Concise Review: Pluripotent Stem Cell-Based Regenerative Applications for Failing β-Cell Function

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ABSTRACT

Diabetes engenders the loss of pancreatic β-cell mass and/or function, resulting in insulin deficiency relative to the metabolic needs of the body. Diabetic care has traditionally relied on pharmacotherapy, exemplified by insulin replacement to target peripheral actions of the hormone. With growing understanding of the pathogenesis of diabetic disease, alternative approaches aiming at repair and restoration of failing β-cell function are increasingly considered as complements to current diabetes therapy regimens. To this end, emphasis is placed on transplantation of exogenous pancreas/islets or artificial islets, enhanced proliferation and maturation of endogenous β cells, prevention of β-cell loss, or fortified renewal of β-like-cell populations from stem cell pools and non-β-cell sources. In light of emerging clinical experiences with human embryonic stem cells and approval of the first in-human trial with induced pluripotent stem cells, in this study we highlight advances in β-cell regeneration strategies with a focus on pluripotent stem cell platforms in the context of translational applications.

INTRODUCTION

Diabetes is a staggering health problem affecting more than 300 million people worldwide. By 2030, an estimated 440 million adults will be afflicted with diabetes [1, 2]. Premature morbidity and mortality create a substantial and escalating burden on the global health system and society. Type 1 diabetes (T1D) is defined by insulin deficiency brought about by autoimmune destruction of islet β cells. Type 2 diabetes (T2D) is defined by the progressive inability of the insulin secretory capacity to match peripheral insulin needs. Defective innate β-cell regeneration, because of either β-cell destruction or insufficient β-cell replenishment, is increasingly recognized as central to the pathobiology of both T1D and T2D (Fig. 1) [3–6].

Pharmacological means that promote insulin production or replace insulin function have comprised a primary line of therapy for insulin insufficiency in T1D and a large proportion of patients with T2D, in particular those with advanced disease. Case in point, injection of exogenous insulin is required for people with T1D and advanced T2D. Glucagon-like peptide-1 (GLP-1) analogs have been designed more recently to augment insulin secretion and preserve β-cell function. Alternatively, DPP-4 inhibitors, which prevent inactivation of GLP-1, have been used to promote endogenous insulin production in T2D [7]. Whereas collectively the spectrum of preventive and palliative approaches has led to improved diabetic care, a fail-safe physiological regulation of systemic blood glucose levels remains challenging.

Frequent fluctuations in blood glucose levels have been implicated as a culprit of heterogeneous (co)morbidities, such as retinopathy, nephropathy, neuropathy, or cardiovascular complications. In this context, various nonpharmacological approaches have been recently explored to restore functionality of the failing β-cell mass. Such regenerative approaches have evolved rapidly, from prototypic cell replacement therapies through pancreas/islet transplantation to the potential use of artificial insulin-producing cells derived from stem cells or pancreatic progenitor cells (Fig. 2).

ADULT CELL-BASED THERAPIES FOR DIABETES

Islet transplantation has provided a foundation for next-generation cell-based therapeutics for diabetes [8]. The Edmonton protocol, a flagship islet transplantation protocol, has achieved long-term islet survival. In early studies, more than 50% of subjects gained insulin independence 1 year post-transplantation and experienced improved glycated hemoglobin levels and protection from hypoglycemia [9]. Twenty percent of islet recipients are insulin therapy-free 5 years after transplantation [10]. Recent studies have shown improvements in primary efficacy, safety outcomes, and insulin independence 3 years post-transplant [11, 12]. Despite notable progress in the field, several issues still need to be addressed. These include the islet isolation methods, sites for transplantation, immunosuppression, or immunosilosisation strategies [13–16]. It is also...
notable that total pancreatectomy with intrahepatic autotransplantation for non-T1D patients results in only 30% insulin independence at 3 years [17]. Although this is in part because of low islet yield in some patients [17], failure to achieve high rates of insulin independence in autologous, autoimmunity-free settings highlights the current limitations of islet transplantation. With further improvement, islet transplantation could provide a long-term therapy for patients with insulin deficiency.

An additional key issue is the persistent shortage of postmortem pancreatic tissue for islet isolation. This will be a major hurdle when islet transplantation becomes the standard therapy for T1D. One solution would be the successful combination of an opt-out system and the priority rule to increase organ donation. Additionally, in an effort to obtain a sustainable source of insulin-producing cells, various alternative sources have been explored, including islet transplantation from living donors [18] and xenogeneic islets [19]. Insulin-producing surrogate cells from diverse stem cell sources have also emerged as alternative biotherapeutic candidates for diabetes care [8, 20]. Among adult stem cells, extensive experience has been obtained with multipotent mesenchymal stem cells (MSCs) [21]. In addition to generation of β-like cells from MSCs [21, 22], their potent anti-inflammatory or immune-suppressive effects have been increasingly evaluated. This includes their potential for immunomodulation and tissue damage protection to suppress autoimmunity in T1D or to enhance islet engraftment and survival [23–25], underscoring versatility in their mechanism of action and benefit potential. Because the current efforts using adult mesenchymal stem cells, and also hematopoietic or pancreatic stem cells, have been extensively reviewed [8, 20, 21, 26, 27], this synopsis highlights natural and bioengineered pluripotent stem cells, underscoring their translational potential for diabetes therapies.

**EMBRYONIC STEM CELL-BASED THERAPIES FOR DIABETES**

The capacity for unlimited self-renewal and pluripotent lineage specification renders embryonic stem cells (ESCs) a unique platform for regenerative medicine. Various protocols have shown successful and efficient differentiation into ectoderm, endoderm, and mesoderm. Currently, there are two main approaches for generating insulin-producing cells from ESCs. The first is to leverage the spontaneous differentiation propensity of pluripotent cells through embryoid body (EB) formation, followed by selection for β-cell/β-cell-progenitor marker-expressing cells. Use of insulin-producing cells from ESC-derived progeny, selected for β-cell-specific gene expression, has been shown to normalize hyperglycemia in diabetic mice [28, 29]. The second strategy involves stepwise, lineage-specific differentiation protocols, largely adapted from in utero β-cell developmental blueprints. In this way, guided differentiation of ESCs has achieved generation of insulin-producing cells [30], although subsequent studies have questioned the authenticity of derived progeny [31]. Through a marriage of both strategies, spontaneous differentiation of EBs and coaxed differentiation of early pancreatic progenitors, successful generation of insulin-producing cells has also been documented [32].

As spontaneous differentiation is limited by inefficiency, guided differentiation has been the primary strategy used for differentiation of human ESC into insulin-producing cells. With renewed focus on decoding embryonic development, the progressive evolution of endoderm into primitive gut tube and ultimately discrete pancreatic β cells has become increasingly defined [33]. The critical first step of guided differentiation protocols is the induction of endoderm from human ESCs [34, 35], which is typically achieved through stimulation with activin A (a Nodal surrogate), Nodal, and/or Wnts, under low serum conditions [36–38]. Derived definitive endoderm expresses markers such as FOXA2, SOX17, and CXCR4 [33]. Further guidance achieves generation of pancreatic multihormonal endocrine cells through foregut, pancreatic endoderm, and endocrine progenitor stages [39]. However, resulting cells demonstrate immature β-cell-like phenotypes [39]; that is, these human ESC-derived β-like cells produce high levels of intracellular C peptide comparable to human islets and respond to insulin secretagogues but fail to respond to high glucose stimulation. Modified or improved protocols have been established using combinations of cytokines and small molecules, such as fibroblast growth factors, noggin, KAAD-cyclopamine Sonic hedgehog pathway inhibitors (KAAD-cyclopamine or SANT-1), retinoic acid, nicotinamide, and GLP-1 (Table 1) [40–51]. Notable improvements in pancreatic differentiation have been reported with use of a small-molecule Indolactam V, which accelerates induction of pancreatic progenitor cells from definitive endoderm through protein kinase C (PKC) activation [52], or suppression of the transforming growth factor (TGF)β/activin/bone morphogenetic protein signaling pathways at specific stages by noggin or SB431542/ALK5 inhibitors [43, 44]. Accordingly, use of noggin, PKC activator (25,55)-(E,E)-8-(5-(4-(trifluoromethyl)phenyl)-2,4-pentadienoylamino)benzolactam (TPB) and TGFβ inhibitor in combination has proven effective.
for induction of pancreatic endoderm and endocrine precursors [46].

**INDUCED PLURIPOTENT STEM CELL-BASED THERAPIES FOR DIABETES**

Despite notable progress, the clinical development of cell replacement therapies using human ESCs has been surrounded by ethical concerns. The use of allogeneic ESC-derived cells is also associated with immunological mismatch. Although a recent study has demonstrated successful generation of personalized human ESCs through somatic cell nuclear transfer (NT-ESCs) [53], derivation of human NT-ESCs remains challenging. In this regard, nuclear reprogramming technology, which allows generation of pluripotent stem cells from adult somatic cells, has opened a new path for generating patient-specific pluripotent stem cells [54, 55]. The induced pluripotent stem cell (iPSC) technology relies on genetic introduction of selected pluripotency-associated factors in adult somatic cell sources, which reprogram cell fate enabling de-differentiation into a pluripotent stem cell state [56, 57]. Derived human iPSC lines show characteristics similar to human ESCs, including morphology, global gene expression profiles, elongated telomeres, and the propensity to differentiate into all three germ layers [58, 59], offering a self-renewable source of new tissues derived from the patient’s own cell pool [60].

**PATIENT-DERIVED iPSCS AND THEIR DIFFERENTIATION INTO INSULIN-PRODUCING CELLS**

Unlike their natural counterparts, iPSCs carry patient-specific genetic traits, providing a unique autologous pluripotent platform. Accordingly, differentiation of patient-derived iPSCs into disease-relevant cell types would allow for patient-specific modeling of disease progression and patient-specific drug screening [59, 61]. Several studies have demonstrated the efficacy of recapitulating disease phenotypes using patient-specific iPSCs [62–64], verifying the utility of iPSC technology for in vitro disease modeling. Patient-specific iPSCs would also create the opportunity for immunosuppression-free, autologous stem cell-based regenerative approaches for degenerative disorders.

Derivation of diabetes-specific iPSCs and their differentiation into functional β cells provide the foundation for new diagnostic and therapeutic applications. Diabetes-specific iPSCs have been derived from both T1D and T2D patients [61, 65–68], which demonstrate similar genome-wide gene expression profiles to those of human ESCs [69]. Importantly, iPSC clones derived from patients of different age groups and sex are capable of generating insulin-producing cells [65, 68, 69], a prerequisite in establishing a broader translational platform for diabetes-specific iPSCs. Patient iPSC-derived β-like cells would enable detailed analysis of patient-specific immunity against β cells at the cellular level, whereas autologous properties would facilitate use as a cell-based therapy for diabetes. A recent study demonstrates that iPSC-derived β cells from subjects with maturity-onset diabetes of the young type 2 (MODY2), characterized by impaired glucokinase activity, recapitulate the β-cell-autonomous phenotypes of MODY2 [70].

**CHALLENGES FOR CLINICAL APPLICATIONS OF DIABETES-SPECIFIC iPSCS**

**Immunogenicity and Epigenetic Abnormalities**

Recent iPSC studies have raised several possible concerns for broader application. In particular, the reprogramming process and subsequent expansion of iPSCs have been associated with potential genetic and epigenetic abnormalities [71–74]. Moreover, iPSC-derived cells have been reported to show abnormal gene expression patterns, capable of inducing T-cell-dependent immunity in syngeneic recipients [75]. Yet, these initial studies need further confirmation as, for example, only limited immunogenicity of transplanted iPSC-derived cells has been reported [76].

**Teratoma Formation**

Another biosafety concern surrounding the therapeutic use of iPSCs or derivatives is the risk of teratoma formation upon transplantation. The primary source of teratoma is the residual
| Reference | Year [Ref.] | ESCs/iPSCs | Differentiation | Definitive endoderm (SOX17, FOXA2) | Pancreatic endoderm (PDX1, NGN3) | Islet-like cells (NKX6.1, INS, GCG) | Insulin positive % | Insulin/C-peptide secretion |
|-----------|-------------|------------|----------------|-----------------------------------|----------------------------------|------------------------------------|-----------------------|----------------------------|
| D’Amour et al. (Viacyte) | 2006 [39] | hESC (CyT203) | 5 stages, ~18 days | ACT, WNT | FGF10, CYC, RA, (DAPT), Ex4 | EX4, IGF1, HGF | 7.3% up to 12% | No glucose responsiveness induced by secretagog |
| Jiang et al. (Geron) | 2007 [41] | hESC (H1, H7, H9) | 4 stages, ~36 days | ACT, Na-butyrate | EGF, bFGF, NOG (in suspension) | NIC, IGFII | 4.1% | 3.5-fold induction at 20 mM glucose |
| Kroon et al. (Viacyte) | 2008 [86] | hESC (CyT49) | 4 stages, 12 days | ACT, WNT | KGF, CYC, RA, NOG | In vivo maturation cells in Gelfoam discs, Tx into epididymal fat pad/renal capsule | Yes, 10–14 weeks after Tx |
| Park et al. | 2008 [61] | T1D iPSC JDM#1 (retroviral) | 4 stages, ~36 days | ACT, Na-butyrate | EGF, bFGF, NOG (in suspension) | NIC, IGFII | 2- to 4-fold induction at 40 mM glucose, clonal variations |
| Tateishi et al. | 2008 [83] | hESC (H9) iPSC #3, #7 (retroviral) | 4 stages, ~36 days | ACT, Na-butyrate | EGF, bFGF, NOG (in suspension) | NIC, IGFII | 2- to 4-fold induction at 40 mM glucose, clonal variations |
| Chen et al. | 2009 [52] | hESC (HUES2, HUES4, HUES8, and HUES9) | 4 stages, ~18 days | ACT, WNT | FGF10, CYC, RA, ILV | | |
| Maehr et al. | 2009 [68] | hESC (HUES8) T1D iPSC (DPS H2.1, H2.4, H1.5) (retroviral) | 4 stages, ~18 days | ACT, WNT | FGF10, CYC, RA, ILV | EX4, DAPT, HGF, IGF1 | 6- to 7-fold induction at 20 mM glucose, clonal variations |
| Ameri et al. | 2010 [40] | hESC (SA181, SA121) | 4 stages, ~23 days | ACT, WNT | FGF10, CYC, RA, ILV | IGF1, Ex4, BMP4, NIC | | |
| Mfopou et al. | 2010 [43] | hESC (VUB01, 02, 07) | 4 stages, ~18 days | ACT, WNT | FGF10, CYC, RA, ILV | GLP1, DAPT, HGF, IGF1 | 1-8-fold induction at 10 mM glucose, clonal variations |
| Thatava et al. | 2011 [66] | iPSC (HCF1, BJRSA, BJ#1) (lentiviral) | 4 stages, ~18 days | ACT, WNT | FGF10, CYC, RA, ILV | GLP1, DAPT, HGF, IGF1 | 1-8-fold induction at 10 mM glucose, clonal variations |
| Nostro et al. | 2011 [44] | hESC (HES3/INS-GFP) iPSC 38-2 (retroviral/transgene-removed) | 4 stages, ~25 days | ACT, WNT | Ebf/ACT, BMP4 bFGF, VEGF | WNT, DKK1, DM, NOG, CYC, RA, FGF10, SB | SB, NOG, ySI-X | ~25% | No glucose responsive |
| Kudva et al. | 2012 [67] | T1D & T2D, integration-free iPSCs | 4 stages, ~25 days | ACT, WNT | Ebf/ACT, BMP4 bFGF, VEGF | WNT, DKK1, DM, NOG, CYC, RA, FGF10, SB | SB, NOG, ySI-X | ~25% | No glucose responsive |
| Micallef et al. | 2012 [49] | hESC (HES3, MEL1/INS-GFP) | Embryoid body (20–30 days) | ACT, BMP4 | NOG, RA, GLP1, NIC | IGF1, NIC | Up to 34% GFP* |
| Basford et al. | 2012 [50] | hESC (HES2) | 5 stages, ~22 days | ACT, WNT, BMP4 VEGF, bFGF | FGF10, WNT, NOG, CYC, RA, DM | SB, NOG, ySI-X | 19.8% | No glucose responsiveness induced by KCl |
| Rezania et al. (Betalogics) | 2012 [46], 2013 [85] | hESC (H1) | 4 stages, ~14 days | ACT, WNT | RA, NOG, SANT-1, (TPB), ALK5, LDN (last step in suspension) | In vivo maturation Tx into renal capsule | 12% (pre-Tx), robust islet formation in vivo | Yes, 32–34 weeks after Tx reversed diabetes in mice |
Definitive endoderm
Pancreatic endoderm
Islet-like cells

48% (in vitro) Yes, 16–18 weeks after Tx

Reference Year [Ref.] ESCs/iPSCs Differentiation
Xie et al. 2013 [51] hESC (CyT49) In suspension, ACT, WNT, FGF10, CYC, RA, ILV, GLP1, DAPT, HGF, IGF1
Thativa et al. 2013 [69] Multiple T1D iPSC lines 5 stages, ~26 days ACT, WNT, EGTA, FGF10, CYC, RA, ILV
Hua et al. 2013 [70] MODY2 (GCK mutation) 4 stages, 12 days ACT, WNT, EGTA, T2D, Type 2 diabetes

Abbreviations: ACT, activin A; ALK5i, ALK5 inhibitor (blocking transforming growth factor
R1 kinase); bFGF, basic fibroblast growth factor; BMP4, bone morphogenetic protein 4; CYC, KAAD-cyclopamine (Sonic
hedgehog inhibitor); DM, dorsomorphin (inhibitor of ALK receptors, blocking transforming growth factor
B/MMP-mediated activation of SMAD proteins); EGF, epidermal growth factor; ESC, embryonic stem cell;
lin-transferrin-selenium; NOG, noggin (blocking TGFb/g controlled growth factor); T1D, type 1 diabetes; T2D, type 2 diabetes;
Vegf, vascular endothelial growth factor.

To this end, derived pancreatic progenitors are transplanted into immune-compromised hosts and allowed to mature into glucose-responsive insulin-secreting cells capable of treating diabetes therapy. The major barrier preventing clinical applications of pluripotent cells in diabetes therapy is the low efficiency in generating insulin-producing islet-like cells. However, human pluripotent stem cell lines show substantial differences in spontaneous differentiation propensities [81–83]. Moreover, intrapatient divergence has been reported in the propensity for pancreatic differentiation among T1D-specific iPSC lines [69]. Such intrapatient variations will in principle impose a translational challenge for individualized applications for diagnostic or therapeutic purposes.

Inefficient Differentiation Into Insulin-Producing Cells

For diagnostic and therapeutic applications of iPSCs, it is critical to achieve reliable and efficient differentiation into insulin-producing islet-like cells. However, human pluripotent stem cell lines show substantial differences in spontaneous differentiation propensities [81–83]. Moreover, intrapatient divergence has been reported in the propensity for pancreatic differentiation among T1D-specific iPSC lines [69]. Such intrapatient variations will in principle impose a translational challenge for individualized applications for diagnostic or therapeutic purposes.

Lack of Glucose Responsiveness in Derived Insulin-Producing Cells

Another limitation is the difficulty in generating glucose-responsive and insulin-producing cells from human pluripotent stem cells. Despite promising results and proof-of-principle studies, most protocols yield populations of β-like cells lacking glucose responsiveness [39, 84]. Such glucose-unresponsive stem cell-derived β cells mirror neonatal immature β cells. For instance, in vitro guided differentiation of human pluripotent stem cells has achieved islet-like cells responsive to insulin secretagogues, but not high glucose stimulation [39]. Necessitating improvement, the field has shifted toward in vivo differentiation/maturation of pancreatic progenitor cells to generate glucose-responsive insulin-producing cells [70, 85]. To this end, derived pancreatic progenitors are transplanted into immune-compromised hosts and allowed to mature into glucose-responsive insulin-secreting cells capable of treating diabetes.
in vitro differentiation steps have impeded rapid translation of iPSCs into scalable diabetes therapy (Table 2).

**TOWARD CLINICAL APPLICATIONS OF iPSCs**

**Genomic Modification-Free iPSCs**

Generation of iPSCs independently from integrating vectors can in principle avoid some of the biosafety concerns. Accordingly, much effort has been made to produce iPSCs without integrating vectors. In this regard, nonintegrating viral vectors (e.g., adenoviral and Sendai viral vectors), nonviral vectors (removable transposon, episomal, and plasmid vectors), or direct transfection of reprogramming proteins or encoding mRNA have been successfully used to derive iPSCs [57, 90–92]. Minimizing the use of pluripotency factors has led to the identification of microRNAs and small molecules with the potential of facilitating reprogramming [93]. Genomic modification-free iPSC generation is also likely to reduce clonal variation among derived iPSCs. Of note, reproducible generation of genomic modification-free, T1D- and T2D-specific iPSCs has been demonstrated with nonintegrating Sendai reprogramming vectors [67].

**Improved Biosafety and Differentiation Propensity Through Selection of Somatic Cell Source for Nuclear Reprogramming**

iPSCs are typically derived from skin-derived fibroblasts. Other cell sources such as keratinocytes, adult stem cells, blood cells, stomach, and liver cultures have been used as somatic cell sources for iPSC derivation [58, 94, 95]. Notably, reprogramming of mature B lymphocytes requires depletion of the key B-cell transcription factor [96], emphasizing the importance of the intracellular environment in cell reprogramming. Reprogramming efficiency, levels of chromosomal damage, or even epigenetic memory of derived iPSCs can all be affected by the properties of the somatic cell source [65]. Conversely, residual epigenetic memory may inform protocols for improved pancreatic differentiation [73]. For instance, the use of endoderm cell sources, such as hepatocytes, rather than ectoderm-derived dermal cells, may at least in principle improve the iPSC differentiation propensity into insulin-producing cells. It is also notable that somatic cell sources can affect the persistence of undifferentiated cells upon differentiation, substantially affecting the teratoma-formation propensity after transplantation of iPSC-derived progeny [97]. It should, however, be underscored that most recent studies have not demonstrated the correlation between somatic sources and derivation of iPSC progeny [98].

**Improved Pancreatic Differentiation and Glucose Responsiveness of iPSC Progeny**

Currently, most promising protocols require lengthy in vivo maturation to obtain glucose-responsive islet-like cells [46]. In this context, it is notable that expression of urocortin 3 (UCN3), which regulates glucose-stimulated insulin secretion in β cells [99], gradually increases during β-cell maturation in vivo [100]. Importantly, UCN3 is also expressed in derived β-like cells after in vivo maturation, but not after in vitro differentiation, suggesting the potential role of UCN3 in achieving glucose responsiveness. Conversely, a lack of induction of key pancreatic factors, PDX1 and NKX6.1, is responsible for poor iPSC differentiation into insulin-producing cells [69] or in vivo maturation of iPSC-derived islets.

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**Table 2. Milestones in translating stem cell-based regenerative technologies into clinical grade practice-conducive products**

| Key stages in the path toward regenerative iPSC therapies for diabetes | Milestones accomplished |
|-------------------------------------------------|------------------------|
| Generation of type 1 diabetes-specific iPSCs    | Yes                    |
| Generation of type 2 diabetes-specific iPSCs    | Yes                    |
| Genomic modification-free, diabetes-specific iPSCs | Yes                 |
| Differentiation of iPSCs into insulin-producing cells | Yes          |
| In vivo generation of glucose-responsive islet-like cells | Yes        |
| Consistent in vitro maturation of iPSC-derived islet-like cells | No         |
| GMP manufacturing of scalable human doses       | No                    |
| Optimization of repair capacity                  | No                    |

Abbreviations: GMP, good manufacturing practice; iPSC, induced pluripotent stem cell.

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**Potential T1D Recurrence After Transplantation of iPSC-Derived Islets**

In the absence of immunosuppression, pancreas transplantation from human leukocyte antigen (HLA)-identical twins or HLA-identical siblings frequently results in T1D recurrence. This secondary T1D is characterized by rapid return of hyperglycemia without pancreatic rejection [87, 88]. Damaged islets demonstrate infiltration of mononuclear cells and selective β-cell destruction, emphasizing cellular mediated autoimmunity in the pathogenicity of T1D. In fact, pre-existing cellular islet autoimmunity prevents islet survival upon transplantation [89]. Together, these observations support the notion that autologous iPSC-derived islets are subject to autoimmune destruction and therefore require an immunosuppressive coregimen for survival of transplanted progeny.

**Complex iPSC Technology/Guided Differentiation for Clinical Grade Manufacturing**

Use of iPSC-derived insulin-producing cell products in the clinic necessitates multiple elaborate steps, including (a) somatic cell preparation, (b) reprogramming through ex vivo gene delivery using good manufacturing practice (GMP)-grade reprogramming vectors, (c) expansion of iPSC lines under GMP protocols, (d) extensive characterization of derived iPSC lines, and (5) preparation of pancreatic progenitor cells for transplantation through stepwise differentiation. Each step in the process requires regulatory agency-approved reagents, such as specific cell culture media, cytokines, small molecules, and animal component-free coating matrices. Thus, the complexity of iPSC technology and multiple drug-induced or pre-existing diabetes [46, 86]. One caveat of this approach is the extended in vivo maturation with a required 5- to 8-month period before achieving definitive glucose responsiveness [46, 85, 86].

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**Pluripotent Stem Cell Applications for Diabetes**

| Table 2. Milestones in translating stem cell-based regenerative technologies into clinical grade practice-conducive products | **Milestones accomplished** |
|-------------------------------------------------|------------------------|
| Generation of type 1 diabetes-specific iPSCs    | Yes |
| Generation of type 2 diabetes-specific iPSCs    | Yes |
| Genomic modification-free, diabetes-specific iPSCs | Yes |
| Differentiation of iPSCs into insulin-producing cells | Yes |
| In vivo generation of glucose-responsive islet-like cells | Yes |
| Consistent in vitro maturation of iPSC-derived islet-like cells | No |
| GMP manufacturing of scalable human doses       | No |
| Optimization of repair capacity                  | No |

Abbreviations: GMP, good manufacturing practice; iPSC, induced pluripotent stem cell.
Thus, pharmacological or genetic induction of UCN3, PDX1, and/or NKX6.1 may gauge the efficiency of generating iPSC-derived insulin-producing cells with authentic glucose responsiveness. Moreover, neonatal \( \beta \) cells do not show typical glucose-responsive insulin secretion and are considered immature [101–103], a property regulable through thyroid hormone signaling [104], offering a physiological means to enhance functional maturation of derived \( \beta \) cells.

**Direct Reprogramming to Insulin-Producing Cells**

An alternative reprogramming approach leverages \( \beta \)-cell-specific factors to directly derive insulin-producing cells without generating iPSCs. Studies have demonstrated that overexpression of a set of three pancreatic factors, PDX1, NEUROG3, and MAFA, can reprogram the fate of hepatocytes, pancreatic exocrine tissues, or liver ductal cells into insulin-producing cells in vivo [105–107]. Although derived insulin-producing cells do not necessarily exhibit complete \( \beta \)-cell phenotypes, those cells are able to control blood glucose levels in diabetic mice, expanding the available regenerative platforms for diabetes care.

**CONCLUSION**

The epidemic of diabetes requires new means to address a rampant global need, ensuring effective solutions beyond the current standard of care. In this context, regenerative technologies offer a radical innovation with potential significant impact in advancing diabetes care. New knowledge in developmental biology and disease pathophysiology has fueled the evolution of management approaches increasingly targeted to addressing the root cause of the problem. Pertinent to the future of diabetes therapy, regenerative modalities aim to restitute pancreatic \( \beta \)-cell structure and function. Such reparative approaches may prove particularly useful with the recognition that diabetes reflects a defective innate \( \beta \)-cell regeneration capacity because of augmented destruction or insufficient replenishment of the existing \( \beta \)-cell pool. Stem cells, including pluripotent platforms highlighted in this work, have the remarkable aptitude to form specialized tissues and promote repair signaling, restoring organ structure and function. Translation of regenerative principles into practice, however, presents significant challenges requiring careful optimization to maximize safe and effective clinical application.

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**AUTHOR CONTRIBUTIONS**

S.J.H.: manuscript writing; A.T. and Y.I.: conception and design, manuscript writing, financial support.

**DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST**

The authors indicate no potential conflicts of interest.

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