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Review Article

Current stem cell based therapies in diabetes

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Abstract: Diabetes is a disease with wide-ranging personal and societal impacts that has been managed medicinally for over half a century. Since the discovery of stem cells, pancreatic islet regeneration has become a central target for clinical application that has the potential to decrease or eliminate the need for insulin administration and adjunctive medications. The discovery of alternative routes to pluripotency that bypass the ethical implications of embryonic stem cells has significantly expanded the horizons of stem cell based therapy. Engraftment of mature insulin producing cells derived from induced pluripotent stem cells may represent the most promising treatment strategy for diabetic patients with impaired β-cell function. These cells are easily accessible and have been shown to closely mimic endogenous β-cell function *in vivo*. While the risks of oncogenesis and transplant rejection are still of great concern, large strides have been made on both fronts with the application of integration free induction strategies and the ongoing development of microcapsules that cloak implanted cells from an autoimmune response. This review will focus on the progress and remaining obstacles in diabetes related stem cell research, and will specifically discuss approaches using embryonic, induced pluripotent, germline and mesenchymal derived stem cells.

Keywords: Stem cells, stem cell therapy, diabetes mellitus, embryonic stem cells, induced pluripotent stem cells, mesenchymal stem cells, germline stem cells

Introduction

Type 1 and Type 2 diabetes mellitus affects over 300 million people worldwide [1]. Patients with diabetes have over twice the medical expenditures of those without diabetes; in the United States alone, cost of care is $176 billion, and productivity losses account for an additional $69 billion [2]. Chronic complications of diabetes include cardiovascular disease, peripheral vascular disease, neuropathy, nephropathy, retinopathy, and endocrine disorders [2-4]. Type 1 diabetes mellitus (T1DM) is characterized by a deficiency of β-cells as result of autoimmune destruction, while Type 2 diabetes mellitus (T2DM) is characterized β-cell dysfunction and insulin resistance in the peripheral tissues [1, 4]. Both result in the inability to regulate plasma glucose [1, 4].

Insulin therapy mimics β-cell function, but does not match the precision of functioning β-cells [4, 5]. When β-cell function declines significantly, whole pancreas transplant surgery may be considered [3, 4]. While patients demonstrate insulin independence postoperatively, drawbacks to this approach include a 1-3% mortality rate associated with the surgery, lifelong immunosuppression, and limited availability of donor pancreases [3, 4]. Islet transplantation is a less invasive alternative, but it is less effective in achieving insulin independence. Furthermore, the issues of immunosuppressant dependence and limited availability of donor islets remain [1, 3, 4]. Many β-cells are lost in the isolation process, compounding the issue of donor availability [5].

As pancreatic β-cells are dysfunctional or altogether absent in diabetic patients, replacement of these cells has become the major target of stem cell research in diabetes [3, 4]. If successful, this treatment would address the shortage associated with the use of donor pancreases and donor islets [4, 5]. There are a number of different sources of stem cells, including embryonic stem cells (ESCs), induced pluripotent stem cells (iPSCs), germ cell derived stem cells,

and mesenchymal stem cells (MSCs). This review will discuss the current evidence and strategy behind these stem cell sources, as well as the advantages and disadvantages of each.

Embryonic stem cells

Table 1 briefly summarizes some of the advantages and disadvantages of current stem cell based methodologies for diabetes. ESCs are considered to be a valuable treatment option in all types of medicine due to their ability to differentiate into any cell type in the human body. Characteristics of ESCs relevant to clinical medicine include pluripotency of gene expression, self-renewal ability, and high proliferative capacity [4, 6]. While this is an exciting opportunity for diseases involving cellular deficiency, harnessing the power of ESCs has proven difficult due to the complicated nature of induction strategies [6].

A variety of both *in vivo* and *in vitro* differentiation protocols have been developed in order to produce functional pancreatic islets. In general, human ESCs (hESCs) are first harvested from the inner cell mass of the blastula 4-5 days post fertilization, when a high level of telomerase activity persists and when cells maintain the ability to differentiate into all three germ layers [4]. Next, hESCs are differentiated into definitive endoderm (DE) and then, through a chain of endodermal of intermediates, into functional β-cells [1, 7]. These techniques involve exposing hESC lines to specific transcription factors, which promote coordinated activation and inhibition of intracellular signaling pathways. Many cell signaling and epigenetic factors involved in the differentiation process are still unknown, although the presence of markers such as *PDX1*, *Isl1,* and *Foxa2* are indicative of pancreatic β-cells [8, 9]. The exact composite and temporal progression of transcription factors present in pancreatic cells is important for identification, as many of these factors are seen in different combinations in other cell lineages [10]. The differentiation process is meant to mimic the embryological development of the pancreas [6, 11].

Final determination of functional islet cells is made by the presence of endocrine hormones insulin, glucagon, somatostatin (SS), ghrelin, and pancreatic polypeptide (PP), and their expression pattern within the islets. Mature β-cells are defined as those cells capable of both secreting insulin and responding to glucose stimulation with appropriate secretion levels. Insulin production is measured by serum concentration of C Peptide, a byproduct of insulin processing, and by proinsulin. This allows endogenous insulin to be distinguished from insulin taken up from the culture medium [6, 10].

In vitro differentiation techniques allow for more discrete manipulation of the cellular environment and transcriptional factor exposure, but recent research has focused on transplantation of hESC grafts prior to complete differentiation into mature β-cell, such as transplantation of pancreatic progenitors or DE cells [11, 12]. Co-transplantation of undifferentiated hESCs with mouse embryonic dorsal pancreas cells was found to result in differentiated, functional human pancreatic insulin producing cells in 100% of experimental cases studying mice [12]. In contrast, transplantation of hESCs with mouse embryonic liver tissue did not show any insulin production [12]. This suggests that there may be important differentiation signals within the pancreatic microenvironment that are currently undiscovered and which play a crucial role in cell lineage development and β-cell function [4, 13].

Clinical trials involving hESC-derived pancreatic insulin producing cells for the treatment of diabetes have yet to be evaluated. However, variable success on this front has been shown using *in vitro* and animal models. D'Amour et al. developed a method for first producing DE from hESCs [14]. In a subsequent paper, the same group was able to extend this protocol to produce insulin positive cells *in vitro*, although the cells were unresponsive to glucose [7]. Many individual cells also co-expressed insulin and other hormones such as glucagon and SS, a characteristic not seen in mature adult pancreatic cells [7]. This study illustrates the difference between insulin positive cells and actual functioning β-cells.

Kroon et al. implanted hESCs that had only been induced to the point of pancreatic endo-

Table 1. Summary of Regenerative Methodologies

	Advantages	Disadvantages
ESCs[1]	Pluripotent differentiation capacity with unlimited multiplicative ability	Ethical constraints Highest risk for teratoma formation Elicits an autoimmune response
iPSCs[2]	β-cell replicates can be generated without ethical controversy Easily accessible stem cell source	Mutagenic potential of some reprogramming methods Barriers to long-term transplant viability and functionality
NT-ESCs[3] and fESLCs[4]	Embryonic stem cell like product without ethical controversy	Limited access to stem cell source Limited exploration of differentiation to β-cells
htESLCs[5]	Embryonic stem cell like product without ethical controversy	Dispute over whether htELSCs are pluripotent
MSCs[6]	Improved β-cell function through immunomodulation Low risk of autoimmune response due to lack MHC II complexes Lower oncogenic risk than that of iPSCs/ESCs due to limited differentiation capacity	Effects are incomplete and temporary; would require chronic administration and adjunct therapy

[1]Embryonic Stem Cells; [2]Induced Pluripotent Stem Cells; [3]Nuclear-transfer Embryonic Stem Cells; [4]Female Embryonic Stem-like Cells; [5]Human Testis Derived Embryonic Stem-like Cells; [6]Mesenchymal Stem Cells.

dermal cells, similar to those found in fetal pancreatic tissue at 6-9 weeks post fertilization, into the fat pads of immunocompromised mice [11]. At three months post implantation, levels of human C peptide both during fasting and after glucose stimulation were similar to those levels found in mice that had been transplanted with adult human pancreatic islet cells, marking a similarity in efficacy between the two approaches [11]. The presence of human C peptide, not normally found in mice, indicated that the injected pancreatic endodermal cells had differentiated into functioning human β-cells capable of both secreting and processing insulin. Mouse β-cells were then ablated using the toxin streptozotocin, and high serum human C peptide persisted, indicating that the transplanted hESCs had differentiated and were capable of regulating mouse blood glucose independently of the host pancreas [11]. The group reported controlled levels of blood glucose up to 200 days after which they excised the hESC-containing implant and demonstrated a return of blood glucose levels to those of untreated diabetic mice [11]. A study with a similar protocol produced comparable results, and reported restored glucose control for greater than six weeks in 30% of streptozotocin treated mice [15].

The preliminary results for treatment of diabetes via transplantation of hESC-derived insulin producing cells show promise, although many hurdles to clinical application still exist. A range of studies claim to have produced functioning mature β-cells, but only a small number of protocols have been reliably reproduced or are able to produce functioning β-cells in large quantities [6, 16]. Additionally, the ethical debate surrounding the harvest of hESCs has made research on this topic controversial, and as a result, the majority of studies focus on animal models [4]. To date, only one clinical trial involving hESCs in the context of diabetes is open, with enrollment having begun in 2014. The goal of the study is to evaluate the safety and efficacy of VC-01, an implant containing hESC derived pancreatic progenitor cells encapsulated by an immune protecting device, which would allow the cells to proliferate and differentiate into mature β-cells *in vivo* [17]. Without encapsulation devices such as this, immunosuppressants, which pose a risk to the patient, would be needed to prevent host attack of transplanted pancreatic cells [6].

Moreover, safety evaluation is necessary because when undifferentiated cells are transplanted, there is the risk of oncogenesis and specifically of formation of teratomas, tumors that contain all three germ layers [6]. Studies have shown the rate of teratoma formation to be between 33-100% depending on the implantation site of undifferentiated hESCs into mice [18]. Kroon et al. reported teratomas in 6.7% (7 of 105) of mice in the study mentioned above, although the rate of teratoma formation is highly variable depending on cell maturation, purity, and implantation techniques [11]. Cell purification techniques, such as fluorescence activated cell sorting, magnetically activated cell sorting, genetic selection, cell surface markers, and reporter ESC lines can help prevent the implantation of undifferentiated cells, thus decreasing risk of oncogenesis [6, 19].

Induced pluripotent stem cells

The use of iPSCs untangles regenerative therapy in diabetes from ethical constraints, but also

poses its own unique challenges. The production of iPSCs from human fibroblasts was first demonstrated by Yamanaka and colleagues through retroviral transduction of four transcription factors (*Oct-3/4, Sox-2, Klf-4,* and *c-Myc*) in a process termed direct reprogramming [20]. In lieu of the high tumorigenic potential of direct reprogramming resulting from genome integration and activation of oncogenic *c-Myc*, additional research proved iPSCs could be produced from somatic cells in the absence of *c-Myc*, but at the expense of efficiency [21].

Retroviral factor delivery has traditionally been preferred for the generation of iPSCs for its high efficiency; however, retroviral integration and the tumorigenesis associated with proto-oncogene factors limit clinical application. In order to prevent insertional mutagenesis associated with direct reprogramming, various integration-free reprogramming methods have been proposed. Episomal plasmids are an area of current study, and have been shown to reliably reprogram fibroblasts and blood cells [22]. Plasmids have the potential to produce clinical grade cells while generating lower aneuploidy rates than retroviral transduction. However, other reagents including the RNA-based Sendai virus (SeV) enable even greater genetic integrity [23]. Methods for SeV reprogramming are efficient and highly reliable, and unlike retroviral vectors, SeV replicates outside of the cell cycle thereby preventing integration into host DNA [23]. However, clinical transition is complicated by unavailability of the virus commercially [23]. Of the methods currently in use, RNA reprogramming generates the lowest rate of iPSC aneuploidy when successful. Unfortunately, RNA reprogramming frequently only produces a limited number of successful iPSCs from fibroblasts and is incapable of producing iPSCs from blood cells. The addition of microRNA has shown promise in improving the outcomes of RNA based pluripotency induction methods [23].

The successful conversion of stem cells into mature insulin producing pancreatic cells is evaluated by the expression of many factors, but importantly, by *PDX1* and *NKX6-1*, which are known to be essential determinants of mature β-cell function [24, 25]. Attempts to produce bona fide human β-cells from iPSCs *in vitro* initially generated insulin positive cells incapable of co-expressing *NKX6-1* and *PDX1*. These differentiated cells expressed insulin, glucagon, and somatostatin simultaneously, and the polyhormonal cells showed poor intracellular insulin concentration and impaired glucose-stimulated insulin secretion. Transcriptional analysis revealed that these polyhormonal cells had a greater degree of similarity to fetal β-cells as opposed to the desired adult β-cells [24, 26]. However, after revision of the methods and soluble inductive signals of the differentiation protocol, monohormonal adult β-cell replicates were generated by Pagliuca et al. [1]. The iPSC derived β-cells (SC-β cells) secreted insulin in similar proportion to cadaveric primary islet cells during *in vitro* sequential glucose challenges. Additionally, intracellular calcium levels of SC-β cells were observed to rise in parallel with cadaveric β-cells under fluorescent microscopy, marking an appropriate enzymatic and channel-mediated response to elevated environmental glucose. Global gene expression analysis revealed SC-β cells expressing marker genes of mature β-cells, including *NKX6-1* and *PDX1*. Although SC-β cells gene expression did not identically match cadaveric β-cells, the SC-β cells produced by Pagliuca et al. represent the closest stem cell derived reproduction to date [1].

Previous *in vivo* tests of iPSC derived β-like cells did not closely replicate normal islet regulation of blood glucose levels. However, the SC-β cells of Pagliuca et al. were demonstrated to retain monohormonal production of human insulin following transplantation into kidney capsules of immunocompromised mice [1]. At two weeks post-transplant, SC-β cell recipients exhibited similar glucose stimulated insulin secretion to mice transplanted with human islet cells [1]. In β-cell devoid murine diabetic models, hyperglycemia was controlled equally well by SC-β cells and cadaveric islet transplantation, with fasting blood glucose less than 200 mg/dl compared to 600 mg/dl for polyhormonal cell transplanted and non-transplanted control mice [1]. Mice receiving SC-β cells displayed continued insulin secretion at 18 weeks and decreased post transplant morbidity and mortality compared to control mice [1].

Although SC-β cells have been shown to be capable of secreting insulin appropriately in order to manage diabetes in immunocompro-

mised murine models, circumventing immune rejection of transplanted cells represents the continued challenge. Most grafts are allogeneic, and while SC β-cells can be generated from a patient's own somatic cells, transplanted cells remain vulnerable in T1DM due to autoimmunity. To increase the longevity of transplanted cells, systemic immunosuppressant agents have been utilized in animal models. However, the complications of long term immune suppression, including increased susceptibility to cancer and infections, negate the benefits of SC-β cells and preclude rapid clinical translation [27]. One early proposed solution aims to implant cells within an artificial macroencapsulation system to isolate the transplanted cells from host immune defenses while permitting the exchange of small molecules including glucose and insulin. There is worry, however, that encapsulation devices would provide insufficient surface area for exchange of nutrients, and that the body's propensity to encase foreign bodies in fibrous scar tissue may further prevent long term graft survival. To combat these challenges, microencapsulation systems that modify the local immune environment have been devised [27].

While the stability and selective permeability of alginate microcapsules provide an ideal platform for bioencapsulation of SC-β cells, the survivability of grafted material *in vivo* has been augmented by incorporating site specific immune modulators. Microcapsules containing SC-B cells have been co-transplanted with mesenchymal stem cells, regulatory T Cells and Sertoli cells in an attempt to dampen graft immunogenicity [27]. Recent immunoevasive strategies involve the incorporation of immune suppressing agents into the microcapsule membrane. Integration of ursodeoxycholic acid (UDCA), a tertiary bile acid determined to inhibit immune activation and phagocytosis of allogeneic tissue, was found to increase encapsulated β-cell viability without negatively impacting the integrity of the microcapsule [28]. In fact, the addition of UDCA was suggested to reduce cell swelling and optimize microcapsule durability [28]. Islet containing microcapsules have also been coated with the chemokine CXCL12 [27]. CXCL12 is strongly chemotactic for immune-suppressive regulatory T cells and repellant of effector T cells, thereby promoting local immune-isolation of the engrafted cells [27]. Chen et al. demonstrated that in murine models of diabetes, CXCL12 coating prolonged encapsulated islet function *in vivo* by effectively shielding allogeneic and xenogeneic islets from acute cell-mediated rejection; however, they were incapable of abating humoral anti-islet antibodies [27]. Coated cells improved site specific protection from chronic immune destruction and may also prove valuable as a protective coating for retrievable devices containing encapsulated SC-β cell populations. Although CXCL12 only hindered eventual islet rejection in the presence of anti-islet antibodies, in the future the chemokine may function in conjunction with other local immune modulators to permit graft survival [27]. Taken together, these studies address the ethical and safety concerns surrounding iPSC therapy as well as its potential efficacy in the treatment of T1DM [1, 21-28].

Germline stem cells

Researchers have explored the possibility of producing β-cells from stem cells derived from oogonia and spermatogonia. Currently, there are two dominant methods involving oogonia. The first involves using the oocyte to transform a somatic nucleus into a pluripotent cell. Yamada et al. successfully reprogrammed an adult somatic nucleus to a diploid pluripotent stem cell by transferring it to an enucleated oocyte [29]. These nuclear transfer ESCs (NT-ESCs) were demonstrated to be pluripotent by expression of surface markers and genes, and the ability to generate cell types of all three germ layers [29, 30]. Further, these NT-ESCs could differentiate into insulin positive cells that secreted insulin when stimulated *in vitro* [29]. Although pluripotent cells have been demonstrated using female germline stem cells, production of functional β-cells in *in vivo* models must be explored [29]. Another hurdle in this field that would likely hinder clinical transition is the availability and cost of acquisition of human oocytes [31].

The second method involving oogonia requires collection of female germline stem cells (FGSCs) from murine ovaries and passaging and expanding these cells in culture [32]. The expanded cell lines are subsequently cultured in ESC-specific conditions to yield female ES-like cells (fESLCs) [32]. The stem-cell-like

nature of these cells has been confirmed by demonstrating their pluripotency through the expression of surface markers, transcription factors, and teratoma formation [32]. Chimera assays of offspring from mice injected with these labeled fESLCs confirmed the potential to differentiate into all three germ layers [32]. The ability to first collect the stem cells and expand them in culture partially addresses the issue of cell availability. However, if this strategy has a place in the treatment of diabetes, further studies demonstrating the feasibility of this protocol using human cells that can be inducted to produce insulin will be necessary.

Spermatogonium stem cells (SSCs) have shown more promise. These cells are located on the basement membrane of the seminiferous tubules in the testes, and within this microenvironment differentiate into mature male gametes [33]. In murine models, it has been shown that when exposed to various growth factors and reagents in culture, SSCs transform into pluripotent ESC-like cells [34-36]. The transformation from SSCs to ESC-like cells was replicated in humans, yielding human testis-derived embryonic stem-like cells (htESLCs) [33, 37]. Unlike iPSCs, generation of htESLCs from SSCs does not require genetic modification [38]. Once formed, htESLCs have the ability to differentiate into any of the three germ layers, including pancreatic endodermal cells capable of producing insulin *in vitro* [33]. Thus, htESLCs may have the same regenerative potential as ESCs without the associated ethical implications [4].

Although htESLCs show promise, there is controversy as to whether these cells are truly pluripotent. Several groups have reported the formation of only small teratomas upon implantation of htESLCs into immunocompromised mice, suggesting a potentially lower risk of teratomas than is the case for ESCs and iPSCs [33, 37]. As teratoma formation is a means of gauging pluripotency, it has come into question whether htESLCs are truly pluripotent because of their limited ability to do so [39]. Furthermore, inadequate expression of pluripotency markers has cast additional doubt on the pluripotent nature of htESLCs [39]. Highlighting this controversy, one of the first efforts to produce htESLCs, published by Conrad et al. in 2008 was later retracted in 2014 [40, 41]. The retraction states, "*Nature* does not dispute the main claim that the cells are pluripotent to some level, but the level of proof of pluripotency shown is not in line with regular criteria for such papers in *Nature*" [40]. Gene expression microarray and teratoma formation analysis suggests that htESLCs more closely resemble MSCs [39]. *In vitro* culturing of select testicular cell types suggests that htESLCs originate from somatic mesenchymal progenitors present in the primary testicular cell cultures, which explains their genetic resemblance [42]. The question over the nature of htELSC potency is yet to be resolved. It is possible that optimization of reprogramming strategies could produce the missing pluripotency markers and increase teratoma formation, thus settling the debate.

The potential role of htESLCs in diabetes therapy remains to be further explored. While several groups have shown htESLCs' ability to form insulin-producing cells upon differentiation *in vitro*, their ability to respond to glucose challenge and viability in an *in vivo* model remains to be demonstrated [33, 41]. In addition, since patient derived htESLCs are produced from testes, it would only be possible to produce patient-matched islet cells for male patients [32, 33]. As we learn more about the development and feasibility of htESLCs, there could be potential implications for the use of ovarian stem cells for use in female patients [4].

There is an additional issue as to whether the derivation of ESC-like cells from SSCs is age limited [43]. Previous studies that derived ESC-like cells from SSCs have done so with young mice [34-36, 43]. A recent study of SSCs in mice showed that though they were able to derive ESC-like cells from SSCs in young mice, as has been done previously, they were unable to derive ESC-like cells from adult mice (8 weeks and older) [36, 43]. Ko et al. used SSCs from mice aged 10 days to 7 months, but it is unclear whether SSCs from all age groups yielded ESC-like cells [36, 43]. A later protocol for the derivation of ESC-like cells from SSCs by this group used only mice less than 5 weeks old [35, 43]. Azizi et al. found that a decrease in transcription factors is associated with aging and may be related to the reduction in differentiation to ESC-like cells, suggesting that there may be a critical window for this derivation [43].

These age limitations may have implications for human applications [43].

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) can be found in a wide variety of adult tissues. Due to their immunomodulatory properties and low immunogenicity due to lack of MHC II complexes, MSCs have been studied by many groups in the context of T1DM [44]. MSCs derived from a broad range of tissues including adipose tissue, bone marrow, and cord blood have been shown to have therapeutic effects in experimental diabetic rodent models [45-48]. Favorable immunomodulatory effects have also been reported in *in vitro* experiments with co-culture of human Type 1 diabetic peripheral blood mononuclear cells with MSCs [49] and in human clinical trials with diabetic patients [50]. The specific mechanism through which MSCs exert these favorable effects is in question, and the answer has implications for the clinical application of this treatment.

Bassi et al. purified adipose derived mesenchymal stem cells (AD-MSCs) from epididymal mouse fat [45]. Injection of these cells into non-obese diabetic (NOD) mice, an accepted model for studying T1DM, demonstrated reversal of hyperglycemia in 78% of cases. These results indicate that the AD-MSCs at least partially attenuated the leukocytic infiltrate of host pancreatic islets which is the key feature of the NOD murine model. While this is of course significant, it should be noted that treated mice did not maintain optimal blood glucose levels in the long term, with a steady climb in glucose levels appearing at week 9 post operatively and culminating at week 12 with an average blood concentration of over 300 mg/dL [45].

In addition to increases in circulating insulin, glucagon, and amylin, which indicate at least partially restored pancreatic function, Bassi et al. demonstrated multiple immunomodulatory effects present in mice treated with AD-MSCs [45]. The group reported a decrease in infiltration of inflammatory cells in pancreatic islets as well as a suppression of CD4+TH1 cells, which mount the autoimmune attack on pancreatic islets in T1DM [45]. This type of immune response regulation was also seen by another group who co-transplanted pancreatic islets with MSCs [46]. Furthermore, Bassi et al. demonstrated an increase in regulatory T-cells (Tregs) [45]. Tregs help maintain homeostasis and self tolerance by inhibiting self reactive effector T-cells [51] and thus have the potential to at least partially mitigate the adverse immune response of the Type 1 diabetic patient. Additionally, an increase in TGF-β1, a cytokine shown to alleviate the autoimmune response in T1DM was reported *in vivo* [45, 50]. Upregulation of Tregs and TGF-β1 was confirmed in *in vitro* co-culture experiments of AD-MSC with T-cells, thus demonstrating a cell-to-cell contact mechanism through which the immunomodulatory effects were elicited.

Kono et al. elucidated specific factors secreted by AD-MSCs *in vitro* while demonstrating their systemic effects *in vivo* [48]. The group purified human AD-MSCs (hAD-MSCs) from healthy donors, transduced the cells with green fluorescent protein (GFP) for tracking experiments, and subsequently injected them into immunodeficient diabetic mice. hAD-MSC treated mice showed elevated insulin levels and a partial reversal in hyperglycemia. Importantly, these therapeutic effects were elicited with human cells, demonstrating the potential translative capacity of this treatment. However, like Bassi et al., complete, sustained reversal of hyperglycemia was not achieved, as blood glucose levels, while reduced well below that of untreated diabetic mice, remained above those of non-diabetic mice throughout the experiment up to the last shown data point at 35 days [45, 48].

Co-culture of islets with hAD-MSCs *in vitro* resulted in significant upregulation of several factors, but chiefly TIMP-1 [48]. As TIMP-1 is a glycoprotein that prevents cytokine-induced death, this was a proposed mechanism for the improved β-cell mass seen in pancreatic histologic sections of hAD-MSC treated mice [48]. These findings support the results of other groups that have shown upregulation of secreted anti-apoptotic factors in MSCs in various injury models [52, 53]. To determine whether this increase in β-cell number came directly from hAD-MSC division, a mitotic stain was performed on pancreatic tissue sections [48]. GFP labeled hAD-MSCs were found in and around pancreatic islets; however, no GFP+ cells were found to be undergoing mitosis. This indicates that the hAD-MSCs did not directly divide to give rise to β-cells. Instead, hAD-MSCs may

have fostered a microenvironment conducive to β-cell proliferation, possibly in part through the aforementioned upregulation of TIMP-1 [48]. Because upregulation of TIMP-1 along with other factors was seen in *in vitro* co-culture of islets with hAD-MSCs, a paracrine cross talk model was proposed wherein secreted factors from AD-MSCs elicit the reported therapeutic effects seen in diabetic models [48]. The next question then was, do the cells themselves have to be present in order to evoke the reported therapeutic effects, or can their secreted factors alone achieve a response?

Gao et al. provide interesting insight to this question [47]. The group extracted and purified bone marrow derived mesenchymal stem cells (BM-MSCs) from mice. They too transduced their BM-MSCs with GFP for *in vivo* tracking experiments. Similar to other groups, they reported a partial reversal of hyperglycemia up to 42 days and an increase in the size and number of pancreatic islets in diabetic mice treated with BM-MSCs [45, 47, 48].

To further illuminate the mechanism through which the BM-MSCs exerted their effects, the group cultured BM-MSCs for several days, collected the cell culture medium and concentrated it, and then injected the concentrated media alone (without cells) into diabetic mice [47]. They saw nearly identical results in reversal of hyperglycemia as well as in the increase in pancreatic islets and β-cells. Their data strongly support the paracrine effect described by Kono et al., but take it one step further by completely removing the cells from treatment [47, 48]. A comprehensive characterization of the content of this concentrated media could prove rewarding because if the cocktail could be efficiently replicated, it represents a promising therapy that would completely avoid the overly reactive immune system of the Type I diabetic patient.

Zhao et al. is one of the few groups to report the effects of postnatal stem cells in T1DM using 'stem cell educator therapy' [50]. The group purified and cultured human cord blood derived stem cells (hCB-SCs). They then co-cultured patients' lymphocytes with hCB-SCs for several hours and returned only the now 'educated', potentially less auto-reactive lymphocytes to the patients' circulation. In patients who received stem cell educator therapy, Zhao et al. reported increases in fasting C-peptide levels to that of normal levels, improved responses to oral glucose tolerance tests and normalization of glycated hemoglobin at 12 weeks post treatment up until the last shown data point of 24 weeks. While patients will continued to be monitored in the long term, no negative side effects have been reported [50].

Similar to Bassi et al., the group reported an increase in the autoimmune regulators Tregs and TGF-β1, thus providing insight into possible immunomodulatory mechanisms [45, 50]. Additionally, hCB-SCs expressed the autoimmune regulator (Aire), which "mediates ectopic expression of peripheral self antigens and deletion of autoreactive T-cells" [50]. Knockdown of Aire using small interrupting RNA (siRNA) reduced the number of Tregs in a co-culture of lymphocytes with hCB-SCs, providing further evidence for the mechanism behind the efficacy of treatment [50]. This therapy could be quite promising because not only is its success in treating hyperglycemia superior to that shown in other MSC models, it has been shown already to work in human diabetic patients. Additionally, cord blood donors do not have to be matched to patients due to autologous nature of the transplant. Corroboration of these data by other groups would help bring this approach to the forefront of clinical trials.

Conclusion

Various groups describe the benefits of MSC therapy in T1DM through indirect, immunomodulatory mechanisms that create a microenvironment in which β-cells are able to regenerate and destructive TH1 cells are at least partially suppressed. Though all the groups discussed in this review reported an improvement in hyperglycemia, with the exception of Zhao et al., none reported blood glucose levels within the normal range. It is likely then that MSCs represent a therapy to be used in conjunction with other treatment strategies. Strong histologic evidence supports the fact that undifferentiated MSCs do not themselves directly give rise to new β-cells. Furthermore, cell-free media collected from MSC culture elicited promising, therapeutic results upon injection into diabetic mice. This method, if optimized, could provide an exciting therapy, which, due to its cell-free nature, avoids the issues of oncogenesis and autoimmunity. If collection techniques were

optimized, MSC media could be used in conjunction with standard therapy to lower insulin dependence or alongside transplant of insulin producing cells derived from other pluripotent sources.

Pluripotent cells derived from hESCs and germline stem cells have a potential role in diabetes therapy, but also come with noteworthy barriers. While recent research shows that hESCs can be induced to become glucose responsive β-cells, the availability of hESCs and the ethical concerns surrounding hESC work represent a significant obstacle to further exploration. Initial experiments show that germline stem cells may be able to produce results similar to hESCs in terms of β-cell regeneration while sidestepping ethical controversy. However, the field is relatively young, and several major strides must be taken to move it forward. First, the debate surrounding the origin and pluripotency of SSCs needs to be resolved before research can advance. Furthermore, the insulin producing cells created from SSCs must be proven functional. Additionally, research on oogonium derived cells such as NT-ESCs and fELSCs must still explore if these pluripotent products can be differentiated into functional β-cells *in vivo*. Given these drawbacks to pluripotent cells derived from hESCs and germline stem cells, iPSCs may be the most promising treatment option for restoring β-cell function to patients with T1DM and T2DM.

Due to their widespread availability, lack of ethical controversy, and ability to differentiate into functional β-cell replicates *in vivo*, iPSC therapy represents the farthest strides in diabetes related regenerative medicine to date. However, safe and successful protocols for their production and engraftment need be established. Integration-free reprogramming with RNA has shown promise in generating clinical grade cells with a low risk of oncogenesis. However, efficiency must be increased before this technique can be put to general use. Immunoevasive microcapsules that prevent graft rejection and simultaneously allow for increased metabolic exchange surface area represent a promising approach to circumvent the immune response seen in islet transplant and in T1DM. While microencapsulation of transplanted cells enhances graft viability, dispersion of the capsules *in vivo* may also make withdrawing the cells more difficult should they become cancerous. However, ongoing research aimed at optimized bioengineering of local immunomodulatory microencapsulation structures may prove promising in making regenerative iPSC therapy for diabetes a reality.

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