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Review Acticle

Effect of adipose-derived stem cell transplantation on the viability of random pattern skin flaps: a meta-analysis

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Abstract: A meta-analysis was conducted to assess the effect of adipose-derived stem cells (ADSCs) transplantation on the viability of random pattern skin flaps and to provide important clues for clinical practice. We performed a random-effect meta-analysis on the skin flap survival ratio, microvessel density and vascular endothelial growth factor (VEGF) expression. Combined results showed that flap survival ratio in the experimental groups used ADSCs therapy was higher than that in the control groups (P<0.00001). The microvessel density was higher in the experimental groups than in the control groups (P<0.001). VEGF expression level was also higher in the experimental groups than in the control groups (P<0.001). Multivariable meta-regression showed that the number of ADSCs transplanted (P=0.049) and disease model (P=0.011) were significantly associated with an increase in flap survival ratio, the number of ADSCs transplanted can explain 40.95% of the heterogeneity, and disease model can explain 65.55% of the heterogeneity. The meta-analysis concluded that ADSCs transplantation could improve the microvessel density, VEGF expression level and survival ratio of skin flaps. This may guide future clinical practice to use the ADSCs therapy.

Keywords: Adipose-derived stem cells, random pattern skin flaps, transplantation, animal experiment, meta-analysis

Introduction

The skin flap is extensively used in plastic and reconstructive surgeries [1-3]. It can be used to repair the tissue defect caused by trauma, tumor resection operation, congenital malformation, necrosis of diabetic skin and soft tissue [4]. However, flap necrosis is a common postoperative complication in surgery. It will be desirable to find effective methods to prevent ischemic necrosis of skin flaps. It has been shown that promotion of neo-vascularization or regeneration of endothelial cells can improve the flap blood supply [5], which may provide a feasible solution to prevent flap tissue necrosis. Recent studies have found that adipose-derived stem cells, which belong to adult stem cells, have the ability to differentiate into vascular endothelial cells [6-8]. Compared with marrow-derived stem cells, ADSCs are abundant in subcutaneous adipose tissue and easily harvested [9, 10]. In addition, ADSCs can produce vascular endothelial growth factor (VEGF) to induce angiogenesis [7, 11].

The results of animal experiments can provide guidance for clinical practice. There are lots of animal experiments studying the effect of ADSCs transplantation on the survival of random-pattern skin flaps. However, the experimental designs and the results are not consistent. We conducted a meta-analysis to assess the effect of ADSCs transplantation on random-pattern skin flap survival and the effective quantity of ADSCs.

Materials and methods

Search strategy

We used the key words (adipose derived stem cell) AND (skin OR flap) to search the electronic

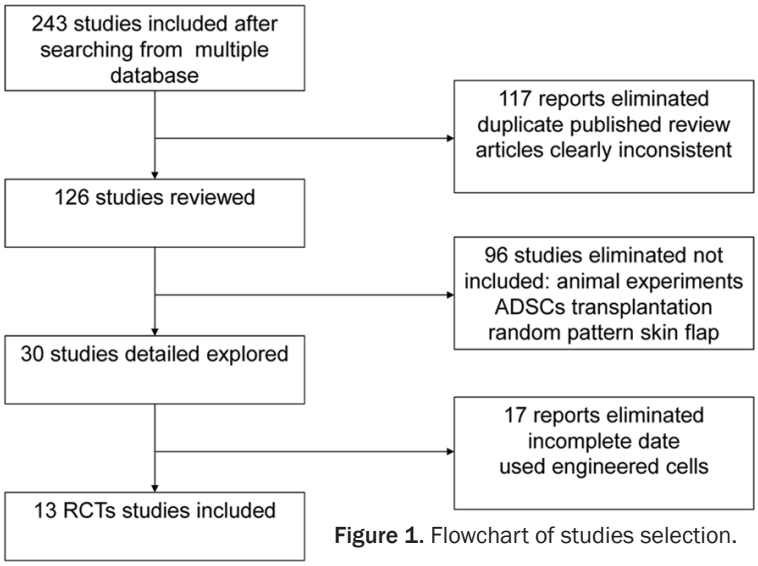

Figure 1. Flowchart of studies selection.

databases of PubMed, EMBASE, Chinese Biomedical Literature (CBM), and China National Knowledge Infrastructure (CNKI). At the same time, the references cited in the publications were also included. The last search was conducted at the end of September 2015.

Eligibility criteria

The eligibilities of the studies were judged independently by two reviewers (HZC and LJP). Eligible studies were RCTs of random-pattern-skin-flap models. Transplanted ADSCs was the only intervention in experimental groups compared with control groups. The eligible studies included flap survival ratio or area, microvessel density, or the VEGF expression level. Reviews, comments, and editorials were excluded.

Data extraction

Full-text articles were screened independently by two reviewers (HZC and LJP). Then information was extracted from each eligible study, including sample entry criteria and sample size, sampling method and processes, basal characteristics, the average number and standard deviation of the continuous index in the outcome, including the flap survival ratio or area, microvessel density, and VEGF expression level. In addition, the number of ADSCs, ADSCs injections, and measurement methods were also extracted. When necessary, data were estimated from the figures of qualified studies [12].

Data analysis

The mean flap survival ratio was different between the experimental and control groups. A random-effect model was selected by the significant heterogeneity ($P<0.01$). We applied multivariable meta-regression analysis to find the source of heterogeneity. Between the experimental animals and control animals, weighted mean differences with 95% confidence intervals (CIs) were estimated continuous variables. In one study, number of experimental-group animals was equal to the number of control group [12, 13]. Statistical hypothesis testing provided the *P*-values at two-sided 0.05 levels.

The subgroups used in multivariate meta-analyses were divided into type of ADSCs (human or mice), animal models (mice or rabbits), number of ADSCs injected ($\leq 10^6$ or $10^6<n<5\times10^6$ or $\geq 5\times10^6$), injection routes (subcutaneous or intravenous), disease model (normal or diseased: the diseased model is diabetic or hypoxia preconditioned rats). The statistically significance in subgroups was analyzed separately.

Between the experimental and control groups, the mean microvascular density and VEGF expression levels were also different. The significant heterogeneity was determined by heterogeneity tests ($P<0.01$). So a random-effect model was applied to find the pooled difference between experimental and control groups. A funnel plot was used to assess publication bias. All analyses were performed with Review Manager Version 5.2 and Stata 12.0.

Results

Study characteristics

A total of 243 reports were identified by the initial search. 117 reports were eliminated, which include duplicate publications, review articles, reports that were clearly inconsistent with the inclusion criteria. 96 reports were eliminated, because they did not include animal experiments, ADSCs transplantation, and random-

Table 1. Characteristics of the studies included in the meta-analysis

First author (year)	N	Type of ADSCs	Animal model	Disease model	Number of ADSCs	Injection route	Method of microvascular density measurement	Method of VEGF measurement
Dong (2014)	8	Human	Mice	Normal	≥5×10^6	Subcutaneous	CD31+	/
Gao (2011)	15	Human	Mice	Diabetic	≥5×10^6	Subcutaneous	CD31+	ELISA
Gong (2014)	29	Human	Rabbits	Normal	≤1×10^6	Subcutaneous	CD31+	/
Cao (2014)	15	Human	Mice	Normal	≤1×10^6	Subcutaneous	vWF	Fluorescence
Li g (2011)	10	Human	Rabbits	Normal	10^6<n<5×10^6	Subcutaneous	CD31+	/
Wang (2013)	8	Human	Mice	Normal	10^6<n<5×10^6	Subcutaneous	vWF	ELISA
Caio (2014)	15	Mice	Mice	Normal	≥5×10^6	Intravenous	/	/
Scott (2012)	7	Mice	Mice	Normal	≥5×10^6	Subcutaneous	/	/
Li (2010)	12	Mice	Mice	Normal	10^6<n<5×10^6	Subcutaneous	vWF	ELISA
Matthias (2012)	8	Mice	Mice	Ischemic	≥5×10^6	Intravenous	CD31+	/
Shang (2012)	48	Mice	Mice	Normal	≥5×10^6	Subcutaneous	/	/
Cagri (2009)	20	Mice	Mice	Normal	≤1×10^6	Subcutaneous	vWF	Fluorescence
Yue (2013)	6	Mice	Mice	Normal	10^6<n<5×10^6	Subcutaneous	CD31+	/

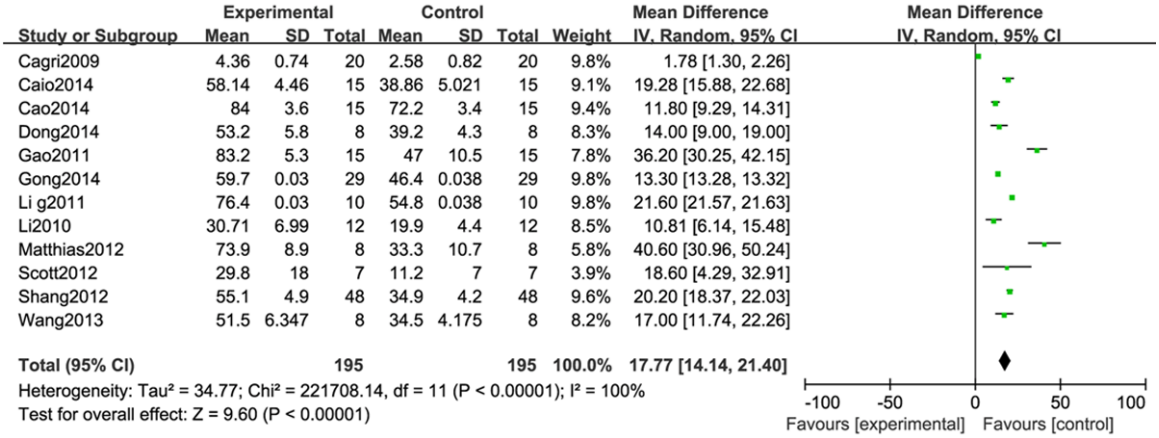

Figure 2. Forest plot for skin flap survival ratio. It showed the impact of ADSCs transplantation on skin flap survival ratio compared with controls, 95% confidence interval.

pattern skin flap. 17 reports were eliminated because of incomplete data or using engineered ADSCs. Therefore, only 13 studies using animal models were included. These studies determined the effect of ADSCs on random-pattern skin flap (**Figure 1**). The studies included 11 normal-animal groups, 1 diabetic group and 1 group of ischemic treatment of skin flaps. The number of studies using human ADSCs (hADSCs) was 6, and the number of studies using mouse ADSCs (mADSCs) was 7. Animals used either mice (n=11) or rabbits (n=2). ADSCs were transplanted through subcutaneous (n=11) or intravenous (n=2). The number of ADSCs injected was ≤10^6 (4 studies), 10^6<n<5×10^6 (4 studies) or ≥5×10^6 (5 studies). Characteristics of the studies included in the meta-analysis were showed in **Table 1**.

Meta-analysis

The continuous variables of the flap survival ratio are presented in mean and standard deviation. Coalescent analysis showed that the level of the flap survival ratio with ADSCs therapy was higher in the experimental groups than in the control groups (pooled difference, 17.77; 95% CI=14.14-21.40; Z=9.60; P<0.00001) with significant heterogeneity (P<0.00001; I Square, 100%; **Figure 2**).

Multivariable meta-regression analysis showed that the number of ADSCs transplanted (P=0.049) and disease model (P=0.011) were associated with a significant increase in the flap survival ratio. These two groups were also used to perform the single factor regression analysis. Subgroups clustered by the num-

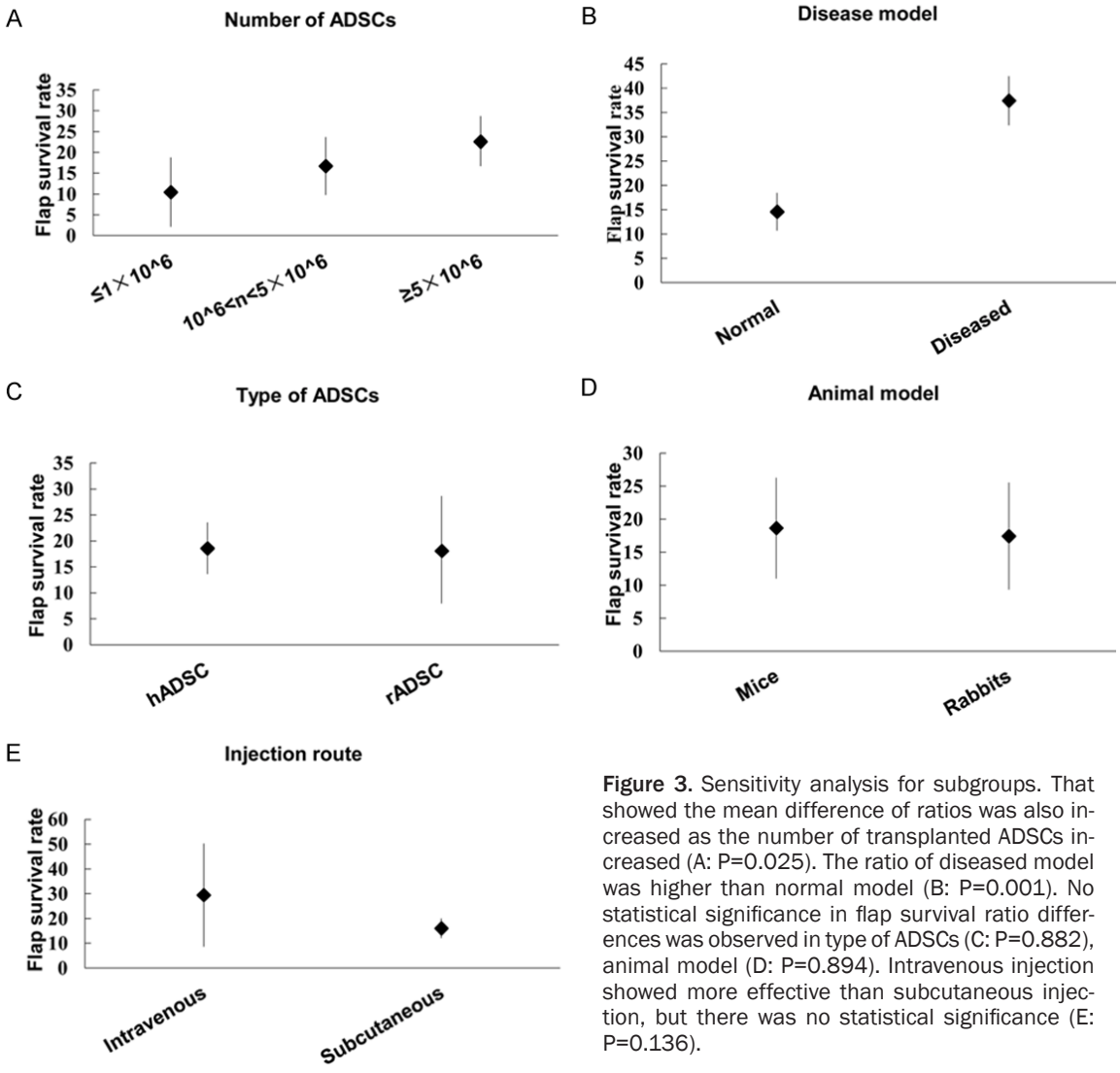

Figure 3. Sensitivity analysis for subgroups. That showed the mean difference of ratios was also increased as the number of transplanted ADSCs increased (A: P=0.025). The ratio of diseased model was higher than normal model (B: P=0.001). No statistical significance in flap survival ratio differences was observed in type of ADSCs (C: P=0.882), animal model (D: P=0.894). Intravenous injection showed more effective than subcutaneous injection, but there was no statistical significance (E: P=0.136).

ber of ADSCs transplanted showed P=0.025, I-squared=99.58%, Adj R-squared=40.95%. Subgroups clustered by disease model showed P=0.001, I-squared=100.00%, Adj R-squared=65.55%.

The results of the subgroup analyses were showed in **Figure 3**. The three subgroups grouped by ADSC number ($\leq 10^6$ or $10^6<n<5\times10^6$ or $\geq 5\times10^6$). The flap survival ratios respectively resulted in 10.44% (95% CI, 2.09-18.78; P< 0.001), 16.74% (95% CI, 9.75-23.72; P<0.001) and 24.99% (95% CI, 18.46-31.53; P<0.001). As the transplanted number of ADSCs increased, the mean difference of ratios was also increased (**Figure 3A**). The subgroup analyses also showed that the flap survival ratio in normal animals was higher than that in the control group (pooled difference, 14.58; 95% CI=10.68-18.4; P<0.001). The flap survival ratio was also significantly high in diseased models (pooled difference, 37.41; 95% CI=32.35-42.48; P< 0.001) (**Figure 3B**). Compared with control groups, the flap survival ratio was significantly high in intravenous injection groups (pooled difference, 29.44; 95% CI=8.57-50.31; P<0.001), and in subcutaneous injection groups (pooled difference, 16.05; 95% CI=12.12-19.98; P< 0.001). But there was no statistically significant difference between the two groups (P=0.136) (**Figure 3E**).

Studies using hADSCs (pooled difference, 18.58; 95% CI=13.64-23.53; P<0.001) or using mADSCs resulted in (pooled difference 18.07; 95% CI=7.49-28.65; P<0.001) a signifi-

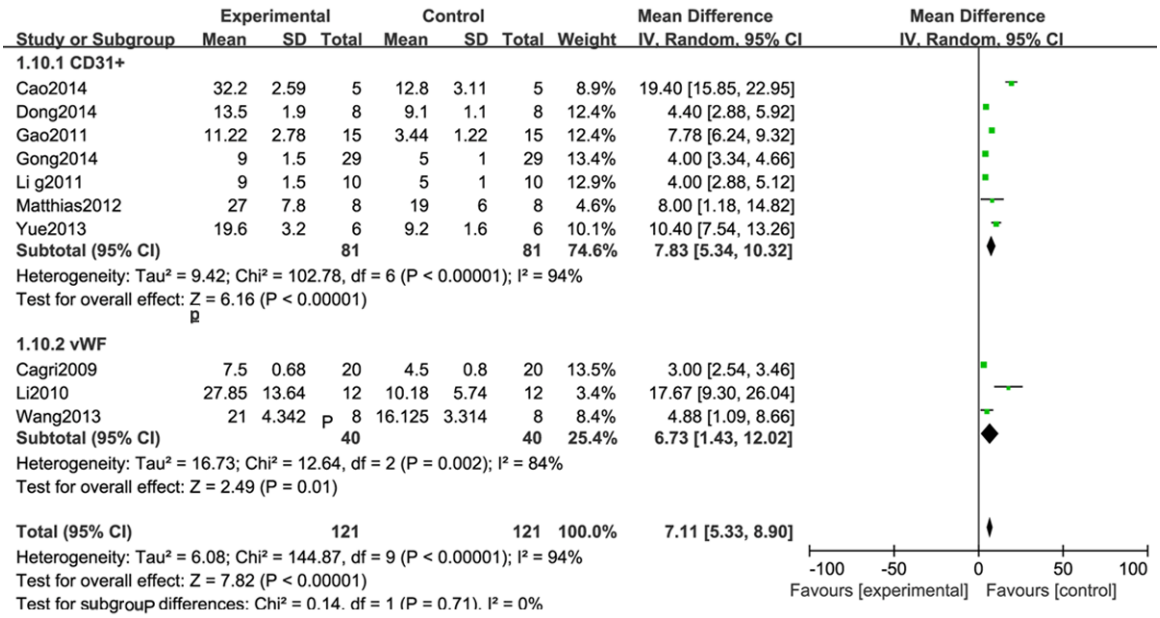

Figure 4. Forest plot for microvessel density. It showed the impact of ADSCs transplantation on microvessel density compared with controls, 95% confidence interval.

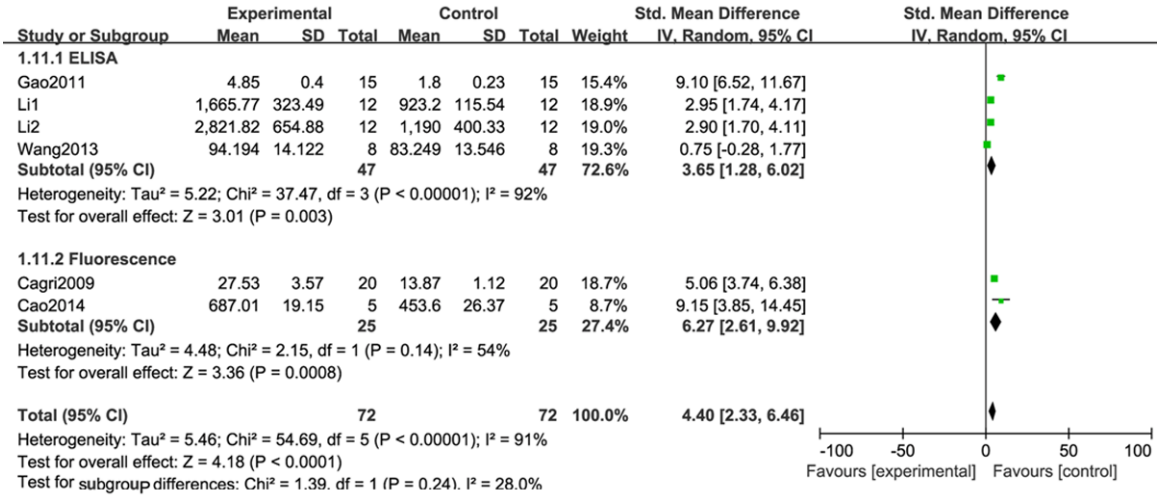

Figure 5. Forest plot for VEGF. It showed the impact of ADSCs transplantation on VEGF, compared with controls, 95% confidence interval.

cantly higher flap survival ratio, compared with the control group (**Figure 3C**). Studies using mice (pooled difference, 18.67; 95% CI=11.03-26.31; P<0.001) and rabbits (pooled difference, 17.45; 95% CI=9.32-25.58; P<0.001) showed a significantly higher flap survival ratio, compared with the control groups (**Figure 3D**).

The mean microvessel density is presented in mean and standard deviation as continuous variables. Pooled analysis showed that the microvessel density (pooled difference, 7.11; 95% CI=5.33-8.90; Z=7.82; P<0.001; **Figure 4**) was significantly higher in experimental groups than in control groups. Microvessel density was measured using CD31+ (pooled difference, 7.83; 95% CI=5.34-10.32; P<0.001) or von Willebrand factor (vWF) method (pooled difference, 6.73; 95% CI=1.43-12.02; P=0.002) (**Figure 4**).

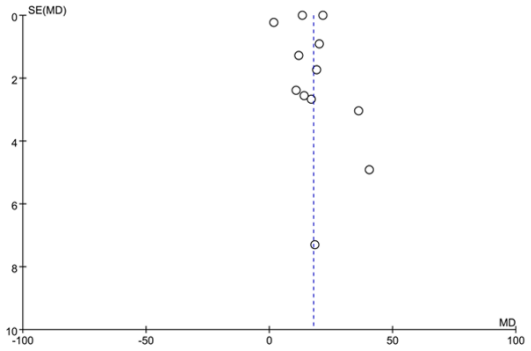

Figure 6. Funnel plot for skin flap survival ratio. Blue-dotted line showed the overall estimated mean difference. No obvious evidence for publication bias was found.

The mean VEGF expression level (presented in mean and standard deviation) was also used as continuous variables. Due to different data units, the method standard mean difference (SMD) was used. **Figure 5** showed ADSCs transplantation could improve VEGF secretion (pooled difference, 4.40; 95% CI=2.33-6.46; Z=4.18; P<0.001), determined by using the enzyme-linked immunosorbent assay (ELISA) or fluorescence method.

Sensitivity analysis

The number of ADSCs transplanted (P=0.025) and the disease model (P=0.001) were significant for the flap survival ratio. They can respectively explain 40.95% and 65.55% of the heterogeneity. As shown in **Figure 3A**, the mean difference of ratios was also increased as the number of transplanted ADSCs was increased. Diseased animals showed a stronger effect than normal animals (**Figure 3B**). No statistical significance in flap survival ratio differences was observed based on types of ADSCs (P=0.882), animal models (P=0.894), or injection routes (P=0.136). The funnel plot of the flap survival ratio showed that values were evenly distributed around the overall estimate, suggesting a lack of publication bias (**Figure 6**).

Discussion

Skin tissue defect can be caused by many diseases, such as trauma, tumor resection operation, congenital malformation and diabetes mellitus [4]. To treat this problem, the flap transplantation was used to repair the defects of organization in clinical settings [1-3]. However, the necrosis of skin flaps is a major concern and a common complication. It affected the survival ratio of the skin flaps [14]. In some pre-clinical experiments, enhancing the blood supply of the flap can improve the survival rate of the skin flap [15]. In 2001, Zuk *et al.* found adipose-derived stem cells after liposuction from adipose tissues, a large number of studies have been carried out on the characteristic and function of ADSCs [16, 17]. Because of the abilities of self-renewal and multi-lineage differentiation, low immunogenic, long-term survivability and reliability, and strong proliferative ability, ADSCs have become the preferred cells for tissue engineering and regenerative medicine [11, 18, 19]. ADSCs are getting more attractive for the advantages of ubiquitous in adipose tissue and easily harvested [8, 10]. Moreover, ADSCs also have the potential of multiple differentiation, including differentiating into osteoblasts, adipocytes, endotheliocytes, insulin cells, cardiomyocytes, neurons, epithelial cells, smooth muscle cells [20-27]. In addition, ADSCs have the ability to secrete VEGF and other pro-angiogenesis factors [7, 11]. VEGF can contribute to angiogenesis by stimulating endothelial cell proliferation, increasing vascular permeability, promoting the migration of endothelial cells, and promoting mobilization from the precursor cells of bone marrow to peripheral blood [28]. Thus, it is assumed that ADSCs can promote blood supply of the skin flap and increase the flap survival ratio by differentiating into endothelial cells and providing the VEGF.

The present meta-analysis analyzed the flap survival ratio, microvessel density, and VEGF expression level. Compared with the control group, the VEGF expression level and the flap microvessel density of the experimental group were increased. The flap survival ratios of ADSCs were higher in transplanted groups than in the control groups. It was concluded that ADSCs can enhance the VEGF secretion and the microvessel density, and then improve the survival ratio of skin flap. In addition, the flap survival ratio results suggest that the survival ratio improved with the increase of transplanted cells number. Thus, this finding indicates that future studies of ADSCs therapy for improving flap survival should use a number more than 5×10^6 of ADSCs for transplantation. But the upper bound of ADSCs needs to be further studied.

The diabetic or ischemic model showed a better effect compared with the normal animal models. Diabetic can cause glucose, fat, protein metabolism disorder which can cause secondary micro-vascular disease. The vessel number and the viability of skin flaps were decreased in diabetic patients, because of increasing the degree of ischemia and hypoxia of the diabetic skin flap [29]. In addition, the ischemic treatment of skin flaps also increased the degree of tissue hypoxia [30]. Under hypoxic conditions, ADSCs can secrete more VEGF [31]. Collectively, this finding suggest that hypoxic environment enhances the ability of ADSCs to increase the skin flap survival ratio, which was confirmed by Yeli Yue [32].

No statistical significance was observed in types of ADSCs and animal models. The differences between the experimental group and control group in the above two subgroups were little, and the 95% confidence intervals had a most overlap. Based on this, we postulate that there may be immunological tolerance to the transferred ADSCs of different species [33]. This is promising for the transplantation of animal ADSCs for human. No statistical significance in flap survival ratio differences was observed on the basis of injection routes. Nevertheless, in order to select the best route of ADSCs transplantation in clinical trials, it is necessary to determine the effect of other methods of ADSC transplantation.

Meta-analyses of animal studies are a useful way to obtain many parameters, including the best therapeutic effect and the optimal dose, which can often guide future research and clinical practice. This meta-analysis evaluated the effects of ADSCs therapy in the skin flap survival, demonstrating that ADSCs transplantation can improve the flap survival ratio. The effect may be better achieved by transplantation of ADSCs of more than 5×10^6 or subjecting ADSCs to hypoxic preconditioning. This may guide future clinical practice to use the ADSCs therapy. Moreover, we analyzed the microvessel density and the VEGF expression level after ADSCs transplantation, which further elaborated the mechanism. However, ADSCs therapies have rarely been tested in other animal species except rats and rabbits. Thus, to ensure more effective and safe use of ADSCs in humans, more animal studies are still needed in the near future.

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Disclosure of conflict of interest

None.

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