The Journal of Clinical Endocrinology & Metabolism, 2023, **108**, 1889–1897
https://doi.org/10.1210/clinem/dgad142
Advance access publication 14 March 2023
Mini-Review

Adipose-derived Mesenchymal Stem Cells Therapy as a new Treatment Option for Diabetes Mellitus

Agnieszka Mikłosz[1] **and Adrian Chabowski**[1]

[1]Department of Physiology, Medical University of Bialystok, 15-222 Bialystok, Poland

Correspondence: Agnieszka Mikłosz, PhD, Department of Physiology, Medical University of Bialystok, Mickiewicza 2C St, 15-222 Bialystok, Poland.
Email: agnieszka.miklosz@umb.edu.pl.

Abstract

The worldwide increase in the prevalence of diabetes mellitus has raised the demand for new therapeutic strategies targeting diabetic symptoms and its chronic complications. Among different treatment options for diabetes, adipose-derived mesenchymal stem cells (ADMSCs) therapy attract the most attention. The therapeutic effects of ADMSCs are based primarily on their paracrine release of immunomodulatory, anti-inflammatory, and trophic factors. Animal models of diabetes as well as human clinical trials have shown that ADMSCs can effectively facilitate endogenous β cell regeneration, preserve residual β cell mass, reduce islet graft rejection, regulate the immune system, and ultimately improve insulin sensitivity or ameliorate insulin resistance in peripheral tissues. Nevertheless, transplantation of mesenchymal stem cells is associated with certain risks; therefore recently much attention has been devoted to ADMSCs derivatives, such as exosomes or conditioned media, as therapeutic agents for the treatment of diabetes. Compared to ADMSCs, cell-free therapy has even better therapeutic potential. This narrative review summarizes recent outcomes and molecular mechanisms of ADMSCs action in the treatment for both type 1 DM and type 2 DM, as well as shows their feasibility, benefits, and current limitations.

Key Words: adipose-derived mesenchymal stem cells, ADMSCs, insulin resistance, insulin secretion, pancreatic β-cells, type 1 diabetes mellitus, type 2 diabetes mellitus

Diabetes mellitus (DM) is a life-threatening disease, and its prevalence is increasing at an alarming rate worldwide. According to the International Diabetes Federation, approximately 537 million adults are suffering from diabetes, and the incidence is expected to rise to 643 million by 2030. This multiple etiology disease in 2019 was considered as the 9th leading cause of death (1). There are 2 major types of diabetes mellitus: type 1 (T1DM) and type 2 (T2DM). Although both types have different pathophysiological mechanisms, they are clinically characterized by hyperglycemia. T2DM, which comprises the majority of all cases, is caused by a combination of genetic and nongenetic factors such as high caloric intake, obesity, central adiposity, sedentary lifestyle, and age that lead to peripheral insulin resistance (IR) and compensatory hypersecretion of insulin from the pancreatic β cells (2). Conventional treatment of T2DM typically begins with lifestyle modification and the usage of hypoglycemic agents, but about 14% to 25% of patients eventually require exogenous insulin injections to control blood glucose level (3). In turn, T1DM (previously known as insulin-dependent, juvenile, or childhood-onset) results from progressive autoimmune destruction of insulin-secreting β-cells in the pancreatic islet, leading to partial or absolute insulin deficiency (4). Maintaining normal glucose and glycosylated hemoglobin (HbA1C) levels is often a challenge for patients with pancreatic insufficiency, resulting in lifetime dependence on insulin therapy. While the discovery of insulin in 1921 was initially considered as a cure for the disease, the exogenous insulin therapy cannot accurately mimic endogenous insulin secretion and properly control the metabolic alterations, further resulting in the development of multisystemic chronic complications (5). In light of this, alternative strategies have been approved to provide superior control of blood glucose and minimize diabetic complications, including cytostatic drugs, monoclonal antibodies, and pancreas/islets transplantation. To date, cadaveric human pancreas and pancreatic islets transplantation remains the most reliable clinical approach for selecting patients with severe DM. Although pancreas transplantation can restore insulin independence in 60% of recipients for 5 years after the procedure, this therapy is associated with a high risk of postoperative complications and mortality (6). However, the scarcity of appropriate pancreatic donors and necessity for chronic immunosuppression significantly limit its widespread application. Another approach, pancreatic islet cell replacement therapy, can save the production of insulin in a glucose-dependent manner but has poor long-term efficacy. An additional drawback of the procedure is the need to transplant a large number of pancreatic islet cells (approximately 340-375 million islet cells per patient) in order to achieve normoglycemia. In practice, 2 to 3 pancreatic islet donors are necessary to transplant procedure (7). In addition, only half of the patients became insulin independent within 5-years after islet transplantation, which was associated with significant loss of β-cell mass (8, 9). Loss of

Received: 8 November 2022. **Editorial Decision:** 6 March 2023. **Corrected and Typeset:** 28 March 2023

transplanted cells is caused by an immediate inflammatory blood reaction, delayed revascularization, and hypoxic stress. Another alternative treatment for diabetes, anti-CD3 monoclonal antibody therapy, has proven effective only in patients with recently diagnosed T1DM or in young patients ages 8 to 11 years. Although considered relatively safe, insulin independence was achieved in only 5% of patients after 2 years of follow-up (10, 11). Despite initially satisfactory results, current treatments are unable to reverse β-cell damage. In addition, available procedures are mainly used for T1DM, and their application in T2DM patients is limited. Therefore, alternative strategies have been designed to cure diabetes and maintain therapeutic effects. In recent years, cell-based therapy using mesenchymal stem cells (MSCs) is of great interest; in particular, adipose-derived mesenchymal stem cells (ADMSCs) are being considered for the treatment of diabetes. This narrative review highlights the most significant therapeutic benefits of ADMSCs and cell-free therapy in the treatment of both T1DM and T2DM and provides insight into the underlying mechanism. Among other issues, we discuss safety and efficacy, as well as limitations and challenges associated with their therapeutic applications.

ADMSCs as a Class of Mesenchymal Stem Cells

Mesenchymal stem cells or mesenchymal stromal cells are fibroblast-like adult stem cells with the capacity to self-renew and differentiate into multiple lineages, ie, mesodermal lineage (adipocytes, chondrocytes, osteocytes, fibroblasts, and myocytes), ectodermal lineage (neurons), and endodermal lineage (hepatocytes) (12). In 1966, Friedenstein and colleagues first discovered the multipotent behavior of precursor cells in the mouse bone marrow (13). Although stem cells are found primarily in the bone marrow, they can originate from different other tissues and organs, such as white adipose tissue, blood, dental tissues, liver, pancreas, spleen, thymus, skeletal muscle, skin, placenta, amniotic fluid, or Wharton's jelly. To standardize MSCs the International Society for Cellular Therapy in 2006 proposed the following minimal criteria: (1) MSCs must be plastic adherent when cultured in vitro; (2) MSCs must express the surface markers CD73, CD90, and CD105 and lack of expression of CD45, CD34, CD14, CD11b, CD79α, CD19, or HLA-DR surface molecules; (3) MSCs must exhibit trilineage potential (be able to differentiate into adipocytes, chondrocytes, and osteocytes) (14).

ADMSCs exhibit typical features of MSCs and are extensively studied for the treatment of a wide range of diseases, such as ischemic stroke, multiple sclerosis, myocardial ischemia, coronary arteriosclerosis, limb ischemia, obesity, fatty liver disease (nonalcoholic steatohepatitis, liver fibrosis, cirrhosis), muscular dystrophy, osteoarthritis, Crohn's disease, cancers, acute kidney injury, chronic skin wounds, and glioma (15-21). Furthermore, ADMSCs may represent a novel diabetes treatment strategy due to their ability to replace damaged β cells and normalize blood glucose level (22-26). The clinical use of ADMSCs has grown over the years, mainly due to their abundance and the overcoming of ethical and legal issues as patients can be treated with their own cells (autologous) or cells from other donors (allogeneic) with minimal risk of cellular rejection. Although autologous ADMSCs therapy is the most attractive treatment option for many diseases, its beneficial effects can be altered by the microenvironment surrounding the cells. Persistent hyperglycemic milieu can impair ADMSCs differentiation potential and proliferation rate, and overall, their functionality (27-29). Nevertheless, transplantation of multipotent stem cells is associated with certain risks, which is why much attention has recently been devoted to ADMSCs derivatives, such as exosomes or conditioned media (CM).

ADMSCs and Pancreatic β-Cell Function

Stem cells have the potential to differentiate into specialized cells, modulate the immune system, and restore β-cell functionality, making them an attractive treatment option for diabetic patients. ADMSCs alleviate hyperglycemia by differentiating into insulin-producing cells (IPCs) and paracrine signaling, thus facilitating endogenous β cell regeneration, preserving residual β cell mass and reducing islet graft rejection (Fig. 1) (30). In addition, ADMSCs have the ability to differentiate into vascular endothelial cells, improving the blood supply to the pancreas. A wide range of paracrine factors have been identified within the ADMSCs secretome, including growth factors, ie, granulocyte colony-stimulating factor, granulocyte macrophage colony-stimulating factor, nerve growth factor, keratinocyte growth factor, vascular endothelial growth factor, fibroblast growth factor, or insulin-like growth factor 1, antiapoptotic, antioxidative, and anti-inflammatory signaling molecules. The paracrine mechanism also plays an important role in immunomodulation. It was shown that ADMSCs can inhibit self-reacting T-cell expansion, development of dendritic cells and β-cell proliferation (24, 31). Their interaction with these innate and adaptive immune cells results in downregulation of proinflammatory cytokines [ie, interleukin-1β, tumor necrosis factor α, interleukin-6 (IL-6)] and upregulation of anti-inflammatory cytokines such as interleukin-10, prostaglandin E2 or indoleamine 2, 3-dioxygenase (32). The effect of ADMSCs on pancreatic β cell function is currently the subject of extensive preclinical and clinical trials (phase I/II), many of which have entered phase III. Next we outline the current understanding of ADMSC-based therapies in DM from the animal model to the clinical trials.

ADMSCs-based Therapy in T1DM

Overall, the therapeutic effect of ADMSCs in the treatment of T1DM falls into 4 categories: (1) efficacy of differentiated IPCs derived from ADMSCs, (2) preservation of residual β cells, (3) engraftment of cotransplanted islet grafts, and (4) supporting the function of precultured islet grafts.

Efficacy of differentiated IPCs derived from ADMSCs

Adult stem cells have developmental plasticity that allows them to adopt an endocrine pancreatic phenotype. The potential of ADMSCs to generate IPCs was discovered in 2003, but initially these cells did not secrete insulin (33). A few years later, Kang et al successfully differentiated human eyelid ADMSCs into fully functional IPCs that released insulin and C-peptide in a glucose-dependent manner (34). Following IPCs transplantation into the renal subcapsular space of streptozotocin-treated immunocompetent mice, normoglycemia was restored. After sacrifice the expression of various human genes including insulin was found in the removed kidney tissues (34). At present, it is evident that the process of ADMSCs differentiation into mature IPCs is tightly controlled by a complex network that depends on transcriptional regulation of genes involved in pancreatic development. The most

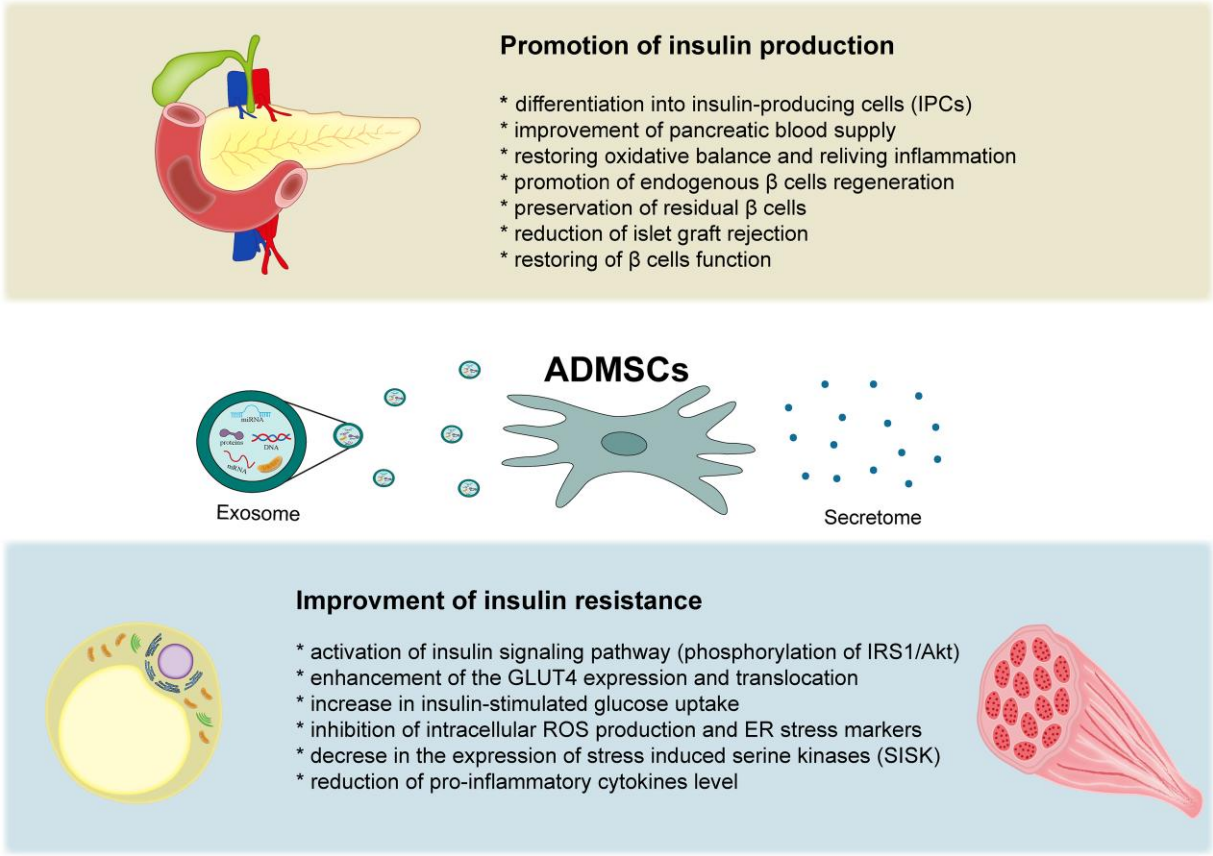

Figure 1. Mechanisms of ADMSCs action in glycemic control. ADMSCs, due to their multilineage differentiation and regeneration potential, promote insulin production and pancreatic islet β-cells vascularization as well as reduce β-cells apoptosis and inflammation. Additionally, ADMSCs improve insulin sensitivity and reduce insulin resistance in peripheral tissues (eg, adipose tissue and skeletal muscles). Abbreviations: ADMSC, adipose-derived mesenchymal stem cell.

specific transcription factors are pancreatic and duodenal homeobox-1, paired box 4, paired box 6, NK2 homeobox 2, NK6 homeobox 1, neurogenin-3, NeuroD, islet1 transcriptional factor, and insulin promoter factor-1 (35-38). Dave et al showed that upregulation of islet1 transcription factor, insulin promoter factor-1, and paired-box 6, without any genetic manipulation, was associated with insulin secretion from human ADMSCs, confirming their ability to differentiate into IPCs (35). Ex vivo generation of functional islet-like cellular aggregates from human ADMSCs has been successfully achieved in other studies (36, 39). Islet-like cellular aggregates transplantation in streptozotocin (STZ)-induced diabetic mice restored normoglycemia within 3 to 4 weeks, offering a reasonable resource of alternative islet therapy in early onset of T1DM (36). Of note, a relatively small number of transplanted ADMSCs can directly transdifferentiate into pancreatic IPCs, which may not be sufficient to fulfill its functions. Therefore, in comparison with ADMSCs, IPCs differentiated in vitro from ADMSCs and then transplanted showed a better therapeutic effect in alleviating hyperglycemia (34, 40, 41). Taken altogether, the ability of ADMSCs to differentiate into mature IPCs could provide an unlimited supply of β cells to replace damaged cells in diabetic patients.

Preservation of residual β cells

The broad immunosuppressive and immunomodulatory effects of ADMSCs along with their antioxidant and antiapoptotic properties effectively reduce autoimmune responses and protect endogenous pancreatic islets. Accordingly, ADMSCs halt β-cell destruction, protect residual β-cell mass, facilitate endogenous β-cell regeneration, and ameliorate islet transplant rejection. It should be emphasized that the method of ADMSCs administration significantly influences their therapeutic potential. Following systemic administration, MSCs are prone to lung microvasculature entrapment and have indirect impact on the pancreas, while intrapancreatic infusion allows them to be physically contacted with pancreatic β cells (42-45). Khatri et al compared the antidiabetic effect of locally administered ADMSCs with their systemic infusion in STZ-induced diabetic NMRI nude mice (46). Direct injection of ADMSCs into the pancreas (0.5×10^6) has been shown to result in better cell protection and regeneration as evidenced by a higher number of replicating islet cells, number of islet cells, or islet area compared to intravenous ADMSCs injection (46). The therapeutic effect of ADMSCs was also confirmed by the Bassi group. They transplanted a single dose of allogenic ADMSCs to non-obese diabetic mice and found an increase in β-cell mass and improvement in hyperglycemia in early-onset autoimmune diabetes (47). Furthermore, the available in vitro and preclinical studies strongly suggest that ADMSCs secretome is also effective in reducing the clinical manifestations of diabetes (48-52). In an animal model of T1DM, intraperitoneal administration of exosomes derived from autologous ADMSCs improved pancreas function and maintained a normoglycemia (48). Similarly, preconditioning of ADMSCs exosomes with miR-146a, which plays an important role in the anti-

inflammatory effect of exosomes, had a beneficial effect on glycemic status (51). In addition to the potential of ADMSCs to restore euglycemia, the infusion of adipose-derived MSCs reduced inflammation by lowering the CD4+ Th1-biased immune response, which promoted Tregs expansion in pancreatic lymph nodes and reduced infiltration of inflammatory cells in the pancreas (47). ADMSCs-derived exosomes also displayed immunosuppressive properties as they increased the population of regulatory T cells along with levels of interleukin-4, interleukin-10, and transforming growth factor-β but decreased the secretion of proinflammatory cytokines (ie, interleukin-17 and interferon-γ) (48). On the other hand, Arzouni et al showed that human ADMSCs have the potential to improve the survival and function of human islet cells in vitro through released products and extracellular matrix, rather than through modification of the immune system (53). However, as 70% to 90% of pancreatic β cells are destroyed at the time of diagnosis, it can be difficult to regenerate a sufficient number of resident islet cells; hence the impact of the strategy to preserve resident pancreatic β cells is limited (54). Moreover, the widespread application of islet transplantation is hampered by an insufficient supply of donors. These drawbacks drastically limit the application of this procedure as a cure for T1DM.

Engraftment of cotransplanted islet grafts

Obtaining a large number of β cells for cellular therapy is a challenge. A possible solution may be a combined islet transplantation together with ADMSCs, ie, hybrid islet transplantation. The effect of pancreatic islets cotransplantation with ADMSCs was investigated by Ohmura et al (55). Although transplantation of 100 or 200 allogeneic islets did not restore normoglycemia, their cotransplantation with 2×10^5 ADMSCs was sufficient to prolong graft survival and establish glycemic homeostasis. In addition, significant revascularization and inhibition of inflammatory cells infiltration, including CD4+, CD8+ T cells, and macrophages, was observed as compared to the islet alone graft group (55). In the other study completed by Gamble et al, human ADMSCs were cocultured with murine and human pancreatic islets. Interestingly, islets cotransplanted with ADMSCs showed reduced cell death and improved islet function and recovery. Moreover, islets cultured alone presented a significant decrease in islets mass compared to the cocultured groups ($22.1 \pm 10.5\%$ islet loss vs $1.1 \pm 0.81\%$ and $2.7 \pm 1.9\%$, for cocultured murine and human pancreatic islets, respectively) (9). Finally, islets cotransplanted with ADMSCs restored normoglycemia at a faster rate (22.3 ± 4.7 days) in comparison with islet transplant group (38.5 ± 7.6 days) (9). Despite encouraging results, a significant limitation was found. Namely, the mice returned to hyperglycemia 60 days after transplantation procedure. Nonetheless, other researchers have shown that engraftment of pancreatic islets with ADMSCs improves the transplant's insulin-releasing function and reduces the required islet mass (56-58). While hybrid islet transplantation has many advantages, several limitations hinder its wide application. The most obvious are (1) the number of ADMSCs that are required to promote pancreatic islet function and (2) time of transplant, taking into account disease progression. Thus there is a need to optimize this cell replacement therapy before it becomes a reliable therapy for diabetic patients.

Supporting the function of precultured islet grafts

The maintenance of viable and functional pancreatic islets is critical for successful islet transplantation. However, pancreatic islet isolation and culture procedures are fraught with dramatic islet cell loss, largely attributed to ischemia, oxidative stress, and an immediate blood-mediated inflammatory response (53, 59). The combination of these factors appears to be responsible, at least in part, for the undesirable loss of large islet mass prior to transplant, leading to a reduction in insulin independence. Following the first 3 days after human islets transplantation into diabetic nude mice, a significant decrease in islet cell survival, insulin content, and β-cell mass was demonstrated (60). As islet function deteriorates with culture, it is important to develop a strategy to maintain the functional viability of the isolated islets prior to in vitro transplantation. Recent studies in animal models have shown that preculture of islets with ADMSCs significantly improves transplant outcomes in STZ-induced diabetic mice (61, 62). The beneficial impact of ADMSCs has been attributed to a secretion of an array of trophic factors that improve islet survival and function during culture (63). In particular, anti-inflammatory and immunomodulatory molecules are useful in improving the longevity of transplanted pancreatic islet cells. Moreover, ADMSCs help to enhance islet cell regeneration by increasing revascularization in a hypoxic environment and maintaining islet integrity and structure. However, available studies do not conclusively determine whether the paracrine molecules secreted by ADMSCs are sufficient to improve islet survival and function (58) or whether physical contact between the stem cells and the pancreatic islets is also needed (61, 64). Yamada et al have found that human ADMSCs have a trophic effect on porcine pancreatic islets in vitro due to paracrine mediators secreted to the medium but not due to the cell-to-cell contact (65). Among different growth factors, vascular endothelial growth factor was conferred as important for cell survival and maintenance (65).

ADMSCs-based Therapy in T2DM

In recent years, the use of ADMSCs has become a potentially safe and effective method of treating T2DM. Possible mechanisms by which ADMSCs improve hyperglycemia include islet β-cell regeneration, modulation of hepatic metabolism toward higher glucose utilization, reduction of inflammation, and amelioration of IR in peripheral tissues (66-70). Nevertheless, improvement in islet function was considered to be the primary mechanism by which adult stem cells could ameliorate hyperglycemia. ADMSCs have the potential to differentiate into IPCs and thus generate an adequate number of islets for insulin production. Nam et al compared the glucose-lowering properties of human adipose-derived MSCs with ADMSCs-derived IPCs (69). Transplantation both cell types into a mouse model with T2DM significantly increased circulating levels of insulin and c-peptide and at the same time lowered inflammatory cytokines, which could ultimately alleviate IR. Interestingly, it turned out that differentiated IPCs had better potential in restoring normal glucose level and improving metabolic parameters than ADMSCs (69). Furthermore, it was found that overexpression of pancreatic and duodenal homeobox-1 successfully induced the differentiation of human ADMSCs into aggregates of insulin-producing cells, which in vivo lowered blood glucose level but did not restore normoglycemia (40). In general, ADMSC-derived IPCs have

the ability to restore euglycemia; however, some problems need to be addressed. To date, neither the long-term stability of ADMSC-derived IPCs nor their number to restore glycemic status have been identified. Therefore, it seems that transplantation of undifferentiated MSCs may be more effective as the administered cells were mainly found in damaged pancreatic tissue. In addition, ADMSCs transplantation could promote β-cells regeneration through recovery of islet beta cells, reduction of apoptosis and inflammation, and improvement of islet vascularization (66, 71). These beneficial effects are attributed to the secretion of angiogenic factors such as vascular endothelial growth factor, insulin-like growth factor 1, hepatocyte growth factor, or von Willebrand factor and the reduction of proinflammatory and apoptotic cytokines, ie, tumor necrosis factor-α, IL-6, interleukin-1β, or caspase-3 in the pancreas (66, 71). In turn, Xie et al reported that 24 hours after ADMSCs infusion, blood glucose level was markedly lowered via promoting hepatic glycogen synthesis and inhibiting hepatic glucose production in T2DM rats (68). Recently, genetic modification has been applied to increase stem cells' ability to promote insulin production. For example, Xue et al found that genetically modified ADMSCs that overexpress fibroblast growth factor 21 and glucagon-like polypeptide 1 could significantly increase insulin level and improve glucose metabolism (72).

ADMSCs-based Therapy in Insulin Resistance

IR is defined as a state of reduced responsiveness of insulin-targeting tissues to physiological levels of insulin, and thus higher than normal insulin level is required to maintain glucose homeostasis (73, 74). Although the molecular mechanism of IR is not yet fully understood, several theories are generally proposed. Among its multiple etiological factors, ectopic lipid accumulation in peripheral tissues, proinflammatory milieu, endoplasmic reticulum stress, mitochondrial dysfunction, and oxidative stress are believed to be major drivers (75). Available therapeutic strategies can ameliorate hyperglycemia or temporally improve insulin sensitivity in target tissues but can never reverse IR. It is now believed that adipose-derived MSCs have the potential to ameliorate IR and thus improve glycemic control in diabetic patients (49, 66, 70, 76). For instance, Hu et al found that ADMSCs promoted insulin releasing and enhanced the expression of glucose transporter 4 (GLUT4) in the liver, adipose tissue, and skeletal muscles in a high-fat diet/STZ-induced T2DM rat model (66). In the same experimental model, ADMSCs were able to improve insulin sensitivity by activating insulin receptor substrate and elevating Akt phosphorylation in skeletal muscles (70). In vitro studies have further demonstrated that ADMSCs-CM secretome has even better therapeutic potential to ameliorate IR, and, importantly, its use is safer than cell-based therapy. Furthermore, compared to stem cells, ADMSCs secretome has even better therapeutic potential to manage T2DM and, again, its use is safer. Recent evidence has shown that ADMSCs-CM controls glycaemia through a broad range of paracrine growth factors, chemokines, and cytokines that could promote islet cell regeneration, increase insulin sensitivity, reduce IR, and regulate the immune system (49, 77-79). Indeed, ADMSCs secretome can effectively increase insulin-stimulated glucose uptake as evidenced by increased Akt phosphorylation and GLUT4 translocation to the plasma membrane in insulin-resistant 3T3L1 and C2C12 cells (76). Similar results were observed by Sanap et al: conditioned medium of ADMSCs significantly increased glucose uptake and decreased the expression of major inflammatory regulators and reactive oxygen species production in insulin-resistant 3T3L1 cells (49). A study by Elshemy and colleagues also found that ADMSCs secretome alleviated hyperglycemia by increasing pancreatic β-cell regeneration and reducing IR in T2DM rats (77). In addition, researchers compared the antioxidative potential of ADMSCs-CM with a conditioned medium of liver-derived mesenchymal stem cell (77). Although both interventions alleviated hyperglycemia, liver-desired-MSCs-CM showed better therapeutic potential. Several lines of evidence suggest that chronic low-grade inflammation contributes to pancreatic islet dysfunction and the development of IR (78). Data obtained from insulin-resistant 3T3L1 cells indicate that ADMSCs secretome reduces stress-induced serine kinases expression, including c-Jun N-terminal kinases, inhibitory-kB kinase β, extracellular signal regulated kinases, or p70S6 kinase and thus alleviates IR (80). Another mechanism by which ADMSCs-CM prevents the development of oxidative stress-induced IR is an inhibition of intracellular production of reactive oxygen species and endoplasmic reticulum stress markers (downregulation of CHOP1 and IRE1) (80). Furthermore, it is well known that the microenvironment around stem cells affects their functionality. For example, culturing ADMSCs in a diabetic microenvironment (preconditioned ADMSCs, pre-ADMSCs) increased the secretion of cytokines related to the polarization of M2 macrophages, ie, IL-6, MCP-1 (79). Interestingly, multiple pre-ADMSCs infusions improved hyperglycemia via ameliorating of IR and increasing pancreatic islet regeneration (79). Similarly, preconditioning of ADMSCs with orexin A, which regulates glucose homeostasis by interacting with pancreatic β cells, was more effective in lowering fasting glucose and insulin levels in diabetic rats compared to rats treated with ADMSCs alone (81). Moreover, preconditioned ADMSCs significantly alleviated dyslipidemia, lowered inflammatory markers, and increased GLUT4 and Akt2 levels, which effectively improved insulin sensitivity of muscle and adipose tissue (81). These data indicate that preconditioning strategies as well as cell-free strategies may represent a unique, innovative therapy to both improve β-cell function and reduce IR (Fig. 1).

Clinical Applications of ADMSCs in DM

Promising results from animal studies have encouraged scientists to use MSCs in clinical trials. Despite MSCs' beneficial potential in regenerative medicine, clinical studies have also looked at the safety and efficacy of MSCs therapies in various populations with various diseases (82, 83). The most commonly reported adverse events after MSCs applications have included transient fever, constipation, headache, fatigue, and arrhythmia (82). Since several factors such as age, race, sex, and underlying diseases of the donor significantly affect the properties of MSCs, they also had an impact on the occurrence of side effects. Recently, the administration of MSC derivatives such as exosomes or MSC-derived CM demonstrates safety and a satisfactory therapeutic effect, most importantly without the risk of cell-specific adverse effects such as malignancy or undesirable differentiation; therefore it may be a candidate for future research.

Currently, the safety and efficacy of ADMSCs therapy in the treatment of DM is studied in several clinical trials (www.ClinicalTrials.gov). The scope of these studies range from long-term diabetic complications (mainly diabetic nephropathy) to newly diagnosed diabetes. Since DM is a multifactorial and multistage disease, it seems that the combination of different therapeutic strategies may achieve better treatment results. In prospective, open-trial studies, patients with recent onset of T1DM (n = 6) received a single infusion of heterologous ADMSCs (1×10^6 cells/kg) together with daily supplementation of cholecalciferol (2.000 UI/day). After a 6-month follow-up, increased serum C-peptide level with a decrease in the insulin requirements was observed (84). In 2020 a prospective, phase II, open-trial, pilot study was performed by the same group. In this study patients with recent onset of T1DM (diagnosis of T1DM for <4 months) received a single dose of allogenic ADMSCs and daily vitamin D (2.000 UI/day) supplementation for 3 months (www.ClinicalTrials.gov: Identifier: NCT03920397). Three months after a combined therapy, the patients had lower insulin requirements (0.22 ± 0.17 vs 0.61 ± 0.26 IU/kg) and lower HbA1c levels ($6.47\% \pm 0.86\%$ vs $7.48\% \pm 0.52\%$). The observed side effects included headache, mild local reactions, tachycardia, abdominal cramps, thrombophlebitis, and mild floaters (85). Furthermore, Thakkar et al evaluated the safety and efficacy of autologous and allogenic adipose-derived insulin secreting mesenchymal stromal cells (IS-AD-MSCs) cotransplantation with bone marrow derived hematopoietic stem cell (BM-HSCs) in treatment of patients diagnosed with T1DM. IS-AD-MSCs together with BM-HSCs were implanted into portal/thyroid circulation. Both stem-based therapies significantly lowered plasma glucose and HbA1c levels, with sustained improvement in C-peptide level and reduced insulin requirements over 2 years of follow-up. Notably, autologous IS-AD-MSCs/BM-HSCs cotransplantation offers better long-term control of hyperglycemia as compared with allogenic infusion (86). Additionally, Dave et al, in a prospective, nonrandomized clinical trial, confirmed the effectiveness of autologous IS-AD-MSCs infusion (along with BM-HSCs) in T1DM patients (87). Over a mean follow-up of 31.71 months after a transplantation, diabetic patients (n = 10) showed increased C-peptide (0.22 ng/mL to 0.92 ng/mL), decreased HbA1c level (10.99% to 6.72%), and lower exogenous insulin requirement (64 IU/day to 39 IU/day) (87). Similarly, another prospective, nonrandomized, open-label clinical trial also investigated the therapeutic effect of autologous IS-AD-MSCs cotransplantation with hematopoietic stem cell in diabetic patients. After a mean follow-up of 7 months, patients had elevated serum C-peptide level and showed a decrease in insulin dependency and HbA1c levels (88). Taken together, ADMSCs have the potential to differentiate into the pancreatic lineage and support the function of resident β cells and therefore may be an ideal replacement therapy in diabetic patients.

Limitations of ADMSCs Therapies

Despite significant progress made in the field of stem cell therapies to treat DM, several challenges need to be addressed. One of the major obstacles for efficient therapeutic use of stem cells is a lack of uniform experimental protocol. The animal experiments differ with regard to the number of stem cell transplants, sources used to harvest ADMSCs, methods used to administer them into the patients (ie, optimal dose, frequency, or route of injection), and the time point of their administration in the course of diabetes. Another hurdle for any stem cell therapy is the poor engraftment and survival of donor cells; thus recently much attention has been paid to developing new strategies to increase their survival and retention. At the time of this review, there are almost 30 clinical trials registered on the website ClinicalTrials.gov using ADMSCs as a therapy for DM and the variety of its chronic complications. Furthermore, the choice of an autologous or allogeneic therapy is an important consideration as the former is immunocompatible and does not raise ethical or legal concerns but may be limited by disease-induced functional impairment and the latter by an immune response to the transplanted cells. In addition, due to the lack of standardization, there are great variations within clinical trials regarding sample size, study design, follow-up period, and onset of diabetes and its progression. Finally, differences in ADMSCs therapeutic efficacy may be related to a specific characteristic of the donor, such as genetic origin or lifestyle. Despite the odds, ADMSCs are expected to become a promising therapeutic agent for treatment of DM; however, further research is needed to address these challenges.

Conclusions

Increasing numbers of preclinical and clinical studies indicate that ADMSCs may be a new regenerative therapy for diabetes mellitus. The mechanisms by which ADMSCs exert their beneficial action include the paracrine secretion of a broad range of cytokines, angiogenic or immunomodulatory factors that promote cell migration to the sites of injury, immunomodulation, and direct regeneration of damaged tissues. In diabetic settings, ADMSCs therapy significantly improved HbA1c and C-peptide levels as well as diminished exogenous insulin requirements. Moreover, ADMSCs' ability to differentiate into various cell types, such as IPCs, myofibroblasts, cardiomyocytes, pericytes, and endothelial cells, makes them promising therapeutic candidates for the treatment of DM. In addition, transplantation of ADMSCs derivatives such as exogenous vesicles or CM and ultimately modification of stem cells may further enhance their clinical benefits. Efforts are now being made to transfer knowledge from in vitro and animal models to the patient's bedside. Although preclinical studies and phase I/II clinical trials have shown that ADMSCs cellular therapy improves the clinical outcomes, further phase III clinical trials are necessary to achieve more reliable and effective benefits prior clinical use.

Funding

This study was supported by the National Science Center (grant no. 2016/23/D/NZ3/01660) and the Medical University of Bialystok (grant no. SUB/1/DN/22/005/1118).

Author Contributions

A.M. participated in study conceptualization, writing of the original draft, and final draft review and editing, visualization, funding acquisition, ensuring communication with the journal. A.C. supervised this project's work, final draft review, and editing.

Disclosures

The authors have nothing to disclose.

Data Availability

Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

References

1. International Diabetes Federation. *IDF Diabetes Atlas Tenth Edition 2021*. Available at: https://idf.org/aboutdiabetes/what-is-diabetes/facts-figures.html.
2. Keane KN, Calton EK, Carlessi R, Hart PH, Newsholme P. The bioenergetics of inflammation: insights into obesity and type 2 diabetes. *Eur J Clin Nutr*. 2017;71(7):904-912. https://doi.org/10.1038/ejcn.2017.45.
3. Lipska KJ, Parker MM, Moffet HH, Huang ES, Karter AJ. Association of initiation of basal insulin analogs vs neutral protamine Hagedorn insulin with hypoglycemia-related emergency department visits or hospital admissions and with glycemic control in patients with type 2 diabetes. *J Am Med Assoc* 2018;320(1):53-62. https://doi.org/10.1001/jama.2018.7993.
4. Refaie AF, Elbassiouny BL, Kloc M, *et al*. From mesenchymal stromal/stem cells to insulin-producing cells: immunological considerations. *Front Immunol*. 2021;12:690623. https://doi.org/10.3389/fimmu.2021.690623.
5. Peng BY, Dubey NK, Mishra VK, *et al*. Addressing stem cell therapeutic approaches in pathobiology of diabetes and its complications. *J. Diabetes Res*. 2018;2018:78064. https://doi.org/10.1155/2018/7806435.
6. Hirshberg B, Rother KI, Digon BJ, *et al*. Benefits and risks of solitary islet transplantation for type 1 diabetes. *Diabetes Care*. 2003;26(12):3288-3295. https://doi.org/10.2337/diacare.26.12.3288.
7. Verhoeff K, Marfil-Garza BA, Shapiro AMJ. Update on islet cell transplantation. *Curr Opin Organ Transplant*. 2021;26(4):397-404. https://doi.org/10.1097/MOT.0000000000000891.
8. Kanak MA, Takita M, Kunnathodi F, Lawrence MC, Levy MF, Naziruddin B. Inflammatory response in islet transplantation. *Int J Endocrinol*. 2014;2014:451035. https://doi.org/10.1155/2014/451035.
9. Gamble A, Pawlick R, Pepper AR, *et al*. Improved islet recovery and efficacy through co-culture and co-transplantation of islets with human adipose-derived mesenchymal stem cells. *PLoS One*. 2018;13-(11):e0206449. https://doi.org/10.1371/journal.pone.0206449.
10. Keymeulen B, Vandemeulebroucke E, Ziegler AG, *et al*. Insulin needs after CD3-antibody therapy in new-onset type 1 diabetes. *N Engl J Med*. 2005;352(25):2598-2608. https://doi.org/10.1056/NEJMoa043980.
11. Sherry N, Hagopian W, Ludvigsson J, *et al*. Teplizumab for treatment of type 1 diabetes (Protégé study): 1-year results from a randomised, placebo-controlled trial. *Lancet*. 2011;378(9790):487-497. https://doi.org/10.1016/S0140-6736(11)60931-8.
12. Ong WK, Chakraborty S, Sugii S. Adipose tissue: understanding the heterogeneity of stem cells for regenerative medicine. *Biomolecules*. 2021;11(7):918. https://doi.org/10.3390/biom11070918.
13. Friedenstein AJ, Piatetzky-Shapiro II, Petrakova KV. Osteogenesis in transplants of bone marrow cells. *J Embryol Exp Morphol*. 1966;16(3):381-390.
14. Dominici M, Le Blanc K, Mueller I, *et al*. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy*. 2006;8(4):315-327. https://doi.org/10.1080/14653240600855905.
15. Bateman ME, Strong AL, Gimble JM, Bunnell BA. Concise review: using fat to fight disease: a systematic review of nonhomologous adipose-derived stromal/stem cell therapies. *Stem Cells*. 2018;36(9):1311-1328. https://doi.org/10.1002/stem.2847.
16. Pan F, Liao N, Zheng Y, *et al*. Intrahepatic transplantation of adipose-derived stem cells attenuates the progression of non-alcoholic fatty liver disease in rats. *Mol Med Rep*. 2015;12(3):3725-3733. https://doi.org/10.3892/mmr.2015.3847.
17. Lee JS, Hong JM, Moon GJ, Lee PH, Ahn YH, Bang OY. A long-term follow-up study of intravenous autologous mesenchymal stem cell transplantation in patients with ischemic stroke. *Stem Cells*. 2010;28(6):1099-1106. https://doi.org/10.1002/stem.430.
18. Llufriu S, Sepúlveda M, Blanco Y, *et al*. Randomized placebo-controlled phase II trial of autologous mesenchymal stem cells in multiple sclerosis. *PLoS One*. 2014;9(12):e113936. https://doi.org/10.1371/journal.pone.0113936.
19. Panés J, García-Olmo D, Van Assche G, *et al*. Expanded allogeneic adipose-derived mesenchymal stem cells (Cx601) for complex perianal fistulas in Crohn's disease: a phase 3 randomised, double-blind controlled trial. *Lancet*. 2016;388(10051):1281-1290. https://doi.org/10.1016/S0140-6736(16)31203-X.
20. Westenfelder C, Togel FE. Protective actions of administered mesenchymal stem cells in acute kidney injury: relevance to clinical trials. *Kidney Int Suppl*. 2011;1(3):103-106. https://doi.org/10.1038/kisup.2011.24.
21. Kharaziha P, Hellström PM, Noorinayer B, *et al*. Improvement of liver function in liver cirrhosis patients after autologous mesenchymal stem cell injection: a phase I-II clinical trial. *Eur J Gastroenterol Hepatol*. 2009;21(10):1199-1205. https://doi.org/10.1097/MEG.0b013e32832a1f6c.
22. Mikłosz A, Nikitiuk BE, Chabowski A. Using adipose-derived mesenchymal stem cells to fight the metabolic complications of obesity: where do we stand? *Obes Rev*. 2022;23(5):e13413. https://doi.org/10.1111/obr.13413.
23. Lee RH, Seo MJ, Reger RL, *et al*. Multipotent stromal cells from human marrow home to and promote repair of pancreatic islets and renal glomeruli in diabetic NOD/scid mice. *Proc Natl Acad Sci U S A*. 2006;103(46):17438-17443. https://doi.org/10.1073/pnas.0608249103.
24. Ezquer F, Ezquer M, Contador D, Ricca M, Simon V, Conget P. The antidiabetic effect of mesenchymal stem cells is unrelated to their transdifferentiation potential but to their capability to restore Th1/Th2 balance and to modify the pancreatic microenvironment. *Stem Cells*. 2012;30(8):1664-1674. https://doi.org/10.1002/stem.1132.
25. Wang L, Zhao S, Mao H, Zhou L, Wang ZJ, Wang HX. Autologous bone marrow stem cell transplantation for the treatment of type 2 diabetes mellitus. *Chin Med J (Engl)*. 2011;124-(22):3622-3628. https://doi.org/10.21037/sci.2016.11.14
26. Guan LX, Guan H, Li HB, *et al*. Therapeutic efficacy of umbilical cord-derived mesenchymal stem cells in patients with type 2 diabetes. *Exp Ther Med*. 2015;9(5):1623-1630. https://doi.org/10.3892/etm.2015.2339.
27. Kočí Z, Turnovcová K, Dubský M, *et al*. Characterization of human adipose tissue-derived stromal cells isolated from diabetic patient's distal limbs with critical ischemia. *Cell Biochem Funct*. 2014;32(7):597-604. https://doi.org/10.1002/cbf.3056.
28. Acosta L, Hmadcha A, Escacena N, *et al*. Adipose mesenchymal stromal cells isolated from type 2 diabetic patients display reduced fibrinolytic activity. *Diabetes*. 2013;62(12):4266-4269. https://doi.org/10.2337/db13-0896.
29. Capilla-González V, López-Beas J, Escacena N, *et al*. PDGF restores the defective phenotype of adipose-derived mesenchymal stromal cells from diabetic patients. *Mol Ther*. 2018;26(11):2696-2709. https://doi.org/10.1016/j.ymthe.2018.08.011.
30. Chhabra P, Brayman KL. Stem cell therapy to cure type 1 diabetes: from hype to hope. *Stem Cells Transl Med*. 2013;2(5):328-336. https://doi.org/10.5966/sctm.2012-0116.
31. Nasef A, Ashammakhi N, Fouillard L. Immunomodulatory effect of mesenchymal stromal cells: possible mechanisms. *Regen Med*. 2008;3(4):531-546. https://doi.org/10.2217/17460751.3.4.531.
32. Kocan B, Maziarz A, Tabarkiewicz J, Ochiya T, Banaś-Ząbczyk A. Trophic activity and phenotype of adipose tissue-derived

mesenchymal stem cells as a background of their regenerative potential. *Stem Cells Int*. 2017;2017:1653254. https://doi.org/10.1155/2017/1653254.
33. Timper K, Seboek D, Eberhardt M, *et al*. Human adipose tissue-derived mesenchymal stem cells differentiate into insulin, somatostatin, and glucagon expressing cells. *Biochem Biophys Res Commun*. 2006;341(4):1135-1140. https://doi.org/10.1016/j.bbrc.2006.01.072.
34. Kang HM, Kim J, Park S, *et al*. Insulin-secreting cells from human eyelid-derived stem cells alleviate type I diabetes in immunocompetent mice. *Stem Cells*. 2009;27(8):1999-2008. https://doi.org/10.1002/stem.127.
35. Dave SD, Vanikar AV, Trivedi HL. In-vitro generation of human adipose tissue derived insulin secreting cells: up-regulation of Pax-6, Ipf-1 and Isl-1. *Cytotechnology*. 2014;66(2):299-307. https://doi.org/10.1007/s10616-013-9573-3.
36. Chandra VGS, Muthyala S, Jaiswal AK, Bellare JR, Nair PD, Bhonde RR. Islet-like cell aggregates generated from human adipose tissue derived stem cells ameliorate experimental diabetes in mice. *PLoS One*. 2011;6(6):e20615. https://doi.org/10.1371/journal.pone.0020615.
37. Fazili A, Gholami S, Zangi M, Seyedjafari E, Gholami M. In vivo differentiation of mesenchymal stem cells into insulin producing cells on electrospun poly-L-lactide acid scaffolds coated with Matricaria chamomilla L. Oil. *Cell J*. 2016;18(3):310-321. https://doi.org/10.22074/cellj.2016.4558.
38. Dayer D, Tabar MH, Moghimipour E, *et al*. Sonic hedgehog pathway suppression and reactivation accelerates differentiation of rat adipose-derived mesenchymal stromal cells toward insulin-producing cells. *Cytotherapy*. 2017;19(8):937-946. https://doi.org/10.1016/j.jcyt.2017.05.003.
39. Okura H, Komoda H, Fumimoto Y, *et al*. Transdifferentiation of human adipose tissue-derived stromal cells into insulin-producing clusters. *J Artif Organs*. 2009;12(2):123-130. https://doi.org/10.1007/s10047-009-0455-6.
40. Lee J, Kim SC, Kim SJ, *et al*. Differentiation of human adipose tissue-derived stem cells into aggregates of insulin-producing cells through the overexpression of pancreatic and duodenal homeobox gene-1. *Cell Transplant*. 2013;22(6):1053-1060. https://doi.org/10.3727/096368912X657215.
41. Sun LL, Liu TJ, Li L, *et al*. Transplantation of betatrophin-expressing adipose-derived mesenchymal stem cells induces β-cell proliferation in diabetic mice. *Int J Mol Med*. 2017;39(4):936-948. https://doi.org/10.3892/ijmm.2017.2914.
42. Carlsson PO, Schwarcz E, Korsgren O, Le Blanc K. Preserved β-cell function in type 1 diabetes by mesenchymal stromal cells. *Diabetes*. 2015;64(2):587-592. https://doi.org/10.3892/ijmm.2017.2914.
43. Phan NK, Duong TT, Pham TL-B, *et al*. Preliminary evaluation of intravenous infusion and intrapancreatic injection of human umbilical cord blood-derived mesenchymal stem cells for the treatment of diabetic mice. *Biomed Res Ther*. 2014;1(3):98-105.
44. Yaochite JNU, Caliari-Oliveira C, De Souza LEB, *et al*. Therapeutic efficacy and biodistribution of allogeneic mesenchymal stem cells delivered by intrasplenic and intrapancreatic routes in streptozotocin-induced diabetic mice. *Stem Cell Res Ther*. 2015;6(1):31. https://doi.org/10.1186/s13287-015-0017-1.
45. Murai N, Ohtaki H, Watanabe J, *et al*. Intrapancreatic injection of human bone marrow-derived mesenchymal stem/stromal cells alleviates hyperglycemia and modulates the macrophage state in streptozotocin-induced type 1 diabetic mice. *PLoS One*. 2017;12-(10):e0186637. https://doi.org/10.1371/journal.pone.0186637.
46. Khatri R, Petry SF, Linn T. Intrapancreatic MSC transplantation facilitates pancreatic islet regeneration. *Stem Cell Res Ther*. 2021;12(1):121. https://doi.org/10.1186/s13287-021-02173-4.
47. Bassi ÊJ, Moraes-Vieira PMM, Moreira-Sá CSR, *et al*. Immune regulatory properties of allogeneic adipose-derived mesenchymal stem cells in the treatment of experimental autoimmune diabetes. *Diabetes*. 2012;61(10):2534-2545. https://doi.org/10.2337/db11-0844.
48. Nojehdehi S, Soudi S, Hesampour A, Rasouli S, Soleimani M, Hashemi SM. Immunomodulatory effects of mesenchymal stem cell–derived exosomes on experimental type-1 autoimmune diabetes. *J Cell Biochem*. 2018;119(11):9433-9443. https://doi.org/10.1002/jcb.27260.
49. Sanap A, Bhonde R, Joshi K. Mesenchymal stem cell conditioned medium ameliorates diabetic serum-induced insulin resistance in 3T3-L1 cells. *Chronic Dis Transl Med*. 2021;7(1):47-56. https://doi.org/10.1016/j.cdtm.2020.09.001.
50. Jin J, Shi Y, Gong J, *et al*. Exosome secreted from adipose-derived stem cells attenuates diabetic nephropathy by promoting autophagy flux and inhibiting apoptosis in podocyte. *Stem Cell Res Ther*. 2019;10(1):95. https://doi.org/10.1186/s13287-019-1177-1.
51. Zhang L, Wang Q, Su H, Cheng J. Exosomes from adipose tissues derived mesenchymal stem cells overexpressing microRNA-146a alleviate diabetic osteoporosis in rats. *Cell Mol Bioeng*. 2022;15(1):87-97. https://doi.org/10.1007/s12195-021-00699-4.
52. Safwat A, Sabry D, Ragiae A, Amer E, Mahmoud RH, Shamardan RM. Adipose mesenchymal stem cells–derived exosomes attenuate retina degeneration of streptozotocin-induced diabetes in rabbits. *J Circ Biomarkers*. 2018;7(1849454418807827). https://doi.org/10.1177/1849454418807827.
53. Arzouni AA, Vargas-Seymour A, Rackham CL, *et al*. Mesenchymal stromal cells improve human islet function through released products and extracellular matrix. *Clin Sci*. 2017;131(23):2835-2845. https://doi.org/10.1042/CS20171251.
54. Achenbach P, Bonifacio E, Koczwara K, Ziegler AG. Natural history of type 1 diabetes. *Diabetes*. 2005;54(Suppl 2):S25-S31. https://doi.org/10.2337/diabetes.54.suppl_2.s25.
55. Ohmura Y, Tanemura M, Kawaguchi N, *et al*. Combined transplantation of pancreatic islets and adipose tissue-derived stem cells enhances the survival and insulin function of islet grafts in diabetic mice. *Transplantation*. 2010;90(12):1366-1373. https://doi.org/10.1097/TP.0b013e3181ffba31.
56. Navaei-Nigjeh M, Moloudizargari M, Baeeri M, *et al*. Reduction of marginal mass required for successful islet transplantation in a diabetic rat model using adipose tissue–derived mesenchymal stromal cells. *Cytotherapy*. 2018;20(9):1124-1142. https://doi.org/10.1016/j.jcyt.2018.06.001.
57. Karaoz E, Okcu A, Ünal ZS, Subasi C, Saglam O, Duruksu G. Adipose tissue-derived mesenchymal stromal cells efficiently differentiate into insulin-producing cells in pancreatic islet microenvironment both in vitro and in vivo. *Cytotherapy*. 2013;15(5):557-570. https://doi.org/10.1016/j.jcyt.2013.01.005.
58. Kono TM, Sims EK, Moss DR, *et al*. Human adipose derived stromal/stem cells (hASCs) protect against STZ-induced hyperglycemia; analysis of hASC-derived paracrine effectors. *Stem Cells*. 2014;32(7):1831-1842. https://doi.org/10.1002/stem.1676.
59. Keymeulen B, Gillard P, Mathieu C, *et al*. Correlation between β cell mass and glycemic control in type 1 diabetic recipients of islet cell graft. *Proc Natl Acad Sci U S A*. 2006;103(46):17444-17449. https://doi.org/10.1073/pnas.0608141103.
60. Davalli AM, Ogawa Y, Ricordi C, Scharp DW, Bonner-Weir SWG. A selective decrease in the beta cell mass of human islets transplanted into diabetic nude mice. *Transplantation*. 1995;59(6):817-820.
61. Rackham CL, Dhadda PK, Le Lay AM, King AJF, Jones PM. Preculturing islets with adipose-derived mesenchymal stromal cells is an effective strategy for improving transplantation efficiency at the clinically preferred intraportal site. *Cell Med*. 2014;7(1):37-47. https://doi.org/10.3727/215517914X680047
62. Rackham CL, Dhadda PK, Chagastelles PC, *et al*. Pre-culturing islets with mesenchymal stromal cells using a direct contact configuration is beneficial for transplantation outcome in diabetic mice. *Cytotherapy*. 2013;15(4):449-459. https://doi.org/10.1016/j.jcyt.2012.11.008.
63. Fuggle SV, Allen JE, Johnson RJ, *et al*. Factors affecting graft and patient survival after live donor kidney transplantation in the UK.

Transplantation. 2010;89(6):694-701. https://doi.org/10.1097/TP.0b013e3181c7dc99.
64. Scuteri A, Donzelli E, Rodriguez-Menendez V, *et al*. A double mechanism for the mesenchymal stem cells' positive effect on pancreatic islets. *PLoS One*. 2014;9(1):e84309. https://doi.org/10.1371/journal.pone.0084309.
65. Yamada S, Shimada M, Utsunomiya T, *et al*. Trophic effect of adipose tissue-derived stem cells on porcine islet cells. *J Surg Res*. 2014;187(2):667-672. https://doi.org/10.1016/j.jss.2013.10.031.
66. Hu J, Fu Z, Chen Y, *et al*. Effects of autologous adipose-derived stem cell infusion on type 2 diabetic rats. *Endocr J*. 2015;62(4):339-352. https://doi.org/10.1507/endocrj.EJ14-0584.
67. Yu S, Cheng Y, Zhang L, *et al*. Treatment with adipose tissue-derived mesenchymal stem cells exerts anti-diabetic effects, improves long-term complications, and attenuates inflammation in type 2 diabetic rats. *Stem Cell Res Ther*. 2019;10(1):333. https://doi.org/10.1186/s13287-019-1474-8.
68. Xie M, Hao HJ, Cheng Y, *et al*. Adipose-derived mesenchymal stem cells ameliorate hyperglycemia through regulating hepatic glucose metabolism in type 2 diabetic rats. *Biochem Biophys Res Commun*. 2017;483(1):435-441. https://doi.org/10.1016/j.bbrc.2016.12.125.
69. Nam JS, Kang HM, Kim J, *et al*. Transplantation of insulin-secreting cells differentiated from human adipose tissue-derived stem cells into type 2 diabetes mice. *Biochem Biophys Res Commun*. 2014;443(2):775-781. https://doi.org/10.1016/j.bbrc.2013.10.059.
70. Deng Z, Xu H, Zhang J, *et al*. Infusion of adipose-derived mesenchymal stem cells inhibits skeletal muscle Mitsugumin 53 elevation and thereby alleviates insulin resistance in type 2 diabetic rats. *Mol Med Rep*. 2018;17(6):8466-8474. https://doi.org/10.3892/mmr.2018.8901.
71. Wang XY, Liu CL, Li SD, *et al*. Hypoxia precondition promotes adipose-derived mesenchymal stem cells based repair of diabetic erectile dysfunction via augmenting angiogenesis and neuroprotection. *PLoS One*. 2015;10(3):1-18. https://doi.org/10.1371/journal.pone.0118951.
72. Xue B, Xiao X, Yu T, *et al*. Mesenchymal stem cells modified by FGF21 and GLP1 ameliorate lipid metabolism while reducing blood glucose in type 2 diabetic mice. *Stem Cell Res Ther*. 2021;12(1):133. https://doi.org/10.1186/s13287-021-02205-z.
73. Nadeau KJ, Regensteiner JG, Bauer TA, *et al*. Insulin resistance in adolescents with type 1 diabetes and its relationship to cardiovascular function. *J Clin Endocrinol Metab*. 2010;95(2):513-521. https://doi.org/10.1210/jc.2009-1756.
74. Kahn SE. The relative contributions of insulin resistance and beta-cell dysfunction to the pathophysiology of type 2 diabetes. *Diabetologia*. 2003;46(1):3-19. https://doi.org/10.1007/s00125-002-1009-0.
75. Lee SH, Park SY, Choi CS. Insulin resistance: from mechanisms to therapeutic strategies. *Diabetes Metab J*. 2022;46(1):15-37. https://doi.org/10.4093/dmj.2021.0280.
76. Shree N, Bhonde RR. Conditioned media from adipose tissue derived mesenchymal stem cells reverse insulin resistance in cellular models. *J Cell Biochem*. 2017;118(8):2037-2043. https://doi.org/10.1002/jcb.25777.
77. Elshemy MM, Asem M, Allemailem KS, Uto K, Ebara M, Nabil A. Antioxidative capacity of liver- and adipose-derived mesenchymal stem cell-conditioned media and their applicability in treatment of type 2 diabetic rats. *Oxid Med Cell Longev*. 2021;2021:8833467. https://doi.org/10.1155/2021/8833467.
78. Esser N, Legrand-Poels S, Piette J, Scheen AJ, Paquot N. Inflammation as a link between obesity, metabolic syndrome and type 2 diabetes. *Diabetes Res Clin Pract*. 2014;105(2):141-150. https://doi.org/10.1016/j.diabres.2014.04.006.
79. Su W, Yu S, Yin Y, *et al*. Diabetic microenvironment preconditioning of adipose tissue - derived mesenchymal stem cells enhances their anti - diabetic, anti - long - term complications, and anti - inflammatory effects in type 2 diabetic rats. *Stem Cell Res Ther*. 2022;13(1):422. https://doi.org/10.1186/s13287-022-03114-5.
80. Sanap A, Bhonde R, Joshi K. Conditioned medium of adipose derived mesenchymal stem cells reverse insulin resistance through downregulation of stress induced serine kinases. *Eur J Pharmacol*. 2020;881:173215. https://doi.org/10.1016/j.ejphar.2020.173215.
81. Boushra AF, Mahmoud RH, Ayoub SE, Mohammed RA, Shamardl HA, El Amin Ali AM. The potential therapeutic effect of orexin-treated versus orexin-untreated adipose tissue-derived mesenchymal stem cell therapy on insulin resistance in type 2 diabetic rats. *J. Diabetes Res*. 2022; 2022(9832212):1-9.
82. Wang Y, Yi H, Song Y. The safety of MSC therapy over the past 15 years: a meta-analysis. *Stem Cell Res Ther*. 2021;12(1):545. https://doi.org/10.1186/s13287-021-02609-x.
83. Lalu MM, McIntyre L, Pugliese C, *et al*. Safety of cell therapy with mesenchymal stromal cells (SafeCell): a systematic review and meta-analysis of clinical trials. *PLoS One*. 2012;7(10):e47559. https://doi.org/10.1371/journal.pone.0047559.
84. Dantas JR, Cabral DA, Pereira K, *et al*. Heterologous adipose-derived mesenchymal stem cells and vitamin D supplementation in patients with recent-onset type 1 diabetes mellitus—six months follow-up. *Diabetes*. 2018;67(Suppl 1):1112-P. https://doi.org/10.2337/db18-1112-P.
85. Araujo DB, Dantas JR, Silva KR, Souto DL. Allogenic adipose tissue-derived stromal/stem cells and vitamin D supplementation in patients with recent-onset type 1 diabetes mellitus: a 3-month follow-up pilot study. *Front Immunol*. 2020;11(993):1-12. https://doi.org/10.3389/fimmu.2020.00993.
86. Thakkar UG, Trivedi HL, Vanikar AV, Dave SD. Insulin-secreting adipose-derived mesenchymal stromal cells with bone marrow-derived hematopoietic stem cells from autologous and allogenic sources for type 1 diabetes mellitus. *Cytotherapy*. 2015;17(7):940-947. https://doi.org/10.1016/j.jcyt.2015.03.608.
87. Dave SD, Vanikar AV, Trivedi HL, Thakkar UG, Gopal SC, Chandra T. Novel therapy for insulin-dependent diabetes mellitus: infusion of in vitro-generated insulin-secreting cells. *Clin Exp Med*. 2015;15(1):41-45. https://doi.org/10.1007/s10238-013-0266-1.
88. Trivedi HL, Vanikar AV, Dave SD, Thakkar UG. Cotransplantation of adipose tissue-derived insulin-secreting mesenchymal stem cells and hematopoietic stem cells: a novel therapy for insulin-dependent diabetes mellitus. *Stem Cells Int*. 2010;2010(582382):1-5. https://doi.org/10.4061/2010/582382.