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Review

# Mesenchymal stem cells for diabetes mellitus treatment: new advances

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## Abstract

Mesenchymal stem cells (MSCs) are the most widely used stem cells of the human body due to ease of successful isolation and expansion for many years. In particular, from 2012 until now, MSCs have been widely clinically used to treat various diseases, including graft versus host disease (GVHD), Crohn's disease, and knee osteoarthritis. In this review, the applications of MSCs in diabetes will be reviewed and discussed. Diabetes mellitus type 1, also known as Type 1 diabetes (T1DM), is an autoimmune disease in which immune cells attack the beta cells in islets of Langerhans (pancreatic islets). Although type 2 diabetes (T2DM) is considered to be a disease related to insulin resistance, several recent studies have shown some relation of immune dysfunction in this disease. Therefore, MSC transplantation may be a beneficial treatment for both T1DM and T2DM. MSC transplantation in preclinical trials and clinical trials for T1DM and T2DM have shown a moderate to significant improvement in diabetes without adverse side effects. In this review, we will discuss some of the updates from preclinical and clinical trials of MSC transplantation for diabetes.

## Keywords

Diabetes mellitus, Mesenchymal stem cells, Stem cell therapy, Stem cell transplantation

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## Introduction

Diabetes mellitus (DM) is a metabolic disorder caused by deficient insulin secretion or insulin dysfunction leading to hyperglycemia as well as chronic metabolic change of carbohydrates, protein and fat (Bastaki, 2005). Chronic hyperglycemia leads to many serious complications, namely kidney failure, heart issue, and eye diseases. Thus, it considered a costly disease because a majority of countries spend 5% to 20% of their health expense on diabetes, according to the International Diabetes Federation (IDF) (IDF, 2015). Moreover, diabetes is a high-incidence disease. In 2004, there were more than 200 million DM patients; the predicted prevalence of DM is expected to double by 2025 (Halban, 2004). Also according to the IDF, of the approximate 7 billion people worldwide, about 415 million adults (aged 20-79) suffered from diabetes in 2015 and that number is expected to rise to 642 million people in 2040 (IDF, 2015). The Western Pacific region of the world has the highest prevalence of diabetes, with 153 million cases which account for about 37% of total worldwide cases.

Diabetes is classified into two main types (type 1 and type 2) which both lead to hyperglycemia (Moorefield, 2012). Diabetes mellitus type 1, or Type 1 diabetes (T1DM), is described as a genetic autoimmune disease. It is caused by a severe deficiency of insulin due to damage of pancreatic islet beta cells (Zhang et al., 2008). T1DM patients have to depend on exogenous insulin injection to stabilize their blood glucose. Diabetes mellitus type 2 (T2DM) accounts for 90-95% of diabetes cases, and is caused by insulin resistance in peripheral tissues. T2DM is related to many risk factors, such as obesity, hypertension, lifestyle, age, family history, past history of impaired glucose tolerance (IGT) and impaired fasting glucose (IFG), and gestational diabetes (GDM). Many of the risk factors can be preventable.

Diabetic specialists have been studying ways to optimize therapy for diabetes since current therapies have two major limitations. The first is the challenge of insulin injection; there is long-term dependency, difficulty in adjusting the exact amount of exogenous insulin appropriate for each moment, and potential of insulin resistance when used long-term. The second limitation is pancreas/ islet/ islet cell transplantation; there is the problem of donor shortage and non-availability, potential of transplant rejection, and difficulty to functionally activate and prolong grafted materials. Therefore, improved treatments for diabetes which can address the aforementioned limitations will have valuable application to both diabetes research and management.

Recently, stem cell therapy has achieved positive results in medicine related to the 3Rs (Replacement, Repair and Regeneration). Of note, 3R therapy uses stem cells and progenitor cells as effective tools to prevent, repair, replace and treat damaged organs. In fact, stem cell therapy has been applied successfully in diabetes, from preclinical to clinical studies (Dave et al., 2014; Dong et al., 2008;

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Gao et al., 2014; Le et al., 2016; Si et al., 2012; Thi-Tung Dang, 2015; Voltarelli et al., 2011; Wu et al., 2015; Wu et al., 2007; Xiao et al., 2013; Xie et al., 2016; Yang et al., 2010; Zhou et al., 2009). Stem cell transplantation is an excellent platform for diabetes therapy due to the fact that it can slow down the progression of diabetes and eliminate the complications of long-term blood glucose homeostatic effect.

The most popular stem cell type that has been evaluated in diabetes mellitus treatments have been mesenchymal stem cells (MSCs). These cells have great potential and it is feasible to isolate them, there is an abundant source, and ethical concerns are minimal. MSCs are isolated from various tissues, including adipose tissue, bone marrow, umbilical cord blood, umbilical cord and dental pulp (Pham et al., 2014; Ren et al., 2016; Van Pham et al., 2016).

## Mesenchymal stem cells

Mesenchymal stem cells (MSCs) have been demonstrated to be involved in the *in vivo* self-repair and self-regeneration processes of animal tissues. These cells can be isolated from different tissues such as bone marrow, adipose tissue, dental pulp, fetal appendages as well as umbilical cord blood, umbilical cord, and placenta. When cultured *in vitro*, they appear as spindle-shaped cells. MSCs express a specific marker profile; they are positive for CD29, CD51, CD73, CD90, and CD105 expression, yet negative for hematopoietic markers such as CD31 and CD45 (Wang et al., 2014). Interestingly, they do not express MHC class II and only express MHC class I at low levels. Moreover, they do not express Fas ligand and costimulatory molecules such as B7 and CD40, thus they have been suggested to be hypoimmunogenic cells (Atoui and Chiu, 2012). Other properties include their ability to self-renew, to create fibroblast colony forming units (CFU-F), and to differentiate into other cells such as bone, cartilage and fat. The aforementioned characteristics are part of criteria used to determine and identify MSCs (Dominici et al., 2006).

Although the term "mesenchymal" defines their origin and differentiation tendency, MSCs are flexible and can change *in vitro* to exhibit features of specific cells or progenitor cells belonging to the endoderm or ectoderm layers. From the culmination of many protocols focused on optimizing their culture, at present it is feasible to efficiently isolate and expand MSCs *in vitro* and *in vivo* (Ducret et al., 2016; Pham et al., 2014; Sensebe et al., 2011). Moreover, MSCs have been shown to play a role in the healing process in applications of MSC-based therapies for several diseases, such as heart dysfunction, neurodegenerative diseases, liver diseases, renal failure, and diabetes. Despite positive results of the therapy, it remains unclear as to the mechanisms exerted by MSCs. In this review, we will explore and discuss the role of MSCs in diabetic treatment.

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**Table 1.** Preclinical-trials of diabetes mellitus treatment by stem cell therapy

| Species | Type of DM | Cell types/ transplant route | Immune suppression | Duration | Results |
|---|---|---|---|---|---|
| Mice (Wang et al., 2011) | 1; NOD | UC-MSC-derived IPC/ retro-orbital vein | No | 23 days | Improved body weights after 7 days;<br>Lowered BG levels after 3 days; improved glucose tolerance at day 14;<br>Prolonged survival of treated mice while NOD mice had died before end point;<br>Human C-peptide and human cell nuclei appeared at the same sites within the lobules of mouse livers. |
| Mice (SCID) (Santamaria et al., 2011) | 1; STZ | ESSC-derived IPC/ renal subcapsular transplant | No | 35 days | BG levels were stablized in IPC-treated mice while ESSC-treated mice had peak increase in BG level at the early week 5 in the diabetic ones;<br>Improved weight loss in IPC-grafted mice;<br>Human insulin was only found in xenograft of IPCs and the serum of IPC-grafted mice with mean concentration 11.9 μIU/ml. |
| Mice NOD/ SCID (Phadnis et al., 2011) | 1, pancreactomize and STZ | BM-MSC-derived IPC/ renal capsule | No | 70 days | Transplanted IPCs could mature and secrete human c-peptide (insulin) in vivo;<br>IPCs transplantation normalized BG levels and maintained for up to 8 weeks thereafter;<br>Weight loss continued in the diabetic mice with 50% mortality by day 40 after the onset of diabetes whereas, IPC-grafted mice exhibited weight gain;<br>After removal of grafted IPC, mouse BG increased to high levels within 3 days after and 93% mice died within 3 weeks; no detectable human C-peptide in plasma; and all mice had BG levels above 350 mg/dL at 2 h of glucose challenge. |
| Mice (Ngoc et al., 2011) | 1, STZ | UCB-MSC-derived IPC/ portal vein | No | 30 days | Improved weight loss: a slight decrease in unencapsulated IPCs-grafted mice versus significant increase in encapsulated IPCs-grafted mice compared with strong decrease in body weight in diabetic control;<br>BG levels: obviously increased in unencapsulated IPC-grafted group whereas encapsulated IPC transplantation almost stabilized the BG levels;<br>Encapsulated IPC alleviated the immune response at day 15 and 30 compared with the unencapsulated IPC-treated group. |
| Mice (Ho et al., 2012) | 1; STZ | BM-MSC/ Tail vein infusion | No | 6 months | Safe;<br>Multiple Intravenous Transplantations were better than single injection;<br>Reduced systemic oxidative stress levels from week 17;<br>Markedly increased production of human insulin from week 11;<br>Blood sugar level normalized at the end of week 15;<br>At the end of 6 months, MSCs were proved to exist in liver tissues of the recipients and 51% of human cells in the recipient liver coexpressed human insulin. |

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| Mice (Kim et al., 2012) | 1; STZ | hAMSC-derived IPCs/ Kidney | No | 210 days | Safe; overcoming immune rejection for a long period;<br>Improved survival rate: 120 days after transplantation: group control: 0/12 alive mice; group hAMSC-treated: 0/12 alive mice and group hAMSC-IPC: 9/16 alive mice;<br>Restored body weight and maintained normoglycemia up to 210 days;<br>Detected human insulin and C-peptide in the blood of normalized mice after 2 months;<br>Human cell genes were detected in the transplanted kidneys. |
| Rat (Tsai et al., 2012) | 1; STZ | UC-MSC-derived IPCs/ portal vein | No | 8 weeks | Significantly decreased in BG levels at 4 weeks;<br>Diabetic rats sustained hyperglycemia;<br>Grafted cells were located in the liver. |
| Mice (Xiao et al., 2013) | 1; STZ | UC-MSC and CB-MNC/ Tail vein infusion | Yes | | Safe; Co-transplanted UC-MSCs and CB-MNCs at a ratio of 1:4 were effectively decreased the blood glucose levels in the first week and stabilized thereafter (7/10 mice);<br>Detected Alu sequence indicated that the human cells had homed into the recipient's pancreas and kidney. |
| Mice (Kanafi et al., 2013) | 1; STZ | SHED and DPSC-derived IPC/ subcutaneous transplantation | No | 10 weeks | 90% transplanted diabetic mice survived and normalized BG levels within 2 weeks and maintained 2 months or prolonged normoglycemia even after graft removal; also their body weight and glucose level in urine became normal;<br>The diabetic mice remained hyperglycemia and reduced body weight and died within 10 – 13 days after STZ injection. |
| Mice (Dao et al., 2014) | 1; STZ | PDPCs or PDPC-derived-IPCs/ Renal capsule | No | 8 weeks | Decreased BG levels and improved glucose tolerance in cell-treated groups at 4 weeks and 8 weeks;<br>Detected human insulin in the serum and kidney sections of IPC-grafted mice. |
| Rat (Boroujeni and Aleyasin, 2014) | 1, Alloxan | UC-MSC-derived IPCs/ intraperitoneal transplant | No | 17 days | The BG levels of MSC-treated rats were decreased at day 10 and increased thereafter;<br>Transduced MSC-treated rats showed normalized BG within 3–4 days.<br>Diabetic rats sustained hyperglycemia and died within 6 months. |
| Rat (Hu et al., 2014) | 2, STZ with HFD | WJ-MSC/ intravenous infusion | No | 10 weeks | Decreased significantly in BG levels at 2 weeks and modestly increased thereafter;<br>Improved fasting C-peptide, glucagon and HbA1c levels after 10 weeks of transplantation;<br>The number of beta cells in MSC-treated rats were more than two-fold compared with diabetic rats. |
| Rat (Tsai et al., 2014) | 1; STZ | BM-MSC-derived IPC/ portal vein | No | 8 weeks | Decreased in BG levels at 1 week, reached <250 mg/dl for 6 weeks;<br>Decrease slowly in the body weights;<br>Grafted cells were localized in the recipient's liver. |
| Mice (Zhang and Dou, 2014) | 1, STZ | BM-MSC-derived IPC/ right-side testis | No | > 80 days | Normalized BG of diabetic mice for at least 80 days following xenograft.<br>Blood glucose of grafted mice rose again after their graft removed.<br>Human insulin existed in recipient mice. |
| Mice (Gabr et al., 2015) | 1, STZ | BM-MSC-derived IPC/renal subcapsular space | No | 12 weeks | The differentiated cells expressed low levels of pancreatic endocrine genes;<br>Increased the percentage of IPCs among transplanted cells from under ≤3% to approximately 18% at 4 weeks;<br>Normalized BG after 8±3 days of transplantation;<br>Increased expression of insulin, glucagon, and somatostatin genes. |

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| Mice (Hu et al., 2015) | NOD | WJ-MSC/ intravenous infusion | No | 18 weeks | Normalized FPG and fed blood glucose in 6-8 days and maintained for 6 weeks;<br>Improved level of fasting C-peptide of these mice;<br>Protected for 8-week delayed onset of diabetes;<br>Increased the number of the $CD4^+CD25^+Foxp3^+$ Tregs in WJ-MSC-treated group;<br>Decreased the levels of IL-2, IFN-c, and TNF-a;<br>Depressed the degree of insulitis. |
| Mice (Tsai et al., 2015) | NOD | WJ-MSC/ retro-orbital vein | No | 23 days | Increased survival rate;<br>Significantly decreased BG three days after transplantation; at day 23, human C-peptide and serum insulin levels increased and glucose tolerance improved;<br>Reduced T helper 1 cells; IL-17-producing T-cells and dendritic cells;<br>Increased Tregs number and anti-inflammatory cytokine levels;<br>The MSC could differentiate into IPC in vivo and performed the function in tissue repair. |
| Rat (Yu et al., 2015) | pancreatectomy | UC-MSC-derived IPCs/ portal vein | No | 56 days | Decreased BG two weeks after transplantation (18.7 ± 2.5 mmol/L in treated group vs. 25.8 ± 1.25 mmol/L in diabetic rats);<br>On day 56, glucose tolerance tests showed that after 45 min, BG levels were significantly lower (12.5 ± 4.7 mmol/L in treated group vs 42.2 ± 9.3 mmol/L in diabetic rats). |
| Rat (Zhou et al., 2015) | 1, STZ | UC-MSC/Tail vein infusion | No | 42 days | BG levels started to decrease at day 6 and modestly lowered thereafter;<br>Restored islet structure: with the size at 63% and cell number at 42% or the normal rat;<br>Human cell existed in the recipient's pancreas and activated the pancreatic PI3K pathway and its downstream anti-apoptotic signal;<br>Induced the trophic effects on islets via the expression of β-cell growth factor genes and proteins. |
| Rat (Xie et al., 2016) | 2, STZ with HFD | UC-MSC/ Tail vein infusion | No | 7 days | Improved glucose homeostasis and promoted insulin sensitivity;<br>Alleviated insulin resistance by producing IL-6 that induced M2 polarization;<br>Obesity and insulin resistance were associated with increased pro-inflammatory adipose tissue macrophages infiltration. |
| Mice (Xin et al., 2016) | 1, STZ | BM-MSC or IPC/ renal sub-capsular space | No | 21 days | BG levels of the IPC-treated mice were normalized within 6 days and maintained throughout 21 days; whereas MSC-treated mice remained hyperglycemia;<br>Transplanted IPCs expressed insulin, C-peptide, and PDX-1 without apparent apoptosis in vivo. |

## Mesenchymal stem cells and diabetes mellitus

It is known that MSCs are active participants in the healing process of damaged tissues. Although they are present and can be isolated from diabetic human and animal tissues, the repair process is limited due to inadequate and impaired functions of MSCs (Cianfarani et al., 2013; Tobita et al., 2015). Adult MSCs can proliferate and differentiate *in vitro* by the effects of several soluble and non-soluble factors in the culture medium. A diabetic or hyperglycemic environment reduces the proliferative potential of MSCs yet increases senescence and

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apoptosis of the cells (Cheng et al., 2016; Cianfarani et al., 2013; Cramer et al., 2010; Hankamolsiri et al., 2016; Kim et al., 2015; Wajid et al., 2015). Moreover, the differentiation and immunomodulation capacity of diabetic or high glucose treated MSCs are distinct from normal cells (Montanucci et al., 2016). A high glucose condition boosts adipogenic differentiation while decreasing osteogenic differentiation (Cramer et al., 2010; Hankamolsiri et al., 2016) and diminishing angiogenic capacity (Kim et al., 2015) of affected MSCs.

Interestingly, a small proportion (6%) of diabetic MSCs express proinsulin and C-peptide and these cells can differentiate rapidly into functional islet-like cells in the presence of a pseudo diabetic milieu (Phadnis et al., 2009). It has been suggested that the diabetic microenvironment may be a condition for MSC differentiation into insulin-producing cells *in vitro* and *in vivo*. Moreover, expression of stem cell specific surface markers on diabetic MSCs decreased (Cianfarani et al., 2013) while expression of pluripotent markers increased (Cheng et al., 2016). Additionally, hyperglycemia conditioned MSCs showed a reduction of cell migration (Cheng et al., 2016; Cianfarani et al., 2013) and of secretion of growth factors related to wound healing (Cianfarani et al., 2013). Therefore, the reduced expression of certain important features, such as the above, warrants more investigations in MSC applications for diabetes treatment.

## Mesenchymal stem cell transplantation for diabetes mellitus

It is known that MSCs play a crucial role in healing damaged tissues. They can differentiate to replace the dead cells as well as secrete stimulant factors to activate surrounding cells in the microenvironment, enhancing the tissue repair process (Wang et al., 2014). Therefore, MSCs can be applied to treat tissues impaired by chronic hyperglycemia. For T1DM, MSC transplantation can theoretically increase beta cell mass via the following effects:

(1) beta cell replacement through *in vitro* or *in vivo* differentiation;

(2) local microenvironment modification by production of cytokines, chemokines and factors to stimulate endogenous regeneration;

(3) reduction or prevention of autoimmunity to beta cells (Ezquer, 2014). Although several MSC transplantation studies have clearly shown the outcome of controlled glucose metabolism, there have been observations of decreased insulin resistance as well as enhanced beta cell function effects. Moreover, the mechanisms of MSC treatment for T2DM still has not been well understood. Some studies have suggested that the immunomodulatory and inflammatory effects of MSCs are what contribute to the resulting reduction of insulin resistance (Liu et al., 2014; Xie et al., 2016).

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## Safety of human mesenchymal stem cell transplantation

The first concern of any therapy is the risk of mortality. Similar to modern generation drugs, MSC-based therapies should be controlled and monitored for safety before their positive effects are determined. In diabetic animal models, graft rejection as well as acute adverse responses or sudden death were not noticed after xenograft MSC and/or differentiated MSC transplantations (Gabr et al., 2013; Ho et al., 2012; Hu et al., 2015; Lee et al., 2006; Xie et al., 2016). Interestingly, graft tolerance was observed when human cells were transplanted into diabetic mice without immunosuppressive treatment (Chao et al., 2008; Zhou et al., 2015). Moreover, MSC treated diabetic rodents survived and showed a prolonged life relative to non-treated diabetic animals (Kadam et al., 2010; Tsai et al., 2015). MSC transplantation was proven to be safe and well-tolerated in a small cohort of T2DM patients although approximately 22% of them had slight transient fevers (Kong et al., 2014).

## Efficacy of human mesenchymal stem cell transplantation

### Preclinical treatment

Many studies have shown that human MSCs (hMSCs) or/and hMSC-derived islet-like cells effectively safety transplanted into diabetic animals (Ho et al., 2012; Kim et al., 2012) (**Table 1**).

Human MSC transplantations lead to alleviated blood glucose levels in diabetic animals. It was confirmed that hMSC infusion could improve blood glucose homeostasis in both type 1 and type 2 diabetic animals. The glucose levels decreased markedly from a few days to 2 weeks after hMSC infusion and this decrease was maintained until 20 days to 10 weeks thereafter (Ammar et al., 2015; Ho et al., 2012; Hu et al., 2014; Hu et al., 2015; Kadam et al., 2010; Lee et al., 2006; Tsai et al., 2015; Xie et al., 2016; Yang et al., 2010; Zhou et al., 2015). Multiple transplantations of hMSCs could improve the effects of hyperglycemia (Ho et al., 2012; Lee et al., 2006). Glucose levels were notably normalized after seven administrations of hMSCs (Ho et al., 2012).

Other research studies have shown the efficiency of hMSC derived insulin-producing cells (IPCs) on diabetic treatments. These cells could be *in vitro* differentiated from many sources of hMSCs and exhibited many characteristics of actual beta cells, including C-peptide expression, insulin production, glucose response ability as well as pancreatic beta cell specific gene expression (Gabr et al., 2013; Kao et al., 2015; Kim et al., 2015; Seyedi et al., 2015; Seyedi et al., 2016; Thi-Tung Dang, 2015; Van Pham et al., 2014; Zhang and Dou, 2014). The IPCs were transplanted into liver (Chao et al., 2008), renal capsule (Hu et al., 2009; Kadam et al., 2010); blood glucose levels were reduced 3 days thereafter and normalized at more than 9 weeks out or until removal of graft (Chao et al., 2008; Kadam et al., 2010; Zhang et al., 2010). However, some results have shown that hIPC transplantations does not lower glucose levels (Hu et al., 2009; Ngoc et al., 2011).

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## Clinical applications

Although many preclinical studies have shown evidence that hMSC therapy has beneficial effects in the treatment of diabetes mellitus, there are still not many clinical applications of MSC therapy for T1DM or T2DM in the world. The first problem is due to the safety issue of MSC transplantation (**Table 2**). However, this can be alleviated by understanding the special characteristics as well as *in vitro* and *in vivo* behavior of MSCs through experimental studies and evidence. MSCs themselves show unchanged morphology and phenotype as well as the normal karyotype. They also express tumor suppressors and oncogenes at normal levels even after they undergo long-term culture. There is no evidence that tumor formation is associated with MSC transplantation. Additionally, most of the clinical trials have proven that MSC transplantation for the treatment of diabetes is safe although there have been a few reported cases of fever. However, it is difficult to elucidate whether the fever was caused by the cell transplantation or from diabetic symptoms or certain infections.

The second problem or issue is whether MSC therapy is effective for the treatment of DM in humans. Although the mechanism has not been clearly demonstrated, MSC transplantation is capable of reducing blood glucose in various periods of follow-up time from a few months to several years. Moreover, it has been suggested that MSC transplantation can normalize or maintain the ameliorated blood glucose levels as well as improve serum insulin levels, C-peptide, HbA1C, and the daily insulin requirement for a long period. Beside evidence of these systemic effects, MSC transplantation has the potential to treat diabetic complications such as foot ulcers, thrombosis, heart failure, kidney failure, and blindness. Therefore, it is meaningful that MSC therapy can be applied to treat early and/or late stages of diabetes as well as relieve the pain of complications and delay or cease the need for amputations. Moreover, based on the successful clinical trials, many MSC therapies continue to be developed to improve the efficiencies of the following:

(1) prolonged time effect;

(2) reduced expenditure on treatment and increase in patients treated; and

(3) upgrade in cell products, from abundance and availability (commercial distribution) to development of diverse sources of MSCs and improvement of identifiable therapeutic characteristics of MSCs (e.g. immunomodulatory potential).

Moreover, MSCs have been suggested as universal therapeutic cells (Atoui and Chiu, 2012) based on their immune privilege and are being developed as ready-to-use products (e.g. Prochymal). Finally, the mechanisms related to MSC therapy, while still unclear, are gradually being discovered through more research findings. As of now, pertinent questions remain such as:

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**Table 2. Clinical applications of stem cell transplantation for diabetes mellitus**

| Type of DM | Cases | Cell types | Methods | Immune suppress | Duration | Status | References |
|---|---|---|---|---|---|---|---|
| 1 | 5 | ASC and cultured BM | Autologou, intraportal | No | 2.9 months | Safe<br>30% to 50% decreased insulin requirements<br>4- to 26-fold increased serum c-peptide levels | (Trivedi et al., 2008) |
| 1 | 15 | UCB | Autologou,Intravenous infusion | No | > 6 months | Safe<br>Provides some slowing of the loss of endogenous insulin production<br>increased Treg populations | (Haller et al., 2008) |
| | 24 | UCB | Autologou, Intravenous infusion | No | 24 months | Completed clinic trial in 2013<br>Safe<br>Increased regulatory T cells (Tregs) and naive Tregs 6 and 9 months after treatment;<br>Fails to preserve C-peptide. | NCT00305344 |
| 1 | 11 | hASC-IPC and cultured BM | Allogenic, omental vein infusion | N/A | mean follow-up of 23 months | Safe<br>Decreased mean exogenous insulin requirement to 0.63 units/kgBW/day;<br>Decreased Hb1Ac to 7.39%;<br>Raised serum C-peptide levels to 0.38 ng/mL;<br>Became free of diabetic ketoacidosis events. | (Vanikar et al., 2010) |
| 1 | 63 | PROCHYMAL® (human MSC) | Allogenic, Intravenous infusion | N/A | 2 years | Complete<br>Phase 2 | NCT00690066 |
| 2 | 10 | Placenta-derived MSC | Allogenic, 3 intravenous infusion | N/A | 6 months | Safe<br>Decreased insulin dose from 63.7±18.7 to 34.7±13.4 IU;<br>Increased C-peptide level from 4.1±3.7 ng/mL to 5.6±3.8 ng/mL<br>The renal function and cardiac function were improved after infusion. | (Jiang et al. 2011) |
| 1 | 15 | UCB-MSC (Stem Cell Educator therapy) | Allogenic, Intravenous infusion | N/A | 40 weeks | Phase 1/ phase 2 study;<br>Safe;<br>Reduced HbA1C values;<br>Decreased the median daily dose of insulin;<br>Increased basal and glucose-stimulated C-peptide levels through 40 weeks;<br>Increased expression of CD28 and ICOS;<br>Increased the number of CD4+CD25+Foxp3+ Tregs, and restoration of Th1/Th2/Th3 cytokine balance | NCT01350219 |
| 1, 2 | 30 | BM-MSC bone marrow enriched CD90+ cells | Autologou, Intramuscular injection | N/A | 45 weeks | safe and feasible<br>Improvements of microcirculation and complete wound healing;<br>18/22 patients showed wound healing after 45 weeks. | NCT01065337 |
| 2 | 118 | BM-MNC | Autologous, Intra-pancreatic injection | N/A | 36 months | Improved C-peptide levels. | (Hu et al. 2012) |

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| 2 | 36 | UCB-MSC (Stem Cell Educator therapy) | Allogenic, Intravenous infusion | | 12 months | Phase 1/ phase 2 study;<br>Safe;<br>HbA1C reduced from 8.61%+/-1.12 to 7.25% +/-0.58 at 12 weeks, and 7.33%+/-1.02 at one year post-treatment;<br>Insulin sensitivity was improved post-treatment;<br>Reversed immune dysfunctions through immune modulation on monocytes and balancing Th1/Th2/Th3 cytokine production. | NCT0141 5726 |
| 1 | 29 | UC-MSC | Allogenic, Intravenous transfusion | N/A | 21 months | Safe and effective;<br>Fasting plasma glucose was not significant difference between treated and control group;<br>HbA1c reached the lowest level at the sixth month (baseline 6.8 ± 0.57%, 6 months 5.5 ± 0.67%);<br>C-peptide achieved the highest at the end of first year and slight decrease afterthat but still remained better than baseline. | (Hu et al. 2013) |
| 2 | 21 | BM-MSC | Autologous | N/A | 12 months | The insulin requirement decreased by 66.7% in the intervention group and 32.1% in controls;<br>9/11 (91%) patients could maintain HbA1c <7% in the intervention group, whereas 6/10 (60%) in the control group. | (Bhansali et al. 2014) |
| 1 | 20 | BM-MSC | Autologou, Intravenous infusion | No | 12 months | Phase 1 complete<br>Safe;<br>C-peptide peak values and C-peptide were preserved or even increased in the MSC-treated patients | NCT0106 8951 |
| 1 | 42 | UC-MSC and BM-MNC | Allogenic UC-MSC and autologous BM-MNC, intra-arterial pancreatic infusion | N/A | 12 months | Phase 1/ phase 2<br>Safe Improved metabolic measures:<br>AUCC-Pep increased 105.7%, insulin area under the curve increased 49.3%, HbA1c decreased 12.6%, fasting glycemia decreased 24.4%.<br>Daily insulin requirements decreased 29.2% | NCT0137 4854 |
| 2 | 18 | UC-MSC | Intravenous transfusion | N/A | 6 months | 4/18 patients (22.2%) had slight transient fever.<br>Safe and well tolerated;<br>Alleviated blood glucose; and increased the generation of C-peptide levels and Tregs in a subgroup of T2DM patients. | NCT0141 3035 |

(i) how do MSCs survive,

(ii) how do MSCs behave in live bodies, and

(iii) what reactions and responses occur after cell transplantation over time. Therefore, studies of MSCs and their application for the treatment of diabetes are valuable to understand the underlying mechanisms.

## What role do hMSCs play after they are administrated in diabetic bodies?

### Survival

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Labeled MSCs have been transplanted into diabetic mice to determine their survival in body tissues. It was confirmed that the grafted cells presented and secreted functional human insulin and C-peptide (Xin et al., 2016; Zhang et al., 2010). Hess *et al.* found approximately 2.5% insulin-positive cells in diabetic mice whereas the cells were absent in the normal pancreas (Hess et al., 2003). Lack of certain surface antigens on MSCs may result in a lowered immune responses in recipients as described above (safety issue).

## Differentiation potential and cell replacement

It has been shown that MSC transplantation can ameliorate blood glucose levels within the follow-up time from few weeks to several years. However, the underlying mechanism of this effect is still unclear. One proposed mechanism is the replacement potential of transplanted MSC-derived cells. MSCs are able to trans-differentiate into insulin-producing cells and have been suggested to compensate impaired beta cells in diabetic animals. It is necessary to carefully determine the surrogate capability of MSCs. Although MSCs can be induced to secrete insulin *in vitro* and/or *in vivo*, few MSCs become fully functional beta cells *in vivo* (only approximately 1.7 – 3% of transplanted cells). Moreover, the transient survival of transfused cells confirms that the replacement potential may not be the only mechanism of the therapeutic effect of MSCs. Sordi *et al.* have suggested the role of MSCs as helper cells when they observed normalized blood glucose levels and neovascular formation after co-transplantation of pancreatic MSCs and islet mass (Sordi et al., 2010).

## Immune modulation - an important property of MSCs

Besides the differentiation potential, MSCs have the unique potential to modulate immune responses via several mechanisms. The *in vitro* immunosuppressive capability of MSCs was investigated since the late 1990s (Wang et al., 2014). When co-cultured with leukocytes, it is found that MSCs are able to alter the proliferation of several immune cells. They are able to inhibit B lymphocytes (Corcione et al., 2006), NK cells (Spaggiari et al., 2008), inhibit differentiation and function of monocyte-derived dendritic cells (Jiang et al., 2005), and suppress the activation and proliferation of T cells (Nauta and Fibbe, 2007). These results have been confirmed by others (Atoui and Chiu, 2012; Mattar and Bieback, 2015; Spaggiari et al., 2008; Wang et al., 2014; Yaochite et al., 2016; Zhao et al., 2010). In particular, MSCs are able to induce macrophages to convert from proinflammatory type 1 to anti-inflammatory phenotype, resulting in alleviation of insulin resistance in T2DM (Wang et al., 2014; Xie et al., 2016). Moreover, it is specifically described that MSCs can alter the ratio of regulatory T cells versus other subtypes of T cells while capable of inducing the generation of regulatory T cells (Tregs) and simultaneously inhibiting the proliferation of T helper 1 (Th1) and Th17 cells (Luz-Crawford et al., 2013). MSCs are capable of suppressing autoimmune responses in T1DM. Interestingly, these effects appear after direct interaction between MSCs and immune cells whereas the microenvironment components do not alter the immunomodulatory capability of MSCs *in vitro* (Wehner et al., 2013).

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It has been proposed that MSCs are not only multipotent adult stem cells but also universal donor cells due to their ability to avoid immune rejection (Atoui and Chiu, 2012). Moreover, systemic administration of MSCs derived from autologous or allogeneic or even xenogeneic sources have been reported which can create non-specific systemic immunosuppression (Nauta and Fibbe, 2007). The transplanted MSCs could survive and differentiate in allogeneic or xenogeneic recipients due to their immunotolerance capability. Atoui *et al.* have suggested that the underlying mechanism of immunotolerance capability of MSCs is due to their hypoimmunogenicity, T cell modulation and microenvironment immune suppression (Atoui and Chiu, 2012). Consequently, transplanted MSCs can be tolerated (in part) in the recipients and can induce local pancreatic stem cells to proliferate, leading to replacement of impaired cells in the diabetic animals.

It has been found that MSCs can produce many cytokines and factors which improve and modulate the surrounding microenvironment. These components include inflammatory cytokines, immunosuppressive molecules and growth factors responsible for the tissue repair process (Ma et al., 2014). The presence of inflammatory cytokines can initiate the immunomodulatory effects of MSCs via immunosuppressive factors such as nitric oxide (NO) in mice and indoleamine 2,3-dioxygenase (IDO) in humans, along with IL-10, TSG6, IL-6, LIF, PGE2, HO-1 and truncated CCL2 (Ma et al., 2014). The immunomodulatory ability of MSCs is supposedly plastic since it depends on the inflammation status and cytokine status (Ma et al., 2014; Wang et al., 2014). Both NO and IDO play a role as a switch; strong inflammation drives MSCs to induce immunosuppression while weak inflammation enhances immune responses induced by MSCs (Wang et al., 2014). Therefore, it is necessary to determine inflammation and cytokine levels as well as immunosuppressants to optimize the procedure of MSC transplantation.

Notably, it is perceived that MSCs derived from various tissues have different capabilities of immunomodulation (Mattar and Bieback, 2015). This difference may derive from their origins or different culture conditions (Menard et al., 2013).

## Conclusion

Mesenchymal stem cells possess unique properties which make them suitable candidates for use in diabetes mellitus treatment strategies. Besides their potential to differentiate into various types of cells (e.g. beta cells), they also possess the ability to modulate immunity and angiogenesis via secreted paracrine factors. For Type 1 diabetes, the effects of MSCs on immune modulation are clearly evident; for Type 2 diabetes, some of the mechanisms exerted by MSCs are still unclear. However, for both T1DM and T2DM, MSC transplantation can dramatically improve the blood glucose levels, while reducing the insulin dose and side effects associated with DM. More importantly, both in preclinical trials and clinical trials, MSC transplantation has been

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demonstrated to be safe; there have been no observed adverse side effects or tumorigenesis. The data thus far have suggested that MSC transplantation is a promising therapy for DM. More controlled and randomized clinical trials are needed to further optimize the stem cell transplantation process.

## Abbreviations

AMSC : Amniotic mesenchymal stem cell; BG: Blood glucose; BM: Bone Marrow; DPSC: Dental pulp stem cells from permanent teeth; DM: Diabetes mellitus; ESSC: Endometrial stromal stem cells; HFD: High fat diet; IPC: Insulin Producing Cell; MNC: Mononuclear Cell; MSC: Mesenchymal Stem Cell; NOD: Non-obese diabetic; PDPC: Periosteum-Derived Progenitor Cells; SCID: Severe Combined Immunodeficiency; SHED : Stem cells from pulps of human exfoliated deciduous teeth; STZ: Streptozotocin: T1DM: Type 1 Diabetes mellitus; T2DM: Type 2 Diabetes mellitus; UC: Umbilical Cord; UCB: Umbilical Cord Blood; WJMSC: Wharton's Jelly Mesenchymal Stem Cell.

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## Author contribution

Ngoc Kim Phan has written the preclinical results showed in Table 1. Kiet Dinh Truong has written the clinical review. Loan Thi-Tung Dang has written the rest and completed and edited the review

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