Cell Transplantation
Volume 31: 1–15

DOI: 10.1177/09636897211051743
journals.sagepub.com/home/cll
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Preclinical and Clinical Amelioration of Bone Fractures with Mesenchymal Stromal Cells: a Systematic Review and Meta-Analysis

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Abstract

Even though reunion of bone fracture confronts clinicians, mesenchymal stromal cells (MSCs) are investigated to be curative in bone fracture. This study aimed to explore the application potential of MSCs for healing bone fractures. By inputting search terms and retrieving studies published up to March 2021, multiple databases, including PubMed, EMBASE, Web of Science, and Cochrane Library, were searched to identify eligible studies. The mean difference (MD) and 95% confidence interval (95% CI) were calculated to analyze the main results in the meta-analysis. Data analysis was performed using Engauge Digitizer 10.8 and R Software. Of the 31 articles, 26 were preclinical studies ($n = 913$), and 5 were clinical trials ($n = 335$). Preclinically, MSCs therapy significantly augmented the progress of bone regeneration [(bone volume over tissue volume (MD7.35, $p < 0.01$)], despite some non-significant effects (on the callus index, bone strength, work to failure, and stiffness). Clinically, the MSC group had a significantly reduced incidence of poor recovery (odds ratio (OR) 0.30, $p < 0.01$); however, a significant decrease in healing time was not observed in the MSC group (MD 2.47, $p = 0.26$). In summary, our data suggest that patients with bone fractures benefited from MSC administration and that MSCs are a potentially useful agent for bone regeneration. Despite these satisfactory outcomes, larger randomised clinical trials (RCTs) are necessary to confirm these findings.

Keywords

mesenchymal stromal cells, bone regeneration, preclinical trials, clinical trials, meta-analysis

Introduction

Bone fractures are the disconnection of intact bones caused by tremendous external force (from a fall, car crash, hit, etc.) and happen most in children and elderly individuals. Each year, approximately 8 million people in the United States suffer traumatic fractures, which can lead to hospitalizations, surgeries, inconveniences and lost work time[1]. Fracture healing is associated with increases in delayed union, non-union, infection and revision surgery[2], and no ideal treatments for accelerating the progression of fracture healing have been recommended. The direct cost of osteoporotic fractures was $19 billion a year in 2005 and is projected to reach $60 billion by 2030[3].

Although several methods to improve fracture healing have been developed clinically, they all have their own limitations. Research on electromagnetic field therapy for bone repair has been carried out for more than 60 years, but the therapeutic effect of this therapy is still conspicuous. Autologous bone marrow transplantation is considered an effective method for bone repair, but the sampling technique and the quality of cell preparation may affect its efficacy[4]. Autologous bone marrow grafting might be an effective strategy for bone repair, but the quality of the harvesting technique and cell preparation might affect the efficacy[5]. Human bone morphogenetic proteins (rhBMPs) 2 and 7 are used to treat non-union and tibial shaft fractures but can lead to adverse reactions such as inflammation and a risk of

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Submitted: April 22, 2021. Revised: August 30, 2021. Accepted: September 21, 2021.

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ectopic bone formation[6]. Recombinant human parathyroid hormone (PTH) 1-34 (teriparatide) has been used off-label in clinical practice[7]. However, the US Food and Drug Administration (FDA) has not approved it for this indication. Therefore, new therapeutic targets are required to treat fractures and shorten the time for bone healing.

Mesenchymal stromal cells (MSCs) are multipotent stromal cells with the ability to self-renew and have the potential to differentiate into muscle cells, chondrocytes, adipocytes, osteoblasts, and other cells[8]. MSCs can be isolated from various sources, such as adipose tissue, bone marrow, tendons, cord blood, foetuses[9]. Moreover, MSCs are also easily cultured and amplified and are immunologically inert[10]. MSCs have recently been reported to treat burns, myocardial infarction, ulcerative colitis, systemic sclerosis and other diseases[11,12,13]. As a reserve force to induce tissue regeneration after injury[14], MSCs have been widely studied for their therapeutic potential in fracture healing and bone regeneration[15]. It has recently been reported that MSCs can enhance bone healing. In the process of bone resorption, active TGF-1 released from the bone matrix induces the aggregation of MSCs to the fracture site through the SMAD signalling pathway[16], and MSCs stimulate angiogenesis, further promoting the fracture healing cascade[17].

In this regard, a number of recent animal studies and clinical trials have reported the benefits of MSCs in the treatment of fracture healing. However, the clinical efficacy of MSCs in bone repair or strengthening has not been established. Moreover, there is little consensus on which cell source, type of cell, dose and method of administration are most beneficial to patients. As such, the purpose of our study was to provide a systematic review of animal and clinical studies on the treatment of bone repair with MSCs.

Methods

Literature Search Strategy

The meta-analysis was performed according to the Preferred Reporting Items for Systematic Review and Meta-Analysis (PRISMA) statement[18] (see additional file 1). We conducted a comprehensive literature search using the online databases PubMed, EMBASE, Web of Science, and Cochrane Library up to March 7, 2021. The terms "mesenchymal stem cells OR mesenchymal stromal cells" and "bone fractures OR bone regeneration OR bone repair" were used to identify the relevant literature. Two investigators independently extracted data, and a third investigator was included when a disagreement occurred. The retrieval strategy is shown in Additional file 2.

Inclusion and Exclusion Criteria

If the studies fulfilled these criteria, they were considered for further evaluation: (1) studies with specific definition of fracture non-union or bone defect; (2) controlled studies estimating the effects of MSCs (including bone marrow-derived MSCs, adipose-derived MSCs and umbilical cord-derived MSCs) in animals by in vivo administration; (3) studies in which the control group received either a placebo or no treatment; (4) studies in which effect estimates and precision measurements (standard deviation or standard error) were considered when reporting data; (5) trials with the full text accessible.

The exclusion criteria were as follows: (1) single-arm studies; (2) trials using non-MSCs (including other mature cells, MSC conditioned media (CM), granules, ointments, Chinese medicines, pulsed ultrasound and surgical treatment); (3) other types of articles, such as reviews, meta-analyses, conference records, editorials, and research letters; and (4) articles presenting irrelevant outcomes, such as historical outcomes (HE staining, immunohistochemical staining) and biochemical indexes (IL-1, IL-6 and TGF-β).

Data Extraction

Two investigators (Hanxiao Yi and Yang Wang) independently identified the titles and abstracts and resolved uncertain data through discussion and consensus. The information extracted from preclinical trials contained the following: (1) first author; (2) year; (3) region; (4) stromal cell type; (5) No. of fractures; (6) model strain/species; (7) sex/age; (8) fracture site; (9) fracture model; (10) dosage; (11) administration; and (12) observation time (post model). For clinical trials, the information included the following: (1) first author; (2) year; (3) study type; (4) patients evaluated; (5) patients included; (6) follow-up; (7) fracture site; (8) source of MSCs; (9) route of delivery; (10) timing; and (11) dose/volume.

Assessment of Study Quality and Bias

The risk of bias was assessed independently by 2 authors (Hanxiao Yi and Yang Wang). Different quality assessment tools were used to assess the bias risk for each selected study. For preclinical trials, the risk of SYRCLE bias (RoB) was used to study the six sections: the title, the abstract, the introduction, the methods, the results, and the discussion. Each item contained several details and was classified as having low, unclear, or high bias risk[19].

For clinical trials, the Cochrane Risk of Bias (Rob) tool for randomized control trials (RCTs)[20] was used, and the individual domains were (1) random-sequence generation; (2) allocation sequence concealment; (3) blinding of participants and personnel; (4) blinding of outcome assessment; and (5) completeness of outcome data. The publication bias of the preclinical trials was assessed using funnel plots and Egger's test.

Outcome Measurements

We refined some data in the original articles to a consensus definitions: bone volume over tissue volume (BV/TV), the percentage of bone volume (BV) over tissue volume (TV); callus width (CW), the maximal outer diameter of the

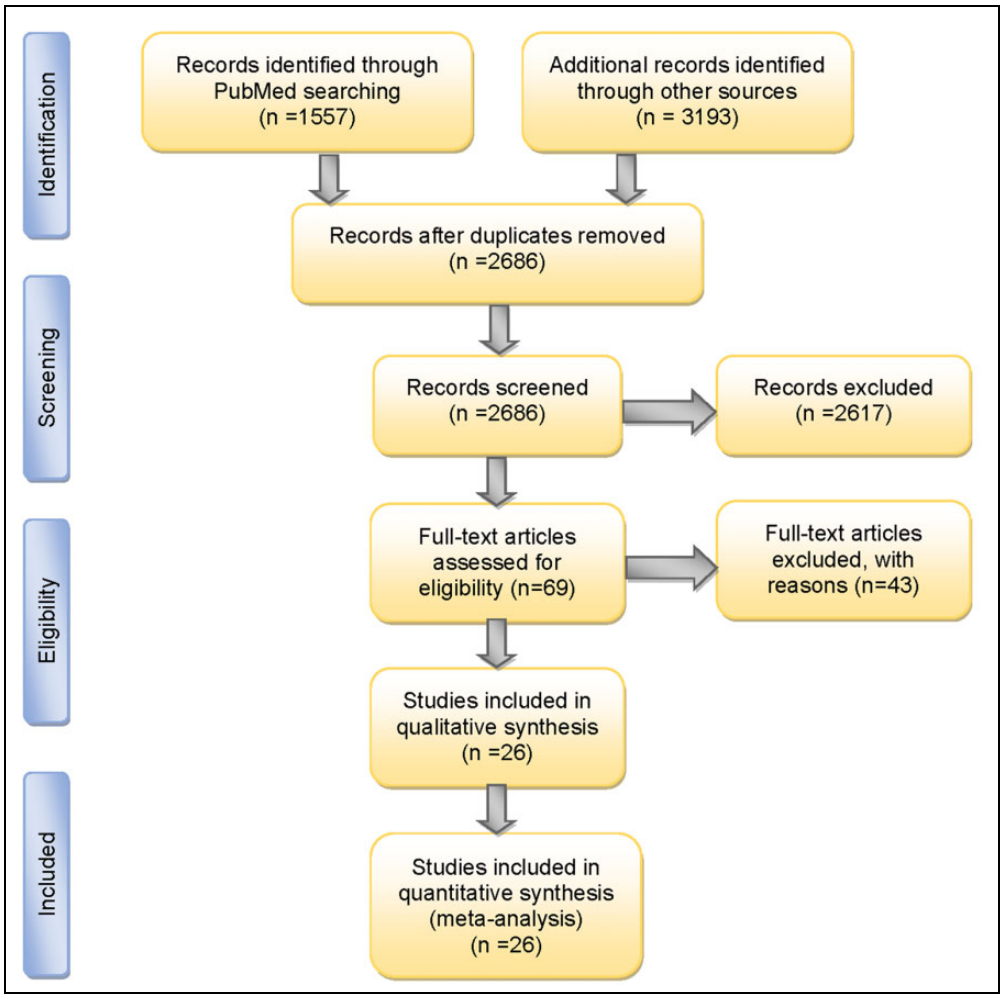

Figure 1. Flow Chart of the Article Screening Process.

mineralized callus minus the outer diameter of the femur; callus area (CA), the sum of the areas of the external mineralized callus; and callus index (Cl.Ind), (the volume occupied by callus including voids—the callus TV)/the volume occupied by callus including voids · 100. Stiffness is the ability to resist elastic deformation under stress and was calculated as the slope of the linear part of the load-displacement curve. Work to fracture was calculated from the area under the load-displacement curve. The elastic modulus (E-modulus), the slope of the stress-strain curve, is known as the tissue stiffness[21]. Trabecular thickness (Tb.Th) was calculated as a measure of the thickness of all bone voxels and the bone mineral density (BMD). Cg.Ar/CI.Ar was calculated as the ratio between the cartilage area and the CA.

Statistical Analysis

Engauge Digitizer (Mitchell 2016) was used to collect data from statistical charts. Data analyses were performed with R software 4.02 (University of Auckland, New Zealand). All continuous data evaluated in this article are presented as the mean difference (MD) with the corresponding 95% confidence interval (95% CI) to eliminate the influence of units and measures. Binary data are presented as odds ratios (ORs) with 95% CIs. Statistical heterogeneity was evaluated with the inconsistency index (I^2). If $I^2 \geq 50\%$, a fixed-effect model was used; otherwise, a random-effect model was employed. Subgroup analysis was used to find potential sources of heterogeneity. Egger's linear regression and Begg's funnel plot were employed to calculate the potential publication bias.

Result

Search Results

A total of 2686 references were retrieved from the PubMed, EMBASE, Web of Science and Cochrane Library databases. After removal of duplicates, other types of articles (reviews, meeting abstracts and comments), and irrelevant articles, 58 potential full-text articles were carefully reviewed. Among the studies screened, 32 records were removed according to different criteria: improper study designs ($n = 11$) and inappropriate result forms ($n = 21$). A total of 26 studies were ultimately included in the meta-analysis (Fig. 1).

Quality of Studies

Experimental trials: According to SYRCLE's RoB Tool, all the preclinical studies had a moderate to high risk of bias. The outcome of the quality assessment showed that 36% of studies had a low risk, 30% of studies had an unclear risk, and 34% of studies had a high risk of bias. None of the studies clearly elucidated the generation of random sequences, and none provided complete baseline information, making it difficult to confirm whether each group of animals were randomly grouped and to accurately extract article characteristics. In all 26 studies, there appeared to be a lack of standard practice for allocation concealment, blindness to study personnel and outcome assessors, and therefore, there was high detection bias. Attrition and reporting bias were low because the results of all 26 articles were clear and sufficient. See Table 1 for details.

Clinical trials: All clinical trials were relatively high quality except one article was marked as having a high risk of bias in terms of allocation concealment and an unclear risk of bias in terms of randomization, blinding, and selection. Most articles performed poorly in allocation, blinding, randomization and blurred deception in their methodologies. However, all studies integrally reported present outcomes from all participants, and no missing data existed (Fig. 2).

Study Characteristics

Experimental trials: Ten of the 26 studies used mice, while 11 studies used rats, and the other 3 studies used pigs and sheep, 4 studies used rabbits. bone marrow-derived MSCs (BMSCs, $n = 20$), umbilical cord-derived MSCs (UC-MSCs, $n = 1$), and adipose-derived MSCs (ADSCs, $n = 5$) were used in these articles. MSCs were administered through different routes, such as local transplantation ($n = 9$) and tail vein injection ($n = 17$). Other information was also recorded. The detailed characteristics of these studies are shown in Table 2.

USA, United States of America; MSC, mesenchymal stem cells; Con, control; AD, administration; R-BMSCs, rat bone marrow mesenchymal stem cells; H-BMSCs, human bone marrow mesenchymal stem cells; S-BMSCs, sheep bone marrow mesenchymal stem cells; R-ADSCs, rabbit adipose derived mesenchymal stem cells; M-BMSCs, mouse bone marrow mesenchymal stem cells; P-BMSCs, pig bone marrow mesenchymal stem cells; H-UCMSCs, human umbilical cord mesenchymal stem cells; M-ADRCs, mouse adipose-derived regenerative cells; R-BMSCs, rat bone mesenchymal stromal cells; W, week; M, month; M, male; F, female; NA, not available; m, minute; h, hour; d, day. IV injection, intravenous injection.

Clinical trials: Five studies ($n = 335$ patients) were included in the meta-analysis. Of these studies, three were RCTs ($n = 124$ patients)[22,23,24] and two were retrospective studies ($n = 211$ patients)[25,26]. Four of the five studies used autologous bone marrow-derived MSCs[22,23,25,26], while the others used allogeneic UC-MSCs[24]. Three of the studies adopted administration through implantation[22,25,26], and the remaining two studies used percutaneous injection[23,27]. The detailed information of these studies is summarized in Table 3.

Preclinical Evidence

Analysis of regenerated bone. The healing status of bone fractures in animals was reported as various measurements; therefore, we reported the results according to the volume and strength of the regenerated bone. The data for BV/TV, which was the most frequently used output of microCT analysis performed on bone and was reported by 13 studies ($n = 446$ animals), was analysed using a random-effect model. We did not find a distinct improvement in BV/TV (1st week, MD 0.06, 95% CI: −0.05 to 0.17, $p = 0.29$; 2nd week, MD 1.52, 95% CI: −1.83 to 4.87, $p = 0.37$) in animals treated with MSCs until 4 weeks after treatment (4th week, MD 7.35, 95% CI: 2.84 to 11.86, $p < 0.01$; 5th week, MD 9.17, 95% CI: 4.51 to 13.83, $p < 0.01$; 8th week, MD 11.79, 95% CI: −1.44 to 25.02, $p = 0.08$) (Fig. 3A). Furthermore, BV, which was reported by 11 studies ($n = 297$ animals) and directly reflects the volume of the regenerated bone, showed distinct differences between the two groups over the observation periods (2nd week, MD 4.12, 95% CI 1.13 to 7.12, $p < 0.01$; 12th week, MD 4.11, 95% CI 1.83 to 6.39, $p < 0.01$) except at the 8th week (MD 12.42, 95% CI 23.52 to 48.35, $p = 0.50$) (Fig. 3A). TV, which is mainly subjectively determined by the researcher, was also analyzed. Four of the 22 studies reported TV ($n = 94$ animals), and a random-effect model was used for the analysis. The animals in the MSC group revealed a significantly higher TV than those in the control group (MD 13.88, 95% CI 3.51 to 24.24, $p < 0.01$) (Fig. 3B).

The callus is also an important indicator of fracture healing. In this section, we mainly focused on alterations in the callus (such as CW, CA, and callus index) that occurred following MSC administration. All 4 studies involving animals reported the effect of MSCs on the CA and CW. Pooled results showed that the CW was larger in the MSC group than the control group over the four weeks following treatment (2nd week, MD 0.59, 95% CI: 0.29 to 0.89, $p < 0.01$; 3rd week, MD 0.56, 95% CI: 0.43 to 0.69, $p < 0.01$; 4th week, MD 0.67, 95% CI: 0.53 to 0.81, $p < 0.01$). However, we the callus index did not significantly change (MD 0.75, 95% CI: −5.33 to 6.82, $p = 0.81$). Additionally, both Cheung[28] and Wei[29] reported changes in the CA ($n = 55$ animals) at 2, 3, and 4 weeks post treatment (Fig. 3C, D). They suggested that at the 2nd week (MD 9.90, 95% CI: 2.53 to 17.28, $p < 0.01$), 3rd week (MD 7.33, 95% CI: 1.06 to 13.60, $p = 0.02$) and 4th week (MD 6.93, 95% CI: 0.98 to 12.88, $p = 0.02$) after treatment, a significant change in the CA was discovered in the MSC group.

Table 1. SYRCLE's Tool for Each Preclinical Trial.

Author	Random Sequence SELECTION BIAS	Allocation Concealment	Baseline Characteristics	Blinding (Study Team) DETECTION BIAS	Random Housing	Random Outcome Assessment REPORTING BIAS	Blinding (Outcome Assessors)	Incomplete Outcome Data ATTRITION BIAS	Selective Outcome Reporting REPORTING BIAS	OTHER BIAS
Chen	?	-	?	–	+	–	+	+	+	+
Cheung	?	–	?	+	?	–	+	+	+	+
Doron	?	–	?	–	?	–	+	+	+	+
Dozza	?	–	?	–	+	–	–	+	+	+
Erdogan	?	–	?	–	+	–	–	–	+	+
Huang (1)	?	–	?	–	+	?	+	+	+	+
Huang (2)	?	–	?	–	+	?	–	+	+	+
Kumar	?	–	?	–	?	–	–	+	+	+
Pelled	?	–	?	–	–	?	–	+	+	+
Qu	?	–	?	–	+	?	+	+	+	+
Augustine	?	–	?	–	?	–	+	+	+	+
Sheyn	?	–	?	–	+	?	+	+	+	+
Tawonsa–watruk	?	–	?	–	–	–	+	+	+	+
Tewari	?	–	?	–	+	–	+	+	+	+
Thomas	?	–	?	–	+	–	–	+	+	+
Rapp	?	–	?	–	+	–	–	+	+	+
Wang	–	–	?	–	?	?	–	+	+	+
Wei	?	–	?	–	?	?	–	+	+	+
Xu	?	–	?	–	?	?	–	+	+	+
Yan	?	–	?	–	–	–	–	+	+	+
Yao	?	–	?	–	–	–	–	+	+	+
Zhang	?	–	?	–	+	–	–	+	+	+
Wang	?	–	?	–	?	?	–	+	+	+
Ramkumar	?	–	?	–	–	?	+	+	+	+
Kim	?	–	?	–	–	?	+	+	+	+
Issei	?	–	?	–	?	?	?	+	+	+

(+) low risk of bias; (-) high risk of bias; (?) unclear risk of bias.

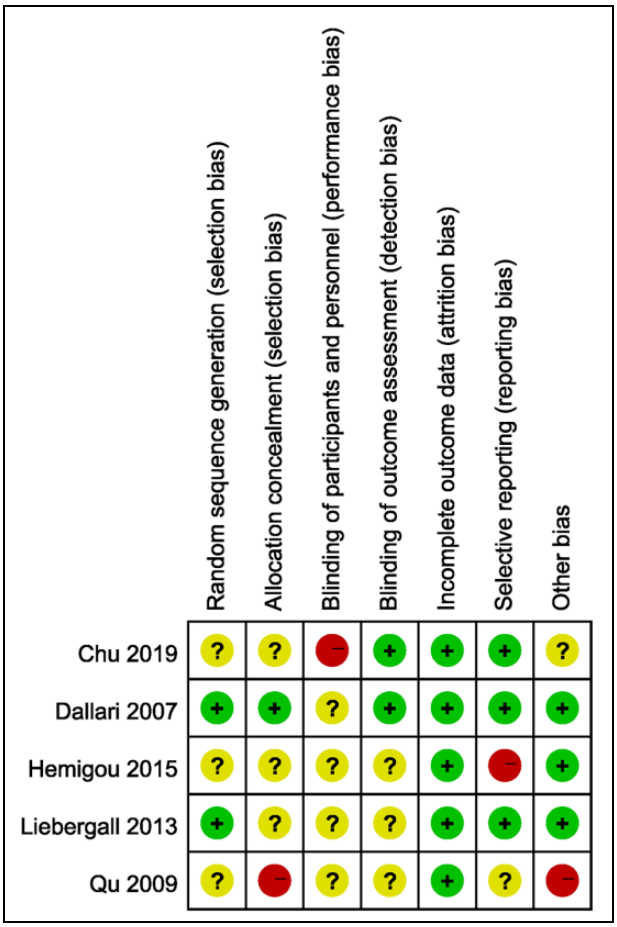

Figure 2. Quality evaluation of the included studies. Quality assessment of included clinical trials using the Cochrane RoB tool.

Analysis of biomechanical tests and secondary indexes of bone regeneration. Mechanical testing is the ultimate method to determine an intervention's effect on fracture healing. Stiffness is the ability to resist elastic deformation under stress and is calculated as the slope of the linear part of the load-displacement curve. Work to fracture is calculated from the area under the load-displacement curve. The E-modulus, the slope of the stress-strain curve, is known as the tissue stiffness[21]. Our study showed that the E-modulus (MD -2666.91, 95% CI: −4094.07 to −1239.75, $p < 0.01$) and stiffness (MD 23.80, 95% CI: 9.36 to 38.25, $p < 0.01$) were significantly increased in animals in the MSC group. However, bone strength (MD −20.05, 95% CI: −45.00 to 4.90, $p = 0.12$) and work to fracture (MD 3.62, 95% CI: −0.99 to 8.23, $p = 0.12$) were not significantly increased in animals in the MSC group (Table 4). Despite some negative results, these data hinted that MSCs actually contribute to increasing the strength of newly regenerated bone.

Cg.Ar/CI.Ar, the ratio between the cartilage area and the callus area; No, number of fracture sites. MD, mean difference.Finally, we summarized the ultimate load according to the observation time. Our results indicated that there were no significant differences between the two groups at the 2nd week (MD 4.33, 95% CI: −0.55 to 9.20, $p = 0.08$) or the 8th week (MD 1.73, 95% CI: −45.34 to 48.81, $p = 0.94$). Interestingly, the ultimate load increased by 2.65 at the 5th week (MD 2.65, 95% CI: 1.14 to 4.16, $p < 0.01$) (Additional file 3). This result may suggest the early effect of MSCs on bone regeneration, but this theory needs to be further confirmed.

Moreover, other indicators, such as fibrous tissue (MD 34.64, 95% CI: 28.30 to 40.99, $p < 0.01$), trabecular thickness (Tb, Th) (MD 0.10, 95% CI: 0.02 to 0.18, $p = 0.01$), BMD (MD 0.94, 95% CI: 0.61 to 1.27, $p < 0.01$), connectivity density (MD 5.33, 95% CI: 2.77 to 7.88, $p < 0.01$), density of BV (MD 39.33, 95% CI: 31.31 to 47.34, $p < 0.01$), the Cg.Ar/CI.Ar ratio (MD −0.12, 95% CI: −0.15 to −0.09, $p < 0.01$) confirmed that MSCs were efficacious in facilitating bone regeneration (Table 4).

Subgroup Analysis

To identify heterogeneity potentially influencing the analysis of the BV/TV, subgroup analysis was conducted based on model species, location, administration route, cell origin and year. At the 4th week, animals in the local administration group had higher BV/TV (MD 7.35, 95% CI: 4.35 to 10.35, $p < 0.01$) than animals in the control group. Furthermore, for animals systemically receiving injection of MSCs (MD 4.11, 95% CI: −1.05 to 7.16, $p < 0.01$) and animals treated with MSCs of human origin ($p < 0.01$), a significant difference in the BV/TV between the two groups were observed. Asian articles also reported a great improvement in BV/TV ($p < 0.01$). Subgroup analysis also indicated that the efficacy of MSCs may be species-specific because mice receiving MSCs had an increased BV/TV (MD 16.23, 95% CI: 7.83 to 24.62, $p < 0.01$), and rats (MD 2.81, 95% CI: 1.03 to 4.59, $p < 0.01$). At the 8th week, no factors were identified to prominently impact the final outcomes (Additional file 4).

Publication Bias

The publication bias related to BMD (Fig. 4 BMD), BV/TV (Fig. 4 BV/TV), BV (Fig. 4 BV) and ultimate load (Fig. 4 ultimate load) was determined by using a funnel plot and Egger's linear regression. BMD (Egger's test, $p = 0.498$), BV/TV (Egger's test, $p = 0.234$), and ultimate load (Egger's test, $p = 0.677$) showed nonsignificant publication bias. Although there was a significant publication bias for BV (Egger's test, $p < 0.01$), this is likely the result of the use of a random-effect model for a small-scale study.

Clinical Evidence

As for the clinical trials, we considered two indicators of interest, namely, healing time and poor recovery, primarily due to the lack of studies reporting clinical trials and lack of indicators that could be included in the meta-analysis. In addition, rapid fracture healing is very important for patients

Table 2. Pre-clinical Study Characteristics.

Author	Year	Region	NO. of Fracture			Strain/Species	Gender/age	Fracture site	Model	Dosage	Cell type	Timing (Post model)	AD
			Total	MSC	Con								
Chen	2017	China	140	22	96	rat/SD	male/adult	distal end of the tibia	oscillating mini saw, 21-gauge needle	NA	R-BMSCs	NA	local transplantation
Cheung	2013	China	60	20	40	rat/SD	female/ 30 months	right femur shaft	a 500 g metal blade, dropping, a height of 35 cm	NA	R-BMSCs	within 1m	left ventricle injection
Doron	2017	USA	36	9	18	rat/Crl:NIH-Foxn1rnu	female/12 weeks	the fifth and sixth rib	a 2-cm-long incision, a 5-mm-long segment	2×10^6 hMSCs	H-BMSCs	3d	IV injection
Dozza	2018	Italy	15	8	7	sheep/ Bergamasca-Massese	female/adult	right tibial	cut 15-mm of periosteum, cut 10-mm of cylindrical bone	NA	S-BMSCs	within 2h	local transplantation
Erdogan	2015	Thailand	36	18	18	rabbits/New Zealand	male/adult	tibia	NA	2×10^6 MSCs	R-BMSCs, R-ADSCs	NA	local transplantation
Huang (1)	2015	China	30	10	20	mice/FVB/N	male/8 weeks	tibia	hand saw transverse osteotomy, a 23-gauge hypodermic	5×10^5MSCs	M-BMSCs	4d	cardiac injection
Huang (2)	2015	China	48	24	24	rat/SD	male/14 weeks	right femur	a 500 g metal blade, dropping, a height of 35 cm	2×10^6 GFP-MSCs	R-BMSCs	4d	intracardiac injection
Kumar	2009	USA	30	6	18	mice/nude	male/10-12 weeks	right tibial	a three-point bending apparatus, a 2–3 mm-long segmental	1×10^6 MSCs	M-BMSCs	NA	tail vein
Pelled	2016	USA	6	3	3	minipigs/ Yucatan	NA/1.5 years	lumbar vertebra	a surgical drill bit, 15-mm in depth and 4-mm in diameter	4×10^6 BMP6 - MSCs	P-BMSCs	NA	local transplantation
Qu	2015	China	80	20	40	rat/SD	NA/6-8 weeks	tibial	NA	3×10^8 MSCs	H-UCMSCs	NA	injected circumferentially
Augustine	2019	USA	28	14	14	rat/SD	male, female/ 10 weeks	right tibial	6-millimeter diaphyseal sized defects	30×10^6 rBMSCs	H-BMSCs	Immediately	local transplantation
Sheyn	2016	USA	28	21	7	mice/NOD/ SCID	female/6-8 weeks	radial fracture	a 1.5-mm defect	1×10^6MSCs	H-BMSCs	NA	local transplantation
Tawonsawatruk	2016	UK	17	5	7	rat/Wistar	NA/NA	tibia	amid shaft osteotomy, a 1 mm gap	5×10^6 MSCs	H-BMSCs	3W	pericyte injection
Tewari	2014	China	30	10	20	rat/SD	female/NA	tibia	NA	1×10^6MSCs	M-BMSCs	NA	tail vein

(continued)

Table 2. (continued)

Author	Year	Region	NO. of Fracture			Strain/Species	Gender/age	Fracture site	Model	Dosage	Cell type	Timing (Post model)	AD
			Total	MSC	Con								
Thomas	2012	USA	48	24	24	mice/C57BL/6	male/6-7 weeks	mid-shaft tibia	a 220 g, dropping, a height of 195 mm	NA	R-BMSCs	24h	tail vein
Rapp	2015	Germany	12	6	6	mice/C57BL/6	male/NA	femur	femur osteotomy model and a non-invasive ulna-loading model	1×10^6MSCs	M-BMSCs	2h	IV injection
Wang	2011	China	25	5	5	rat/SD	NA/10 weeks	middle third of the fibula	a 2 mm defect	NA	H-BMSCs	NA	local transplantation
Wei	2016	China	40	10	30	rat/SD	female/NA	mid-shaft of the femur	cut 15-mm of periosteum, cut 10-mm of cylindrical bone	1×10^6MSCs	R-BMSCs	3d	intracardiac injection
Xu	2014	China	10	5	5	rat/SD	NA/NA	tibia	a sagittal saw, a 1.1 mm Kirschner wire	2×10^5Sox11-MSCs	R-BMSCs	4d	tail vein
Yan	2011	USA	26	11	15	mice/FVB syngenic	female/8-12 weeks	tibia	a three-point bending device with a standardized force	1×10^6MSCs	M-BMSCs	NA	tail vein
Yao	2016	USA	64	32	32	mice/NA	NA/8 weeks	right femur	a drop-weight blunt guillotine device	3×10^5 ADSCs	M-ADSCs	1d	tail vein
Zhang	2017	China	NA	NA	NA	mice/Osx-mCherry	male,female/ NA	right femur	a drop-weight blunt guillotine device	3×10^5 ADSCs	M-ADSCs	NA	intramuscular injection
Wang	2019	China	24	6	18	white rabbits/ New Zealand	NA/4 month	femur	a 4 mm in width,8 mm in depth defect	1×10^6MSCs	R-BMSCs	3d	local transplantation
Ramku-mar	2019	USA	NA	NA	NA	mice/C57BL/6	NA/6-8 weeks	calvarial	a 4 mm full-thickness defect	1×10^6 MSCs	M-ADSCs	NA	local transplantation
Kim	2013	Korea	14	7	7	rabbits/New Zealand	male/NA	mandible	a 7 cm inferior border of the mandible	2×10^6 MSCs	H-BMSCs	1d	transcutaneously injected
Issei	2014	Japan	60	20	40	rat/Wistar	NA/10 weeks	left femurs	NA	1×10^6 ADSCs	M-ADRCs	Immediately	pericyte injection

Table 3. Clinical Study Characteristics.

Author	Year	Study type	Patients evaluate (n [% male])		Patients included	Follow-up (M)	Fracture site	Source of MSCs	MSCs Route	Timing	Dose/volume
			MSC	Con							
Chu	2019	historical control	16(62.5)	23(60.8)	39	30.5 ± 5.3	tibial	autologous	implantation	intraoperative	13186.9 ± 4301.1 cells/13–26 ml
Dallari	2007	RCT	10(60.0)	18(44.4)	28	12	tibial	autologous	implantation	intraoperative	(13.0–34.5) × 10^3 cells/NA
Hernigou	2015	historical control	86(44.2)	86(40.7)	172	12	ankle	autologous	percutaneous implantation	NA	(6.1 ± 1.8) × 10^4 cells/NA
Liebergal	2013	RCT	12(91.6)	12(83.3)	24	12	distal tibial	autologous	percutaneous injection	3–6 WPP	1.03 × 10^8 cells /8 ml
Qu	2009	RCT	36(75.0)	36(77.7)	72	13.2 ± 4.6	femoral, tibial	allogeneic	percutaneous injection	NA	1 × 10^6-10^7 cells/NA

WPP, week post procedure; NA, not available; RCT, randomized controlled trial; M, months

treated with MSCs to achieve good recovery. Satisfactory recovery can well reflect the effect of MSCs on bone regeneration, which is also the goal of surgical treatment.

Healing time. The healing time was reported in two studies including 111 patients ($n = 52$, MSCs; $n = 59$, control). Our pooled outcome showed that the healing time in the MSC group was not significantly decreased (MD −2.47, 95% CI: −6.8 to 1.86, $p = 0.26$) compared with that in the control group (Fig. 5A).

Poor recovery. Poor recovery at the final follow-up was reported by three studies[23,25,26], and a total of 235 patients were included ($n = 114$ receiving MSCs, $n = 121$ receiving control treatment). Contrary to the outcome of healing time, MSC therapy significantly reduced the number of patients with poor recovery (OR 0.30, 95% CI: 0.16 to 0.57, $p < 0.01$) (Fig. 5B).

Discussion

Fractures are the most common form of trauma hospitalization, with more than 150,000 people admitted to the hospital in Australia each year[30]. In the United Kingdom (UK), non-union hospital treatment alone is estimated to cost between £7,000 and £7,900[31]. Fractures result in a considerable number of patients with disabilities, inconveniences and increased financial burden. Therefore, we first conducted a systematic review and meta-analysis to summarize the efficiency of MSCs in the treatment of bone repair, summarizing evidence from preclinical and clinical trials. Ultimately, 22 preclinical and 5 clinical studies were included.

To assess the risk bias of included articles investigating animals, quality assessment was conducted using SYRCLE's RoB scale. SYRCL's RoB tool was proposed by Hooijmans et al[19] in 2014 and is an adaptation of the Cochrane RoB tool, which is a tool used to assess animal research evidence that helps to improve the efficiency of translating animal research into clinical practice. We observed that most of the studies showed high and unclear risk of selection bias and detection bias, which was mainly due to the lack of randomization, concealment of allocation, baseline characteristics of the groups of animals, and blinding of the researchers and assessors to the results. At present, most of the animal research published globally has a high risk of bias in these aspects, suggesting the necessity to improve the methodological quality of animal research. In clinical studies, most studies lack specific methods to describe allocation concealment and blinding of participants and personnel, and thus, they have a high risk of bias. This information is crucial because selection bias and performance bias are associated with exaggerated effect sizes.

In terms of the effect of MSCs on bone regeneration, the results of our meta-analysis showed that MSCs could promote bone regeneration in animals. However, the callus index, bone strength and work to fracture of the MSC

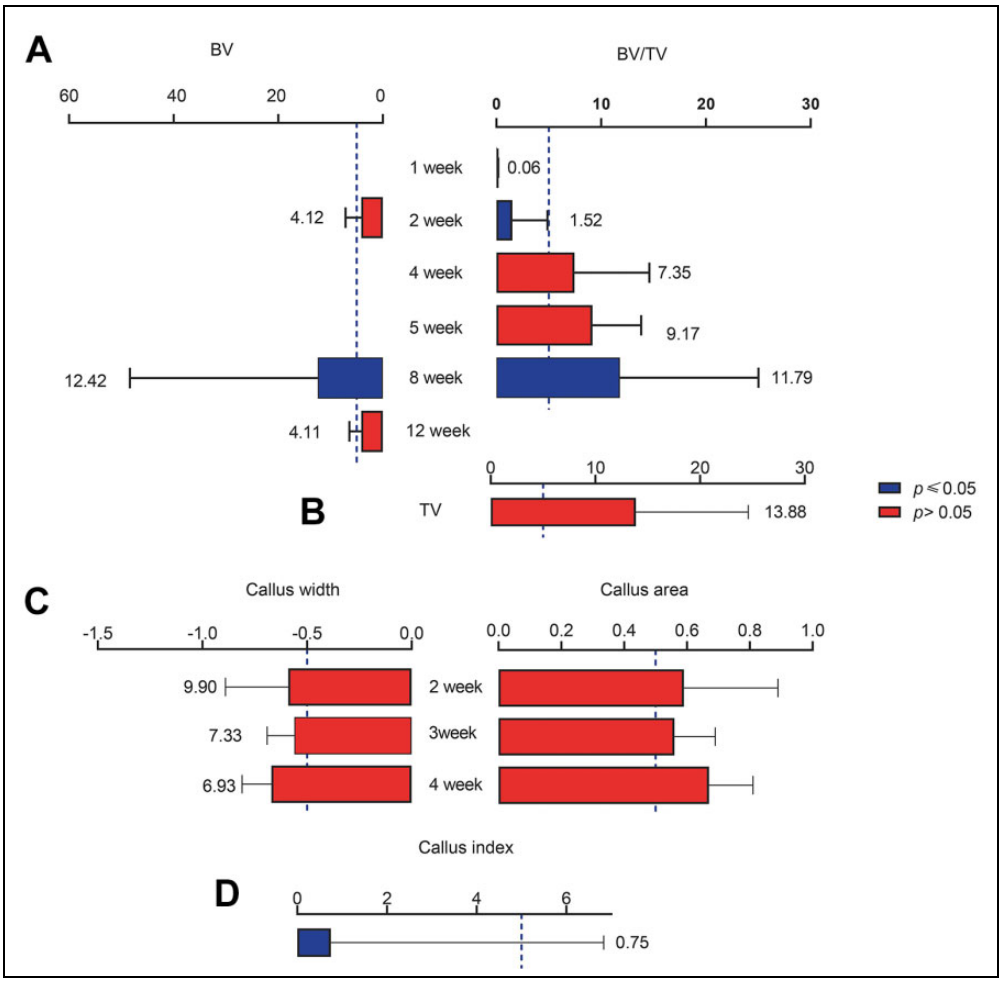

Figure 3. Subgroup analysis for BV, BV/TV, TV, callus width, callus area and callus index. (A) Subgroup analysis of BV at the 2nd, 8th, and 12th weeks and BV/TV at the 1st, 2nd, 4th, 5th, 8th, and 12th weeks. (B) Pooled analysis of TV. (C) Subgroup analysis of the callus width and callus area at the 2nd, 3rd, and 4th weeks. (D) Pooled analysis of the callus index. All analyses were conducted by using a random- or fixed-effects model with a 95% confidence interval. BV, bone volume; TV, tissue volume.

Table 4. Analysis of Biomechanical tests and Secondary Indexes of Bone Regeneration.

Outcome	No.	MD	95% CI	*P* value
Biomechanical test				
E-modulus	158	−2666.91	[−4094.07; −1239.75]	***<0.01***
Bone strength	20	−20.05	[−45.00; 4.90]	0.12
Work-to-fracture	38	3.62	[−0.99; 8.23]	0.12
Stiffness	166	23.80	[9.36; 38.25]	***<0.01***
Secondary indexes of bone regeneration				
Bone mineral density	208	4.29	[2.84 to 5.74]	***<0.01***
Fibrous tissue	22	34.64	[28.30 to 40.99]	***<0.01***
Connectivity density	46	5.33	[2.77 to 7.88]	***<0.01***
Density of BV	42	39.33	[31.31 to 47.34]	***<0.01***
Trabecular thickness	72	0.10	[0.02 to 0.18]	***0.01***
Cg.Ar/Cl.Ar	27	−0.12	[−0.15 to 0.09]	***<0.01***

treatment group showed no improvement compared to those in the control group, which was inconsistent with the results of Doron et al[32] and might be explained by the small number of studies included. The pooled outcomes of poor recovery from clinical trials greatly decreased (OR = 0.3, $p < 0.01$) after MSC transplantation, suggesting that MSC transplantation is associated with improved prognosis. Nevertheless, patients treated with MSCs did not show reduced healing

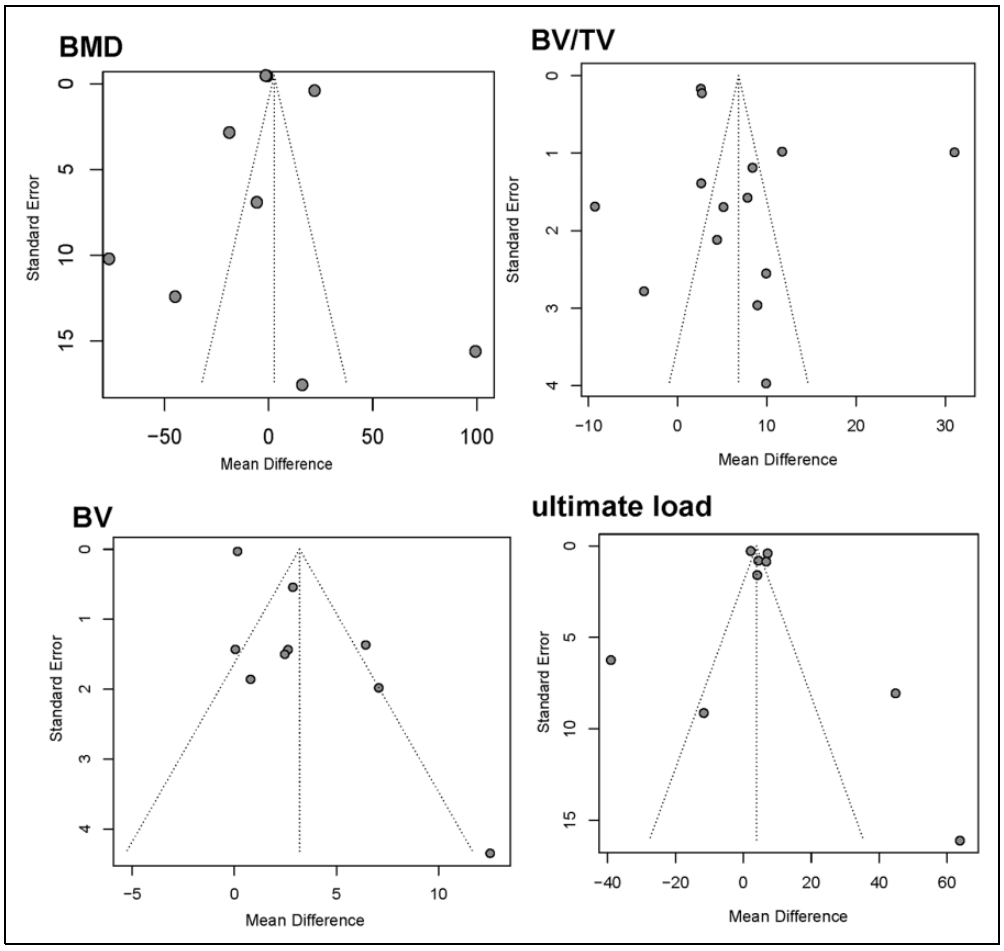

Figure 4. Funnel plots of primary and secondary outcomes. Funnel plots were generated for BMD, BV, BV/TV, and ultimate load.

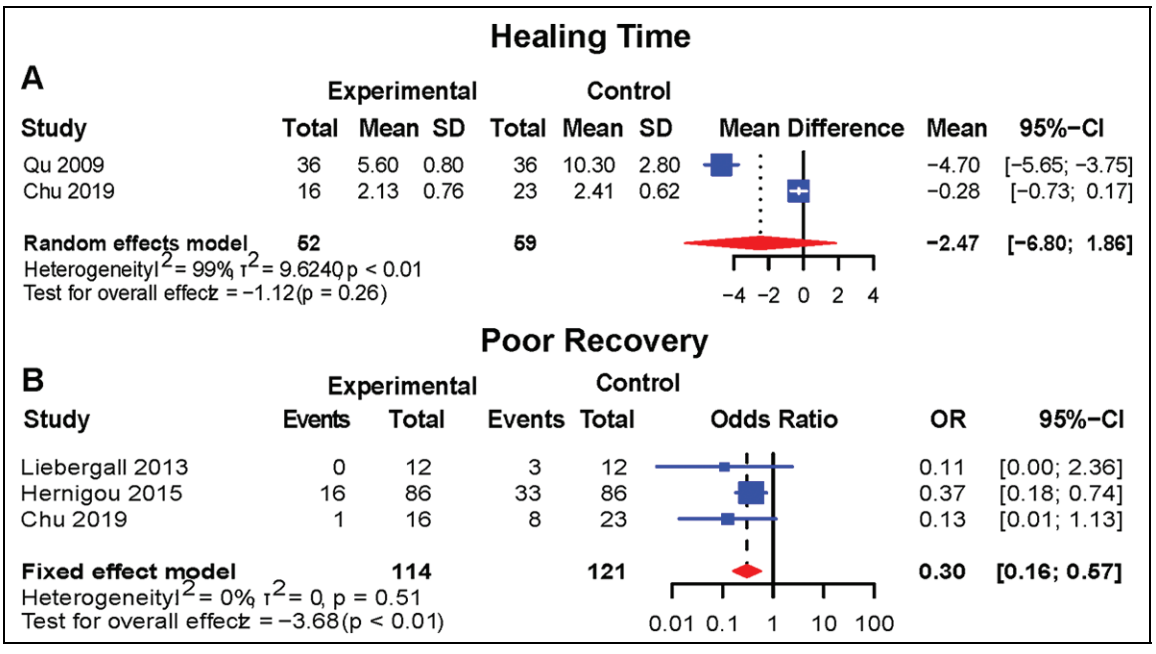

Figure 5. Clinical outcomes of healing time and poor recovery. Pooled analysis of (A) the healing time and (B) the rate of poor recovery.

time as anticipated. Overall, our results demonstrated a strongly positive relationship between MSC transplantation and the prognosis of bone fractures preclinically and clinically. To explore the source of heterogeneity, we performed a subgroup analysis of administration route, cell origin, model species and location. The results indicated that these variables (administration route and model species) were potential sources of heterogeneity and are highly important for future clinical applications. With regard to the delivery route of MSCs, different methods may lead to opposite outcomes, and systemic administration was preferred. However, Huang et al[33] reported that in a mouse fracture model, both systemic and local use of allogeneic BMSCs were effective. Therefore, this point should be further studied in future clinical research.

Due to the small number of clinical trials retrieved[22,23,25,26,27], we were unable to conduct a meta-analysis of adverse events. Inspiringly, no studies reported life-threatening adverse events. The results from these trials showed that patients with bone non-union treated with MSCs had a shorter treatment time, a higher healing rate, and a significantly lower number of complications (such as the incidence of infection, neuropathy, skin necrosis, amputation, and Charcot joint disease) than those not treated with MSCs ($p < 0.05$). In contrast, Chu et al[26] reported significant pain and treatment-related complications during fracture healing. One single-arm study[34] ($n = 8$) and 3 case reports[35,36,37] ($n = 8$), including 16 patients with bone non-union, also reported the efficacy of MSCs in treating bone fractures. The researchers found that all 16 fracture patients treated with MSCs healed successfully without complications such as excessive tissue growth, tumour formation, wound infection, etc. Currently, another phase III, multicentre, open-label, RCT (NCT03325504) is currently underway in France to compare two doses (100×10^6 cells versus 200×10^6 cells) of bone marrow autologous MSCs and biomaterials with an iliac crest autologous graft for bone healing in non-union after long bone fractures. The results are expected on December 30, 2021.

In addition to MSCs, MSC-CM and other cell-based therapies in bone regeneration are also under investigation. Maria[38] and Augustine[2] concluded that the application of MSC-CM to treat bone defects had a favorable effect on the repair and regeneration of bone tissue. This finding suggests that paracrine effects are of great importance in MSC treatment. In addition to MSCs, MSC-like cells cultured from induced pluripotent stromal cells (iPSCs) have the ability to form mature mineralized substances histologically similar to bone[39]. Even human induced pluripotent stromal cells (hiPSCs) potentially differentiate into functional osteoblasts and subsequently form calcified bone stroma in the healing of critical bone defects without tumorigenesis[40]. Chen et al[41] observed that local transplantation of a new calcium phosphate cement, chitosan-RGD biomaterial derived from human embryonic stromal cells (hESCs), could induce attachment, proliferation and bone mineral synthesis in vitro. Kim et al[42] also discovered a significant improvement in bone formation in immunodeficient mice by subcutaneous inoculation of osteoblasts differentiated from human embryonic stromal cells seeded onto three-dimensional porous poly (d, l-lactic-co-glycolic acid)/hydroxyapatite composite biomaterials. However, the specific mechanisms by which cell therapy induces bone fracture healing are still unknown, and future research should focus on the mechanistic study of cell-based therapy.

Though many cell types are utilized in treating bone non-union, MSCs have great advantages over other types of cells. MSCs are clonogenic and have the potential to differentiate into various tissues, such as myocytes, chondrocytes, adipocytes and osteoblasts[8]. MSCs are also easily expanded in culture, immunologically inert and can be isolated from various sources, such as adipose tissue, bone marrow, tendons, cord blood, foetuses[10]. Hence, this flexibility in the MSC collection site avoids the ethical issues associated with the use of embryonic stromal cells. UC-MSCs have been demonstrated to be a promising alternative source because these MSCs are more readily available[43], proliferate faster in vitro and are less immunogenic than other MSCs[44]. Moreover, adult-type MSCs seem to have stronger immunosuppressive effects than foetal-type MSCs[45]. Over the past 10 years, the clinical use of MSCs derived from umbilical cord-derived MSCs and adipose tissues has increased by more than 30%[46]. The repair of bone fracture is a postnatal regenerative process, and fracture healing involves an anabolic phase of increased TV and the formation of new bone tissue, followed by an extended catabolic phase in which the tissue is remodelled into its original structure[47]. Fracture healing and endochondral bone formation are regulated by fibroblast growth factor 2 (FGF-2)[48], BMPs[49], PTH-related protein[50], transforming growth factor β (TGF-β)[51], parathyroid hormone (PTH)[50], wingless morphogenetic factors, and Wnt protein[52], some of which can be released by MSCs and participate in the interactive feedback loops of bone morphogenesis. Future studies involving MSCs in clinical trials are urgently needed to ensure MSC-related safety. Although various types of these cells are reported to promote bone regeneration, it is necessary to select the single most suitable cell type for fracture healing[5].

This meta-analysis has several advantages. First, this is the first study to analyze clinical and preclinical trials of MSCs for bone regeneration. Second, a systematic literature search was conducted, a published research protocol was followed to ensure a rigorous review process, and the quality of the included literature was evaluated according to different literature characteristics. Third, we analyzed the potential sources of heterogeneity and conducted an analysis, and the results enabled us to remind researchers of which variables they should pay attention to in prospective studies.

However, some weaknesses still exist. First, the enrolled animal studies had a small sample size, and thus the conclusions should be treated with caution. Second, the number of included clinical trials was limited, and some articles had a

small sample size, few bone indicators and low methodological quality. Furthermore, some adverse events (infection, skin necrosis and coverage) were unable to be analyzed in this article but were reported by some studies. Finally, different sources of cells, delivery methods, and doses were used in the preclinical and clinical trials, which potentially impacted the pooled outcomes despite being analyzed by subgroup analysis or meta-regression. In summary, more clinical bone indicators and widely acceptable indicators in experimental studies should allow a strict assessment of the efficacy of MSC therapy in bone healing. In addition, more expanded RCTs are warranted to evaluate the efficacy and safety of MSC therapy in the future.

Conclusion

MSCs are a promising therapy for patients with bone nonunion, but more RCTs are needed to support this finding.

Authors' Contributions

XQ M presented the idea and designed the whole outline of this article. YW and HX Y were responsible for all data extraction and analysis. The final version was revised by HX Y and QY L. YW, HX Y, XQ M, and QY L were all responsible for the final submission. All authors approved this submission and this statement.

Hanxiao Yi, Yang Wang, and Qunying Liang contributed equally to this work.

Ethic Approval

This search is a review paper and does not entangle any ethic problems. All authors approved this submission and this statement.

Statement of Human and Animal Right

This review does not contain any studies with human and animals. All authors approved this submission and this statement.

Statement of Informed Consent

There are no human subjects in this article and informed consent is not applicable. All authors approved this submission and this statement.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Nurturing funds for nursing young talents of Sun Yat-sen University (No. N2020Y06). All authors approved this submission and this statement.

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Supplemental Material

Supplemental material for this article is available online.

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