Administration of mesenchymal stem cells in diabetic kidney disease: a systematic review and meta-analysis

Wenshan Lin¹†, Hong-Yan Li²†, Qian Yang¹, Guanyong Chen¹, Shujun Lin¹, Chunling Liao¹ and Tianbiao Zhou¹*

Abstract

Background: Mesenchymal stem cell (MSC) therapy shows great promise for diabetic kidney disease (DKD) patients. Research has been carried out on this topic in recent years. The main goals of this paper are to evaluate the therapeutic effects of MSCs on DKD through a meta-analysis and address the mechanism through a systematic review of the literature.

Method: An electronic search of the Embase, Cochrane Library, ISI Web of Science, PubMed, and US National Library of Medicine (NLM) databases was performed for all articles about MSC therapy for DKD, without species limitations, up to January 2020. Data were pooled for analysis with Stata SE 12.

Result: The MSC-treated group showed a large and statistically significant hypoglycemic effect at 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, and 6 months. Total hypoglycemic effect was observed (SMD = −1.954, 95%CI = −2.389 to −1.519, p < 0.001; I² = 85.1%). The overall effects on serum creatinine (SCr) and blood urea nitrogen (BUN) were analyzed, suggesting that MSC decreased SCr and BUN and mitigated the impairment of renal function (SCr: SMD = −4.838, 95%CI = −6.789 to −2.887, p < 0.001; I² = 90.8%; BUN: SMD = −4.912, 95%CI = −6.402 to −3.422, p < 0.001; I² = 89.3%). Furthermore, MSC therapy decreased the excretion of urinary albumin. Fibrosis indicators were assessed, and the results showed that transforming growth factor-β, collagen I, fibronectin, and α-smooth muscle actin were significantly decreased in the MSC-treated group compared to the control group.

Conclusion: MSCs might improve glycemic control and reduce SCr, BUN, and urinary protein. MSCs can also alleviate renal fibrosis. MSC therapy might be a potential treatment for DKD.

Keywords: Mesenchymal stem cell, Diabetic kidney disease, Animal study, Clinical trial, Meta-analysis, Systematic review
Introduction
Diabetes mellitus (DM) is a chronic metabolic disease with a rising incidence rate, and its microvascular and macrovascular complications are associated with a large global burden of morbidity and mortality [1]. Diabetic kidney disease (DKD) is a serious kidney-related complication that is present in approximately 40% of patients with DM [2], and patients with DKD have an increased risk of cardiovascular events and all-cause mortality [3]. Abnormal blood glucose status leads to oxidative stress and induces the release of inflammatory mediators, resulting in glomerular lesions in DM patients. The current evidence indicates that it is very difficult to prevent the progression of DKD. The creatinine clearance rate (CCr), serum creatinine (SCr), blood urea nitrogen (BUN), microalbuminuria, urinary albumin excretion, etc. are important indicators to assess the renal damage associated with DKD.

Mesenchymal stem cells (MSCs) are being used systemically or locally to treat many diseases, as they exhibit great self-renewal and differentiation potential [4, 5]. Stem cells are self-renewing, self-replicating pluripotent cells and can be classified according to their origin: embryonic stem cells, adult stem cells, and induced pluripotent stem cells. Among them, adult stem cells, the undifferentiated cells in differentiated tissues, can be isolated from the bone marrow, adipose tissue, umbilical cord blood, and deciduous teeth. MSCs have been used for tissue regeneration and repair [6], treatment of inflammatory disease [7], prevention of transplant rejection [8], and other clinical applications.

At present, there are some data indicating that MSCs might improve complications from DM [9–11]. We conducted this systematic review and meta-analysis to evaluate the effects of MSC therapy on DKD.

Search strategy
We searched the Embase, Cochrane Library, ISI Web of Science, PubMed, and US National Library of Medicine (NLM) databases through January 2020 for original papers that assessed the effects of MSC administration on DKD animal models or patients without language restrictions. Keywords in this research included the following: (mesenchymal stem cells OR MSC OR multipotent stromal cells OR mesenchymal stromal cells OR mesenchymal progenitor cells OR Wharton jelly cells OR adipose-derived mesenchymal stem cells OR bone marrow stromal stem cells) AND (diabetic nephropathy OR DN OR diabetic kidney disease OR DKD).

Randomized controlled trials, comparative studies, or controlled trials that assessed the efficacy or safety of MSC therapy for treatment as an intervention in DKD animal models (without species limitations) or patients with DKD were included. The included studies were required to contain biochemical data on renal function or adverse events and to report albuminuria and impaired renal function in patients or animals with DM. The precise distinction between DKD and diabetic nephropathy (DN) was outside the scope of this paper, and both were included. Reviews, case reports, meta-analyses, comments, and letters were excluded. Articles that studied embryonic stem cells, induced pluripotent stem cells, or MSC components (rather than actual MSCs) for the treatment of DKD were excluded. In addition, studies that lacked a control arm or did not provide essential data such as renal function and sample size were excluded. We also searched for additional relevant reports by browsing the references of the articles.

Data extraction
The main features of the included studies were summarized, and the data were extracted independently by two authors using a standardized datasheet. Adverse events and biochemical indicator data were extracted from the articles, such as blood glucose, CCr, SCr, BUN, U-albumin/U-creatinine ratio (U-ACR), microalbuminuria, urinary albumin excretion, urine protein/Cr, kidney weight, body weight, and kidney weight/body weight ratio. If a paper contained no specific information, data were obtained by measuring the chart in the paper or by contacting the primary authors. Any disagreements in the extracted data were resolved by the third author.

Validity and quality assessment
For clinical trials, quality assessment was performed using 4 items based on the Jadad scale [12]: randomization, concealment of allocation, blinding method, and description of withdrawals and dropouts. A total score of ≥3 was considered high quality.

For animal studies, the methodological quality assessment was carried out using a risk of bias (RoB) tool by the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE), which is based on the Cochrane RoB tool and adjusted for animal experiments. The following ten items were assessed. (1) Sequence generation: Were the subjects randomly assigned to the case or control groups with an adequately generated allocation sequence? (2) Baseline characteristics: Were the baseline characteristics of the two groups comparable? (3) Allocation concealment: Was the allocation of all the subjects adequately concealed? (4) Random housing: Were all the subjects randomly housed in the same environment during the experiment? (5) Researcher blinding: Were the researchers blinded to which subjects had received treatment (in this case, MSC treatment)? (6) Random outcome assessment: Were the animals selected in random order for outcome assessment? (7) Blinding of outcome assessors: Were the outcome assessors
blinded to the group information? (8) Incomplete outcome data: Were incomplete outcome data or dropouts adequately addressed? (9) Selective outcome reporting: Was the study free of selective outcome reporting for significant results? (10) Other sources of bias: Was the study apparently free of other problems that could result in a high risk of bias, such as contamination of MSCs, inappropriate influence of the funder, errors in units of analysis, design-specific risk of bias, and additional animals to replace dropouts? An answer of “yes” means a low risk of bias, while “no” means a high risk of bias, and “unclear” means the risk of bias cannot be assessed for the lack of sufficient information. Disagreements were resolved by consensus-oriented discussion.

**Statistical analysis**

Stata SE 12 was used for statistical analysis. For continuous variables, standard mean differences (SMDs) were obtained by pooling the mean values, standard deviations, and sample sizes. For binary data, the odds ratio (OR) was calculated. Moreover, 95% confidence intervals (95%CIs) were calculated between the MSC-treated groups and the control groups. If there were multiple MSC-treated groups in an article, the data in the control group were reused. Heterogeneity across studies was quantified using $I^2$ and was considered significant at a $p$ value of $< 0.1$. The data were pooled using a fixed-effect model without heterogeneity or a random-effect model. A $p$ value of $< 0.05$ was regarded as statistically significant for all analyses. Potential publication bias was assessed with Begg’s test, Egger’s test, and the trim-and-fill method.

**Results**

**Search results**

In total, 33 studies in 29 publications were included, among which 28 publications were based on animal studies [13–40] and 1 was based on a clinical trial [41]. In addition, there are 4 ongoing clinical trials registered with the NLM.

Among 32 animal studies, 24 studies used rat models, 7 used mouse models, and 1 used a rhesus macaque model. A single method or a combination of multiple methods was used to induce DM, including streptozotocin (STZ) injection, high-fat diet dietary induction, nephrectomy, and natural development of models. However, the dosage and frequency of STZ injection and the time when the animals were tested for the establishment of DN were different. Although MSCs were used in all the included studies, the details of the source, dosage, frequency, administration, and point in time varied. The sources of MSCs were bone marrow mesenchymal stem cells (BM-MSCs) in 22 studies, adipose-derived stem cells (ADSCs) in 4 studies, human umbilical cord blood-derived mesenchymal stem cells (hUCB-MSCs) in 5 studies, and stem cells from exfoliated deciduous teeth in 1 study. Allogeneic administration was used in 23 studies, xenoplastic administration was used in 8 studies, and autologous administration was used in 1 study. The characteristics of the included animal studies are summarized in Table 1.

The only clinical trial was a multicenter, randomized, double-blind, dose-escalating, sequential, placebo-controlled study, finished in 2016. Thirty patients were randomized to receive one of two doses of mesenchymal precursor cells or placebo, and the efficacy and adverse events were observed. The main features of the clinical trial are shown in Table 2.

None of the animal experiments reported the occurrence of graft rejection after administration, but 2 MSC-treated human patients developed antibodies specific to the donor HLA in the clinical trial, one of these cases occurred transiently, whereas the other presented at baseline and persisted throughout the observation period without the appearance of adverse events. Strangely, however, antibodies specific to the donor HLA were also found in one placebo-treated patient. Six animal experiments specified the deaths or dropouts. Lang and Dai [27] reported the deaths of 6 model rats during the construction of the diabetes model (21.4%, 6/28), and Wang et al. [21] reported 1 death each in the MSC-treated group (8.3%, 1/14) and the DN group (10%, 1/10) as well as 2 deaths because of anesthesia. In the study of Li et al. [32], no rat died in the DN group (0.0%, 0/14), and 2 died in the MSC-treated group (18.2%, 2/11). During a 12-week observation, the MSC-treated group (25%, 3/12) had lower mortality than the DN-treated group (66.7%, 8/12) [33]. Similarly, Xian et al. [34] found 2 deaths in the hUCB-MSC group (16.7%, 2/12), making for a markedly lower mortality rate than the T1DM group (40%, 6/15) at the end of the study. An et al. [39] found no marked change in the immune system of rhesus macaque DN models in response to hUCB-MSC treatment.

**Quality assessment**

Quality assessments of animal experiments and clinical trials were performed (Tables 3 and 4). Table 3 shows a number of “unclear” judgments in the quality assessment of animal experiments; in particular, outcome assessment in a random order, concealment of allocation and blinding of outcome assessors in all included experiments were rated “unclear,” largely due to a lack of awareness of randomization and blinding methods in animal experiments. As shown in Table 4, a total score of 7 suggested the high methodological quality of the included clinical trial.
Table 1 Main features of included studies (animal studies)

| PMID | Year | Author     | Country | Sample size | Model features | Comparison | Stem cell species | Intervention | Observed indicators                                                                 | Duration |
|------|------|------------|---------|-------------|----------------|------------|-------------------|--------------|--------------------------------------------------------------------------------------|----------|
| 1    | 2008 | Ezquer et al. | Chile   | 16          | STZ was injected intraperitoneally at a dose of 40 mg/kg, for 5 consecutive days. | STZ + MSCs vs STZ + vehicle | C57BL/6 mouse bone marrow (allogeneic transplantation) | Twenty-five days after the first STZ dose, mice received 0.5 × 10⁶ MSCs or the vehicle via the tail vein. | Blood glucose; urinary glucose; intraperitoneal glucose tolerance test; urine albumin/creatinine ratio; pancreas and kidney histopathology | 62 days  |
| 2    | 2009 | Ezquer et al. | Chile   | 16          | C57BL/6 mice received intraperitoneally 200 mg/kg STZ. | STZ + MSCs vs STZ + vehicle | C57BL/6 mouse bone marrow (allogeneic transplantation) | Thirty and 51 days after STZ injection, animals received via the tail vein the vehicle (untreated) or 0.5 × 10⁶ MSC (MSC treated). | Blood glucose; urinary glucose; insulinemiasa serum creatinine; urine albumin/creatinine ratio; pancreas and kidney histopathology; kidney weight/body mass ratio | 90 days  |
| 3    | 2009 | Zhou et al.  | China   | 32          | The male rats received a single intraperitoneal injection of STZ (60 mg/kg). | STZ + MSCs vs STZ + vehicle | SD rat bone marrow (allogeneic transplantation) | 2 × 10⁶ labeled MSCs per animal in 0.2 mL SFM were given via the left cardiac ventricle. Control diabetic animals were treated identically but infused with 0.2 mL SFM instead of cells. | Blood glucose; kidney weight/body mass ratio; urine albumin/creatinine ratio; blood pressure; creatinine clearance rate; kidney histopathology | 2 months |
| 4    | 2009 | Zhou et al.  | China   | 24          | A single intraperitoneal injection of STZ (60 mg/kg) was given to SD rats. | STZ + MSCs vs STZ + vehicle | SD rat bone marrow (allogeneic transplantation) | 2 × 10⁶/200 μL MSCs were given via the left cardiac ventricle. A week later, the second intracardiac injection of the MSCs was performed. Control diabetic animals received 200 μL serum-free DMEM-LG. | Blood glucose; body mass; urine protein; kidney/body mass ratio; creatinine clearance rate; kidney histopathology | 2 months |
| 5    | 2012 | Fang et al.  | China   | 24          | Rats were injected intraperitoneally with 40 mg/kg body weight of STZ for 5 consecutive days. | STZ + MSCs vs STZ + vehicle | SD rat adipose tissue (autologous transplantation) | Intravenous infusion of autologous ADMSCs (1.0 × 10⁷) was performed 4 weeks after the onset diabetes via the tail vein. Animals in the vehicle group received an equal volume of culture medium at the same time. | Blood glucose; insulinemia; cholesterol; triglycerides; BUN; creatinine; malondialdehyde; TNF-α; IL-1β; IL-6; renal morphology | 12 weeks |
| 6    | 2012 | Park et al.  | Korea   | 14          | Experimental diabetes was induced by intravenous injection of STZ (50 mg/kg). | STZ + MSCs vs STZ + vehicle | Human umbilical cord blood (xenotransplantation) | hUCB-SC (1 × 10⁶ cells/rat) were infused through the tail vein 4 weeks after the STZ injection. Both diabetic and diabetic rats treated with hUCB-SC were injected subcutaneously with insulin (2 U/day/rat) to maintain blood glucose levels of 350 to 500 mg/dL. | Blood glucose; body mass; kidney weight; creatinine; urinary protein; fibronectin; a-SMA; E-cadherin | 1 month  |
| 7    | 2012 | Park et al.  | Korea   | 14          | Experimental diabetes was induced by injecting 50 mg/kg STZ through the tail vein. | STZ + MSCs vs STZ + vehicle | Human umbilical cord blood (xenotransplantation) | hUCB-MSC were infused at a dose of 5 × 10⁶ cells/rat through the tail vein 2 days after the STZ injection when blood glucose was > 350 mg/dL. | Blood glucose; body mass; kidney weight; creatinine; urinary protein; kidney histopathology; BMP-7; TGF-β1; fibronectin; a-SMA; E-cadherin | 1 month  |
| PMID | Year | Author | Country | Sample size | Model features | Comparison | Stem cell species | Intervention | Observed indicators | Duration |
|------|------|--------|---------|-------------|----------------|------------|-------------------|-------------|-------------------|----------|
| 8 23, 295, 166 | 2013 | Wang et al. | China | 17 | Diabetes was induced by a single intraperitoneal injection of streptozotocin (STZ) 65 mg/kg in the rats following overnight fasting. | STZ + MSCs vs STZ + vehicle | SD rat bone marrow (allogeneic transplantation) | MSC-treated DN rats were injected with 2 × 10^6 MSC via the left renal artery. All the diabetic rats received daily injections of insulin to maintain blood glucose levels between 16 and 28 mmol/L. | Blood glucose; body mass; kidney weight; kidney/body mass ratio; creatinine clearance rate; urine albumin/creatinine ratio; creatinine; renal morphology; nephrin; podocin; VEGF; BMP-7 | 2 months |
| 9 23, 762, 850 | 2013 | Zhang et al. | China | 20 | SD rats were anesthetized and received a single intraperitoneal injection of 60 mg/kg STZ. | STZ + MSCs vs STZ + vehicle | SD rat bone marrow (allogeneic transplantation) | MSCs (1 × 10^6) were resuspended in 2 mL of PBS and administrated to anesthetized rats through tail vein. | Blood glucose; insulinemia; insulin; kidney and pancreas histopathology; VCAM-1; TGF-β; IL-10; synaptopodin | 8 weeks |
| 10 23, 791, 972 | 2013 | Lv et al. | China | 32 | Diabetes was induced in the female Wistar rats by a single intraperitoneal injection of STZ (60 mg/kg) after one-night fasting. | STZ + MSCs vs STZ + vehicle | Wistar rat bone marrow (allogeneic transplantation) | MSCs were transplanted via the tail vein at a concentration of 2 × 10^6 in 0.5 mL serum-free media once a week for 2 continuous weeks. | Blood glucose; urinary albumin excretion; creatinine clearance rate; kidney/body mass ratio; kidney histopathology; ED-1; MCP-1; collagen I; fibronectin; IL-1β; IL-6; TNFα; HGF | 8 weeks |
| 11 24, 513, 119 | 2013 | Lv et al. | China | 24 | Following one night of fasting, a single injection of STZ (60 mg/kg) was given intraperitoneally to induce diabetes. | STZ + MSCs vs STZ + vehicle | Wistar rat bone marrow (allogeneic transplantation) | MSCs were transplanted via the tail vein at a concentration of 2 × 10^6 in 0.5 mL serum-free media once a week for 2 continuous weeks. | Blood glucose; urinary albumin excretion; creatinine clearance rate; kidney/body mass ratio; renal histopathology; collagen I; FN; TGF-β; MDA; SOD; ROS | 8 weeks |
| 12 24, 606, 996 | 2014 | Abdel Azz et al. | Egypt | 40 | Diabetes of female albino rats was induced by a single intraperitoneal injection of STZ (60 mg/kg) body weight. | STZ + MSCs vs STZ + vehicle | White albino rat bone marrow (allogeneic transplantation) | DN rats received MSCs in a single dose of 10^6 cells per rat by intravenous injection in the rat tail vein. | Blood glucose; BUN; creatinine; urinary albumin excretion; body weight; renal histopathology; TGF β; TNFα; bcl2; Bax; VEGF | 4 weeks |
| 13 24, 845, 071 | 2015 | Lv et al. | China | 24 | A single injection of STZ (60 mg/kg) was given via intraperitoneal injection to induce diabetes following one-night fasting. | STZ + MSCs vs STZ + vehicle | Wistar rat bone marrow (allogeneic transplantation) | MSCs were transplanted via the tail vein at a concentration of 2 × 10^6 in 0.5 mL serum-free media once a week for 2 continuous weeks. | Blood glucose; urinary albumin excretion; creatinine clearance rate; kidney/body mass ratio; renal histopathology; TGF-β; collagen I; a-SMA; E-cadherin; BMP-7; Smad2; Smad3 | 8 weeks |
| 14 27, 018, 336 | 2016 | Lang et al. | China | 20 | After fasting 12 h, SD rats were given STZ 55 mg/kg by i.p. injection. | STZ + MSCs vs STZ + vehicle | SD rat bone marrow (allogeneic transplantation) | For rats in the MSC group, intravenous infusion of autologous MSCs (2 × 10^6/mL) was performed 4 weeks after the onset of diabetes via the tail vein. | Blood glucose; body weight; urinary protein; creatinine; renal mass index; kidney histopathology; MMP-9; PAI-1; TGF-β1; Smad3 | 12 weeks |
| 15 27, 721, 418 | 2016 | Nagaishi et al. | Japan | 12 | Diabetes was induced via an HFD containing 60% lard (high-fat diet 32) for 28 weeks. | HFD + MSCs vs HFD + vehicle | C57BL/6-GFP transgenic mouse bone marrow (allogeneic transplantation) | C57BL/6J mice were administrated 1.0 × 10^6 MSCs/g body weight 4 times (HFD-MSC) every 2 weeks. | Blood glucose; urinary albumin/creatinine ratio; kidney histopathology; ICAM-1; TNFα; megalin; TGF-β; ZO-1 | 8 weeks |
| PMID | Year | Author | Country | Sample size | Model features | Comparison | Stem cell species | Intervention | Observed indicators | Duration |
|------|------|--------|---------|-------------|---------------|------------|-----------------|-------------|---------------------|----------|
| 16   | 27, 721, 418 | 2016 Nagaishi et al. | Japan | 12 | Diabetes was induced by a single intraperitoneal injection of STZ (150 mg/kg). | STZ + MSCs vs STZ + vehicle | C57BL/6GFP-transgenic mouse bone marrow (allogeneic transplantation) | C57BL/6J mice were administered 1.0 × 10^7 MSCs/g body weight 2 times (STZ-MSC) every 4 weeks. | Blood glucose; urine albumin/creatinine ratio; kidney histopathology; ICAM-1; TNF-α; megalin; TGF-β; ZO-1 | 8 weeks |
| 17   | 27, 774, 826 | 2016 Hamza et al. | Egypt | 20 | Albino Wistar rats were given a single intraperitoneal injection of a mixture of 70 mg/kg STZ. | STZ + MSCs vs STZ | Albino rat bone marrow (allogeneic transplantation) | Rats were given a single-dose intravenous treatment of 10 × 10^6 cells per subject. | Blood glucose; insulinemia; BUN; creatinine; uric acid; serum total protein; serum albumin; urinary urea, urinary creatinine; urinary albumin; kidney histopathology; HO-1; AGEP; FGF; PDGF; TGF-β; IL-8; MCP-1 | 3 weeks |
| 18   | 28, 814, 814 | 2016 Nagaishi et al. | Japan | 8 | C57BL/6 mice were given a single intraperitoneal administration high dose (150 mg/kg) of STZ. | STZ + MSCs vs STZ + vehicle | STZ rat bone marrow (xenoplastic transplantation) | Mice were administered 4 times with 1.0 × 10^6 MSCs/g body weight via the tail vein every 2 weeks. | Blood glucose; urine albumin/creatinine ratio; kidney histopathology | 8 weeks |
| 19   | 28, 814, 814 | 2016 Nagaishi et al. | Japan | 10 | SD rats were given a single tail vein injection of 55 mg/kg of STZ. | STZ + MSCs vs STZ + vehicle | STZ rat bone marrow (allogeneic transplantation) | Rats were administered 1 × 10^6 MSCs/g body weight via the tail vein. | Blood glucose; urine albumin/creatinine ratio; kidney histopathology | 8 weeks |
| 20   | 28, 814, 814 | 2016 Nagaishi et al. | Japan | 16 | OLETF rats developed diabetes and DN with natural course. | OLETF + MSCs vs OLETF + vehicle | LETF rat bone marrow (allogeneic transplantation) | Rats were administered 1 × 10^6 MSCs/g body weight via the tail vein. | Blood glucose; urine albumin/creatinine ratio; kidney histopathology | 8 weeks |
| 21   | 29, 425, 466 | 2018 Rashed et al. | Egypt | 20 | Diabetes was induced by a single intraperitoneal injection of STZ (50 mg/kg). | STZ + vehicle vs STZ + MSCs | Wistar strain Albino rat bone marrow (allogeneic transplantation) | DN rats treated with a single injection of 1 × 10^6 labeled MSCs per animal in 0.5 mL serum-free medium into the tail vein. | Blood glucose; insulinemia; BUN; creatinine; creatinine clearance rate; urinary albumin excretion; kidney histopathology; TNF-α; IL-10; SOD; TGF-β; Bedlin-1 | 2 weeks |
| 22   | 29, 484, 379 | 2018 Li et al. | China | 25 | Diabetes was induced by a single intraperitoneal injection of 55 mg/kg STZ. | STZ + MSCs vs STZ + vehicle | SD rat bone marrow (allogeneic transplantation) | 2, 4, 5, and 7 weeks after successful establishment of the diabetes model, MSCs were transplanted via the tail vein at a concentration of 5 × 10^6 cells. | Microalbumin; urine albumin/creatinine ratio; BUN; kidney histopathology; MCP-1; IL-18; TNF-α; ICAM-1; CDD8; TGF-β; fibronectin; IL-1α; IL-2; IL-6; EGF; IL-10; TNF-α; IFN-γ; GRO; VEGF | 8 weeks |
| 23   | 31, 023, 998 | 2019 Bai et al. | China | 24 | Diabetes was induced by a single intraperitoneal injection of 60 mg/kg STZ after 1-night fasting. | STZ + MSCs vs STZ + vehicle | SD rat bone marrow (allogeneic transplantation) | MSCs were transplanted via the tail vein at a concentration of 5 × 10^6 in 0.5 mL PBS once a week for 2 continuous weeks. | Blood glucose; creatinine; BUN; microalbumin; albumin/creatinine ratio; kidney histopathology; TGF-β; Smad2/3; phosphorylated Smad3; Smad3; IFN-γ; IL-6; TNF-α | 12 weeks |
| 24   | 31, 150 | 2019 Xian et al. | China | 19 | Healthy female NOD mice were purchased. When two NOD-T1DM + MSCs vs NOD- | Human umbilical cord | 1 × 10^6 hUCMSCs suspended in 0.3 mL of phosphate-buffered | Blood glucose; weight; creatinine; BUN; urinary albumin excretion; | 8 weeks |
| PMID | Year | Author | Country | Sample size | Model features | Comparison | Stem cell species | Intervention | Observed indicators | Duration |
|------|------|--------|---------|-------------|---------------|------------|------------------|-------------|---------------------|----------|
| 720  |      |        |         |             | consecutive tests showed a blood glucose level > 16.6 mmol/L, the mouse was diagnosed with T1DM. | T1DM       | derived MSCs (xenoplastic transplantation) | saline (PBS) were injected into the tail vein on the 3rd day after diabetes onset (only once). | MCP-1; RAGE; nephrin; WT1; NF-xB | 12 weeks |
| 25   | 2019 | Cai et al. | China  | 20          | Wistar rats were provided with a prepared HFD for 6 weeks and then injected with 60 mg/kg STZ for 2 weeks. | HFD + STZ + MSCs vs HFD + STZ + vehicle | Rat bone marrow stromal cells (allogeneic transplantation) | Rats treated with 2 × 10^6 labeled MSCs per animal via the tail vein. | Blood glucose; creatinine; BUN; alanine aminotransferase; 24 h urine volume; urine protein; urinary albumin excretion; renal histopathology; p-cadherin; synaptopodin; FSP-1; α-SMA; snail; fibronectin; collagen I | 12 weeks |
| 26   | 2019 | Lee et al. | Korea  | 14          | CD1 mice were intraperitoneally injected with STZ 80 mg/kg for 3 days. | STZ + MSCs vs STZ + vehicle | Human umbilical cord-derived MSCs (xenoplastic transplantation) | Five weeks after the induction of diabetes, human umbilical cord blood-derived MSCs were administered to mice three times. | Blood glucose; creatinine; BUN; urine albumin/creatinine ratio | 19 weeks |
| 27   | 2019 | Takemura et al. | Japan  | 10          | SDF fatty rats formed a spontaneously obese type 2 diabetes model and were subjected to right nephrectomy. | Nephrectomy + MSCs vs nephrectomy | EGFP rat subcutaneous adipose tissue (allogeneic transplantation) | 1 mL of the adipose-derived mesenchymal stem cell suspension (6.0 × 10^6 cells/mL) was administered via the femoral vein. | Albuminuria; proteinuria; urinary creatinine; podocalyxin; L-FABP; KIM-1; TNF-α; IL-6; renal histopathology | 2 weeks |
| 28   | 2019 | Takemura et al. | Japan  | 11          | SDF fatty rats formed a spontaneously obese type 2 diabetes model and were subjected to right nephrectomy. | Nephrectomy + MSCs vs nephrectomy | EGFP rat subcutaneous adipose tissue (allogeneic transplantation) | Adipose-derived mesenchymal stem cell sheets were laminated in three layers under the renal capsule using a cell sheet transfer device. | Albuminuria; proteinuria; urinary creatinine; podocalyxin; L-FABP; KIM-1; TNF-α; IL-6; renal histopathology | 2 weeks |
| 29   | 2019 | Yu et al. | China  | 12          | SD rats were fed a HFD for 8 weeks, followed by an STZ injection at a single dose of 25 mg/kg. HFD feeding was maintained in the newly diabetic rats for 24 weeks. | HFD + STZ + MSCs vs HFD + STZ + vehicle | SD rat adipose tissue (allogeneic transplantation) | Rats were treated through the tail vein with a single infusion of 3 × 10^6 ADSCs once a week for 24 weeks. | Blood glucose; creatinine; BUN; urine albumin/creatinine ratio; ALT; AST; ALP; LDL-C; TC; TG; renal histopathology; insulin; glucagon; collagen I; α-SMA; CD163; albumin; SP-C; CD206; P38K; p-AKT; IL-1β; IL-6; IL-10; TNF-α | 25 weeks |
| 30   | 2019 | An et al. | China  | 12          | Rhesus macaques were administered a single high dose of STZ (80 mg/kg) intravenously. Insulin was used to maintain the FBG level at 15–20 mmol/L. | STZ + MSCs vs STZ + vehicle | Human umbilical cord-derived MSCs (xenoplastic transplantation) | MSCs from a single donor were suspended in 100 mL normal saline and delivered at a density of 2 × 10^6 cells/kg to one DN rhesus macaque at an infusion rate of 45–50 drops/min. A total of four times of MSC transplantation were performed during 2 months. | Blood glucose; serum creatinine; BUN; uric acid; LDL-C; HDL-C; TC; TG; HbA1C; eGFR; microalbumin; urinary creatinine; urine albumin/creatinine ratio; body weight; renal histopathology; IL-1β; IL-16; IL-8; IL-6; IL-10;TNF-α; TGF-β; CCL-5; SGLT-2 | 1 year |
| 31   | 2019 | Rao et al. | China  | 18          | GK rats were given a HFD for 2–4 weeks. | HFD + MSCs vs HFD + vehicle | Human bone marrow MSCs from donors aged 16–20 | A total of 4 × 10^6 MSC cells were administered via the tail vein to each rat. | Blood glucose; body weight; serum cholesterol; serum triglycerides; urinary albumin; kidney/body mass ratio; renal | 8 weeks |
Table 1 Main features of included studies (animal studies) (Continued)

| PMID | Year | Author | Country | Sample size | Model features | Comparison | Stem cell species | Intervention | Observed indicators | Duration |
|------|------|--------|---------|-------------|----------------|------------|------------------|-------------|---------------------|----------|
| 32   | 31, 871, 464 | Rao et al. | China | 20 | GK rats were given HFD for 2–4 weeks. | HFD + MSCs vs HFD + vehicle | Human exfoliated deciduous tooth stem cells from donors aged 6–8 (xenoplastic transplantation) | A total of 4 x 10^6 MSC cells were administered via the tail vein to each rat. | Blood glucose; body weight; serum cholesterol; serum triglycerides; urinary albumin; kidney/body mass ratio; renal histopathology; α-SMA; Col I; fibronectin; lamininβ; nephrin; synaptopodin; IL-1β; IL-6; IL-10; TNF-α; TGF-β; HGF | 8 weeks |

SD Sprague-Dawley, SFM serum-free medium, HFD high-fat diet, BUN blood urea nitrogen, NOD non-obesity diabetes, OLETF Otsuka Long-Evans Tokushima Fatty, LETO Long-Evans Tokushima Otsuka, MDA malondialdehyde, SOD superoxide dismutase, ICAM-1 intracellular adhesion molecule-1, ZO-1 zona occludens protein-1, HO-1 heme-oxygenase-1, AGEP advanced glycation end product, FGF fibroblast growth factor, PDGF platelet-derived growth factor, EGF epidermal growth factor, RAGE advanced glycation end products, FSP-1 fibroblast-specific protein-1, L-FABP liver-type fatty acid-binding protein, KIM-1 kidney injury molecule-1, ALT alanine aminotransferase, AST aspartate aminotransferase, ALP alkaline phosphatase, LDL-C low-density lipoprotein cholesterol, TC total cholesterol, TG triglyceride, SP-C pro-surfactant protein C, eGFR estimated glomerular filtration rate, SGLT-2 Na + glucose cotransporter 2
Table 2 Main features of included studies (clinical trials)

| Author, year | Study design | Treatment strategies | Detailed scheme | Patient characteristics | Main outcome measures | Adverse events | Study duration |
|--------------|--------------|----------------------|-----------------|------------------------|----------------------|---------------|---------------|
| Packham et al., 2016 | Multicenter, randomized, double-blind, dose-escalating, sequential, placebo-controlled trial | Vehicle vs MPC | Two rexlemestrocel-L (allogeneic mesenchymal precursor cells) doses (150 x 10^6 or 300 x 10^6) or saline placebo were suspended in 100 mL normal saline and infused with filtration over 45 min. | The study population was male and female patients ≥45 and ≤85 years old with type 2 diabetes and advanced diabetic nephropathy (e.g., eGFR20–50 mL/min/1.73 m²), who were receiving a stable, standard of care therapeutic regimen of the maximum tolerated recommended dose of an angiotensin converting enzyme inhibitor (ACEI) or a angiotensin 2 receptor blocker (ARB) for at least 3 months prior to screening. | Adverse events; serum creatinine; creatinine clearance; albumin-creatinine ratio; protein-creatinine ratio; cystatin-C; HbA1c; triglycerides; systolic blood pressure; diastolic blood pressure; hs-CRP; IL-6; TNF-α | Edema peripheral; lower respiratory tract infection; urinary tract infection; cataract; anemia; fall; acute myocardial infection; anemia; asthma; cardiac failure congestive; cardiac failure constrictive; cardiac failure congestive; cardiac failure constrictive; cardiac failure congestive; cardiac failure constrictive; cardiac failure congestive; cardiac failure congestive; cardiac failure congestive; cardiac failure congestive | 60 weeks |

Assessment of glucose
Glucose was detected after MSC treatment in all but 2 studies [32, 37]. Sixteen studies measured glucose once at the end of the experiment [18–21, 23–29, 31, 33–36]. Seven studies conducted blood glucose monitoring at several points in time [14–17, 22, 30, 40]. Five studies, 7 studies, 5 studies, 12 studies, 17 studies, 7 studies, and 2 studies were included to assess the effect of treatment on blood glucose levels at 1 week, 2 weeks, 3 weeks, 1 month, 2 months, 3 months, and 6 months, respectively, all of which showed a highly significant hypoglycemic effect in the MSC-treated group (1 week: SMD = -1.484, 95%CI -2.586 to -0.381, p < 0.001; I² = 80.6%; 2 weeks: SMD = -2.312, 95%CI -3.743 to -0.882, p = 0.002, I² = 89.6%; 3 weeks: SMD = -4.007, 95%CI -6.472 to -1.541, p = 0.001, I² = 92.1%; 1 month: SMD = -1.740, 95%CI -2.660 to -0.821, p < 0.001, I² = 83.8%; 2 months: SMD = -1.830, 95%CI -2.633 to -1.028, p < 0.001; I² = 86.0%; 3 months: SMD = -1.649, 95%CI -2.838 to -0.461, p = 0.007; I² = 84.6%; 6 months: SMD = -3.045, 95%CI -5.895 to -0.195, p = 0.036; I² = 76.4%). The total hypoglycemic effect was also analyzed (SMD = -1.954, 95%CI -2.389 to -1.519, p < 0.001; I² = 85.1%) (Fig. 1).

Assessment of serum creatinine
There were 4 studies, 2 studies, and 5 studies that assessed Scr at 1 month, 3 months, and 3 months, respectively. All of them showed significantly reduced creatinine values in the MSC-treated group (1 month: SMD = -4.126, 95%CI -7.936 to -0.315, p = 0.034; I² = 94.9%; 2 months: SMD = -3.505, 95%CI -4.746 to -2.264, p < 0.001; I² = 1.8%; 3 months: SMD = -6.736, 95%CI -10.311 to -3.162, p < 0.001; I² = 89.0%). The total effect on Scr was also analyzed, suggesting that MSCs decreased Scr and improved renal function (SMD = -4.838, 95%CI -6.789 to -2.887, p < 0.001; I² = 90.8%) (Fig. 2).

Assessment of blood urea nitrogen
BUN was evaluated at 5 different time points, each of which was used by relatively few studies. At 2 weeks (2 studies included), 3 weeks (2 studies included), 1 month (2 studies included), and 3 months (4 studies included), BUN decreased in the MSC-treated group, although no statistical significance was seen at 3 weeks or 1 month (2 weeks: SMD = -2.514, 95%CI -3.582 to -1.447, p < 0.001; I² = 37.3%; 3 weeks: SMD = -4.432, 95%CI -9.220 to -0.356, p = 0.070; I² = 92.0%; 1 month: SMD = -10.392, 95%CI -21.247 to 0.464, p = 0.061; I² = 95.6%; 2 months: SMD = -3.389, 95%CI -6.679 to -0.099, p = 0.044; I² = 89.8%; 3 months: SMD = -5.902, 95%CI -8.988 to -2.815, p < 0.001; I² = 85.0%). The total effect on BUN was also analyzed, suggesting that MSCs decreased BUN (SMD = -4.912, 95%CI -6.402 to -3.422, p < 0.001; I² = 89.3%) (Fig. 3).

Assessment of creatinine clearance rate
The data of six studies were pooled to evaluate CCr at 2 months after MSC treatment; CCr was significantly decreased in the MSC-treated group compared to the DKD group (2 months: SMD = -1.881, 95%CI -2.842 to -0.921, p < 0.001; I² = 79.7%) (Fig. 4).

Assessment of blood insulin level
Two studies assessed insulinemia. The insulin level decreased at 3 months after MSC treatment, although the...
| Study    | PMID   | Sequence generation | Baseline characteristics | Allocation concealment | Random housing | Researchers blinding | Random outcome assessment | Outcome assessors blinding | Complete outcome data | Outcome reporting | Other source of bias |
|----------|--------|---------------------|--------------------------|------------------------|----------------|---------------------|---------------------------|--------------------------|---------------------|------------------|------------------|
| Ezquer, 2008 | 18, 489, 988 | Yes | Yes | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| Ezquer, 2008 | 19, 822, 294 | Yes | Yes | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| Zhou, 2009 | 19, 951, 572 | Unclear | Yes | Unclear | Yes | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| Zhou, 2009 | 20, 067, 112 | Yes | Unclear | Unclear | No | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| Fang, 2012 | 22, 552, 764 | Yes | Unclear | Unclear | Yes | No | Unclear | Unclear | Unclear | Yes | Yes |
| Park, 2012 | 22, 564, 642 | Yes | Unclear | Unclear | Unclear | No | Unclear | Unclear | Unclear | Yes | Yes |
| Park, 2012 | 23, 026, 513 | Yes | Unclear | Unclear | Yes | No | Unclear | Unclear | Unclear | Yes | Yes |
| Wang, 2013 | 23, 295, 166 | Yes | Yes | Unclear | Yes | Unclear | Unclear | Unclear | Yes | Yes | Yes |
| Zhang, 2013 | 23, 762, 850 | Yes | Yes | Unclear | Yes | No | Unclear | Unclear | Unclear | Yes | Yes |
| Lv, 2013 | 23, 791, 972 | Yes | Yes | Unclear | Yes | No | Unclear | Unclear | Unclear | Yes | Yes |
| Lv, 2013 | 24, 513, 119 | Unclear | Unclear | Unclear | Yes | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| Abdel Aziz, 2014 | 24, 606, 996 | Unclear | Yes | Unclear | Yes | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| Lv, 2015 | 24, 845, 071 | Yes | Yes | Unclear | Yes | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| Lang, 2015 | 27, 018, | Yes | Yes | Unclear | Unclear | Unclear | Unclear | Unclear | Yes | Yes | Yes |
| Study | PMID | Sequence generation | Baseline characteristics | Allocation concealment | Random housing | Researchers blinding | Random outcome assessment | Outcome assessors blinding | Complete outcome data | Outcome reporting | Other source of bias |
|-------|------|---------------------|--------------------------|------------------------|----------------|---------------------|--------------------------|--------------------------|----------------------|------------------|---------------------|
| 15 Nagaishi, 2016 | 27, 721, 418 | No | Yes | Unclear | Yes | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| 16 Nagaishi, 2016 | 27, 721, 418 | No | Yes | Unclear | Yes | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| 17 Hamza, 2016 | 27, 774, 826 | No | Unclear | Unclear | Unclear | No | Unclear | Unclear | Unclear | Yes | Yes |
| 18 Nagaishi, 2016 | 28, 814, 814 | Unclear | Yes | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| 19 Nagaishi, 2016 | 28, 814, 814 | Unclear | Yes | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| 20 Nagaishi, 2016 | 28, 814, 814 | Unclear | Yes | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| 21 Rashed, 2018 | 29, 425, 466 | Yes | Yes | Unclear | Yes | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| 22 Li, 2018 | 29, 484, 379 | Yes | Yes | Unclear | Yes | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| 23 Bai, 2019 | 31, 023, 998 | Yes | Yes | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| 24 Xian, 2019 | 31, 150, 720 | Yes | Yes | Unclear | Yes | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| 25 Cai, 2019 | 31, 190, 436 | Yes | Yes | Unclear | Yes | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| 26 Lee, 2019 | 31, 285, 429 | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | No | Yes |
| 27 Takemura, 2019 | 31, 622, 047 | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
| 28 Takemura, 2019 | 31, | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Unclear | Yes | Yes |
Table 3  Quality assessment of animal intervention studies by SYRCLE (Continued)

| Study   | PMID Generation       | Baseline characteristics | Allocation concealment | Random housing | Researchers blinding | Random outcome assessment | Outcome assessors blinding | Complete outcome data | Outcome reporting | Other source of bias |
|---------|-----------------------|--------------------------|------------------------|----------------|---------------------|--------------------------|--------------------------|----------------------|---------------------|---------------------|
| 2019    | 622, 047              | Yes                      | Yes                    | Yes            | Yes                 | Unclear                  | Unclear                  | Yes                  | Yes                  | Yes                  |
| 29 Yu, 2019 | 31, 747, 961       | Yes                      | Yes                    | Unclear        | Yes                 | Unclear                  | Unclear                  | Yes                  | Yes                  | Yes                  |
| 30 An, 2019 | 31, 791, 397         | Yes                      | Yes                    | Unclear        | Yes                 | Unclear                  | Unclear                  | Yes                  | Yes                  | Yes                  |
| 31 Rao, 2019 | 31, 871, 464        | Yes                      | Yes                    | Unclear        | Yes                 | Unclear                  | Unclear                  | Yes                  | Yes                  | Yes                  |
| 32 Rao, 2019 | 31, 871, 464        | Yes                      | Yes                    | Unclear        | Yes                 | Unclear                  | Unclear                  | Yes                  | Yes                  | Yes                  |
significance was not notable (3 months: SMD = 3.051, 95%CI −0.091 to 6.193, p = 0.057; \(I^2 = 90.3\%\)).

Assessment of urine protein

The measurement of urine protein varied in the included studies. Microalbuminuria, urinary albumin excretion, the urinary albumin/urinary creatinine ratio, and the urinary protein/creatinine ratio were used to assess urine protein excretion in the DKD animals. Urinary albumin excretion levels at 1 month (2 studies included) and at 2 months (7 studies included) were observed to be lower in the MSC-treated group than in the DKD group, although no significance at 1 month was observed (1 month: SMD = −6.507, 95%CI −17.935 to 4.921, p = 0.264; \(I^2 = 98.3\%\); 2 months: SMD = −4.386, 95%CI −5.891 to −2.881, p < 0.001; \(I^2 = 85.5\%\)). The total effect on urinary albumin excretion was also analyzed, suggesting that MSCs decreased urinary albumin

Table 4 Quality assessment of clinical trials by Jadad score

| Author, year | Type | Randomization | Concealment of allocation | Double blinding | Withdrawals and dropouts | Jadad score |
|--------------|------|---------------|---------------------------|-----------------|--------------------------|-------------|
| Packham et al., 2016 | Multicenter, randomized, double-blind, dose-escalating, sequential, placebo-controlled trial | An Interactive Voice Response System/Interactive Web Response System (IVRS/IWRS) was accessed to randomize eligible patients. All infusions were prepared by an unblinded pharmacist at the phase 1 unit who provided to the blinded clinical staff visually identical infusion products comprising reixelmestrocel-L or saline suspended in 100 mL normal saline. | Patients, investigators, and the sponsor were blinded to the treatment allocation through the entire 60-week study. | Yes | 7 |

Fig. 1 The effect of MSC treatment on glycemic control. 1w, 1 week; 2w, 2 weeks; 3w, 3 weeks; 1m, 1 month; 2m, 2 months; 3m, 3 months; 6m, 6 months
Fig. 2 The effect of MSC treatment on serum creatinine. 1m, 1 month; 2m, 2 months; 3m, 3 months

Fig. 3 The effect of MSC treatment on blood urea nitrogen (BUN). 2w, 2 weeks; 3w, 3 weeks; 1m, 1 month; 2m, 2 months; 3m, 3 months
excretion (SMD = −4.830, 95%CI −6.602 to −3.058, p < 0.001; \( I^2 = 92.5\% \)).

Microalbuminuria was detected at 3 weeks and 3 months; each of these time points was addressed by 2 studies that satisfied the inclusion criteria. Microalbuminuria was found to be decreased in the MSC-treated group at 3 months (3 weeks: SMD = −9.112, 95%CI −21.627 to 3.404, p = 0.154; \( I^2 = 95.3\% \); 3 months: SMD = −4.431, 95%CI −5.771 to −3.091, p < 0.001; \( I^2 = 0.0\% \)). The total effect on microalbuminuria was analyzed, suggesting that microalbuminuria was significantly lower in the MSC-treated group than in the DKD group (SMD = −5.791, 95%CI −8.681 to −2.901, p < 0.001; \( I^2 = 86.3\% \)).

The urinary albumin/urinary creatinine ratios at 1 month (6 studies included) and at 2 months (10 studies included) were observed to be significantly lower in the MSC-treated group than in the untreated DKD group (SMD = −5.791, 95%CI −8.681 to −2.901, p < 0.001; \( I^2 = 86.3\% \)). The kidney weight and the kidney weight/body weight ratio were used to assess kidney hypertrophy. No significant intergroup difference in kidney weight was found between the MSC and untreated DKD groups at 1 month (2 studies included; SMD = −0.674, 95%CI −2.052 to 0.704, p = 0.337; \( I^2 = 67.0\% \)).

The kidney weight/body weight ratio was found to be significantly decreased in the MSC-treated group at 2 months (8 studies included, SMD = −1.364, 95%CI −2.164 to −0.565, p = 0.001; \( I^2 = 79.7\% \)), while no significant difference was found between the two groups at 3 months (2 studies included, SMD = −10.012, 95%CI −29.753 to 9.729, p = 0.320; \( I^2 = 97.0\% \)).

The total effect on the kidney weight/body weight ratio was analyzed, and the analysis suggested that a reduced kidney weight/body weight ratio was found in the MSC-treated group (SMD = −1.624, 95%CI −2.594 to −0.655, p = 0.001; \( I^2 = 86.9\% \)).

### Assessment of kidney weight

Kidney weight and the kidney weight/body weight ratio were used to assess kidney hypertrophy. No significant intergroup difference in kidney weight was found between the MSC and untreated DKD groups at 1 month (2 studies included; SMD = −0.674, 95%CI −2.052 to 0.704, p = 0.337; \( I^2 = 67.0\% \)).

The kidney weight/body weight ratio was found to be significantly decreased in the MSC-treated group at 2 months (8 studies included, SMD = −1.364, 95%CI −2.164 to −0.565, p = 0.001; \( I^2 = 79.7\% \)), while no significant difference was found between the two groups at 3 months (2 studies included, SMD = −10.012, 95%CI −29.753 to 9.729, p = 0.320; \( I^2 = 97.0\% \)). The total effect on the kidney weight/body weight ratio was analyzed, and the analysis suggested that a reduced kidney weight/body weight ratio was found in the MSC-treated group (SMD = −1.624, 95%CI −2.594 to −0.655, p = 0.001; \( I^2 = 86.9\% \)).

### Assessment of body weight

There were 3 studies and 5 studies that assessed body weight at the 1-month and 2-month time points, respectively. No significant difference in 1-month body weight was found between the two groups (SMD = 2.634, 95%CI −0.730 to 5.999, p = 0.125; \( I^2 = 95.5\% \)). At 2 months, the body weight of the MSC-treated groups significantly increased compared to that of the DKD groups (SMD = 0.903, 95%CI 0.346 to 1.459, p = 0.001; \( I^2 = 40.2\% \)). An overall effect of MSC treatment on body
weight was also found (SMD = 1.499, 95%CI 0.461 to 2.536, \( p = 0.005; I^2 = 87.3\% \)).

**Assessment of renal fibrosis**

Four included studies evaluated the percentage of glomerulosclerosis at 2 months after MSC treatment, and no significant difference was found (SMD = −0.350, 95%CI −4.173 to 3.473, \( p = 0.858; I^2 = 96.2\% \)).

Transforming growth factor-β (TGF-β) was measured at different time points using different methods. According to polymerase chain reaction (PCR) assays at 1 month (2 studies included) and 2 months (3 studies included) as well as western blot (WB) assays at 2 months (2 studies included), TGF-β was significantly decreased in the MSC-treated group (1-month PCR: SMD = −3.281, 95%CI −4.225 to −2.337, \( p < 0.001; I^2 = 42.2\% \); 2-month PCR: SMD = −7.594, 95%CI −13.274 to −1.915, \( p = 0.009; I^2 = 93.6\% \); 2-month WB: SMD = −9.329, 95%CI −11.569 to −7.089, \( p < 0.001; I^2 = 16.2\% \)). The same was true for the total expression of TGF-β (SMD = −6.839, 95%CI −9.367 to −4.312, \( p < 0.001; I^2 = 90.5\% \)).

Collagen I (Col-I) was detected by immunohistochemistry (IHC) and PCR. According to PCR at 2 months (3 studies included), Col-I was significantly decreased (SMD = −11.856, 95%CI −14.887 to −8.826, \( p < 0.001; I^2 = 41.3\% \)) in the MSC-treated group, although no significant intergroup difference was found by IHC at 2 months (2 studies included; SMD = −4.714, 95%CI −10.670 to 1.242, \( p = 0.121; I^2 = 95.3\% \)). An overall effect on Col-I expression was also found (SMD = −9.081, 95%CI −14.233 to −3.929, \( p < 0.001; I^2 = 95.1\% \)).

Three included studies evaluated fibronectin (FN) by IHC at 2 months after MSC treatment, and a statistically significant decrease was found in the MSC-treated group (SMD = −7.781, 95%CI −10.680 to −4.881, \( p < 0.001; I^2 = 71.3\% \)).

Two studies evaluated α-smooth muscle actin (α-SMA) by WB at 1 month after MSC treatment, and 3 studies quantified its expression by PCR at 2 months. Both measures of α-SMA expression were significantly decreased in the MSC-treated group (1-month WB: SMD = −2.514, 95%CI −3.550 to −1.479, \( p < 0.001; I^2 = 0.0\% \); 2-month PCR: SMD = −2.098, 95%CI −3.721 to −0.476, \( p = 0.011; I^2 = 83.4\% \)). An overall effect of MSC treatment on the expression of α-SMA was found (SMD = −2.249, 95%CI −3.311 to −1.186, \( p < 0.001; I^2 = 72.1\% \)).

E-cadherin was quantified by WB at 1 month (2 studies included) after MSC treatment; the treatment was associated with a significant and notable decrease in E-cadherin deposition (SMD = 3.600, 95%CI 2.338 to 4.861, \( p < 0.001; I^2 = 0.0\% \)).

**Assessment of inflammatory mediators**

Monocyte chemokine protein-1 (MCP-1) was detected by IHC at 2 months (2 studies included) after MSC treatment, and no significant difference was found between the two groups (SMD = −8.913, 95%CI −20.994 to 3.167, \( p = 0.148; I^2 = 93.1\% \)).

Tumor necrosis factor-α (TNF-α) was detected by enzyme-linked immunosorbent assay (ELISA) at 2 weeks (3 studies included) and by PCR at 1 month (2 studies included) after MSC treatment, both of which showed statistically significant decreases in the MSC-treated group (2-week ELISA: SMD = −3.853, 95%CI −7.207 to −0.499, \( p = 0.024; I^2 = 90.4\% \); 1-month PCR: SMD = −4.369, 95%CI −6.835 to −1.903, \( p = 0.001; I^2 = 57.5\% \)). An overall effect of MSC treatment on the expression of TNF-α was found (SMD = −4.027, 95%CI −5.955 to −2.098, \( p < 0.001; I^2 = 84.9\% \)).

**Risk of bias**

Given sufficient data to assess publication bias, 2-month blood glucose was used for measurement. There was some degree of bias, indicated by a moderate asymmetry of the funnel plot, and Egger’s test showed \( p = 0.013 \). However, the trim-and-fill method did not identify any missing studies (Fig. 5).

**Discussion**

Meta-analysis of medication in clinical trials is essential for clinical decisions in evidence-based medicine. Before medications are put into clinical use, preclinical experiments to explore their efficacy and safety must be performed, and these studies can be costly. In addition, in the absence of compelling evidence, testing directly on humans is both highly risky and unethical. A meta-analysis based on animals may provide a good reference to predict the outcomes of clinical trials. To evaluate the therapeutic effects of MSCs on DKD and review the mechanisms involved, we carried out this study. In this study, we performed a literature search with no species restrictions, yielding 32 animal studies in 28 publications and 1 clinical trial; on this basis, we conducted a meta-analysis of the animal studies and a systematic review.

The concept of DKD was proposed to replace DN in the Kidney Disease Outcomes Quality Initiative (K/DOQI) by the National Kidney Foundation (NKF) in 2007 and has been used to specify renal lesions caused by DM. DN is characterized by proteinuria ≥300 mg/day in a diabetic patient, with or without diabetic retinopathy and hypertension. However, with a new pathological classification of diabetic kidney lesions involving lesions of the tubules, interstitium, and/or vessels as determined by renal biopsy, the concept of DN has shifted to DKD in recent years focusing on clinical diagnosis. Because of the conceptual update, the precise distinction between DN and DKD was considered outside the scope of this study to avoid confusion, and both clinical entities were included.
Fig. 5 Publication bias. a Funnel plot. b Egger's test. c Trim-and-fill method.
In this paper, we found that MSCs might improve diabetic status, islet function, and glucose levels, as well as provide reno-protection. MSCs appeared to be effective in the treatment of diabetes, mitigating diabetic symptoms such as weight gain and decreased urine output and enhancing pancreatic islet function to improve inclusion secretion and glycemic control. Regarding the therapeutic effect of DKD, reductions in SCR, BUN, Ccr, urinary protein, and renal hypertrophy were found in the MSC-treated group. In addition, molecular detection showed that MSCs might reduce the expression of renal fibrosis-related indicators, such as TGF-β, Col-I, FN, α-SMA, and E-cadherin, and the expression of inflammatory mediators such as MCP-1 and TNF-α.

To the best of our knowledge, this study is the first attempt to systemically evaluate MSC administration in DKD without species limitations. El-Badawy and El-Badri [42] conducted a meta-analysis of the therapeutic effects of different sources of stem cells in T1DM and T2DM by evaluating C-peptide, HbA1c, insulin requirements, and adverse effects, showing improved outcomes with stem cell therapy, especially CD34+ hematopoietic stem cell therapy. According to the study, the incidence of adverse effects was 21.72%, and no deaths were reported. To assess and quantify stem cells in animal studies of chronic kidney disease (CKD). Papazova et al. [43] performed a systematic review and meta-analysis and reported notable improvements in plasma creatinine, plasma urea, urinary protein, glomerular filtration rate (GFR), and blood pressure. Wang et al. [44] screened and pooled the data from small animal models of acute kidney injury (AKI) and CKD treated with MSCs and confirmed that impaired renal function was improved.

For glucose at 2 months, the moderate funnel plot asymmetry suggested the presence of bias, and Egger’s test showed $p = 0.013$; however, the trim-and-fill method did not show any missing studies. We detected significant heterogeneity, one of the inevitable drawbacks of animal meta-analyses; its causes may have included the following: different construction methods of animal models, different MSC treatment schemes, and different detection methods.

The therapeutic effects of MSC treatment seemed to be promising in animal studies, but the lone human investigation appeared to tell another story. That trial, a randomized, double-blind, placebo-controlled study of MSCs published in 2016, primarily assessed the safety over a 60-week follow-up and the efficacy over a 12-week follow-up. Regarding safety and tolerance, neither adverse events associated with MSCs nor persistent donor-specific anti-HLA antibodies were observed in the trial. However, except for interleukin-6 values and GFR stabilization, no significant difference from placebo was found in any other treatment outcome: urinary protein, Ccr, lipid profile, HbA1c, blood pressure, TNF-α, adiponectin, TGF-β, uric acid, and fibroblast growth factor 23. Nevertheless, the results are not convincing, as they come from a single trial with a small sample size ($N = 30$).

Previous studies have indicated that MSCs can improve some other renal diseases. Chang et al. [45] assessed the effects of MSCs in an anti-Thy1.1-induced rat model of glomerulonephritis and found that intrarenal transplantation of MSCs with hypoxic preconditioning could reduce glomerular apoptosis, autophagy, and inflammation. Barbado et al. [46] conducted a clinical study in patients with lupus nephritis and found that MSC therapy dramatically improved proteinuria levels at the end of the first month, and the ameliorations were sustained throughout the follow-up period. Song et al. [47] conducted a study in rats with nephropathy induced by adriamycin (ADR) and showed that MSCs attenuated ADR-induced nephropathy by inhibiting NF-kB to diminish oxidative stress and inflammation and improve glomerulosclerosis and interstitial fibrosis.

How do MSCs improve renal lesions? MSC therapy has been reported to exert beneficial effects on renal impairment in animal models and patients [48–50]. However, the exact mechanisms of nephroprotection of MSCs remain unclear at present. To date, several potential mechanisms have been proposed. Immunoregulation is one important aspect, encompassing anti-inflammatory, antiapoptotic, and antioxidant action [51, 52]. In addition, one cannot ignore the inhibition of extracellular matrix accumulation, which may be achieved by promoting the secretion of antifibrotic factors and reducing the expression of renal fibrosis-related indicators [27, 53]. Protection of renal cells such as podocytes [51] and renal tubular epithelial cells [52] also deserves a place on the list. Proangiogenic potential is one of the functional characteristics of MSCs and may play a part in kidney repair [54]. Furthermore, attention should be paid to the homing of exogenously administered MSCs to specific parts or organs owing to the number of cells that come into play [15], and with the dedifferentiation of tubular cells into stem-like cells, there is a possibility of organ regeneration in AKI with MSC therapy [52].

In this study, a sensitivity analysis was performed, and we found that the results for sensitivity analysis were similar to those of non-sensitivity analyses. It might indicate that the results might be robust to some extent.

**Limitations**

Only one clinical trial was included in this study, meaning that human data were seriously lacking. As for animal experiments, notable heterogeneity and bias left the conclusions uncertain. Because of the limited longevity of animals, the included animal experiments generally
had short observation periods. Heterogeneity was observed in this meta-analysis due to the factors such as the experimental models of DM (e.g., animal species, method used to induce diabetes, and type of diabetes) and MSC treatment (e.g., source, dosage, frequency and route of administration, and timing of administration in relation to the onset of diabetic kidney disease). Sensitivity analysis should be performed by omitting each individual study. Concomitant effects of glycemia confound interpretations about direct therapeutic effects on renal injury. MSC-related adverse events were also limited. Overall poor quality of the experimental studies incorporated in the meta-analysis was found, and further attention should be paid to the design methodology as well as animal experiments with higher quality and larger samples in the future. If preclinical experiments yield sufficient evidence of efficacy and safety, it is expected that more human investigations will be conducted in the future.

Conclusions
In animal models of DKD, MSCs might improve body weight, glycemic control, and pancreatic islet function to secrete insulin and reduce the SCr, BUN, Ccr, urinary protein, and renal hypertrophy. MSCs can reduce the expression of inflammatory mediators and alleviate renal fibrosis. MSC therapy might be a potential treatment for DKD.

Abbreviations
MSC: Mesenchymal stem cell; DKD: Diabetic kidney disease; NLM: National Library of Medicine; SYRCLE: Systematic Review Centre for Laboratory Animal Experimentation; SCr: Serum creatinine; BUN: Blood urea nitrogen; Ccr: Creatinine clearance rate; UACR: Urinary albumin/urinary creatinine ratio; TGF-β: Transforming growth factor-β; Col-I: Collagen I; FN: Fibronectin; α-SMA: α-Smooth muscle actin; TNF-α: Tumor necrosis factor-α; PCR: Polymerase chain reaction; WB: Western blot; IHC: Immunohistochemical; ELISA: Enzyme-linked immunosorbent assay; GFR: Glomerular filtration rate; DM: Diabetic mellitus; DN: Diabetic nephropathy; RoB: Risk of bias; SMDs: Standard mean differences; OR: Odds ratio; CI: Confidence intervals; ST2: Streptozotocin; BM-MSCs: Bone marrow mesenchymal stem cells; ADSCs: Adipose-derived stem cells; HUCB-MSCs: Human umbilical cord blood-derived mesenchymal stem cells; KDQI: Kidney Disease Outcomes Quality Initiative; NKF: National Kidney Foundation; CKD: Chronic kidney disease

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Statement of human and animal rights
This article does not contain any studies with human or animal subjects.

Statement of informed consent
There are no human subjects in this article and informed consent is not applicable.

Authors’ contributions
TBZ contributed to the conception and design of the study, WSL, HYL, and TBZ were responsible for the collection of data and performed the statistical analysis and manuscript preparation. SJL, QY, GYC, and CLL were responsible for checking the data. All authors were responsible for the drafting of the manuscript and read and approved the final version.

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Competing interests
The authors declare that they have no competing interests.

Author details
1Department of Nephrology, The Second Affiliated Hospital of Shantou University Medical College, No. 69 Dongsha Road, Shantou 515041, China. 2Department of Nephrology, Huadu District People’s Hospital of Guangzhou, Southern Medical University, Guangzhou, China.

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