protocols. Randomized controlled trials should be done to assess the clinical potency, stem cells efficacy, and adverse effect in the treatment of burns before general conclusions can be drawn.

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