Adipose stem cell-based regenerative medicine for reversal of diabetic hyperglycemia

Hyun Joon Paek, Courtney Kim, Stuart K Williams

Abstract
Diabetes mellitus (diabetes) is a devastating disease that affects millions of people globally and causes a myriad of complications that lead to both patient morbidity and mortality. Currently available therapies, including insulin injection and beta cell replacement through either pancreas or pancreatic islet transplantation, are limited by the availability of organs. Stem cells provide an alternative treatment option for beta cell replacement through selective differentiation of stem cells into cells that recognize glucose and produce and secrete insulin. Embryonic stem cells, albeit pluripotent, face a number of challenges, including ethical and political concerns and potential teratoma formation. Adipose tissue represents an alternative source of multipotent mesenchymal stem cells, which can be obtained using a relatively simple, non-invasive, and inexpensive method. Similarly to other adult mesenchymal stem cells, adipose-derived stem cells (ADSCs) are capable of differentiating into insulin-producing cells when co-transplanted. Additionally, anti-inflammatory and immunomodulatory effects of ADSCs can protect donor islets during the early phase of transplantation and subsequently improve engraftment of donor islets into the recipient organ. Although ADSC-therapy is still in its infancy, the potential benefits of ADSCs are far reaching.

INTRODUCTION
Diabetes mellitus (diabetes) is a chronic disease, affecting over 347 million people globally[1-8]. Due to diets with high fat and high sugar accompanied by sedentary lifestyles, the global epidemic of diabetes is expected to rise. Furthermore, the economic burden imposed by diabetes and its complications easily exceeds $100 billion annually[9].

The most common treatment for type 1 and type 2 diabetes is insulin therapy. Intensive insulin treat-
ment can maintain normoglycemia, and control acute hypoglycemia as well as long-term complications, however, fails to achieve normal hemoglobin A1c levels. Advances in commercial glucose monitors, insulin formulation, and insulin pumps are also providing improved control of diabetic symptoms. However, even with widely available insulin therapy, the life expectancy of diabetic patients is approximately 12 years shorter on average than that of non-diabetic individuals. Additionally, those with child-onset type 1 diabetes have a significantly increased risk of retinopathy, nephropathy, neuropathy, and various cardio-, cerebro- and peripheral vascular diseases.

More definitive treatment options for type 1 diabetes, which is characterized by autoimmune destruction of insulin-producing β-cells in pancreatic islets of Langerhans, are pancreas or pancreatic islet transplantation. Over a century ago, pancreas extracts were the first transplants tested in diabetic patients. Modern-day pancreas and pancreatic islet transplantsations are relatively effective in normalizing fasting and postprandial blood glucose levels, hemoglobin A1c levels as well as restoring insulin and C-peptide production. However, the severe shortage of available donors limit the widespread adoption of this form of therapy, and thus, appear to only benefit less than 0.5% of type 1 diabetes. Additionally, life-long requirement of immunosuppression and adverse effects caused by immunosuppressants, such as nephrotoxicity, hypertension, and hypersensitivity to infection, often leads to patient non-compliance. Lastly, reoccurring autoimmune against pancreatic β-cells continues to be a major challenge associated with transplantation therapies.

Recent advancements in stem cell isolation and differentiation methodologies have resulted in production of cell lines with the capability to synthesize, package, and subsequently secrete insulin in response to glucose. Albeit pluripotent, embryonic stem (ES) cell differentiation often leads to the development of multiple cell lineages, resulting in a mixed population of cells along with target cells. Definitive endodermal markers are also absent in ES cells, and undifferentiated teratogenic ES cells may pose serious risks as well. Due to ethical and legal concerns and risks of teratoma formation, embryonic stem cells face austere challenges in becoming a clinically viable solution although cellular isolation device may provide a method to implant embryonic stem cells with insulin producing capabilities.

Multipotent progenitor cells are now known to be localized in many different organs. Although multipotent, adult stem cells provide a relatively reliable source of mesenchymal stem cells for cell-based therapies. Recently, adult stem cells from bone marrow, umbilical cord blood, pancreatic duct, periosteum, and adipose tissue have shown a capacity to differentiate into insulin-producing cells.

Among the many tissue sources for adult stem cells, adipose tissue is particularly attractive based on its stem cell abundance and ease of tissue procurement through a minimally invasive and relatively inexpensive procedure. Mesenchymal stem cells from bone marrow and adipose tissue share similar cell populations, along with cell characteristics. Adipose tissue has also been reported to contain a significantly greater number of mesenchymal stem cells than bone marrow per unit weight. In this review, adipose-derived stem cells will be specifically examined for their utility in developing treatments for diabetes and diabetic complications.

**Direct differentiation into pancreatic hormone producing cells**

Kodama et al. proposed four mechanisms of pancreatic regeneration: (1) replication of mature β-cells; (2) differentiation of stem cells; (3) cell fusion; and (4) transdifferentiation of one stem cell type to another. Most studies on cell-based therapies focus on direct differentiation of stem cells into insulin-producing β-cells.

Mesenchymal stem cells derived from adipose tissue exhibit characteristics well suited for transdifferentiation into a pancreatic endocrine lineage, which is of the endodermal origin. Freshly isolated adipose-derived stem cells (ADSCs) also expressed stem cell factor (SCF) and its receptor (c-kit), but not ABCG2, nestin, Thy-1, and Isl-1. Lin et al. reported that ADSCs constitutively expressed glucagon and NeuroD as well as insulin. The proliferative ADSCs, on the other hand, expressed the transcription factor Isl-1 and Pax-6, which are critical transcription factors required for β cell development, as a previous study showed that formation of insulin- and glucagon-positive cells were found inhibited during development of Isl-1 knock-out mice. Therefore, the intrinsic expression of Isl-1 in ADSCs provides a considerable advantage for generating insulin-producing cells. Proliferative ADSCs also express stem cell markers nestin, ABCG2, SCF, and Thy-1. Nestin was originally thought to be a neural stem/progenitor cell marker but was recently reported to be a multipotent pancreatic stem cell marker as well, detected within pancreatic islets. ABCG2 has also shown to be associated with pancreatic islet-derived precursor cells and neural stem cells. Koijima et al. demonstrated that extrapancreatic insulin-producing cells, which were positive for proinsulin and insulin, were present in the adipose tissue of streptozotocin-induced diabetic rodents. Based on these intrinsic characteristics, ADSCs can serve as a promising source of pancreatic hormone-producing cells following differentiation.

Derivation of insulin producing cells from stem cells is made possible through the understanding of key steps during embryonic development and the coordinated activation of intracellular transcription factors. Similar to embryonic stem cells, derivation of insulin-producing cells from ADSC is executed through a progressive multi-stage differentiation protocol: starting from definitive endoderm into pancreatic endoderm and finally into pancreatic hormone-expressing cells. Outlines the culture conditions used by various groups to stimulate ADSCs into an insulin-producing cell lineage.

All of the differentiated cell populations reported
were stained positively for dithizone, indicating the presence of endogenous insulin. Furthermore, these stem cell-derived insulin producing cells exhibited abundant expression of Pdx-1, C-peptide, insulin, glucagon, somatostain, pancreatic polypeptide, and Glut-2\(^{22,44,56}\). Enhanced expression of Isl-1, Pax-4, Ngn-3, Ipf-1, Pax-6, Nkx-2.2, Nkx-6.1, Foxa2, GLP-1 receptor, and glucokinase was also confirmed in differentiated cells, implicating pancreatic lineage\(^{22,44,45,60}\). Interestingly, transcription of leptin and adiponectin was also well maintained in differentiated cells, still demonstrating adipose tissue characteristics. Additionally, expression of visfatin, which activates insulin receptors and has a blood glucose lowering effect similar to insulin, was significantly upregulated following differentiation into an insulin producing phenotype\(^{60}\).

Following transplantation of human ADSC-derived insulin producing cells into streptozotocin-induced diabetic mice, a significant level of human C-peptide was detected in subjects, demonstrating successful insulin production \textit{in vivo}. Although these differentiated cells demonstrated a capacity to lower blood glucose levels, the insulin secretion level compared to mature pancreatic islets was significantly lower, and they failed to restore normoglycemia in STZ-induced diabetic mice\(^{64,44,74}\).

The ability of ADSCs to differentiate into insulin-producing cells akin to mature native pancreatic cells also remains under question. Dot et al\(^{79}\) used a genetic lineage tracing method to determine whether pancreatic stem cells contribute to pancreatic β-cell replenishment during adult life. In this study, they demonstrated that terminally differentiated mature β-cells maintain their proliferative capacity and serve as a major source of new β-cells in mice, contrary to previously reported studies\(^{71-74}\). Although this study directly rejected pluripotent adult stem cells’ role in replacing β-cells \textit{in vivo} following partial pancreatectomy, it does not directly refute the utility of insulin-producing cells, differentiated from adult stem cells \textit{in vitro}, as a potential new treatment option for diabetics as demonstrated by a number of studies previously reported\(^{71-74}\).

**Engraftment of transplanted islets**

Success of pancreatic islet transplantation depends on successful engraftment into the recipient liver where donor islets are transfused through the hepatic portal vein. However, apoptosis, inflammation and ischemia frequently interfere with successful engraftment\(^{78}\), and therefore two or more pancreata are frequently required to procure sufficient numbers of islets for each transplant. This is a major limitation to the widespread use of this therapy, considering the acute shortage of donor organs. Due to unavoidable destruction of native islet structures, including intraskeletal vascularity, during isolation, islet engraftment could take up to several weeks\(^{76,67}\). Further deterioration of islets and β-cell death can occur due to ischemia and inflammation, ultimately leading to graft failure\(^{78,79}\). A mean to improve engraftment of transplanted islets will lead to a reduction of the required number of pancreata and more positive clinical outcomes.

Adipose-derived stem cells have been reported to possess inherent regenerative angiogenic potential and anti-apoptotic capability through their secretion of trophic factors\(^{80-82}\). ADSCs also have anti-inflammatory and immunomodulatory properties, including suppression of T-cell proliferation\(^{82,83}\). Therefore, ADSCs can potentially allow improved engraftment of transplanted islets with enhanced vascularization and suppression of inflammation.

Ohmura et al\(^{79}\) tested hybrid islet transplantation by co-transplanting allogeneic mouse pancreatic islets along with autologous ADSC under the kidney capsule of recipient mice and demonstrated that autologous murine ADSCs were able to significantly prolong allogeneic islet survival and achieve normoglycemia for up to 14 d. Allogeneic islets alone could not survive under the kidney capsule for longer than 2 d, and normoglycemia was never achieved. The islets following hybrid transplantation showed well-preserved islet architecture and were surrounded by endothelial cells compared to islet grafts transplanted without ADSCs, suggesting vascularization had been improved. Infiltration by CD4+/CD8+ T cells and CD68+ macrophages were also markedly reduced, suggesting successful anti-inflammation and immunomodulation by ADSCs and prolonged graft islet retention when ADSCs were co-transplanted with donor islets\(^{79}\). Although it is still uncertain whether this hybrid transplantation method will work in a clinical model, which utilizes the hepatic portal vein route for islet transplantation rather than the kidney capsule, the potentially enormous benefits of ADSCs in islet engraftment is clearly promising.

Veriter et al\(^{84}\) also showed the utility of ADSCs by co-encapsulating xenogeneic porcine islets with autologous primate ADSCs in semipermeable capsules and transplanting them in primates. Compared to islets encapsulated alone, improved oxygenation, graft survival and function, and glycated hemoglobin correction, as well as greater vasculogenesis were observed in co-encapsulated implants, consequently reducing the cellular stress immediately following transplantation\(^{89}\).

It is widely accepted that a significantly large number of pancreatic islets are lost during the first 10-14 d following infusion into human liver through the portal vein\(^{90}\), even in the presence of immunosuppression. Furthermore, 60% of transplanted islets were reported to die during this period even in syngeneic animal models\(^{86}\). An ability to prevent such early death immediately following transplantation, as demonstrated by Ohmura et al\(^{79}\), Veriter et al\(^{84}\) and Cavallari et al\(^{82}\), using ADSCs, may prove to be enormously beneficial to the successful engraftment of transplanted islets.

**Challenges and opportunities for ADSCs in diabetes**

Several uncertain factors in stem cell-based cell therapy for diabetes still remain: (1) the absence of gold-standard,
Adipose-derived stem cells and diabetes

reproducible differentiation protocol for generating insulin-producing cells from adult stem cells; (2) an exact dosage of stem cell-derived β-cells to reverse diabetic conditions and feasibility of producing such dosage in vitro; (3) proliferative capacity and maintenance of differentiated insulin-producing cells; (4) sensitivity to counter-regulatory hormones; (5) potential adverse effects of undifferentiated adult stem cells; and (6) potential in vivo migration of differentiated cells following implantation. Consensus of investigators on the criteria for transdifferentiation and plasticity to avoid confusion with cell fusion, contaminating stem cell populations, and to prevent over interpretation of the data, is necessary.

A major challenge also lies in imitating the physiological mechanism of insulin secretion. Insulin secretion occurs through complex regulatory systems, involving multiple hormonal feedback mechanisms and neurological stimulation, within the islet of Langerhans. For instance, insulin secretion by β-cells can inhibit glucagon secretion by α-cells. Somatostatin secreted by δ-cells also regulates insulin secretion by β-cell. In order to mimic normal or near normal metabolic control, differentiated cells must be able to interact with existing pancreatic endocrine cells. Another mechanism of controlling insulin release is through the secretion of incretin hormones, including glucose-dependent insulinotropic peptide and glucagon-like peptide 1. These intestinal tract signaling hormones have shown to be responsible for up to 70% of glucose-induced postprandial insulin secretion. An ability to respond to these signals is also a critical characteristic that stem cell-derived β-cells need to possess in order to closely mimic physiological processes. Lastly, insulin secretion is a pulsatile rather than a constant release, and such pulsatility may be significant in its action. Stem cells differentiated into a pancreatic lineage that simply produces insulin, even in a glucose-responsive manner, without capability to accommodate these complex interactions, will unavoidably fail to reverse diabetic conditions.

The general architecture of natural pancreatic islets also poses another challenge for the efficacy of differentiated insulin-producing cells. Individual islets are highly vascularized and innervated. The endothelial cells comprising the microvasculatures of pancreatic islets of Langerhans may even be glucose responsive. Stem cell-derived islet-like structures thus far have not shown to contain any intrinsic vascularity within them when derived in vitro, and therefore rely on the circulation external to the cell aggregates. The distance between β-cells and capillaries can potentially affect the kinetics of insulin release, and non-physiological integration of islet-like structures to circulation may in turn affect the engraftment, survival, and efficacy of implants. Insulin release by β-cells is affected not only by increased blood glucose level but also by nervous control (cerebral phase) mostly through cholinergic neurons during meal ingestion. Even with whole organ or pancreatic islet transplantation, complete restoration of the cerebral phase of insulin secretion will fail due to a lack of innervation. These structural challenges are critical to overcome for stem cell-derived β-cells or islets to be clinically viable in the future.

Nearly all of the insulin-producing cells derived from adult stem cells co-express glucagon, somatostatin, pancreatic polypeptide along with insulin, all of which are characteristic of immature pancreatic islets of Langerhans. This suggests an incomplete differentiation of stem cells, and could be one of the main reasons why these cells were unable to achieve normoglycemia in diabetic animals. Further differentiation and maturation are required to achieve a more mature substitute capable of functioning similarly to a normal pancreas. However, others also argue that terminally differentiated mature β-cells might not be required for treatment of diabetes. Konno et al and Kajiyama et al. reported that transplantation of adipose-derived stem cells overexpressing Pdx-1 ameliorated hyperglycemia and improved survival rate. Furthermore, ecto-pancreatic transplantation enabled normalization of hemoglobin A1c levels and subsequently attenuated or partially reversed nerve and kidney damages caused by diabetes. Achieving normal hemoglobin A1c levels may also prove to be critical for future stem cell-based therapies.

Diabetic conditions present a uniquely detrimental environment to various cell types. The proliferative capability of mesenchymal stem cells isolated from adipose tissue of streptozotocin-induced type 1 and 2 diabetic rats was reported to be compromised. When ADSCs were exposed to high glucose concentration in vitro prior to implantation into a hindlimb ischemia model, their proliferative capacity and ability to reverse hindlimb ischemia were significantly and irreversibly reduced, compared to ADSCs cultured at a normal glucose concentration. In type 1 diabetic patients, however, autoimmunity did not seem to fundamentally influence the regenerative capability of islets and their progenitor cells. Hess et al. demonstrated that bone marrow derived stem cells initiated pancreatic regeneration and reversed hyperglycemia by stimulating proliferation of the recipient’s innate pancreatic progenitor cells and β-cells. It is highly possibly the same mechanism can be utilized for ADSCs, and therefore, warrants further investigation as well. Improving the relative regenerative capacity of pancreatic islets using ADSCs would potentially benefit diabetic patients.

Transplantation of islet-like cells or pancreas-like tissues generated from stem cells in vitro may be accompanied by graft rejection, graft hypertrophy with subsequent chronic hypoglycemia, and potentially malignant transformation. The intrinsic immunomodulatory capabilities of ADSCs have shown to enhance engraftment of multiple types of tissues when co-transplanted. Vanikar et al. reported that transfusion of ADSCs may reduce the need of immunosuppression during renal transplantations. The ability to reduce the required dosage of immunosuppressants would subsequently minimize complications caused by these agents and improve the clinical uptake of islet-like cells or islets.
outcome of islet transplantation.

Approximately 90% of people with diabetes are suffering from type 2 diabetes. However, only a few cases of stem cell-based research were performed recently,[118-122] to develop a therapeutic option for type 2 diabetes, as type 1 diabetes has stood as the forefront. Deriving insulin-secreting β-cells from stem cells for treatment of type 1 diabetes seems relatively straightforward compared to developing an alternative treatment option for type 2 diabetes. Further research on the complex disease mechanisms of type 2 diabetes in association with the potential utility of stem cells may improve the quality of life for hundreds of millions of patients.

CONCLUSION

It is now undeniable that the utility of ADSCs in the treatment of diabetes is extremely promising. The abundance of available source tissue, high frequency and multipotency of adipose-derived mesenchymal stem cells, its trophic and regenerative capabilities, all serve as valuable solutions to the ever-increasing diabetic population and associated health crises observed around the world. Understanding of ADSCs and the development of ADSC-based treatments for diabetes are still considered to be in their infancy, and numerous challenges and opportunities still lie ahead. The exact mechanism of generating insulin-producing cells from ADSCs as well as further maturation of those cells into functional pancreatic islets still needs to be further explored. Sustainability of differentiated insulin-producing cells is still under investigation. Autoimmune attack on β-cells, which is a fundamental disease mechanism of type 1 diabetes, has not been completely resolved and can make any future cell-based therapy unfeasible.

Current therapies for diabetes ranging from insulin injection to pancreatic islet transplantation are not truly the best options for patients. Stem cells that are theoretically limitless in numbers and multipotent will provide hopes and viable therapies for millions of diabetic patients in the future. However, if all stem cell-based therapies only eliminate the need for glucose monitoring and insulin injection for convenience and modestly improve diabetic symptoms, it would not justify the adoption of these therapies in the future. Therefore, stem cell-based therapies must be able to provide fundamentally improved multifaceted metabolic controls and concomitantly improve long-term prognosis in diabetic patients to be widely accepted as a clinically viable therapy.

REFERENCES


16. Paroni R, Fermo I, Fiorina P, Cighetti G. Determination of asymmetric and symmetric dimethylarginines in plasma...
26 Williams PW. Notes on diabetes treated with extract and by grafts of sheep’s pancreas. BMJ 1894; 2: 1303-1304


83 Ghannam S, Boufi C, Djouad F, Jorgensen C, Noel D, Im...

84 Keyser KA, Beagles KE, Kiem HP. Comparison of mesenchymal stem cell homing from different tissues to suppress T-cell activation. Cell Transplant 2007; 16: 555-562 [PMID: 17708345]


101 Perksen N. The in vivo regulation of pulsatile insulin secretion. Diabetologia 2002; 45: 3-20 [PMID: 11845219]


104 Ahren B, Holst JJ. The cecal insulin response to meal ingestion in humans is dependent on both cholinergic and noncholinergic mechanisms and is important for postprandial glycemia. Diabetes 2001; 50: 1030-1038 [PMID: 11334405]


113 Hess D, Li L, Martin M, Sakano S, Hill D, Strutt B, Thysen


P- Reviewers: Fiorina P, Panchu P S- Editor: Ma YJ L- Editor: A E- Editor: Liu SQ

Paek HJ et al. Adipose-derived stem cells and diabetes