Therapeutic approaches for Type 1 Diabetes: Promising cell-based approaches to achieve ultimate success

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A B S T R A C T

Type 1 Diabetes mellitus (T1DM) is a chronic metabolic disorder characterized by pancreatic β-cells destruction. Despite substantial advances in T1DM treatment, lifelong exogenous insulin administration is the mainstay of treatments, and constant control of glucose levels is still a challenge. Endogenous insulin production by replacing insulin-producing cells is an alternative, but the lack of suitable donors is accounted as one of the main obstacles to its widespread application.

The research and trials overview demonstrates that endogenous production of insulin has started to go beyond the deceased-derived to stem cells-derived insulin-producing cells. Several protocols have been developed over the past couple of years for generating insulin-producing cells (IPCs) from various stem cell types and reprogramming fully differentiated cells. A straightforward and quick method for achieving this goal is to investigate and apply the β-cell specific transcription factors as a direct strategy for IPCs generation. In this review, we emphasize the significance of transcription factors in IPCs development from different non-beta cell sources, and pertinent research underlies the marked progress in the methods for generating insulin-producing cells and application for Type 1 Diabetes treatment.

1. Introduction

Diabetes Mellitus (DM) is a chronic metabolic disorder marked by excessive blood glucose levels. Over the last decade, the global incidence of diabetes has increased rapidly among all age categories, imposing a significant financial burden on families and society [1]. Type 1 Diabetes Mellitus (T1DM) is characterized by the autoimmune destruction of pancreatic β-cells, resulting in impaired insulin production and secretion [2]. Long-term blood sugar dysregulation triggers multiple diabetic complications [3]. Diabetic nephropathy, neuropathy, retinopathy and cataracts, and cardiovascular disease are all caused by uncontrolled blood glucose levels in diabetes [3,4].

Exogenous insulin administration and insulinotropic medications are the most popular therapies to control glucose metabolism in T1DM [5]. A more intensified metabolic control can prevent or postpone the progression of secondary complications, though it is associated with higher risks of hypoglycemia and weight gain [6,7].

Due to improved surgical and islet harvesting techniques, pancreatic islet transplantation has become another T1DM therapy aiming at better metabolic control via restoring endogenous insulin production. An acceptable rate of 90 percent success in one-year allograft function and insulin independence was reported in most operating centers throughout the last two decades. The widespread application of this approach is limited due to the absence of enough donor islet cells and the need for long-term use of immunosuppressive drugs to control allograft rejection and maintain islet function [8].

Therefore, other β-cell replacement strategies, especially the utilization of stem cells as an unlimited source of insulin-producing cells, are currently being tested in clinical trials. In this regard, human pluripotent stem cells (hPSCs), including embryonic stem cells (ES) and induced pluripotent stem cells (iPSCs), offer a potentially unique way for lineage commitment into insulin-producing cells (IPCs) [4].

The use of embryonic stem cells requires suppressing the host immune system, which is especially important in T1DM patients with an activated immune system. Thus, the patients-origin stem cell-derived IPCs and the cell encapsulation may be able to eliminate this requirement [9,10]. Recent findings demonstrated that the endocrine cells of the endoderm layer, specifically the liver and gut, can be reprogrammed into β-like cells. The transdifferentiation of cells into β-like cells requires the activation of β-cell-specific genes involved in insulin synthesis,
maturation, and secretion, as well as the repression of silent genes in mature β-cells [11]. Most protocols for stem cell differentiation into IPCs or transdifferentiation are based on a comprehensive knowledge of the molecular mechanisms underlying the pancreas and beta-cell development in animal models, fetus tissues, or isolated human islets [4,12]. Transcription factors, epigenetic modifications, and non-coding RNAs regulate gene expression toward beta cell identity [13,14]. Many groups have developed procedures for generating IPCs using soluble signaling agents such as growth factors, transcription factors, cytokines, as well as steroids, and other small molecules that recapitulate the development of pancreatic β-cells [4,15]. Besides the intrinsic difficulty of this inducing process pharmacologically, the poor reproducibility of the protocols is an important drawback, particularly in the induction of patient-derived sources to insulin-producing cell generation [11,16]. Application of transcription factors in IPCs production can be a more straightforward and efficient strategy. This review highlights the specific transcription factors and their interconnections, orchestrating pancreas development and beta-cell differentiation. A particular focus is providing insights into the prime cell candidates and techniques for IPC generation.

2. Pancreas development

The pancreas consists of three parts: exocrine, ductal, and endocrine. The digesting enzyme-producing cells, acinar cells, are closely connected with ductal cells, which secrete ions to the gut through the branched duct system. The islets of Langerhans are functional units of the endocrine pancreas containing the cells that produce glucagon (α-cells), insulin (β-cells), somatostatin (5-cells), pancreatic polypeptide (PP-cells), and ghrelin (ε-cells). The adult pancreas is comprised of acinar cells (98%) with scattered islet cells (2%) throughout the organ’s core sections [17].

Human embryogenesis is a process that is divided into 23 stages, according to the Carnegie classification system, which was proposed by O’Rahilly and Müller. These stages are identified based on the external features of the developing human embryo. The formation of the human pancreas begins during stage 9 or 25–27 days post-conception (dpc) (Table 1). This process is initiated by patterned foregut epithelium [18]. Rachel et al. reported that the stages of pancreas development are the same in mice and humans. However, there are timing and expression profile discrepancies between the two species [19].

Dorsal and ventral buds, containing pancreatic progenitors, are the primary structures during pancreas development. Both dorsal and ventral pancreatic buds appear by 30–33 dpc [19,20]. The ventral pancreas is generated in the region of the ventral foregut with low cardiac FGF signaling. Dorsal pancreatic development necessitates RA signaling and secreted proteins from the notochord and dorsal aorta, such as Activin and FGF2, which suppress Shh expression in the dorsal pancreatic epithelium [21]. The endoderm becomes anteriorized, causing whole pancreas agenesis in the retinoic acid-deficient Raldh2 mutant mice [22].

Pancreatic progenitors of both dorsal and ventral buds express different transcription factors, and buds’ microlumens eventually form the luminal network of the branching pancreas [19,20]. Fusion of dorsal and ventral buds occurs 6–7 weeks post conception (wpc), and both buds undergo a phase of outgrowth and branching [23].

Multipotent pancreatic progenitors then form bi-potent progenitors and pro-acinar cells that will differentiate into endocrine progenitors, duct cells, and acinar cells, respectively. The transitory expression of the transcription factor Neurogenin3 (NGN3) is a hallmark of endocrine differentiation in both mice and humans. Subsequently, the number of NgN3-expressing cells increases slightly, and the cells gradually interperse throughout epithelial clusters [19]. At 7.5–8 wpc, insulin-producing cells are the first endocrine cells to appear in the human pancreas. They are the most common endocrine cell type in the first trimester of pregnancy [24]. Glucagon and somatostatin-expressing cells develop at eight wpc, while pancreatic polypeptides and ghrelin-expressing cells appear at nine wpc [24]. The next stage is the differentiation of acinar cells, which begins around 11 wpc. In the second trimester, embryonic islet formation occurs through budding from embryonic ducts. Islet vascularization and innervation are started at 12–13 wpc and completed at 20 and 28 wpc, respectively [19,25,26].

3. The transcription factors in pancreas development

Different transcription factors are orchestrated in pancreas development (Table 1). The nuclear PDX1, FOXA2, and SOX17 are present in the presumed duodenal-pancreatic endoderm [19]. FOXA2 appears in the dorsal foregut endoderm, where it acts as a regulatory factor to promote the expression of the PDX1 transcription factor at 29 dpc [20]. Nuclear FOXA2 was found in all endodermal epithelial cells, but SOX17 was only found in the area lacking SHH.

Transcription factors are also the key players in the dorsal and ventral buds’ commitment to pancreatic fate through the establishment of pancreatic lineage and domain identity. In the current lineage models of pancreatic cell differentiation, PDX1/PTF1A/SOX9/NKX6.1 transcription factors are the hallmark of multipotent progenitors, while NKX6.1/SOX9 and PTF1A transcription factors are the markers of bipotent progenitor and pro-acinar cell respectively. Although, as mentioned above, NGN3 represents an endocrine progenitor, SOX9, NFATC1/INS, and ARX/GLUC represents duct cell, β-cell, and α-cell, respectively [27].

Self-renewing multipotent progenitor cells (MPCs) appear after dorsal and ventral endodermal commitment to the pancreatic fate [28]. PDX1, PDX1, HNF6, HNF1B, FOXA2, HNF1B, SOX9, GATA4/6, and HES1 transcription factors mediate pancreatic progenitor cell expansion and maintain pancreatic identity [29,30]. During this process, the transcription factors PTF1A and NKX6.1/6.2 act as the master regulators, but they are expressed in two distinct domains. PTF1A promotes tip

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**Table 1**

<table>
<thead>
<tr>
<th>Gestational day (d)/week (w)</th>
<th>Carnegie stage</th>
<th>Events/processes</th>
<th>Involved transcription factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>G25–27d</td>
<td></td>
<td>Distal Foregut development</td>
<td>FOXA2, SOX17 (dorsal endoderm)</td>
</tr>
<tr>
<td>G30d - 33d</td>
<td>12–14</td>
<td>Pancreatic specification, dorsal and ventral bud formation (Multipotent progenitor)</td>
<td>PDX1, SOX17 (ventral), SOX9, GATA4, GATA6, NKX6.1, FOXA2</td>
</tr>
<tr>
<td>G6–7 w</td>
<td>18–20</td>
<td>Fusion of dorsal and ventral buds</td>
<td>PDX1, SOX9, NKX6.1, FOXA2</td>
</tr>
<tr>
<td>G7w</td>
<td>20</td>
<td>Initiation of tip-trunk compartmentalization process</td>
<td>PDX1, SOX9, NKX6.1, FOXA2 (Trunk progenitor), PDX1, SOX9, NKX6.1, FOXA2, GATA4 (Tip progenitor)</td>
</tr>
<tr>
<td>G7.5 w - G8W</td>
<td>21–23</td>
<td>Appearance of NGN3+/endocrine progenitors and first endocrine cells (insulin + cells)</td>
<td>NGN3, PDX1, NKX6.1, PDX2, NKX2.2, RXF6, MAFA, MAFB, PAX6, ISL1, Insulin (Fetal β-cells)</td>
</tr>
<tr>
<td>G14–19W</td>
<td></td>
<td>Islet formation and β-cell maturation</td>
<td>PAX6, NeuroD1, Insulin, FOX1, NKX6.1, FOXA2, NKX2.2, NEUROD1, MAFA, MAFB, PAX6, ISL1, Insulin</td>
</tr>
<tr>
<td>Postnatal</td>
<td></td>
<td>Mature postnatal β-cell</td>
<td>FOX1, NKX6.1, FOXA2, NKX2.2, NEUROD1, MAFA, MAFB, PAX6, ISL1, Insulin</td>
</tr>
</tbody>
</table>

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identity, while NKX6.1/6.3 promotes trunk identity [30]. PDX1 and SOX9 are expressed in the trunk of the pancreatic ductal tree at various phases of development [31]. Lineage-tracing experiments revealed that the trunk domain is bipotential and predominantly gives rise to ductal and endocrine cell lineages, whereas the tip is quickly restricted to an acinar fate. Notch activity promotes the trunk while suppressing the identity of the tip. Moreover, the loops among transcription factors participate in regulation of domain identity. The double-negative feedback loop among NKX6.1/6.3 and PTF1A, controlled by Notch signaling, causes tip/trunk separation [30].

High Notch signaling promotes both Hes1 and Sox9 expression, resulting in Ngn3 repression and ductal cell fate, while low Notch activity promotes only Sox9 expression, allowing for Ngn3 activation and endocrine fate [27,32]. NGN3 has been detected as early as week eight, increases around week 11, and is not expressed in mature β-cells [19]. Even though individual endocrine precursors are unipotent, NGN3 cells generate all five distinct pancreatic endocrine cell types [33]. The expression of PAX4, ARX, RFX6, NeuroD1, PAX6, ISL1, and IA2, MAFB, NKX2.2 factors was induced by NGN3− endocrine precursors. PDX1, MAF, NeuroD1, and PAX6 are important β-cell transcription factors that control the expression of genes involved in insulin synthesis and glucose-stimulated insulin secretion (GSIS). Thus, they play an important role in regulating β-cell identity and maturation [5].

4. Renewable sources of insulin-producing cells

There have been numerous attempts to find an alternative therapy for islet transplantation due to the lack of its source [34]. An attractive alternative for achieving this goal is the generation of a self-renewable β-cell supply [35] (Table 2). In this regard, pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) and patient-derived induced pluripotent stem cells (iPSCs), are potentially limitless cell sources for generating IPC [36]. In both cases, the efforts at IPC generation are based on extensive knowledge of pancreatic-cell development [4].

The use of PSC in IPC generation raises immunogenicity and teratogenicity issues. So other cell sources for IPC generation are widely investigated. Consequently, insulin-producing cells from mesenchymal stem cells because of immunomodulatory effects, patient-derived pancreatic cells other than β-cell, and any other cell with close lineage to β-cell are suggested.

Nevertheless, consistent long-term 2D cultures cannot replicate the characteristics of complex multicellular tissues [37]. In this context, 3D-based models such as organoids and spheroids have been recently developed with the potential to evolve as in vitro alternatives for the long-term culture of insulin-producing cells [37,38].

4.1. Pluripotent stem cell-derived IPC

A renewable source of human insulin-secreting cells that physiologically respond to glucose is desired for the success of cellular therapy in diabetes [39]. Moreover, a high-proliferative stem cell population is required to generate functional insulin-producing cells. Embryonic stem cells (ESCs) are now the most efficient cell population to achieve this goal [39]. Previous research has shown that the development of pluripotent stem cells (PSCs) to the pancreatic progenitor cell followed by kidney capsule transplantation results in the creation of beta-like cells and the lowering of the blood glucose in the mice [15]. Many groups have developed procedures to generate PSCs-derived IPCs using soluble signaling agents such as growth factors, transcription factors, cytokines, as well as steroids, and other small molecules that recapitulate the development of pancreatic β-cells [4,15] (Fig. 1).

Formation of the embryoid body is the most common approach during embryonic stem cell differentiation toward different cell

Table 2
Therapeutic strategies in current clinical and preclinical studies for Type 1 Diabetes.

<table>
<thead>
<tr>
<th>ID</th>
<th>Therapeutic strategy</th>
<th>Transplanted cell type</th>
<th>Sponsor and collaborators</th>
<th>Trial status</th>
<th>Phase</th>
<th>Country</th>
</tr>
</thead>
<tbody>
<tr>
<td>NTC 04,786,262</td>
<td>VX-880/Safety, Tolerability, and Efficacy</td>
<td>Stem cell-derived, fully differentiated islet cells</td>
<td>Vertex Pharmaceuticals</td>
<td>ongoing</td>
<td>phase I/II</td>
<td>USA</td>
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<tr>
<td>NCT02239354</td>
<td>PEC-Encap/Safety, Tolerability, and Efficacy</td>
<td>Pluripotent stem-cell-derived pancreatic endoderm cells (PECs)</td>
<td>ViaCyte</td>
<td>ongoing</td>
<td>phase I/II</td>
<td>Canada</td>
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<td>NCT03162926</td>
<td>PEC-Direct/Safety and Tolerability</td>
<td>Pluripotent stem-cell-derived pancreatic endoderm cells (PECs)</td>
<td>ViaCyte</td>
<td>completed</td>
<td>phase I</td>
<td>Canada</td>
</tr>
<tr>
<td>NCT01652911</td>
<td>Sernova’s Cell Pouch/Safety, and Efficacy</td>
<td>Human islets Allotransplantation</td>
<td>University of Alberta</td>
<td>completed</td>
<td>phase I/II</td>
<td>Canada</td>
</tr>
<tr>
<td>NCT03513939</td>
<td>Sernova’s Cell Pouch/Safety, and Efficacy</td>
<td>Induced pluripotent stem cell (iPSC)</td>
<td>Sernova Corp</td>
<td>ongoing</td>
<td>phase I/II</td>
<td>USA</td>
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<tr>
<td>NCT03920397</td>
<td>Stem Cell Infusion plus Oral Vitamin D Supplementation</td>
<td>Human allogenic Adipose tissue-derived stem cell</td>
<td>Universidade Federal do Rio de Janeiro</td>
<td>completed</td>
<td>Not Applicable</td>
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<td>completed</td>
<td>phase I/II</td>
<td>USA</td>
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<td>NCT02064309</td>
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<td>ongoing</td>
<td>phase I</td>
<td>Sweden</td>
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<tr>
<td>NCT00790257</td>
<td>Subcutaneous Islets Allotransplantation/Safety and Efficacy</td>
<td>Human islets Allotransplantation</td>
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<td>phase I</td>
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<td>NCT00133809</td>
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<td>Nicole Turgeon MD, Emory University</td>
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<td>NCT02420439</td>
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<td>Human islets Allotransplantation</td>
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<td>NCT03835312</td>
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<td>ongoing</td>
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<td>Rezzania et al.</td>
<td>2014</td>
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<td>- Intraprothetile/Alginate plus</td>
<td>Stem cell-derived β-like cell</td>
<td>Alagapulina et al.</td>
<td>2019</td>
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<tr>
<td>- Subcutaneous/Mouse</td>
<td>Stem cell-derived pancreatic progenitor</td>
<td>Pepper et al.</td>
<td>2017</td>
<td>preclinical</td>
<td>–</td>
<td></td>
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</table>
populations, preparing a cellular structure that can develop into ectoderm, mesoderm, and endoderm precursors (Fig. 2). A couple of intermediate stages should be fulfilled to generate insulin-producing cells from the embryoid body. Lumelsky et al. reported a five-stage mouse EB-based process for creating insulin-producing cells, which express pancreatic β-cell fate markers, rapidly vascularized after being injected into mice and preserved an islet-like structure [40].

As a pivotal moment of cell-based approaches for diabetes treatment, through a series of endodermal intermediates similar to those that take place during pancreatic development in vivo, D’Amour et al. established a five-stage differentiation process. They convert human embryonic stem (hES) cells into endocrine cells capable of synthesizing insulin, glucagon, somatostatin, pancreatic polypeptide, and ghrelin [41]. Following investigations have demonstrated that pancreatic cells produced from human embryonic stem cells are capable of more advanced maturation and insulin release in vivo, as well as protecting against streptozotocin-induced hyperglycemia [39,42]. Rezania et al. have optimized a 4-stage strategy for converting pluripotent hESCs into a highly enriched PDX1+ and NKX6.1+ pancreatic progenitor cell population, which continued to develop in vivo to mature pancreatic endocrine cells. Thirty weeks after transplantation, these cells release the proper level of c-peptide in response to glycemic challenges [43]. Additionally, in a similar study, they added particular components to the fourth stage of differentiation to produce two populations of progenitor cells that were rich in NKX6.1 (80 %) and low in NKX6.1 (25 %). Compared to insulin-positive cells from NKX6.1-low grafts, they found that NKX6.1-high grafts had a greater percentage of post-transplant insulin-positive cells, and these cells displayed advanced maturation and insulin secretion [44].

A newly designed methodology consists of a six-stage planar differentiation protocol for ESC-derived IPC that secretes high amounts of insulin in response to glucose stimulation. At the first stage, more than 90 % of ESCs converted to FOXA2+/SOX17+(endoderm markers) cells. Primitive gut tube formation was followed by emerging PDX1+/NKX6.1+ pancreatic progenitors in the culture medium. The endocrine cell formation was conducted by Notch signaling inhibitors in combination with RA for NGN3+ cell fate [45].

Every stage of differentiation toward insulin-producing cells is usually characterized by a transcription factor marker of cells, indicating the paramount role of transcription factors in IPC formation. Most
attempts to produce IPC are based on activating a specific transcription factor indirectly using activating a signaling pathway or directly applying them as a supplement or overexpressing through gene manipulation. The last approach has demonstrated a short and straightforward one with considerably higher efficacy that dominantly substitutes the former complicated and laborious multi-step methods [46]. According to Blyszczuk et al.’s study, the persistent presence of ectopic PAX4 and, to a minor extent, PDX1 expression in the ES increased the percentage of Nestin-positive cells, which can differentiate into insulin- but not glucagon-producing cells in vitro. The production of insulin-producing cells was boosted from 10% to 60% when PAX4 was expressed continuously. The transplantation of PAX4-derived insulin-producing cells into the renal capsule maintained the blood glucose levels of diabetic mice normal [47].

It has been demonstrated that IPC generation was even more successful using a couple of key transcription factors. PDX1-expressing ESCs in concert with additional transcription factors such as NKX6.1 and NGN3 is suggested in different studies [48, 49]. A team of researchers was successful in producing IPCs from iPSCs by adenovirally induction of PDX-1, NeuroD1, and MAFA. They noticed that insulin production and secretion were increased, and insulin-producing cells responded to glucose in a dose-dependent manner in vitro. The in vivo findings of this study demonstrated that fasting blood glucose levels of diabetic mice returned to normal within seven weeks [50]. Similarly, a recent experiment revealed another method for generating IPCs from PDX1+/NKX6.1+/NEST+ progenitors derived from human pluripotent stem cells [51].

Moreover, a three-step differentiation process was designed by Huiming and colleagues to create insulin-secreting cells from mouse embryonic stem cells. At various phases of differentiation, they applied diverse combinations of ectopic expression of transcription factors using adenoviral vectors. Specifically, in the third and final stage of differentiation, they discovered that the co-expression of Pdx1 and Mafa with either Ngn3 or Neurod1 considerably improved the differentiation efficiency [52].

Many different other studies reported the production of ESC- and iPSC-derived β-cells (SC-b) that exhibit many characteristics of β-cells, such as their function in vitro and in vivo, the expression of b-cell marker genes, and the capacity to regulate glucose levels in diabetic mice [10, 53]. Several issues remained to be solved for applying these sources in β-like cell generation. Diversity in differentiation efficiencies across various PSC lines is one of the main challenges. A study reported that NKX6.1 induction varied between 37 and 84 percent when the same pancreatic progenitor differentiation method was applied to human pluripotent stem cells of eight different subjects [8]. In addition, stem cell-derived β-like cells do not fully mature compared to native mature human β-cells because of low expression of the beta-cell maturation markers, i.e., UCN3, MAFA, and G6PC2 [54]. Another issue is that the functional hPSCs-driven β-cells are contaminated with non-committed progenitor cells, non-functional polyhormonal cells, and other cell types, ultimately leading to poor insulin secretion [55]. Finally, ethical, teratogenic, and immunogenic issues still exist for ESC sources application [56].

4.2. Reprogramming cells to insulin-producing fate

Cell reprogramming, also known as trans-differentiation, is the phenotype transformation of cells from a specific tissue into other cell types, mainly by ectopic expression of particular transcription factors [57]. As previously mentioned, trans-differentiation into insulin-producing cells depends on the activation of β-cells-specific genes, such as those involved in the synthesis, maturation, and secretion of insulin [11]. Different cell sources, including pancreatic endocrine and exocrine and non-pancreatic cells, as well as mesenchymal stem cells, are candidate sources of β-like cell generation (Fig. 3).

Pancreatic acinar cells, ductal cells, endocrine alpha, delta, and
epsilon cells are several cell sources that can be converted into insulin-producing cells [16,58–60]. A potential cell source is exocrine pancreatic cells, which can be applied for IPC generation through over-expressing crucial transcription factors of β-cells. For example, ectopic adenovirus-mediated transduction of acinar cells with Pdx1, Mafa, and Ngn3 genes can conduct insulin-producing phenotype in immune-deficient mice [16]. A different research team demonstrated that Ngn3 and Mafa expression suppresses the acinar cell fate during exocrine cell reprogramming into insulin-producing cells [58]. The duct cells can be transformed into endocrine progeny by ectopic expression of the islet developmental regulators NGN3, MAFA, PDX1, and PAX6 [60]. Azzarelli et al. successfully generated insulin-producing cells from ductal organoids via overexpressing Pdx1, Mafa, and wild-type or phosphomutant Ngn3. They demonstrated that only a small percentage of cells could be successfully reprogrammed into IPCs by wild-type Ngn3, whereas utilizing the phosphomutant Ngn3, preventing further NGN3 phosphorylation at kinase-targeted sites, led to approximately four-fold endocrine fate reprogramming [61].

The potential of pancreatic endocrine cells to regenerate and convert into pancreatic β-cells was studied by Wei et al. [62]. Endocrine cells, such as α- and ε-cells, are potential candidates for pancreatic progenitor cell reprogramming [57]. Numerous in vivo and ex vivo attempts were made to transform α-cells into insulin-producing cells. Zhang et al. reported that transduction of the αT1C.9 cell line using a viral vector containing the Pax4 gene resulted in the activation of insulin production and glucagon reduction. Pax4 expression resulted in the up-regulation of PMN (PDX1, NGN3, and MAFA) and NKX6.1 transcription factors in the αT1C.9 cells [63]. More recently, the in vivo reprogramming of α-cells into functional beta-like cells was conducted by the infusion of adeno-associated virus (AAV) Pdx1- and Mafa-expressing cassette into the pancreatic duct. The correction of blood glucose in both STZ-induced diabetic mice and autoimmune NOD mice was observed by this method [64].

Non-pancreatic tissues, especially the ones with an endodermal origin, are other sources of IPC generation. The overexpression of beta-cell-related transcription factors, particularly PMN factors, causes the development of insulin-positive cells in non-pancreatic tissues and cells, such as the liver [65,66], stomach [67], and intestine [67,68] and mesenchymal stem cells [69].

The pancreas and the liver are derived from the foregut endoderm and are connected during development. Liver cells have been widely investigated as a prospective source for trans-differentiation into insulin-producing cells [11]. STZ-induced hyperglycemia in mice was reversed after adenoviral transduction of murine liver cells by the PDX1 transcription factor, specifically its super-active version (PDX1/VP16) [65]. The findings from a related study indicated that lentiviral-transduced hepatocytes express β-cells-specific and glucose-sensing proteins, including NeuroD1, NKX6.1, and MAFA [66].

The gastrointestinal (GI) tract and the pancreas are also given rise developmentally from the same origin. The gastrointestinal and pancreatic endocrine cells share many features, including some gene expression patterns and cell markers. The α-cell of the pancreas and K/L enteroendocrine cells are all critically involved in maintaining glucose homeostasis by producing hormones belonging to the glucagon superfamily. These features emphasized the promise of GI epithelium application to create a renewable source of insulin-producing cells. The intestinal cells can be converted into insulin-producing cells, either via deletion of the FOXO1 transcription factor [70] or overexpression of PMN factors [67,68]. However, overexpression of PMN factors seems safer because of the critical role of Foxo1 in beta cell protection against cellular stress [71,72]. Chen et al. converted intestinal crypts to insulin-positive cells by inducing PMN factors. The generated cells reverse hyperglycemia in STZ-induced diabetes mouse. However, they reported incomplete reprogramming of the cells [68].

Ariyachet et al. demonstrated that inducible expression of PMN in triple transgenic mice can create insulin-positive cells from gut endocrine cells throughout the GI tract with 40% greater efficiency at gastric antrum. Gastric antrum insulin-positive cells were independently adequate to reverse hyperglycemia. Despite beta cells of the pancreas, these cells can regenerate three weeks after STZ treatment. A comparative transcriptional assessment of GI tract enteroendocrine cells revealed more similarity between antral cells and beta cells. The resultant antral-derived insulin-positive cells had greater similarity accordingly [67].
Accordingly, multiple 3D culturing techniques such as organoids, spheroids, or device-based cultures have been investigated for effective differentiation and maturation of pancreatic β-cells [38,86].

Many attempts have addressed the development of pancreas organoids of liver and gut origin for IPC generation. Wang et al. reported that the 3D-differentiated cells showed greater insulin secretion (about 5-fold), C-peptide production, and GLUT-2 expression compared to cells developed in a monolayer or 2D platform [87]. Islet organoids demonstrated an increased expression of the transcription factors and markers of mature cells, including PDX1, Insulin, GLUT2, NGN3, and MAFA [88].

Comparing the transcriptional profile of progenitor cells in the liver and pancreas of the mouse embryo, the transcriptional regulator, TGFβ (Three-Amino-acid-Loop-Extension (TALE) homeobox TG-interacting factor 2 ), revealed to function as a developmental regulator between two tissues [89]. It is sufficient to induce liver-to-pancreas fate conversion both ex vivo and in vivo. Nuria et al. Developed pancreatic organoids by the ectopic expression of TGFβ2 into the adult mouse hepatocytes composed of SOX9- and PDX1-positive cells [90]. Using lentiviral-mediated transduction of mouse pancreatic ductal organoids, researchers have demonstrated that co-expression of Ngn3, Pdx1, and Mafa is required and sufficient to generate cells that express insulin [61].

According to the developmental viewpoint, the intestine and pancreas both originated from the same gut tube. Therefore, the intestine is a readily available and plentiful source for IPC generation [68,91]. According to a published study, inhibition of the FOXO1 transcription factor in the gut organoids causes the gain-of-function of three transcription factors, i.e., Neurogenin 3, MAFA, and PDX1 (PMN factors), resulting in the conversion of the gut organoid into IPCs [70]. Likewise, A recent study has shown that overexpression of PMN factor in human intestinal organoids promotes the transition of intestinal epithelial cells into β-like cells [68].

In 2019, Tao et al. proposed a novel and effective method to produce human islet organoids from hiPSCs in a perfusable organ-on-chip platform. This platform was developed using a multi-layered microfluidic chip that allowed the circulation of different media and the exposure of organoids to uniform fluid stress. The generated islet organoids displayed proper tissue morphology and multicellular intricacy similar to human pancreatic islets in vivo [92]. These organoids demonstrated improved expression of genes and proteins associated with mature β-cells, increased insulin secretion levels, and the capacity to Ca²⁺ flux in response to glucose in perfused cultures conditions [92].

As we previously noted, islets of Langerhans are intricately units with various cell types and a highly vasculared and innervated architecture. Accordingly, it’s indeed preferable to co-culture supporting cells like Mesenchymal stem cells, Endothelial cells, and Amniotic epithelial cells (AECs) in addition to the main cell source to limit the post-transplantation effects of 3D aggregates [83]. Takahashi and colleagues fabricated self-organized heterogeneous multi-compositional pancreatic organoids through co-cultured MIN6 (Murine β-cell line) with human vascular endothelial cells (HUVECs) and mesenchymal stem cells (MSCs) in a 3D platform with vasculature system that effectively reduced hyperglycemia after being transplanted into diabetic mice [93].

This vasculature is crucial for supplying nutrients and oxygen while boosting angiogenesis-related signals for further growth and differentiation [38]. In a related study, scientists created a novel hydrogel (Amikagel)-based platform for 3D islet organoids. They co-seeded hiESC-FP cells with HUVECs on Amikagel. The cells spontaneously co-aggregated to form multicellular 3D organoids that expressed the INS1 gene and the C-peptide protein. Following maturation, islets exhibited in vitro glucose-stimulated insulin secretion (GSIS) [94].

Spheroids, self-assembling cell aggregates, are regarded as another 3D culture technique [95]. In a recent study, Yonela et al. developed a reproducible technique for 3D culture of INS-1 (pancreatic β-cell) spheroids. The spheroids displayed in vivo-like features of β-cells with the GSIS feature. Unlike the monolayer cultures, which needed sub-culturing after 72–96 h, the 3D INS-1 spheroids persisted in culture.
Lee et al. have developed trans-differentiation of the human liver cells into IPC spheroids via adenovirally *PDX1, NEUROD1*, and *MAFA* genes transduction. The spheroids displayed higher levels of insulin, glucagon, somatostatin, and amylin, as well as pancreas-specific transcription factors, such as PDX1, ISL1, FOXA2, NGN3, NEUROD1, NKX2.2, NKX 6.1, and MAFA. Accordingly, compared to 2D IPCs, spheroids had improved insulin expression, decreased glucagon expression, and reduced blood glucose levels significantly after transplantation into diabetic mice [86].

Adipose tissue-derived MSCs (AT-MSCs) spheroids are a perfect candidate in regenerative medicine, as in 3D IPC generation, due to the availability and quantity of adipose tissue. AT-MSCs-derived insulin-producing spheroids displayed pancreatic β-cell-specific gene expression and enhanced insulin production in response to glucose stimulation. However, the *in vivo* results showed that the subcutaneous or intramesenteric graft of IPCs did not improve the hyperglycemic condition in diabetic mice [96].

### 5. Encapsulation of β-Cells for replacement therapy

Despite the promising results in developing insulin-producing cells, their therapeutic application is still restricted because of safety concerns about teratoma formation and both the allo- and autoimmune reactions following cell transplantation [97]. Islet loss occurs soon after portal infusion because of blood-mediated inflammatory response and ischemia [98]. Furthermore, the survival of transplanted cells and their controlled release of therapeutic factors are crucial for establishing durable function and effective cell-based therapy [99]. Cell encapsulation has emerged as an approach to potentially eliminate this challenge without requiring the overuse of immunosuppression while offering an ample cell mass transfer to the host, a supportive environment for transplanted cells, and ensuring favorable insulin secretion kinetics [100]. In the case of diabetes cell-based therapies, encapsulating materials often create a semipermeable membrane between the host and the implanted beta cells to protect them from the host immune system while permitting the transmission of oxygen, nutrient agents, and smaller molecules like insulin and glucose [100–102] (Table 2). Additionally, the ideal cell encapsulation system should be biocompatible and provide enough blood flow to support the survival and function of beta cell mass and maintain the euglycemia condition [100,103].

Many different forms of encapsulation devices have been created during the recent decades, mainly for xenogenic and allogenic islet transplantation and falling into the micro- and macro-encapsulation categories [102]. Microencapsulation devices are micron-sized polymeric matrices or membranes commonly constructed of hydrogels such as alginates that wrap only a few cell clusters, implying that many capsules are required to deliver [104].

Various encapsulating materials with different properties have been used for islet encapsulation. A highly purified PAMs-free (pathogen-associated molecular patterns) alginate is used in many transplantation studies with a series of modifications and designing. Vegas et al. demonstrated that implantation of hPSC-derived beta-like cells covered with immunomodulatory alginate derives into the intraperitoneal space caused euglycemia in the STZ-induced diabetic mice for 174 days [10]. Alginate microbeads containing BetaGraft cells of porcine origin were conducted into trial in 2021, subcutaneously in T1DM patients by Beta-cell NV (Beta-cell.com).

Most modifications for developing a suitable alginate-based microcapsule are desired to improve mechanical properties against rupture or cell protrusion, permeability against immune cells and proteins, and prevent fibrosis. Intraperitoneal transplanted human islets, encapsulated in a microbead of sodium alginate and poly-L-ornithine, have demonstrated no immune responses in T1DM patients, though limited the bi-directional transport of oxygen, nutrients, and insulin [105]. A similar technology, named IMMUPEL, which comprises two layers of Ca²⁺ cross-linked alginate that sandwich a poly-L-ornithine layer, was applied by LCT to develop DIABECELL. DIABECELL passed clinical approval in Russia in 2010. ViCapsys Inc. has designed a compatible polymer release gradually CXCL12-incorporating microcapsules named VICAPSYN-eluting alginate that potentially immuno-isolated islet graft. It also facilitated vascularization at the transplant site [106]. Sigilon Therapeutics has successfully applied an immunoprotective sphere using a modified alginate called Afigrometer in a preclinical study [107]. A clinical trial is going to be set with Eli Lilly’s collaboration.

In contrast, macro-encapsulation devices are larger devices that contain a considerable population of cells, requiring a single or a few devices to give a therapeutic cell dose [99,104]. They can be implanted intravenously or extravascularly and facilitate surgical transplantation, retrieval, and cell replenishment procedures [77,108]. The devices of macro-encapsulation are made from biocompatible materials to avoid fibrolast overgrowth and enhance engraftment. The cell reservoirs are designed to protect the cells from mechanical stress and exclude immune cells and molecules while enabling the interchange of oxygen and nutrients in order to maintain the viability and functionality of the cells [102,109,110]. Microfabrication, nanofluidic, and 3D-printing technologies are also a hallmark of device customization for size and format, improving device personalization, mechanical robustness, and biocompatibility [77,108]. Other issues, such as the transplantation site, can determine the prerequisites of successful transplantation.

Baxter Healthcare (Theraocyte) is one of the pioneers in macro-encapsulation technology. The designated device provides mechanical strength, vascularization, and immune protection via an outer Teflon membrane and inner hydrogel semipermeable membrane, respectively, in a double membrane planar device [111–113]. The ViaCyte’s PEC-EncapTM and PEC-Direct devices have been implanted subcutaneously in diabetes patients with about one year of successful graft function [114,115]. The immunoprotective membrane of the first and the polymeric membrane of the second device can promote vascularization to improve oxygen and nutrient exchange.

Beta cells are particularly vulnerable to hypoxia, and the lack of sufficient oxygen to maintain cellular viability and functionality is one of the difficulties with many encapsulation techniques [100,116]. This significant obstacle can be solved by the βAir device. Beta-O2 Technologies Ltd has developed βAir to improve cell oxygenation by embedding a gas chamber within an alginate/hydrophilized PTFE device. The device implantation normalized blood glucose, glucose tolerance, and HbA1c levels in streptozotocin-induced diabetic rats for about six months [117]. Consequently, a phase I/II clinical trial (NCT02064309) is examining the safety of the implanted βAir device containing human islets.

Sernova Corporation (Canada) introduced Cell Pouch technology to be vascularized at a subcutaneous site a couple of weeks before the replacement of the cells within the device. The device is verified in the murine model [118] and clinical trial (NCT01652911). The device has no compartmentalization properties to immuno-isolate the transplanted cell, thus making immunosuppression necessary in recipients.

Despite decades of intensive study, it is still challenging to determine whether micro- or macro-encapsulation is the most suitable method for xeno- or allogenic islet transplantation because of each technique’s drawbacks. For example, from a biosafety perspective, macroencapsulation devices are preferred to microencapsulation techniques because they prevent the diffusion of stem cells throughout the body and enable easier explant recovery [102]. However, many groups are struggling to expel the drawbacks of each strategy, and the results are promising to be applied to insulin-producing cells of different origins.

### 6. Conclusion

Type 1 Diabetes Mellitus (T1DM) is a global chronic problem with current short-term exogenous insulin therapy. Constant endogenous insulin production is required for more prolonged blood glucose
stability. Solid pancreas or islets allograft transplantation as an endogenous source of insulin production accompanied by significant limitations, namely, host immune response and the lack of enough donors. Encapsulation strategies to compartmentalize graft from surroundings to block access of immune cells and proteins and prevent fibrosis while enabling bi-directional transport of nutrients and insulin neither eliminate the lack of donor source nor create lifelong rehabilitation. Therefore, an unlimited source is required to meet the worldwide need for insulin-producing cells.

Despite extensive research aimed at the induction of insulin-producing cells from stem cells or somatic cells through both the ectopic expression of β cell-specific transcription factors and various small molecules/growth factors, there are still a lot of problems ahead of achieving clinical translation. For instance, improving the efficiency and reproducibility of differentiation protocols, considering large-scale production of insulin-producing cells and their cost-effectiveness along with the safety of implantation, an adequate amount of insulin secretion in response to glucose, proper encapsulation, and graft survival are challenges to the clinical application of these cells.

To the best of our knowledge, ViaCyte’s cells, i.e., PEC-01, are the only clinically approved stem cell-derived insulin-producing cells used in trials. PEC-01 is a mixture of hESC-derived multipotent pancreatic progenitors implanted subcutaneously before full maturation (NCT02239354, NCT03162926) with two different encapsulations (PEC-Encap, PEC-Direct).

Small molecule-based approaches for IPC generation are very complex and labor-intensive, multistep, and time-consuming, with the need for precise application of a high amount of growth factor/small molecule in specific time points to comply with the rhythm of TFs regulation in β-cell specification and GSIS. Diversity in differentiation efficiencies across various PSC lines, deficiency in full maturation, and variation of GSIS-related gene induction (e.g., NKK6.1) are observed when the same differentiation protocol is used.

Therefore, considerable efforts have been implemented to eliminate these challenges over the last decade. Cumulatively, progress has been made in recruiting direct β-cell-fate specific transcription factors, especially in preclinical and animal studies. Researchers also take advantage of the 3D cell structure to improve the efficiency of insulin-producing cell generation and function.

Vertex Pharmaceutical generated islet-like organoids that tested preclinically on non-human primates and showed a 60 % reduction in the required insulin dosage (International Society for Stem Cell Research (ISSCR) Annual Meeting 2019). Sigilon Therapeutics has developed an engineered insulin-producing cell and verified its efficiency in a preclinical study, and a clinical trial will be launched shortly in cooperation with Eli Lilly company.

Directly introducing the essential transcription factors into stem cells or other somatic cells seems a more effective, fast, and straightforward approach for generating IPC. Moreover, there is a possibility to induce specific insulin-related genes to modify a phenotype. However, due to the involvement of viral vectors in genetic manipulation methods and safety concerns around it, small molecule techniques are more interesting from the point of view of clinical application. Therefore, more attempts at safe gene manipulation strategies must be implemented.

Using sources other than pluripotent stem cells to generate insulin-producing cells can alleviate ethical and safety concerns. The differentiation of mesenchymal stem cells toward IPC and transdifferentiation of the cells that shared the developmental origin with pancreatic beta cells are promising strategies to eliminate host immune response and other above-mentioned challenges ahead of beta cell-like replacement therapy that deserve more in vivo application studies.

In the case of transdifferentiation, physiological ramification among cell candidates creates the need to identify the key transdifferentiation regulator. The gene expression profile of the cell candidate and resultant insulin-producing cell is a promising approach to uncovering these molecular knots, as Ar iyach et al. suggest that Cdx2 constrains the reprogramming of the intestinal cells into beta cells.

In conclusion, we mentioned significant progress in the generation methods of insulin-producing cells and their transplantation for Type 1 Diabetes treatment. Multiple ongoing clinical trials and efforts plan to reduce immunological rejection and improve graft viability and in vivo insulin secretion; however, there are still challenges that ought to be solved. Identification of new key transcription factors and regulatory networks in the β-cell development holds great promise for diabetes cell replacement therapy.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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