Efficiency of stem cell based therapy in the treatment of diabetic foot ulcer: a meta-analysis

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Abstract. Diabetic foot ulcer is a chronic, refractory, frequent complication in diabetic patient. Its treatment often requires multidisciplinary joint efforts, diverse strategies have been adopted to address this annoying issue, including stem cell-based therapy/acellular dermal matrix/negative pressure wound therapy etc. However, consensus has not been reached. To assess the current evidence regarding the efficiency and potential advantages of stem cell-based therapy compared with conventional standard treatment and/or placebo in the treatment of diabetic foot ulcer. A comprehensive search in PubMed, EmBase, Cochrane Central and Web of Science databases was conducted during December 2016 and a systematic review and meta-analysis of all relevant studies were performed. A total of 7 studies that involved 224 diabetic foot patients, classified as Wagner grades 1–5, were analyzed. The pooled results confirmed the benefits of using the stem cell treatment. Partial and/or complete healing were significantly higher in the stem cell group compared with the control group (77.4% vs. 31.9%; RR: 2.22; 95% CI, 1.65–2.98). Subgroup analysis on ABI and TCP02 also confirmed the results. The present meta-analysis indicates that stem cell-based therapy can enhance the healing of diabetic foot ulcers and is associated with lesser pain, lower amputation rate and improved prognosis compared with normal treatment. Well-designed randomized controlled trials are required in the future in order to confirm and update these findings.

Key words: Diabetic foot ulcer, Stem cell-based therapy, Wound healing, Meta-analysis

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DIABETES MELLITUS (DM) comprises a group of common metabolic diseases which results in common social and economic burden. According to Boulton et al. in 2002, the direct and indirect expenditures that were attributable to diabetes were estimated to 132 billion dollars [1]. It is believed that the number of diabetic patients worldwide will rise to 430 million by 2030 [2]. One of the serious chronic complications of DM is the diabetic foot. Among all diabetic patients, approximately 20% to 25% will develop foot problems in their lifetime. These symptoms are usually accompanied by a majority of complications, such as chronic pain at rest, lower extremity weakness, intermittent claudication, ulcer, recurrence of ulcer, and/or even amputation in some rare cases [3]. The cause of diabetic foot ulcer is multifactorial and neuropathy and/or peripheral vascular abnormalities are considered as the main etiological factors [4].

Currently, the standard care for treating diabetic foot ulcer includes offloading, control of foot infection, control of ischemia, wound debridement, and wound dressing [5]. However, diabetic foot ulcers require a long time period for successful healing, and the majority of the patients fail to heal, resulting in foot ulcer, gangrene and limb amputation [6]. Thus, an effective method to accelerate the healing of diabetic foot ulcer is required. Stem cell-based therapy has been considered as a novel treatment for diabetic foot disease both in animal models and human experimentation [7-9].

As a branch of regenerative medicine, stem cell therapy is the use of stem cells to repair and even replace damaged tissues or organs based on ex vivo/in vivo stim-
ulation of stem cell expansion and differentiation into functional progeny [10]. Stem cells can be indentified and separated from many kinds of tissues such as adipose, bone marrow, embryonic, peripheral blood, umbilical cord and so on [11]. But only when the kind of stem is massive, easy to obtained, induced in a controllable manner, safety and effectively, that they be applied in clinical care. The application of embryonic stem cells was restrained due to ethical consideration, while induced pluripotent stem cells (iPSCs) are limited due for safety and effectively uncertainties [12]. At present, the most common used stem cells are bone marrow derived stem cells and adipose derived stem cells. Since 1968 the first successful bone marrow transplantation stem cell based therapies have existed. Over the 50 years it has been all along considered as the future of medicine [13]. However even numerous basic experiments have focus on the subject but unfortunately only a little of then come in to clinical practice, and even lesser became a standard clinical care. Several clinical trials [14-16] have evaluated the efficacy of stem cell treatment, although these trials were conducted on small sample sizes. Therefore, a systematic review was conducted in order to evaluate the effectiveness of stem cell-based therapy in the treatment of diabetic foot ulcers.

Methods

Eligibility criteria

Studies were eligible if they adhered to the following criteria: (1) Publication in English language, (2) Inclusion of human subjects, (3) Involvement of clinical trials and originality of the study, (4) Report of outcomes (5) Comparison of stem cell therapy with placebo and/or conventional treatment. (6) The incidence of diabetic foot ulcers in the subjects. The participants who were candidates for either other stem cell treatments and/or endovascular revascularization studies were excluded. All participants included should not show evidence of improvement in response to standard therapy four weeks and/or longer prior to the initiation of the study. Studies on animals, reviews, case reports and non-original studies were excluded. There was no age restriction.

Information sources and search strategy

A thorough search of the PubMed, EmBase, Cochrane CENTRAL and Web of Science databases was conducted for related studies. The first search was conducted on the 11th of December 2016 and the final search was on the 20th of December 2016. Controlled vocabulary (e.g. medical subject headings terms) supplemented with keywords was used to define the concepts of stem cell therapy and diabetic foot ulcers. The following search terms were used: 1) diabetic foot ulcers, diabetic foot ulcer, diabetic foot, diabetic ulcer, DFU; paired with 2) stem cell, stem cell therapy, progenitor cell, bone marrow stem cells, cell treatment, stem cell transplantation. No restrictions were imposed. A manual search for the references of the original and review articles was conducted for additional, possibly relevant studies.

Study selection

Following removal of the duplicate studies, two of the authors working independently screened the titles and abstracts for eligibility according to the pre-defined inclusion criteria. Disagreements were automatically upgraded to the next level of screening. Based on the title and/or abstracts, full texts of articles that potentially met the inclusion criteria were obtained and carefully screened in duplicate. Disagreements at this level were resolved by consensus and/or discussion with the third author.

Data collection

A total of two authors independently extracted and collected specific information for each study that was included according to the inclusion criteria selected. The information was as follows: name of the author, countries of the studies, year of publication, study design, characteristics of the included patients, Number of participants, type and duration of diabetes, data of ulcers, interventions received, follow-up duration, healing rate, baseline of transcutaneous arterial oxygen tension (TcPO2), ankle brachial index (ABI) and incidences of adverse events. The discrepancies between the two authors were then resolved by a second review of full-text, a thorough discussion and a finalized consensus. Additional information was requested from the study author for studies that presented results by charts and/or graphs. In case of a poor response provided by the author, the Dig XY software (version 1.2) was used in order to measure the graphs and obtain the data. However, the accuracy of such data may be considered poor and susceptible to bias.

Statistical analysis

A total of two reviewers independently assessed the quality of the included studies. The assessment of the
study quality is summarized in Fig. 1. We used the ‘Risk of bias’ tool as described in the ‘Cochrane Handbook for Systematic Reviews of Interventions’ in order to complete the evaluations. The domain assessment included criteria concerning aspects of assessment allocation, sequence generation, blinding, incomplete outcome data, selective reporting and potential threats to the validity of the study. The primary outcome for evaluating efficacy was a partial and/or complete healing rate. The change in ABI and TcPO2 was further analyzed as representative parameters of revascularization, since both were noninvasive and easily reproducible [17]. The Risk ratios (RRs) with 95% confidence intervals (CIs) were applied for the analyses of dichotomous data, whereas continuous data were presented as the standardized mean difference with 95% CIs. A random-effects model was used to estimate the merged result in order to minimize the influence of potential heterogeneity [18]. I^2 analysis was conducted in order to test for heterogeneity among studies. An I^2 of higher than 50% (I^2 > 50%) was considered to indicate statistically significant heterogeneity [19]. All statistical analyses were conducted using Review Manager Software (Rev Man) [Computer program]. (Version 5.3. Copenhagen: The Nordic Cochrane Centre, the Cochrane Collaboration, 2014).

Results

Study selection

A total of 406 studies were identified by the initial search. Following careful screening of titles and abstracts, 399 articles were excluded leaving 7 articles that fulfilled the predefined inclusion criteria and were included in the final analysis [20-26]. A total of 6 publications were full-text articles out the 7 studies [20, 22-26], whereas 1 publication was a meeting conference [21]. The detailed inclusion and exclusion criteria are shown in Fig. 2.

Characteristics of eligible studies

The basic characteristics of the 7 included studies are presented in Tables 1, 2. These studies were published between 2009 and 2016. A total of 2 studies were conducted in China, whereas the remaining 5 were conducted in Iran, Egypt, Germany, Czechoslovakia and India, respectively. It is important to note that 3 studies (Lu 2011, Karina 2012, Dubsky 2013) reported 2 different types of stem cell interventions. Accordingly, 2 comparisons were derived. According to the Cochrane handbook’s recommendation, the same size control group was equally distributed to the 2 types of interventional groups in order to overcome a unit of analysis error. A total of 224 diabetic foot patients with or without ulcers were included, of which 122 were included in the experimental group and 102 in the control group. A total of 59 patients out of the 122 in the experimental group were treated with mesenchymal stem cells (3 bone marrow-derived and 1 granulocyte colony stimulating factor (G-CSF)-mobilized umbilical cord-derived), 40 were treated with bone marrow-derived mononuclear cells, 11 were treated with peripheral blood progenitor cells and 12 were treated with marrow-enriched tissue repair cells. The mean patient age in the included studies ranged from 30 to 78, and partial and/or complete healing ranged from 29% to 86%. The follow-up duration period varied from 12 to 45 weeks.
Adverse events

A total of 4 out of the 7 included studies reported adverse events, such as death, leg edema, sudden stroke or major amputation [23-26]. However, no evidence indicated that these adverse events were stem cell therapy-related. In the Dubsky’s study, one transient elevation of CRP in the bone marrow mononuclear cell-group was observed [25]. The most common adverse event was major amputation and it was noted at a higher incidence in the control group compared with the stem cell group. The incidence of the remaining adverse events exhibited no significant difference between the stem cell and control groups. The detailed information is summarized in Table 3.

Quality assessment

The risk of bias of the included studies was evaluated by the Cochrane assessment tool (Figs. 2, 3). The overall quality of the included studies was moderate, with the quality of the included studies ranging from low to moderate. A total of 6 of the included studies were randomized controlled studies, with the exception of 1 study (Dubsky 2013) that was not randomized due to the ethical considerations.

A total of 3 studies adequately reported the detailed methods of sequence generation, and one study described allocation concealment. A total of 6 studies reported withdrawal and dropout details, whereas 2 studies may had potential bias of selective outcome, and some were
uncertain due to the lack of a protocol publication that described their performance. A total of 5 studies reported partial and/or complete healing rate in both the stem cell and control groups, whereas 2 studies reported ABI and TcPO2 changes as a healing indicator. Additional potential sources of bias were not identified based on the present assessment. No baseline imbalance was mentioned in the studies examined.

**Effect of stem cells on diabetic foot ulcer healing**

The pooled data from 5 studies that assessed healing rate in 150 patients indicated that partial and/or complete healing were significantly higher in the stem cell group compared with the control group (77.4% vs. 31.9%; RR: 2.22; 95% CI, 1.65–2.98) (Fig. 4). No significant heterogeneity was detected. A total of 3 comparisons, involving 47 patients evaluated the effects of stem cells on the mean size of the ulcer. A random-effects model indicated that stem cell-based therapy may enhance ulcer size reduction compared with the control group. Among the 3 studies investigated, the mean difference ranged from $-2.98 \text{ cm}^2$ to $8.06 \text{ cm}^2$, with a combined mean difference of $-3.48 \text{ cm}^2$ (95% CI, $-4.62$ to $-2.33$) (Fig. 5). A total of 3 studies reported that the change noted in ABI in 106 patients, as demonstrated by the ABI index, was significantly higher in the stem cell group (mean difference, 0.17; 95% CI, 0.12–0.22, $p < 0.0001$) (Fig. 6). By contrast, the 3 aforementioned studies reported that the change of TcPO2 in 135 patients was accompanied with a heterogeneity that was noted following the pooling of the studies ($x^2 = 85.09; \text{ DF} = 4, p < 0.001; \Gamma^2 = 95\%$) (Fig. 7). The pooled studies indicated a significant difference that was in favor of the stem cell group (MD, 17.11 mmHg, 95% CI, 5.96–28.27).

**Sensitivity analysis and publication bias**

A total of 7 studies were included in the sensitivity analysis. No significant change was noted with regard to the outcomes investigated, with the exception of the degree of heterogeneity in the TcPO2 group that was decreased following the removal of the study by Qin et al. [20]. The funnel plot was not used to assess publication bias as it was deemed inappropriate, as recommended by the Cochrane handbook due to the small number of studies included [27].

**Discussion**

Wound healing is a complex and dynamic process. Following skin injury, the mutual interaction between the cells and extracellular matrix (ECM) is an important factor in the process of healing. The soluble factors contribute to injury healing. This process involves certain key events, such as angiogenesis, neovascularization and the release of growth factors [28, 29] that are impaired in diabetic patients. An acute wound develops into a non-healing ulcer in diabetic foot patients, due to the damaged peripheral microvasculature and nerves. This symptom is further associated with several complications namely, chronic resting pain, lower extremity weakness,
<table>
<thead>
<tr>
<th>Author, year</th>
<th>Group/subgroup</th>
<th>Number of Patients</th>
<th>Mean Age (years, mean SD)</th>
<th>Male %</th>
<th>DMtype</th>
<th>DM duration (years)</th>
<th>TcPO2 baseline (mmHg)</th>
<th>Ulcer Baseline (cm²)</th>
<th>ABI</th>
<th>Pain-free walking distance</th>
<th>Rest pain</th>
<th>Healing percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dash, 2009</td>
<td>Bmmscs</td>
<td>3</td>
<td>40 ± 10</td>
<td>1 or 2</td>
<td>—</td>
<td>—</td>
<td>7.26 ± 1.41</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3</td>
<td>40 ± 10</td>
<td>1 or 2</td>
<td>—</td>
<td>—</td>
<td>4.24 ± 0.76</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Lu, 2011</td>
<td>Bmmscs</td>
<td>11</td>
<td>63 ± 8</td>
<td>1 or 2</td>
<td>10.3 ± 5.6</td>
<td>42.7 ± 10.1</td>
<td>4.2 ± 2.9</td>
<td>0.55 ± 0.10</td>
<td>1.1 ± 0.4</td>
<td>3.4 ± 0.6</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Bmmncs</td>
<td>11</td>
<td>65 ± 10</td>
<td>1 or 2</td>
<td>9.8 ± 5.0</td>
<td>44.5 ± 10.5</td>
<td>4.3 ± 3.1</td>
<td>0.53 ± 0.10</td>
<td>1.2 ± 0.4</td>
<td>3.5 ± 0.5</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>21</td>
<td>68</td>
<td>1 or 2</td>
<td>43.5 ± 10.0</td>
<td>4.5 ± 2.3</td>
<td>0.54 ± 0.09</td>
<td>1.1 ± 0.4</td>
<td>3.5 ± 0.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Kirana, 2012</td>
<td>Bmmncs</td>
<td>12</td>
<td>68.5 ± 1.5</td>
<td>1 or 2</td>
<td>20.9 ± 4.3</td>
<td>—</td>
<td>9.6 ± 4.2</td>
<td>—</td>
<td>—</td>
<td>83</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Bmtrcs</td>
<td>12</td>
<td>70.9 ± 1.7</td>
<td>1 or 2</td>
<td>20.5 ± 3.9</td>
<td>—</td>
<td>7.7 ± 2.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Dubsky, 2013</td>
<td>Bmmncs</td>
<td>17</td>
<td>60.7 ± 8.9</td>
<td>2</td>
<td>23.1 ± 15.2</td>
<td>16.3 ± 11</td>
<td>5.2 ± 1.6</td>
<td>—</td>
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<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Ppbe</td>
<td>11</td>
<td>63.4 ± 10.4</td>
<td>2</td>
<td>21.5 ± 9.4</td>
<td>16.4 ± 9.8</td>
<td>5.5 ± 1.7</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>22</td>
<td>63.3 ± 9.1</td>
<td>2</td>
<td>19.8 ± 9</td>
<td>14.6 ± 9.6</td>
<td>5.9 ± 2</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Mohamm-Adzadeh, 2013</td>
<td>Autologous MSCs</td>
<td>7</td>
<td>63.5 ± 7.8</td>
<td>—</td>
<td>16.5 ± 8.7</td>
<td>14.2 ± 4.1</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>86</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>14</td>
<td>64.2 ± 7.8</td>
<td>—</td>
<td>14.2 ± 8.5</td>
<td>15.8 ± 17.0</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>Qin, 2016</td>
<td>HUCMSCs</td>
<td>28</td>
<td>75 ± 3</td>
<td>60.7</td>
<td>12.8 ± 7.2</td>
<td>24 ± 3</td>
<td>—</td>
<td>3 ± 1.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>78.9</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>25</td>
<td>73 ± 5</td>
<td>60</td>
<td>13.1 ± 4.6</td>
<td>22.5 ± 6</td>
<td>—</td>
<td>2.85 ± 4.5</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Albehairy, 2016</td>
<td>Mscs</td>
<td>10</td>
<td>—</td>
<td>50</td>
<td>16.40 ± 5.31</td>
<td>4.66 ± 2.22</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>10</td>
<td>—</td>
<td>70</td>
<td>13.30 ± 6.36</td>
<td>3.72 ± 1.87</td>
<td>—</td>
<td>—</td>
<td>—</td>
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</tbody>
</table>

Abbreviations: ABI, ankle brachial index; TcPO2, transcutaneous oxygen pressure.
Table 3  Summary of adverse events

<table>
<thead>
<tr>
<th>Author, year</th>
<th>Group</th>
<th>Number of patients</th>
<th>Infection</th>
<th>Major amputation</th>
<th>Death</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dubsky, 2013</td>
<td>Bmncs</td>
<td>17</td>
<td>1</td>
<td>2</td>
<td>1</td>
<td>4 leg edema</td>
</tr>
<tr>
<td></td>
<td>Pbcps</td>
<td>12</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>3 leg edema 1 temporary pain</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>22</td>
<td>0</td>
<td>10</td>
<td>2 (1 severe arterial bleeding 1 cardiac failure)</td>
<td>Unclear</td>
</tr>
<tr>
<td>Karina, 2012</td>
<td>Bmncs</td>
<td>12</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>1 stroke</td>
</tr>
<tr>
<td></td>
<td>Bmtrcs</td>
<td>12</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>Unclear</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>6</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>Unclear</td>
</tr>
<tr>
<td>Dash, 2009</td>
<td>Bmncs</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Unclear</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>Unclear</td>
</tr>
<tr>
<td>Lu, 2011</td>
<td>Bmnc</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>Unclear</td>
</tr>
<tr>
<td></td>
<td>Bmnc</td>
<td>11</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>Unclear</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>21</td>
<td>0</td>
<td>6</td>
<td>2</td>
<td>Unclear</td>
</tr>
</tbody>
</table>

Fig. 3  Risk of bias graph: The authors’ judgements with regard to each risk of bias item were presented as percentages across all included studies.

Fig. 4  Forest plot and meta-analysis depicting risk ratios of complete and/or partial healing of diabetic foot ulcer for stem cell therapy group and control groups; M-H = Mantel-Haenszel method; CI = confidence interval. (Vertical line represents the point of no difference between the stem cell therapy group and control group. Squares = risk ratios; diamonds = pooled risk ratios for all studies. Horizontal lines = 95% CIs).
intermittent claudication, infection, recurrence, ulceration and major amputation. Conventional standard treatment, such as debridement and control of glucose, always take a long while to repair wound or failed to repair, or even wore lead to wound deterioration such as infection, local necrosis or amputation. A previous review showed with good standard wound care, in 12 weeks only 24 percentages wound healed [30]. Standard DFU treatment to some extent keep a moist wound to promise tissue regeneration, but it cannot repair the damaged microcirculatory system and/or the impaired release of growth factors [31]. Stem cells are known for their multi-potent ability to differentiate and for their extensive self-renewal. These features could overcome the aforementioned complications [32]. Several meta-analyses have shown that stem cell therapy may improve critical limb ischemia, although the analysis of the effect of stem cell therapy on diabetic patients has not been investigated to date. Thus, the present study was conducted in order to analyze the association between stem cell-based therapies and improved healing with reduction in the size of diabetic foot ulcers. This meta-analysis comprised 7 studies, involving 224 diabetic foot patients and aimed to compare the efficacy of stem cell-based therapy and conventional treatment. The results indicate that stem cell therapy is a more effective therapeutic strategy. Stem cell therapy can accelerate the healing of chronic skin ulcers in the diabetic foot patients and it can improve the microvascular regeneration around the wound area. In addition, as stated in the GCP guidelines for the conduct of clinical trials, the safety and well-being of the trial subjects and/or the patients are the most important considerations and should prevail over interest of science and society. The

Fig. 5 Forest plot and meta-analysis of mean difference between the stem cell therapy and the control groups, change of ulcer size area (cm²). CI, confidence interval; SD, standard difference.

Fig. 6 Forest plot and meta-analysis of the mean difference of the change in ABI between the stem cell therapy and the control groups. CI, confidence interval; SD, standard difference.

Fig. 7 Forest plot and meta-analysis of mean difference of change in TcPO2 between the stem cell therapy and the control groups, CI, confidence interval; SD, standard difference.
significant difference of the amputation rate between the stem cell and the conventional groups indicates that this novel treatment strategy provides greater safety. It is important to note that the prior- and -after treatment changes of the two commonly used prognostic indicators- ABI and TcPO2- were also significantly different between these two groups, suggesting that this procedure may have improved long-term effects. A total of 6 types of stem cells were included in the present analysis. The results that were derived from the study conducted by Dubsky et al. indicated that the bone marrow was a preferable source compared with the peripheral blood for the isolation of stem cells. Despite this advantage, peripheral blood progenitor cells are isolated more readily compared with bone marrow mononuclear cells. A subgroup analysis of different stem cells was attempted, although this type of analysis was restricted by the small number of studies. Since 1968 the first successful bone marrow transplantation stem cell based therapies have existed. Over 50 years numerous basic experiments had emerged. But only a little of them came into clinical care. In our analysis, we only obtained researches form restricted areas. There may be some reasons prevent form stem cells’ bench to besides. First, the clinical use of stem cell derived stem cell derived products are varies substantially between countries. For example in USA, Canada and EU the clinical trial are tightly regulated. In USA, to perform a clinical trial of human cell based therapies, permission from FDA is needed. The FDA considered autologous stem cells similarly to allogeneic stem cells, both of them are required multiple pre-clinical test of safety and efficacy [33]. The detailed regulation is in Title 21, part 1271 of the Code of Federal Regulations (C.F.R.). Second, the amounts of obtained stem cell form participants before the treatment are usually small. To enrich cell concentration, cell culture, passage and induced differentiation in vitro are necessary. That requires a relatively high-level laboratory technique which may restrain the application of such researches in some undeveloped areas. Besides, some stem cell may lose function or mutate due to improper induced process or too many passages. That’s a potential threat to the safety and efficacy of the treatment [12].

Third, religions such as Catholic and some Christian groups are object to stem cell use. Especially allogenic stem cells and embryonic stem cells, they believe that such produce offends the sanctity of life (Catholic doctrine 162). Considered nearly 25 percent of Americans believe the Catholic, the religion belief may be a potential barrier in the clinical research. Fourth, as the technique of stem cell therapy rapidly developing, several ethnic issues been raised too, that’s also the main reason hinder the application of embryonic stem cells. For other kind of MSCs, the main challenges was how to minimization the harm, how to face over expectations, how to solve therapeutic misconception and so on [34]. To all, the clinical use of stem cells is limited not only because of the issues of safety and efficacy, the political, legal, religion, socioeconomic and ethical factors should also take into consideration. The moving forward of stem cell therapy is the bench of creativity and caution, even make controversies exists, the development of it is essential and will benefit both science and patients in the future.

Although our analysis indicates the benefits of autologous stem cell-based therapy for diabetic foot ulcers, it cannot be confirmed that the optimized procedure is fully functional due to lack of data. Hence, larger sample sizes and thorough clinical studies are required in order to determine whether the source, the dose and/or the delivery method of stem cells are critical factors for the successful conduct of the procedure.

The limitations of the present meta-analysis areas were as follows: 1) the small number of studies included, which limits the analysis of publication bias; 2) the included studies did not distinguish diabetic foot from critical limb ischemia. Although all the patients included exhibited varying characteristics of foot pathologies namely, ulceration, neuropathic osteoarthropathy and peripheral arterial disease, the etiology and pathophysiology of the ulcers caused by critical limb ischemia and DM were different; 3) The criteria of healing were different among the 7 studies, with 5 of 7 using complete healing rate and the remaining 2 using ulcer size reduction as the measurement of healing. Some of the key data were missing, which did not allow us to analyze the wound healing baseline; 4) certain studies were poorly designed. Although the authors referred to their studies as random controlled trials, the random sequences were not mentioned and/or clearly described. In one study, not all participants experienced ulcers, whereas the number of patients with ulcers was not clarified. This could cause bias in the analysis. An additional potential limitation was that the failure to report the patient’s foot care practice and data by the studies investigated. Despite the exposed therapy, proper foot self-care agents, such as off-loading shoes and antimicrobial agents, can reduce the risk of ulcers and enhance wound closure [35, 36].
**Conclusion**

The currently available evidence derived from the studies analyzed confirms that stem cell therapy is a promising novel treatment strategy for diabetic foot patients [37]. It further provides evidence that stem cells may be associated with shorter recovery time, rapid tissue regeneration and lower limb amputation. Future studies should focus on optimizing the procedure for stem cell therapy, such as identifying the optimal dose, and the primal subgroup of stem cells. Furthermore, a larger sample size and well-designed, comprehensive clinical trials are required to confirm and update the findings of this analysis.

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**Conflict of Interest**

None of the authors have any potential conflicts of interest associated with this research.

**References**


