

Mesenchymal Stem Cells in the Treatment of Type 1 Diabetes Mellitus

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Abstract Diabetes mellitus type 1 is a form of diabetes mellitus that results from the autoimmune destruction of insulin-producing beta cells in the pancreas. The current gold standard therapy for pancreas transplantation has limitations because of the long list of waiting patients and the limited supply of donor pancreas. Mesenchymal stem cells (MSCs), a relatively new potential therapy in various fields, have already made their mark in the young field of regenerative med-

icine. Recent studies have shown that the implantation of MSCs decreases glucose levels through paracrine influences rather than through direct transdifferentiation into insulin-producing cells. Therefore, these cells may use pro-angiogenic and immunomodulatory effects to control diabetes following the cotransplantation with pancreatic islets. In this review, we present and discuss new approaches of using MSCs in the treatment of diabetes mellitus type 1.

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Introduction

Diabetes mellitus type 1 (also known as diabetes type 1 or T1DM) is a form of diabetes mellitus that results from the autoimmune destruction of the insulin-producing beta cells in the pancreas. This is a typical chronic condition that demands permanent daily insulin administration to control blood glucose levels in the range of acceptable limits. T1DM affects 5–10 % of the total diabetic population. Insulin injection, the predominant treatment for T1DM, is effective to ameliorate hyperglycemia, but not sufficient to relieve the autoimmunity associated with T1DM or to facilitate the regeneration of lost islets. The resulting lack of insulin leads to hyperglycemia and its devastating complications, including cardiovascular disease, blindness, kidney failure [1], and amputations [2]. One possible treatment option is pancreatic transplantation. Indeed, whole organ pancreas transplantation is very effective in restoring normoglycemia and maintaining long-term physiological glycemic control. Simultaneous pancreas and kidney transplantation is presently considered the standard therapy for patients with T1DM with end-stage renal failure. However, there is a long waitlist of individuals awaiting pancreas

transplant, and the procedure itself requires the administration of immunosuppressive therapy, which is associated with several major side effects. Islet transplantation is a less invasive procedure compared to the transplantation of the whole pancreas and is associated with a shorter hospital stay and lower morbidity [3]. Islet cell transplantation is based on the isolation of islets from their surrounding tissue and subsequent implantation in the recipient liver [4, 5]. The method entails the enzymatic and mechanical separation of islets from the rest of the organ [6]. Because of the different densities of islet cells in comparison with acinar tissue, one can use the centrifugation procedure to enrich the layers of high purity that are infused intraportally into the liver of the patients. Here, they lodge and can revascularize in a few weeks [4, 5, 7].

Clinical islet cell transplantation may result in a reduced requirement for insulin injections and may decrease hypoglycemic episodes without the morbidities associated with surgery. Unfortunately, islet cell transplantation was not able to achieve results comparable to those of solid organ transplantation until the Edmonton protocol (steroid-free immunosuppression) was devised; this protocol permitted islet cell transplantation to be an appropriate alternative to pancreatic transplantation. Significant advances in islet purification techniques and novel immunomodulatory agents have since renewed the interest in islet cell transplantation. On the other hand, islet cell transplantation is still limited by problems with the supply of islet cells, inadequate engraftment, and the deleterious effects of chronic immunosuppression [8].

As another alternative, the implantation of stem cells may be a potential treatment of T1DM [9, 10]. Stem cells can be prepared from embryonic or adult stem cells. However, the use of embryonic stem cells is controversial, since the collection of such cells is achieved by killing embryos. For this reason, the acquisition of embryonic stem cells is banned in many states or excluded from public funding. Interestingly, Klimanskaya and co-workers have developed a single-cell biopsy method that will not cause embryonic death [11]. This may provide an alternative method to avoid such regulatory issues. Moreover, while embryonic stem cells have great potential to differentiate into insulin-producing cells, they also have tumorigenic potential when transplanted [12]. Mesenchymal stem cells (MSCs), derived from the bone marrow or other sources, are being extensively investigated in the clinical setting for their immunomodulatory and tissue regenerative properties [13, 14]. MSCs have already been tested in some feasibility studies in the context of islet transplantation [15] and can be used to improve engraftment of pancreatic islets by suppressing inflammatory damage and immune-mediated rejection. In addition to their immunomodulatory effects, MSCs are known to provide a supportive microenvironmental niche by secreting paracrine factors and depositing extracellular matrix [16].

Mesenchymal Stem Cells

MSCs are multipotent, self-renewing cells that not only are primarily localized in the bone marrow but can also be isolated from other tissues, including adipose tissue [17], skeletal muscle [18], amniotic fluid [19], and umbilical cord blood [20]. Moreover, MSCs can be expanded for several passages without losing their self-renewing capacity [21]. The International Society for Cellular Therapy has provided criteria for defining MSCs. MSC populations are able to adhere to plastic in culture and exhibit expression of cell surface markers, such as CD105, CD73, and CD90. MSCs also lack the expression of CD45, CD34, CD14 or CD11b, CD79a, or CD19 and HLA-DR surface molecules [22, 23].

MSCs have been well characterized for their ability to differentiate into several cell types of mesenchymal origin, such as osteoblasts, adipocytes, and chondrocytes [22]. However, MSCs have also been shown to be able to differentiate into cell types of endodermal and ectodermal lineages [23], including renal tubular cells [24], lung epithelial cells [25], skin [26], neural cells [27], hepatocytes [28], and insulin-producing cells [29]. Despite this multipotency, the contribution of MSCs to the formation of functional tissue through transdifferentiation processes is still controversial [30].

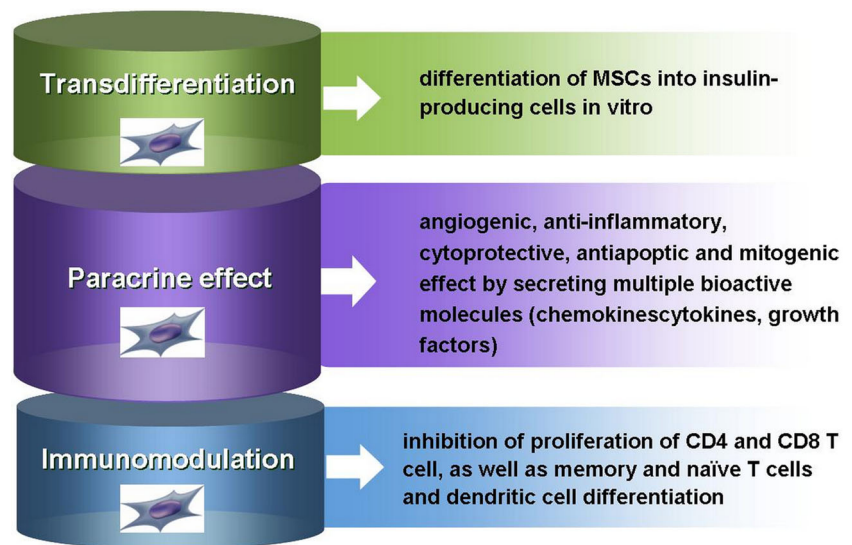
Recently published studies have shown promising results with intraportal transplantation of bone marrow MSCs. Indeed, MSCs have been shown to stimulate liver regeneration after extensive liver resection, thereby ameliorating liver fibrosis and acute liver failure [31–33]. Because the harvesting of bone marrow MSCs is relatively simple, these MSCs have tremendous therapeutic potential in tissue repair and organ regeneration [34, 35]. Therefore, MSCs can also exert important reparative effects. These effects are often achieved through migration to the site of injury [36] and the release of paracrine factors that affect the migration, proliferation, and survival of the surrounding cells [37].

In addition, MSCs have been shown to contribute to repair processes through the secretion of pro-angiogenic molecules, thus promoting the formation of new blood vessels *in vivo* [38]. MSCs have emerged as a useful cell population for immunomodulation therapy due to their ability to secrete a large amount of bioactive molecules that affect immune and inflammatory responses [23, 39, 40]. The combination of immunomodulatory or immunosuppressive activity and tissue regenerative potential remains a significant scientific and clinical interest, and β -cell replacement therapy via stem cell transplantation may be a promising treatment for T1DM (Fig. 1).

Mesenchymal Stem Cell Sources and Safety

In order to utilize MSCs in a translational fashion, it is important to expand these cells *ex vivo* in order to obtain sufficient

Fig. 1 A schematic model demonstrating the biological properties of MSCs in cell therapy of T1DM



quantities for therapeutic use. Compared to other stem cell sources, such as hematopoietic stem cells (HSCs) or stem cell populations from the liver, spleen, and pancreas, MSCs seem to be a promising source for overcoming autoimmunity in T1DM because of their ability to transdifferentiate into various different lineages, including endodermal lines, and their particular immunosuppressive capacities [41]. MSCs from human bone marrow and adipose tissue (AT) represent very similar cell populations with comparable phenotypes [42]. The discovery of multipotent MSCs within AT has established a second major source of MSCs. Thus, MSCs with the potential to adopt a pancreatic endocrine phenotype could also exist in human AT. Besides having a comparable degree of mesodermal differentiation potential, AT-derived MSCs also appear to have higher frequencies and a high potential for angiogenesis or vasculogenesis compared to that of bone-marrow-derived MSCs [43]. Although these were only preliminary results, Timper et al. reported that insulin-producing cells could be generated from adipose-derived MSCs [44]. Finally, the human umbilical cord blood is another source of stem cells with the potential to develop into insulin-producing cells, even in vivo [20].

Human MSC transplantation could be practiced with minimal risks compared with HSC or bone marrow transplantation due to the hypo-immunogenicity of MSCs. This supports the hypothesis that MSCs are immune-privileged, which may be explained by their low expression of MHC proteins and T cell costimulatory molecules [45]. Allogeneic MSCs also have some advantages, such as immediate availability, no limitations in numbers, the possibility of donor selection based on different parameters, rapid performance of quality control tests, and product stability. Ongoing clinical trials are evaluating the effects of bone-marrow-derived MSCs for engraftment and survival of transplanted islets as well as the possibility of preventing complications from type 1 and type 2 diabetes

[46]. In addition to their immunomodulatory effects, MSCs are able to participate in islet regeneration on the basis of their capacity to generate insulin-producing cells [47, 48]. Thus, the regenerative and immunomodulatory properties of allogeneic MSCs make them natural candidates for the treatment of diabetes [40].

Stem cells possess some features of cancer cells, including long lifespan, apoptosis resistance, and the ability to replicate for extended periods of time with the same control mechanisms. Therefore, stem cells may undergo malignant transformation, which is often a key obstacle to the safe use of stem-cell-based medicinal products. MSC-based therapy has also already been used in human disease settings, such as graft-versus-host and cardiac disease, with initial reports indicating a good safety profile. No tumors have been found in human recipients of MSCs thus far, and remarkably, even aneuploid MSCs have not been shown to give rise to tumors [49]. These findings indicate the lack of solid evidence for malignant transformation in vivo following the implantation of MSCs for clinical use. Further studies will be required to determine whether MSCs can prevent tumor formation and related mechanisms. MSCs with chromosomal alterations did not show any sign of malignant transformation either in vitro or in vivo [49]. However, the effects of MSCs on the regulation of tumor growth in animal and in vitro models are not as clear [50, 51]. Thus, whether MSCs stimulate growth of other tumors is still controversial, and no clinical evidence has validated this hypothesis [52].

Mesenchymal Stem Cells in Treatment of Diabetes Mellitus Type 1

One strategy for treating T1DM is to induce the differentiation of stem cells into insulin-producing cells in vitro; such a

strategy may include the use of specific growth factors and an analogous microenvironment *in vitro* [53]. Recent research results on stem cells have shown that stem cell functions are controlled by intrinsic genetic programs and extracellular cues [54, 55]. Because the tissue-specific microenvironment plays a major role in the differentiation of pluripotent cells, MSCs found in the pancreas could represent an ideal source for β -cell generation. During the last few years, many research reports have described the existence of pancreatic tissue-specific MSCs having the general characteristic properties of MSCs, including the ability to differentiate into osteocytes, adipocytes, and neural cells on treatment with appropriate induction media [56]. However, several studies have presented controversial data regarding the origin of pancreatic MSCs and their ability to differentiate into insulin-producing cells (IPCs) [36, 57, 58]. Several problems prevent the translation of MSC-derived IPCs to clinical use, including the low proportion of differentiation to IPCs, restricted proliferation of IPCs, and lack of glucose responsiveness. While a few groups have claimed that these MSCs originated from the bone marrow, many reports have suggested that these cells originated from the pancreas itself. Moreover, one report demonstrated that mesenchymal proliferating cells are derived from a reversible epithelial-mesenchymal transition of pancreatic cells and can be redirected to IPCs *in vitro* [59]. However, using cell lineage tracing experiments in mice, several groups have published contradictory data, attributing the origin of these MSCs to preexisting pancreatic connective tissue rather than the epithelial-mesenchymal transition process [60, 61].

Many research groups have been working on the generation of IPCs from the transdifferentiation of MSCs *in vitro* [62]. Moreover, crosstalk has been observed between MSCs and the pancreas in diabetic animals, although the mechanisms mediating this process are unclear. MSCs express a set of chemokine receptors that may play critical roles in pancreatic homing/regeneration [36]. Systemic administration of MSCs increases β -cell mass and reverts hyperglycemia in streptozotocin-induced diabetic mice [63]. However, it is not clear whether MSCs can directly transform into β -cells. Dor and co-workers suggested that new β -cells can only be generated from preexisting β -cells instead of MSCs and concluded that MSCs only served as a “tropic mediator” to support islet function in an indirect manner, such as promoting angiogenesis [64]. However, some reports did show that IPCs can be regenerated from the transdifferentiation of other cells, pancreatic duct cells, and acinar cells [65–67].

Chen and collaborators [53] first reported the *in vitro* transdifferentiation of MSCs from rats into the functional insulin-producing islet-like cells that actively control blood glucose level in diabetic rats. Pancreatic and duodenal homeobox 1 (Pdx-1) is one of the most critical factors involved in the transdifferentiation of MSCs to β -cells [68, 69]. Other transcription or soluble factors, such as neurogenin 3 (Ngn3),

paired box gene 4 (Pax4), aristaless related homeobox (Arx), glucagon-like peptide-1 (GLP-1), and epidermal growth factor (EGF), have also been shown to play a role in the regeneration of β -cells [56, 70, 71]. Further studies have demonstrated that MSCs also have the potential to transdifferentiate into glucagon- and somatostatin-expressing cells [44]. Several groups have also reported the formation of islet-like clusters from *in vitro*-cultured MSCs, given proper stimulation [72]. On the other hand, these reports failed to confirm the regeneration of whole functional islets, containing α -cells, β -cells, other cell types, and functional vasculature. Moreover, most of these studies failed to generate a sufficient amount of islets for human transplantation and long-term stability. Recent progress in tissue engineering suggested that a biocompatible scaffold may be necessary for the *in vitro* generation of artificial islets with functional vasculature from stem cells [73]. In our recent study [74], we confirmed that the administration of MSCs into the pancreatic microenvironment can indeed improve the symptoms of diabetes. We also aimed to determine whether different pancreatic microenvironments influenced the improvement of hyperglycemia and insulin deficiency. At 28 days after MSC transplantation, we observed a statistically significant decrease in blood glucose levels in rats and found that diabetic rats gained more weight than control rats during the course of the study. However, diabetic rats treated with MSCs did not exhibit significantly different blood glucose levels or body weight. Consequently, our results suggested that the transplantation of MSCs can improve diabetes in the pancreatic microenvironment in an animal model with streptozotocin (STZ)-induced diabetes. The different pancreatic areas into which the MSCs were implanted had no significant influence on the improvement of hyperglycemia and insulin deficiency (Fig. 2).

Gao and collaborators described the paracrine mechanism of MSCs [75] by testing the cytoprotective effects of conditioned medium from cultured MSCs on isolated islets exposed to STZ *in vitro* and in mice islets after the experimental induction of diabetes *in vivo*. The authors found that the transplantation of MSCs can ameliorate hyperglycemia in diabetic mice by promoting the regeneration of β -cells. Both β -cell replication and islet progenitor differentiation contribute to β -cell regeneration. MSC transplantation results in an increased phosphorylation of Akt and Erk by islets *in vivo*, and treatment with MSC-conditioned media promotes islet cell proliferation, concurrent with the increased phosphorylation of Akt and Erk *in vitro*. The induction of β -cell proliferation by MSC-conditioned media is completely blocked by treatment with the PI3K/Akt inhibitor LY294002 but not by treatment with the MEK/Erk inhibitor PD98059. Together, these data suggest that the PI3K/Akt signal pathway plays a critical role in β -cell proliferation after MSC transplantation [75].

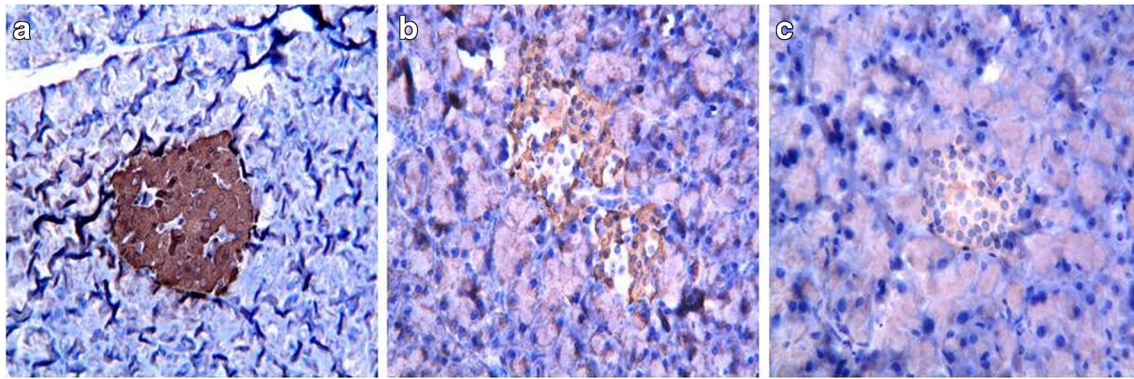


Fig. 2 Immunohistochemistry of pancreas with anti-insulin monoclonal antibody from **a** normal control rats, **b** diabetic rats treated with MSCs into the whole pancreas, and **c** diabetic control rats without MSC treatment

Potential Role of Mesenchymal Stem Cells in Pancreatic Islet Transplantation

In a recent study [23], Figliuzii and collaborators analyzed an approach via which MSC may help the success of pancreatic islet transplantation and addressed some of the current problems associated with islet transplantation. MSCs may exert pro-angiogenic and immunomodulatory effects if they are transplanted together with pancreatic islets. The observed pro-angiogenic effects result from the release of angiogenic factors from MSCs that have been shown to improve islet vascularization and graft function in islet transplantation. The immunomodulatory possibilities of MSCs may help to reduce inflammatory damage to the islets. MSCs may also reduce autoimmunity through their capacity to inhibit T cell proliferation and suppress the differentiation and maturation of dendritic cells [23]. Several other approaches have been attempting to identify immune privileged sites, in order to minimize the role of autoimmunity [62, 76].

As reported by Sakata and collaborators, bone marrow can promote angiogenesis following the cotransplantation with islets. The authors found that blood glucose was lower and serum insulin was higher in mice administered both islet cells and bone marrow MSCs. Moreover, significantly more new peri-islet vessels were detected in these mice as compared with mice implanted with islet cells alone. Vascular endothelial growth factor (VEFG) was expressed at higher levels in mice implanted with bone marrow MSCs than in mice implanted with islets. PDX-1-positive areas were identified in bone marrow cells with increased staining over time. However, there were no normoglycemic mice and no insulin-positive cells in mice implanted with bone marrow alone. These results suggest that the cotransplantation of bone marrow cells with islets is associated with enhanced islet graft vascularization and function [15].

Based on our current understanding, bone marrow stem cells are perhaps the most enticing candidates of non-pancreatic origin for various reasons; in particular, clinical protocols for their collection and use have already been

established, they have been shown to differentiate into pancreatic β -cells, and they have been proven to be effective at stimulating β -cell regeneration in damaged pancreatic tissue. In vivo murine studies have consistently shown that, even without differentiating into β -cells, bone marrow stem cell transplantation causes a reduction in plasma glucose levels and an increase in systemic insulin through a variety of mechanisms. Because a crucial concern for any cell-based T1DM therapy is preventing the autoimmune destruction of β -cells that create the original pathology and because bone-marrow-derived MSCs have been shown to inhibit T cell-mediated immune responses against newly formed β -cells, bone marrow stem cell therapy may provide the best treatment [9].

Human islet transplantation following the Edmonton protocol may be a permanent treatment for T1DM if the transplanted islets were not subjected to blood-mediated inflammatory reactions [77], acute rejection [78], islet toxicity by immunosuppressive agents [79], and ischemia caused by poor vascularity at transplantation [15]. As MSCs are well documented to possess better immunosuppressive and angiogenic properties, many studies have examined the effects of cotransplanting islets with MSCs and have found improved islet function and recovery [80–82].

Clinical Applications of Mesenchymal Stem Cells in Diabetes Mellitus Type 1

The first clinical trial using MSCs was carried out in 1995 [83]. In this study, 15 hemato-oncology patients became the recipients of autologous bone-marrow-derived MSCs. Since then, the use of MSCs has been further explored. By October 2014, the clinical trial database (<http://www.clinicaltrials.gov>) showed 352 clinical trials using MSCs for a variety of clinical disorders, including acute myocardial infarction, stroke, liver cirrhosis, amyotrophic lateral sclerosis, graft-versus-host disease (GVHD), solid organ transplant rejection, and autoimmune disorders. MSCs were used for the treatment of diabetes in 13 clinical trials, including eight trials investigating T1DM.

In general, MSCs appear to be well tolerated, with most trials reporting lack of adverse effects in the medium term, although a few showed mild and transient peri-injected effects [84].

In an ongoing phase I–II clinical trial, patients with T1DM received umbilical MSC (UC-MSC) infusions through the pancreatic artery. The investigators hypothesized that the infusion of UC-MSCs may provide multiple signals for β -cell regeneration and even redifferentiate into local tissues in patients with diabetes mellitus. The use of autologous umbilical cord stem cells in children with T1DM resulted in insignificant differences in daily insulin doses and caused a decline in C-peptide levels after 1 year of follow-up [85]. In another study, Hu et al. assessed the long-term effects of the implantation of Wharton's jelly-derived MSCs (WJ-MSCs) from the umbilical cords of 29 patients with newly onset T1DM. The authors demonstrated that this therapy can restore the function of islet β -cells over a longer time, although the precise mechanisms are still unknown. Thus, these data suggested that the implantation of WJ-MSCs may be an effective strategy for the treatment of T1DM [86]. Another form of cell therapy was performed by Mesples and co-workers using stem cells from the patient's own bone marrow, including a conglomerate of MSCs and HSCs [87]. Bone marrow mononuclear cells were injected by arterial catheterization directly into the patient's pancreas. This therapy was performed in 20 T1DM patients, and the follow-up results showed a significant increase in pancreatic secretion of C-peptide and a decrease in the daily dose of exogenous insulin. This effect partially disappeared by the 3-year follow-up visit without increases in the levels of ICA and GAD antibodies [87].

There are several challenges in the application of MSCs in patients with newly diagnosed T1DM, the most important of which is that the length of the clinical response (insulin independence) and the relapse mechanism must still be investigated.

The Limitations of Mesenchymal Stem Cell-Based Therapy in Diabetes Mellitus Type 1

Promising preliminary and preclinical studies have led to phase I and phase II clinical trials with a therapeutic use of MSCs for T1DM treatment. However, some reports suggest that stem cells are unlikely to be used as a stand-alone therapy, because it may not be efficiently reverse autoimmunity of T1DM. They could be applied only for reducing immunosuppressant doses [88, 89]. The timing of cell delivery, number of cells, engraftment, and cell manufacture are very important; however, there is still a lack of information in this regard [90]. There is a need to tightly control the microenvironment and engraftment of the transplanted cell. Detailed investigations of how the microenvironment affects the homing and immunosuppressive effects of MSC are still lacking. An added

complication in the scale-up of MSC-based technologies in T1DM is the lack of an effective route of cell administration. It was demonstrated that 70 % of MSCs injected intravenously stopped in the lungs, and only a small amount of cells arrived to the heart, kidneys, and liver [91]. The choice of an autologous or allogeneic approach is also an important consideration, and the option may be limited by disease-induced cell dysfunction and by an immune response to the transplanted cells. Another challenge in cell therapy is the large-scale production of MSCs under GMP conditions to prevent contamination and avoid MSC heterogeneity. Furthermore, the methods for the transportation of MSC-based products without affecting their viability and efficacy are an important subject, along with the issues related to cryopreservation.

Conclusions

The main goal of the present review was to present recent studies on the use of MSCs in the treatment of T1DM. We hypothesized that the paracrine function of transplanted MSCs rather than cell transdifferentiation may play a crucial role in hyperglycemia reversal in diabetic animal models. Through this paracrine action, the transplantation of MSCs would ameliorate T1DM. The best results have been obtained by the cotransplantation of MSCs with islet cells. These new innovative tools have provided tremendous opportunities for moving from the bench to the clinic in achieving successful long-term functional islet graft survival.

There is an increased need to describe the mechanisms of MSC-mediated cell therapy in detail, and challenges are still present in terms of engraftment, persistence, tissue targeting, and cell manufacture. Successful management of these challenges and the outcome of clinical trials using MSCs to treat T1DM or their complications will decide the future of cell-based therapy for this devastating disease.

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Conflict of Interest The authors declare no conflict of interest.

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