Review Article

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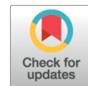

Organoid

Developments in endoderm and pancreatic β-cell differentiation from human pluripotent stem cells

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Human pluripotent stem cells (hPSCs) can differentiate into any cell type in the body, including cells or tissues from ectodermal, mesodermal, or endodermal lineages. Type 1 diabetes is characterized by the selective destruction of β cells in the islets of Langerhans within the pancreas, a process driven by autoimmunity. This destruction leads to the inability to produce and secrete insulin, resulting in poor blood glucose control. Patients with type 1 diabetes require exogenous insulin and regular blood glucose monitoring. However, achieving strict glycemic control solely through this approach is challenging, and there is a risk of side effects such as hypoglycemia when insulin levels rise following exogenous insulin administration. Thus, there is a pressing need for treatments that can overcome these limitations. Stem cell-derived pancreatic β cells are produced through a complex multi-step differentiation process, which involves the application of various growth factors and small molecules to precisely control intracellular signaling pathways at specific times. Our review primarily focused on significant studies concerning the differentiation of hPSCs into highly functional pancreatic β cells, covering key stages of pancreatic development such as definitive endoderm, primitive gut tube, posterior foregut/pancreatic progenitor cells, and pancreatic endocrine cells. Generating insulin-producing functional pancreatic β cells from hPSCs could be a viable and renewable cell source for alternative cell therapy to treat type 1 diabetes.

Keywords: Pluripotent stem cells; Insulin-secreting cells; Endoderm; Islets of Langerhans; Diabetes mellitus

Introduction

Embryonic stem cells, derived from the inner cell mass of the blastocyst stage of an embryo, represent a promising source for cell transplantation or cell-based therapies aimed at repairing damaged cells. These cells are cultured to maintain their ability to self-renew and can proliferate indefinitely while remaining undifferentiated [1]. Embryonic stem cells can differentiate into any cell type in the body, including cells and tissues of the ectoderm, mesoderm, and endoderm lineages. A key advantage of human embryonic stem cells (hESCs) lies in their consistent ability to self-renew and differentiate while in culture [2]. Induced human pluripotent stem cells (hiPSCs) offer nearly identical advantages, with the added benefit of being producible from an individual's own cells [1].

The endoderm gives rise to organs including the lungs, thyroid, thymus, gastrointestinal tract, liver, and pancreas. Diseases associated with organs originating from the endoderm encom-

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pass type 1 diabetes, pneumonia, fatty liver disease, chronic hepatitis, gastroenteritis, colitis, thyroiditis, and various cancers [3]. Of these, type 1 diabetes is considered a promising candidate for cell-based therapy, primarily due to the loss of specific cell types such as pancreatic β cells and the limitations associated with conventional treatments [4].

The pancreas secretes insulin and glucagon, which regulate blood glucose levels [5]. After a meal, when blood glucose levels rise, insulin is released from the pancreatic β cells, allowing glucose to enter cells and be utilized as an energy source. Insulin serves as a crucial regulator of energy metabolism across the body by managing the utilization of the 3 major nutrients: carbohydrates, fats, and proteins [6]. The dynamic balance between energy demand and supply, influenced by diet and activity, significantly affects both the secretion of insulin from pancreatic β cells and its concentration in the blood.

Type 1 diabetes results from the autoimmune destruction of β cells in the islets of Langerhans within the pancreas, leading to a cessation of insulin production and secretion that in turn causes a loss of blood glucose regulation [4]. Consequently, the elevated blood glucose levels during meals cannot be controlled, preventing glucose from entering cells. This impairment hinders the utilization of glucose and significantly affects the synthesis of proteins and fats. Therefore, patients with type 1 diabetes must manage their condition through the administration of exogenous insulin and regular blood glucose monitoring to maintain proper glucose and energy levels [4].

Insulin injections are very helpful for managing blood glucose levels and play a crucial role in patients' survival. However, this method differs from the natural secretion of insulin, which increases in response to a meal and adjusts as blood glucose levels fluctuate with physical activity [4]. Despite frequent monitoring and insulin injections, maintaining strict blood glucose control can be challenging. Type 2 diabetes develops when the insulin secretion from β cells is inadequate due to increased insulin resistance. Reduced insulin secretion capacity of pancreatic β cells necessitates insulin administration, and in cases of β cell dysfunction, continuous insulin administration becomes essential, similar to the management of type 1 diabetes [7].

Cell and gene therapies have been applied as complementary and alternative approaches to treatment for several incurable diseases [2]. A more fundamental physiological treatment strategy for type 1 diabetes involves replacing damaged β cells with new cells, tissues, or organs, enabling the transplanted cell clusters to secrete insulin in response to changes in blood glucose levels [4]. Cell therapy may also serve as a potential treatment for type 2 diabetes in patients who require insulin injections [7].

Transplantation of islets of Langerhans from human donors is technically feasible and can restore glucose control. However, the requirement for islets from multiple donors and the limited availability of donor pancreases are significant barriers to this treatment approach [8,9]. To facilitate cell therapy for damaged pancreatic β cells, it is essential to establish a protocol for differentiating pancreatic β cells from human pluripotent stem cells (hPSCs) into functional insulin-secreting cells. Additionally, developing a method for large-scale production and using these cells as an alternative source is crucial. Moreover, mechanical and genetic manipulation is necessary to prevent immune rejection following transplantation.

Based on the differentiation process and mechanisms, pancreatic β cells derived from hPSCs were induced and differentiated from hESCs and hiPSCs into the endoderm and pancreatic lineage. These differentiated pancreatic β cells are capable of secreting insulin in response to glucose and can regulate blood glucose levels after being transplanted *in vivo* [10–12]. This achievement was made possible by gradually differentiating the cells through each developmental stage, thereby mimicking the *in vivo* developmental processes of the pancreas. The generation of functional pancreatic β cells from hPSCs *in vitro* could serve as a practical and renewable cell source for replacement therapy. The therapeutic potential of these cells is further underscored by their transplantation into patients with type 1 diabetes [13]. However, several challenges persist in generating and transplanting these stem cell-derived pancreatic β cells [7]. The objective is to produce highly functional stem cell-derived pancreatic β cells that can not only synthesize and store insulin but also release it effectively in response to glucose stimulation, while being protected from immune responses. Specifically, it is essential to have a small number of endocrine cells other than pancreatic β cells, to ensure adequate insulin secretion, and to achieve gene expression and chromatin maturation similar to that of pancreatic β cells [14,15]. To protect transplanted stem cell-derived pancreatic β cells from immune attacks, strategies such as gene targeting/engineering, immune suppressants, MHC matching, macroencapsulation, microencapsulation, preconditioning, and transcriptional memory are being explored [16].

These challenges can be addressed through several strategies, including the improvement of differentiation protocols, biomaterial engineering, and genome editing. As technologies for differentiating high-functioning stem cells and developing biomaterials suitable for *in vivo* transplantation rapidly advance, genome editing tools, particularly those based on the CRISPR/Cas9 system, show promise. These tools can permanently modify the genome sequence, thereby altering the phenotype of cell

therapeutics [17,18].

This review explores the essential growth factors and small molecules involved in the differentiation of hPSCs into pancreatic β cells, as well as how this differentiation process can be applied to treat type 1 diabetes. Stem cell-derived pancreatic β cells are generated through a multi-step differentiation process that involves the application of various growth factors and small molecules. These agents precisely regulate intracellular signaling pathways at critical stages. The process and the compounds utilized are illustrated in Fig. 1. This review not only covers major research findings on the differentiation of hPSCs from definitive endoderm to pancreatic endocrine cells but also includes studies aimed at enhancing differentiation protocols, signaling mediators of differentiation, and omics analysis of differentiated single cells, starting from the initial studies on definitive endoderm differentiation. This research could contribute to the development of superior hPSC-derived pancreatic endodermal or endocrine cells, potentially improving therapeutic approaches for patients with type 1 diabetes in future research.

Definitive endoderm differentiation

Ethics statement: This study constituted a comprehensive analysis of previously released studies and thus, was not subject to the approval of the institutional review board.

The definitive endoderm is formed from the inner cell mass through the process of gastrulation in the embryo, during which epiblast cells differentiate into 3 germ layers. The definitive endoderm is distinct from the primitive endoderm, which gives rise to both the visceral and parietal endoderm. The definitive endoderm generates various tissues and cells that are essential for the development of major organs such as the lungs, pancreas, liver, stomach, intestines, colon, bladder, thyroid, and thymus [3]. Cells derived from the definitive endoderm of hPSCs can differentiate into any endoderm derivatives, including pancreatic β cells, lung alveolar cells, and liver hepatocytes. To generate therapeutic endoderm cells, it is necessary to differentiate hPSCs into the endoderm lineage.

1. Activin A-induced definitive endoderm

To differentiate pancreatic β cells from stem cells, it is crucial to identify and control endoderm progenitor populations. Signals that regulate endodermal differentiation during normal embryonic development can guide hPSCs to determine their endodermal fate. In a study aiming to induce differentiation of definitive endoderm, a high concentration of activin was used in the embryoid bodies (EBs) of mouse ESCs. This led to the differentiation of the mesendoderm population expressing brachyury into definitive endoderm expressing Foxa2 [19]. Activin A mimics the action of Nodal, a ligand for the transforming growth factor-β (TGF-β) superfamily. It activates a series of

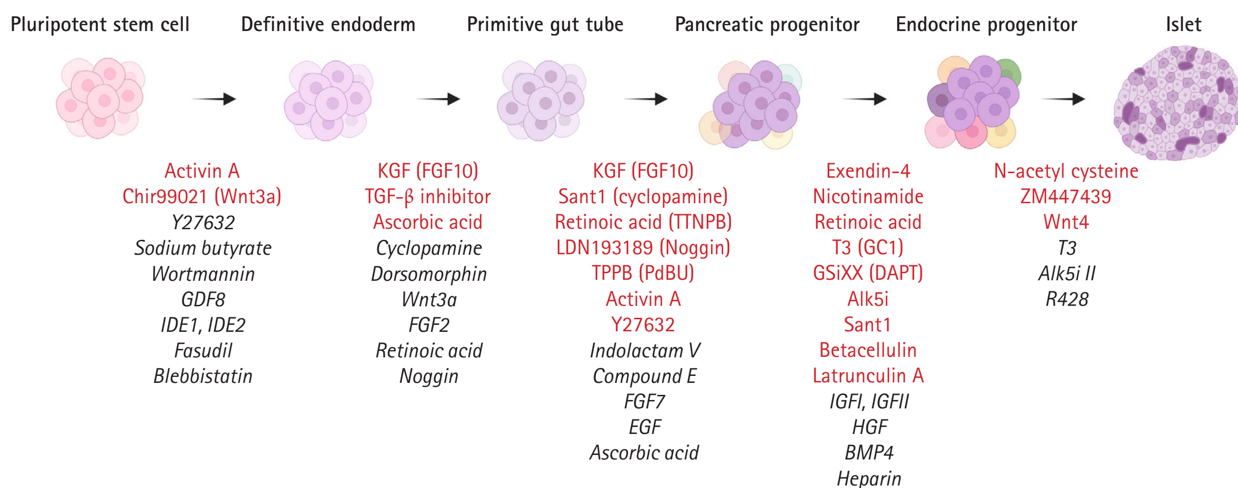

Fig. 1. Compounds used for generating definitive endoderm, primitive gut tube, and pancreatic β cells from human pluripotent stem cells (hPSCs). The compounds used in the differentiation process are presented by differentiation stage (definitive endoderm, primitive gut tube, pancreatic progenitor, endocrine progenitor, islet). Compounds shown in red have been used in many articles, while those shown in black are used in fewer articles. Alk5i, Alk5 receptor inhibitor; BMP4, bone morphogenetic protein 4; EGF, epidermal growth factor; FGF, fibroblast growth factor; GDF8, growth differentiation factor 8; HGF, hepatocyte growth factor; IDE, inducer of definitive endoderm; IGF, insulin-like growth factor; KGF, keratinocyte growth factor; T3, triiodothyronine; GSiXX, γ-secretase inhibitor XX. Figure created with BioRender.com.

downstream signaling events and transcriptional networks regulating definitive endoderm development [20]. EBs stimulated by activin were predominantly composed of endoderm, with over 50% of the cells expressing Foxa2, while lower levels of activin A induced mesoderm [19]. FOXA2 is highly expressed in both mesendoderm and definitive endoderm, serving as a crucial transcription factor during the differentiation of definitive endoderm and pancreas [21]. SOX17 is expressed in the later stages of endoderm development. Definitive endoderm progenitor cells express both FOXA2 and SOX17, which are essential for the differentiation of endoderm-derived cell types.

Mouse ESCs cultured as a monolayer were induced into definitive endoderm by treatment with activin A, and Gsc and Sox17 markers were used to separate $Gsc^{+}Sox17^{+}$ mesendoderm-derived definitive endoderm from $Gsc^{-}Sox17^{+}$ visceral endoderm [22]. High concentrations of activin A led to the efficient differentiation of hESCs into definitive endoderm [23]. Specifically, hESCs were differentiated into definitive endoderm by culturing them as a monolayer with activin A in a low concentration of fetal bovine serum (FBS) (0% for the first 24 hours, 0.2% for the second 24 hours, and 2.0% for days 3 and 4). Among these differentiated cells, 80% were $FOXA2^{+}SOX17^{+}$ cells, and CXCR4 expression was also increased. The mesendoderm is selectively induced from ESCs under serum-free media with activin A, expressing Gsc, E-cadherin, PDGFRα and Foxa2. The mesendoderm is differentiated into definitive endoderm, expressing Gsc, E-cadherin, Foxa2, and Sox17, or mesoderm, expressing Gsc, PDGFRα, Cad11, VEGFR2, and PDGFRβ [24].

2. Activin A and Wnt3a–induced definitive endoderm

Nodal/TGF-β signaling, activated by activin A, along with Wnt signaling, plays a crucial role in patterning the definitive endoderm. Activation of the Wnt pathway using Wnt3a or glycogen synthase kinase-3β (GSK-3β) inhibitors has been shown to induce the differentiation of ESCs cultured under monolayer conditions into mesendoderm [25]. These cells express brachyury, Flk-1, Gsc, and Foxa2, and constitutively express markers of both mesodermal and endodermal cell lineages. Differentiation of mouse ESCs into an anterior streak is achieved with Wnt and high levels of activin, while Wnt combined with low levels of activin leads to differentiation into the posterior primitive streak. Continuous activin signaling further differentiates these cells into endoderm [26]. Mesendoderm differentiation is also enhanced by culturing hESCs as a monolayer with Wnt3A and activin A in a low concentration of FBS, and definitive endoderm differentiation is achieved by culturing hESCs with activin A for an additional 2 days [27]. This resulted in increased expression of SOX17 and CXCR4, as well as the anterior definitive endoderm markers CER1 and FOXA2 [27]. Treatment with Chir99021 (a GSK-3β inhibitor) instead of Wnt, resulted in the differentiation of hESCs into primitive streak, or mesendoderm, within 24 hours, and treatment with activin A led to effective differentiation into definitive endoderm. Treatment with Chir99021 alone caused the cells to differentiate into mesoderm [28]. Activin A and Wnt3a were replaced with growth differentiation factor-8 (GDF8), a TGF-β family member, and GSK-3β inhibitors to promote differentiation into definitive endoderm [29]. Based on these studies, in the first step of pancreatic β cell differentiation, activin A and Chir99021 are used to activate the Wnt pathway, thereby causing the differentiation of hPSCs into definitive endoderm stem cells along the endoderm differentiation process.

3. Definitive endoderm induced by other factors

After the replacement of serum with bone morphogenetic protein 4 (BMP4), basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF), the $CXCR4^{+}CD117^{+}$ population was induced in both serum free EBs and monolayer culture [30]. Two days later, the EBs became definitive endoderm, but were able to generate more cells. In another study, only fibroblast growth factor 2 (FGF2) was used instead of serum [31]. Small molecules—namely, inducer of definitive endoderm (IDE) 1 and IDE2—were identified as analogs of activin A by screening a chemical library. These compounds successfully generated $SOX17^{+}$ definitive endoderm from mouse ESCs and hESCs in monolayer cultures [32]. These small molecules could serve as alternatives to activin A in generating definitive endoderm. Through large-scale, high-throughput screening, small molecules capable of inducing FoxA2 were identified. Among these, fasudil, a ROCK inhibitor, effectively directed mouse ESCs and hESCs into definitive endoderm, which then differentiated into anterior definitive endoderm and PDX^{+} pancreatic progenitor [33]. Other ROCK inhibitors, including RKI1447, hydroxyfasudil (HA1100), Y-27632, and thiazovivin, similarly promoted the differentiation of hPSCs into definitive endoderm, and the myosin II inhibitor blebbistatin also showed the same effect. ROCK is a target of the small GTPase RhoA, which regulates cell contraction, and ROCK inhibition is a novel mechanism for inducing definitive endoderm by modulating the actin cytoskeleton.

A method for differentiating mouse ESCs into definitive endoderm and mesoderm was developed by leveraging the expression of Nodal, which promotes the formation of mesendoderm

precursor cells expressing CXCR4, VEGFR2, and PDGFR-α [34]. SOX17-overexpressing hESCs were induced into a stable definitive endoderm precursor without activin A treatment and differentiated into liver and pancreas derivatives [35]. Sox17$^+$ cells induced by activin A were selectively enriched using fluorescence-activated cell sorting to improve the yield of definitive endoderm, while simultaneously reducing the presence of mesoderm and ectoderm cells [36].

4. Signaling and culture types

Cellular and molecular biological analyses of the differentiation process can be performed using definitive endoderm derived from hESCs. Particularly in the context of mesendoderm, signaling pathways such as Wnt, BMP, or FGF play a critical role in the differentiation of the primitive streak or mesendoderm. Subsequently, high levels of activin A and low levels of FGF guide the differentiation of hPSCs into endoderm, while BMP and Wnt signaling direct their differentiation into mesoderm lineage [3]. Activin A or Nodal activates a heteromeric complex of TGF-β receptors, which then phosphorylates SMAD2/3. The phosphorylated SMAD2/3 translocates to the nucleus and enhances the expression of FOXA2, SOX17, and GSC, which are key in inducing differentiation into the endoderm lineage. While most differentiation protocols have been developed using monolayer (planar) cultures, some protocols have utilized EBs [19,30] and suspension cultures [10,37].

Table 1 shows the protocols of *in vitro* definitive endoderm differentiation, with a detailed comparison of chemicals and conditions [10,11,14,15,19,23,27–33,37–54]. The protocols for inducing definitive endoderm can be broadly categorized into 4 groups: (1) activin A, (2) activin A with Wnt3a, (3) activin A with Chir99021, and (4) other methods. During the definitive endoderm differentiation process, sodium butyrate and the PI3K inhibitor wortmannin have been used in conjunction with activin A. Alternatively, the ROCK inhibitor Y-27632, GDF8, Nodal, IDE1 or IDE2 can be used instead of activin A.

Primitive gut tube differentiation

Precise patterning of the anterior-posterior axis in the definitive endoderm leads to the formation of a primitive gut tube. This primitive gut tube, derived from the definitive endoderm, induces the differentiation of associated organs including the pharynx, esophagus, stomach, duodenum, small and large intestines, as well as the thyroid, parathyroid, thymus, lung, pancreas, and liver along the anterior-posterior axis [55]. The anterior portion of the foregut in the primitive gut tube develops into

Table 1. Protocols for definitive endoderm differentiation

Chemicals	Duration (day)	Culture type	Reference
Activin A			
Activin A		EB	[19][a]
FBS 0%–2% Activin A	5	Monolayer	[23]
BMP4 (days 1–3) bFGF (days 2–5) VEGF (day 5) Activin A (days 2–3, day 5)	5	EB	[30]
Activin A+Wnt3a			
FBS 0–0.2% Activin A Wnt3a (day 1)	3	Monolayer	[27,38–44]
FBS 0.2%–0.5% Activin A Wnt3a (day 1)	3	Monolayer	[11]
FBS 0.2% Activin A Wnt3a (day 1)	2	Suspension	[37,45]
Activin A+Chir99021			
FCS 0.2% [28] Activin A Chir99021 (day 1) [28,46–48] Chir99021 (days 1–2) [15]	3 [15,28] 4 [46–48]	Monolayer	[15,28,46–48]
Activin A Chir99021 (day 1)	3	Suspension	[10,14,48–51]
Activin A bFGF (days 2-3) Wnt3a (day 1) or Chir99021 (day 1)	3	Monolayer	[31]
Activin A Chir99021 (days 1–3) Y-27632 (day 1)	4	Monolayer	[52]
Others			
Activin A Sodium butyrate	7	Monolayer	[53]
Activin A Wortmannin	4	Monolayer	[54]
GDF8 GSK-3β inhibitor (MCX-928)	3	Monolayer	[29]
FBS 0.2% Activin A or Nodal Wnt3a (day 1)	4–6	Monolayer	[32][a]
FBS 0.2% IDE1 or IDE2	4–6	Monolayer	[32][a]
Fasudil or RKI1447 or HA1100 or Y-27632 or thiazovivin or blebbistatin	4	Monolayer	[33][b]

EB, embryoid body; FBS, fetal bovine serum; BMP4, bone morphogenetic protein 4; bFGF, basic fibroblast growth factor; VEGF, vascular endothelial growth factor; GDF8, growth differentiation factor 8; IDE, inducer of definitive endoderm.

[a]Mouse embryonic stem cells (ESCs), [b]mouse ESCs and human ESCs.

the thyroid, lung, esophagus, and stomach, while the pancreas, liver, and duodenum originate from its posterior portion. The midgut and hindgut of the primitive gut tube are responsible for forming the small and large intestines, respectively. Expression of NKX2-1 and SOX2 markers occurs in the anterior foregut, HHEX and PDX1 in the posterior foregut, and CDX in both the midgut and hindgut [55,56].

1. Differentiation protocols

In the process of differentiating definitive endoderm derived from hESCs into pancreatic lineage endocrine cells, the stages of the primitive gut tube and posterior foregut were introduced [27]. This protocol replicates pancreatic organogenesis by progressing through intermediate stages of each endoderm (primitive gut tube endoderm, pancreatic endoderm), mirroring those observed during natural pancreatic development. The differentiation into primitive gut tube was initiated by adding 2% FBS and removing activin A from the culture for 2 to 4 days. The removal of activin A facilitated the transition from definitive endoderm to primitive gut endoderm, which expressed the primitive gut tube markers HNF1B and HNF4A. The addition of fibroblast growth factor 10 (FGF10) and the hedgehog-signaling inhibitor KAAD-cyclopamine further differentiated the primitive gut tube into pancreatic cells characterized by high insulin expression [27]. Keratinocyte growth factor (KGF) has also been used instead of FGF10 [38].

FGF regulates the differentiation of the primitive gut tube from definitive endoderm [39,40]. As the concentration of FGF2 increases, hepatocyte differentiation is suppressed, while differentiation into pancreatic cells is promoted. Higher concentrations of FGF2 enhance the specification of midgut endoderm into small intestine progenitor cells and promote the differentiation of anterior foregut into lung tissue. Retinoic acid and FGF4 together induce the differentiation of definitive endoderm from hESCs into $PDX1^+$ foregut endoderm cells [40]. These endoderm cells can further differentiate into the pancreas, posterior stomach, or duodenal endoderm. Administering FGF10 for 3 days induces the primitive gut tube and patterns the activin A-induced definitive endoderm toward a posterior foregut fate [30]. In addition to FGF10, the inhibition of BMP signaling by Dorsomorphin and the activation of Wnt signaling by Wnt3a (via DKK1 blockade of endogenous Wnt signaling) are required for generating insulin-expressing pancreatic progenitors [30]. Early-stage administration of FGF7 with ascorbic acid (vitamin C) regulates low expression of NGN3 in pancreatic progenitors [29]. The TGF inhibitor SB431542, or TGF-β inhibitor IV, is sometimes administered on the first day of step 2 [37,41,45]. A combination of the hedgehog inhibitor cyclopamine, BMP inhibitor noggin, and retinoic acid—excluding FGF10—significantly reduces differentiation into the liver and increases differentiation efficiency into the pancreas [42]. Based on these studies, in the second stage of pancreatic β-cell differentiation, definitive endoderm cells are induced into the primitive gut tube using KGF, also known as FGF7. To enhance this differentiation, chemicals such as Wnt3a, TGF-β inhibitor IV, and ascorbic acid are added.

Table 2 shows the protocols of *in vitro* primitive gut tube differentiation from definitive endoderm, with a detailed comparison of chemicals and conditions [10,11,14,15,27,29–31,37–52]. The protocols for inducing the primitive gut tube are broadly categorized into 4 groups: (1) FGF10, (2) KGF, (3) KGF combined with a TGF-β inhibitor or ascorbic acid, and (4) other methods.

Table 2. Protocols for primitive gut tube differentiation

Chemicals	Duration (day)	Culture type	Reference
FGF10			
FBS 2%	2–4	Monolayer	[27,43,44]
FGF10	2 [43,44]		
Cyclopamine			
FGF10	3	Monolayer	[30,31]
DKK1 [30]			
Dorsomorphin			
Wnt3a			
KGF (FGF7)			
FBS 2%	3	Monolayer	[11,38]
KGF			
KGF	2 [46–48,52]	Monolayer [46–48,52]	[10,14,46–52]
	4 [52]		
	3 [10,48–51]	Suspension [10,48–51]	
KGF (FGF7)+TGF-β inhibitor or ascorbic acid			
FBS 2%	3	Monolayer	[41]
KGF			
SB431542 (day 1) or TGF-β inhibitor IV (day 1)			
FBS 0.2% [37], 0.2%–0.4% [45]	3	Suspension	[37,45]
KGF			
TGF-β inhibitor IV (day 1)			
FGF7	2 [29]	Monolayer	[15,29]
Ascorbic acid	3 [15]		
Others			
FGF2	6–8	Monolayer	[39]
FBS 2%	4	Monolayer	[40]
FGF4			
Retinoic acid			
FBS 2%	4	Monolayer	[42]
Noggin			
Cyclopamine			
Retinoic acid			

FGF, fibroblast growth factor; FBS, fetal bovine serum; KGF, keratinocyte growth factor; TGF-β, transforming growth factor-β.

Pancreatic progenitors (posterior foregut) and pancreatic endocrine

In the pursuit of treatments for type 1 diabetes, numerous technologies have been developed to differentiate hPSCs into pancreatic β cells. However, these differentiated cells often lack the physiological and metabolic functions characteristic of normal adult pancreatic β cells in the human body. While various protocols have been introduced to address this issue, significant challenges persist in enhancing the functionality of these cells in patients with type 1 diabetes. Notably, there have been major advances in protocols aimed at generating pancreatic β cells with high physiological functionality.

In the initial stages of insulin discovery in ESCs, insulin-secreting cells were isolated from mouse ESCs using a cell-trapping system, and the emergence of insulin-expressing cells was noted [57]. EBs derived from mouse ESCs were used to select a nestin^{+} precursor population, from which insulin-producing cells were developed using serum-free media, B27, bFGF, and nicotinamide [58]. These differentiated cells expressed insulin mRNA and C-peptide, released insulin in response to glucose, and ameliorated hyperglycemia following transplantation into diabetic rats [59]. To enhance the yield of insulin^{+} cells, EBs were treated with activin βB, nicotinamide, and exendin-4 [60]. Mimicking the primary stages of early pancreatic differentiation seen in embryonic development, differentiation was sequentially induced in 3 stages with the following treatments: activin A and retinoic acid in step 1, FBS and bFGF in step 2, and N2, B27, laminin, bFGF, and nicotinamide in step 3, resulting in the differentiation of EBs into insulin-producing cells [61]. Although spontaneous differentiation could generate pancreatic progenitor cells and insulin-producing cells, the quantity of these cells was very low.

1. Early differentiation protocols mimicking pancreatic organogenesis

Protocols for hESCs have been developed that replicate the essential developmental stages of pancreatic organogenesis observed *in vivo* [27,38,54]. These stages include the specification and maturation of mesendoderm, definitive endoderm, primitive gut tube endoderm, posterior foregut, pancreatic endoderm, and endocrine precursors, ultimately leading to the generation of pancreatic β cells capable of secreting insulin. The protocol suggests using 3 key factors—FGF10, retinoic acid, and cyclopamine—to induce the differentiation of primitive gut tube into posterior foregut. hESCs derived from the primitive gut tube were treated with these factors and cultured under serum-free conditions for 3 days, resulting in posterior foregut cells that significantly expressed PDX1, HNF6, and SOX9 [27]. These cells were then cultured in serum-free media containing DAPT and exendin-4, which enhanced insulin secretion and facilitated their differentiation into pancreatic endoderm cells expressing NKX6-1, NKX2-2, NGN3, and PAX4 [27]. Next, insulin-like growth factor 1 (IGF1) and hepatocyte growth factor (HGF) were added to create pancreatic endocrine cells that expressed pancreatic hormone but did not secrete insulin in response to glucose. The protocol was adjusted by removing FGF10 and adding noggin. Posterior foreguts were then induced by culturing in a serum-free medium with cyclopamine, noggin, and retinoic acid, followed by maintenance without inducers to differentiate into pancreatic endoderm and endocrine precursors [38]. When these differentiated pancreatic endocrine cells were transplanted into mice, their maturation into β cells was expedited, and they functioned as glucose-responsive insulin-secreting cells. Compared to forming EBs, monolayer culture is more efficient in differentiating hESCs into pancreatic endocrine progenitor cells, and three-dimensional culture appears to be more effective in maturing pancreatic β-cell progenitors into functional cells [55].

Since this differentiation protocol was developed, it has undergone several modifications and applications across the major developmental stages of pancreatic endocrine cells [11,30–32,37,40–45,54]. The key modifications include: (1) The discovery of indolactam V through high-content chemical screening, which increases the number of PDX1-expressing cells. This compound targets the definitive endoderm to predominantly differentiate into PDX1-expressing pancreatic progenitors, which are precursors to all pancreatic cell types [32,43]. Additionally, pancreatic endocrine differentiation was induced by applying indolactam V after the pancreatic progenitors had formed [44]. (2) The addition of ascorbic acid and the BMP inhibitor noggin to the combination of FGF10, retinoic acid, and cyclopamine, which are known to induce the primitive gut tube into pancreatic endoderm, thereby promoting the formation of PDX1^{+} pancreatic precursors [30]. Without BMP inhibition, there was a significant reduction in intracellular insulin levels, and BMP signaling was found to regulate hepatic differentiation in the ventral foregut endoderm. However, some protocols exclude BMP inhibition [45]. (3) The induction of the posterior foregut was achieved by treating it with a base combination of cyclopamine, noggin, and retinoic acid, followed by treatment with FGF10, compound E, and exendin-4 to differentiate into pancreatic endoderm and endocrine precursors [42]. Substitutions with small molecules include: 1) the use of TTNPB, a

retinoid analog, as a substitute for retinoic acid [37,45], and 2) the use of Sant1 in place of cyclopamine [11,31]. Additionally, differentiation into PDX$^+$ foregut endoderm was attempted using only FGF4 and retinoic acid [40], and differentiation into PDX$^+$/NKX6-1$^+$ pancreatic progenitors was induced using TTNPB, epidermal growth factor (EGF), and KGF [45].

In addition to this protocol, there have been attempts using EBs to obtain more mature pancreatic cells. A 4-step serum-free protocol involved converting definitive endoderm into cell aggregates, which were then treated with a combination of EGF, bFGF, and noggin to form pancreatic endoderm and pancreatic endocrine cells. In the final step, nicotinamide and IGF2 were applied to mature the aggregates [53]. The definitive endoderm was treated with retinoic acid, bFGF, and nicotinamide, with EBs formed in the final step to mature the pancreatic islet [62]. Definitive endoderm was differentiated in the EB state, with retinoic acid applied to further differentiate it into pancreatic endoderm cells [63]. Definitive endoderm was treated with retinoic acid, noggin, and FGF7 to differentiate it into pancreatic precursors. Expansion was promoted with EGF, and maturation was achieved with nicotinamide, bFGF, exendin-4, and BMP4 [54]. This protocol yielded 25% insulin$^+$ cells that released insulin and C-peptide in response to glucose stimulation. Since changes in the timing and intensity of intracellular signals are crucial in pancreatic cell differentiation, the combination and timing of administration of substances that induce pancreatic differentiation continue to be improved.

2. Recent advances in differentiation protocols

Although this differentiation protocol has been shown to generate stem cell-derived pancreatic β cells, it produces a low yield of insulin-producing cells. Additionally, these cells do not secrete insulin appropriately in response to glucose stimulation, among other limitations. To address these issues, numerous improved protocols are being developed, and novel small molecules have been employed to generate more mature pancreatic β cells by modulating intracellular signaling pathways. A protocol to generate pancreatic β cells from hESCs and hiPSCs, which functionally secrete insulin like adult pancreatic β cells, has been reported [10,29]. Based on this earlier protocol, differentiation into pancreatic β cells is induced according to the stage of embryonic development and progresses through intermediate cell types corresponding to various developmental stages. The process now includes 6 to 7 steps and takes about a month. To the 3 basic factors— KGF, retinoic acid, and Sant1—LDN193189, a BMP type 1 receptor inhibitor, and phorbol 12,13-dibutyrate (PdBU), a PKC activator, have been added [10,46,47]. Activation of protein kinase C through the retinoic acid signaling pathway, a fundamental signaling molecule in endoderm development, is further enhanced by treatment with TPPB, a protein kinase C activator, aiding in the generation of pancreatic progenitor cells. LDN193189, an inhibitor of the BMP pathway, promotes differentiation to pancreatic progenitor cells by inhibiting signal transduction. Sant1, a sonic hedgehog (SHH) pathway inhibitor, also promotes the specification of pancreatic progenitor cells. In the subsequent pancreatic endocrine stage, triiodothyronine (T3), γ-secretase inhibitor XXI, ALK5 inhibitor (Alk5i), heparin, and betacellulin are administered in addition to retinoic acid and Sant1 [10]. Alk5i, an inhibitor of TGF-β type I receptor kinase (ALK5), and the thyroid hormone T3 are used to generate insulin$^+$ β-like cells from endocrine progenitor cells in the late stage of differentiation [10]. Betacellulin also inhibits the differentiation of exocrine and alpha cells while increasing insulin production [64].

Ascorbic acid has been utilized to induce differentiation of pancreatic endoderm, while a combination of gamma-secretase inhibitor, N-acetyl cysteine, and the tyrosine kinase receptor AXL inhibitor facilitated the differentiation of pancreatic β-cells [29]. Treatment with ascorbic acid, known for its role in extracellular matrix production, led to the generation of PDX1$^+$/NKX6-1$^+$ pancreatic progenitors, with an upregulation of NGN3 expression following subsequent treatments with Alk5i, BMP receptor inhibitor, and T3. The administration of Alk5i, BMP receptor inhibitor, T3, and gamma-secretase inhibitor XX, which blocks the notch pathway, resulted in the formation of PDX1$^+$/NKX6-1$^+$/NEUROD1$^+$ cells. The inhibition of notch expression in PDX1$^+$ progenitor cells is crucial for pancreatic differentiation, necessitating the use of a notch inhibitor. Additionally, R428, an AXL inhibitor, in combination with Alk5i and T3, promoted MAFA expression, while N-acetyl cysteine, an antioxidant, enhanced nuclear localization of MAFA [29]. EGF and nicotinamide further supported the production of PDX1$^+$/NKX6-1$^+$ pancreatic progenitors by inhibiting the BMP pathway [31].

Activin A and a ROCK inhibitor were added to the protocol to induce pancreatic progenitors [48–51]. The addition of activin A to the pancreatic endoderm increased the proportion of NKX6-1$^+$ cells [31]. By incorporating the ROCK inhibitor Y-27632 into the posterior foregut, pancreatic endoderm expressing PTF1A and NKX6-1, along with KGF, noggin, and EGF, was successfully induced [52]. The inclusion of these 2 factors maintained cluster integrity and shortened the duration of the differentiation step [50]. In the maturation stage of pancreatic differentiation, insulin secretion was significantly

enhanced by resizing clusters and excluding Alk5i and T3, highlighting the importance of TGF-β signaling in the maturation of stem cell-derived pancreatic β cells. Previously, stem cell-derived pancreatic β cells differentiated using earlier protocols exhibited a low insulin secretion response to increased glucose levels. However, subsequent protocols improved biphasic dynamic insulin secretion in response to glucose [50,65]. Using I-BET151, a selective inhibitor of BET, PDX1$^+$/NKX6-1$^+$ pancreatic progenitors can be maintained in a proliferative and expandable state, facilitating the continued growth of pancreatic cells [66]. This inhibitor activates Notch signaling, which is crucial for expressing key genes in pancreatic progenitors and provides an intermediate stage for generating stem cell-derived pancreatic β cells, ensuring a sufficient supply of cells. Aurora kinase A is known to promote the proliferation of pancreatic β cells by upregulating Nkx6-1 [67]. Treating immature stem cell-derived pancreatic β cells with ZM447439, an antiproliferative aurora kinase inhibitor, not only reduced cell proliferation but also improved glucose-stimulated insulin secretion and promoted maturation [15]. Additionally, adding Wnt4 during this pancreatic maturation step enhanced insulin secretion in response to glucose [68].

3. Signaling and culture types

Wnt signaling is activated not only during differentiation into mesendoderm but also after differentiation into definitive endoderm. Endoderm cells expressing the CD177/NB1 glycoprotein tend to differentiate into the pancreatic lineage due to the activation of noncanonical Wnt signaling. In contrast, endoderm cells expressing the T-cell costimulatory ligand CD275/ ICOSL upregulate canonical Wnt signaling and differentiate into liver cells [69]. The former group secretes Wnt, NODAL, and CERBERUS1, which leads to pancreatic differentiation. Sorting and differentiating CD177-expressing cells among endoderm cells enhances the maturity and glucose-responsive function of stem cell-derived pancreatic β cells [69]. Additionally, during the pancreatic endocrine differentiation process, inhibiting the Wnt signaling pathway increases the expansion of pancreatic endocrine cells and improves the yield of pancreatic β cells [70]. During the differentiation of pancreatic β cells from hPSCs, canonical Wnt signaling inhibits the proliferation of pancreatic endocrine cells, while noncanonical Wnt signaling promotes differentiation into the pancreatic lineage and maturation of β cells. Regulating Wnt signaling is a crucial intracellular signal for inducing differentiation of pancreatic β cells.

Although pancreatic precursors can generally be efficiently generated from hPSCs in flat cultures, according to the differentiation protocol, cells are either aggregated from the beginning of differentiation [10,14,37,45,48–51] or before the formation of endocrine progenitor cells [15,53] and then cultured in suspension. This process facilitates the efficient generation of endocrine progenitor cells from pancreatic progenitor cells. Three-dimensional cell clusters of pancreatic endoderm, formed prior to endocrine specification, were generated at an air-liquid interface, efficiently producing pancreatic endocrine cells [29]. The arrangement of cells in 3 dimensions is either as suspension clusters [10,50] or as aggregates on transwell inserts [29]. In some instances, cell clusters are formed after differentiation into pancreatic endocrine cells [11,29]. Aggregation occurs at the stage of pancreatic precursor differentiation, with pancreatic precursors cultured in suspension during the pancreatic endocrine stage [15]. Collecting insulin-positive cells and aggregating them to form islet-sized enriched β-clusters not only improved dynamic insulin secretion but also enhanced calcium signaling, mitochondrial respiratory function, and mitochondrial energization [65]. After dispersing into single cells, sorting based on the surface marker CD49a (ITGA1), and then reconstituting into a stem cell-derived pancreatic β cell cluster, the proportion of C-peptide expressing cells increased to 80% [14].

This differentiation protocol, which utilizes cell aggregation, can produce pancreatic endocrine precursors more efficiently than the generation of progenitors in traditional planar culture. This suggests that there may be challenges in differentiating pancreatic cells in a monolayer culture. Latrunculin A, an actin filament destabilizer, is employed in planar cultures to specify pancreatic endocrine precursors [48]. The polymerized cytoskeletal state resulting from planar culture inhibits NEUROG3-mediated induction of pancreatic endocrine cells in PDX1$^+$/NKX6-1$^+$ pancreatic precursors. By using latrunculin A, cytoskeletal polymerization is inhibited, thereby increasing NEUROG3 expression [48]. In flat culture, pancreatic progenitor cells differentiate into endocrine precursors that secrete various hormones and develop into stem cell-derived pancreatic β cells with enhanced insulin secretion. This demonstrates that planar culture can be effective without the need for cell aggregation, a three-dimensional step in endocrine differentiation [48]. Among stem cell-derived β cells produced by the flat culture method and those produced by the cell aggregation method, the former exhibited more improved insulin secretion. Additionally, the flat culture method is more suitable for inducing β cells using a broad range of stem cells with diverse genetic backgrounds [47].

Table 3 shows the protocols for *in vitro* differentiation of posterior foregut and pancreatic progenitor cells from the primitive

Table 3. Protocols for differentiation of posterior foregut/pancreatic progenitor cells

Chemicals	Duration (day)	Culture type	Reference
FGF10+cyclopamine+retinoic acid			
FGF10 Cyclopamine Retinoic acid	2–4 [27] 4 [43]	Monolayer	[27,43]
FGF10 Cyclopamine (days 1–4) Retinoic acid (days 1–4) Indolactam V (days 5–8)	8	Monolayer	[44]
Ascorbic acid Cyclopamine Retinoic acid Noggin FGF10	3	Monolayer	[30]
Ascorbic acid FGF10 Cyclopamine or Sant1 Noggin Retinoic acid	4	Monolayer	[31]
Cyclopamine (Sant1)+noggin+retinoic acid (TTNPB)			
B27 Cyclopamine or Sant1 [11] Noggin Retinoic acid or TTNPB [37]	3 [37,38,41] 4 [11]	Monolayer [11,38,41] Suspension [37]	[11,37,38,41]
B27 Cyclopamine Noggin Retinoic acid	4	Monolayer	[42]
KGF Cyclopamine Noggin TTNPB Y-27632 (day 3)	3	Monolayer	[52]
Retinoic acid FGF7 Noggin EGF	4 5	Monolayer	[54]
KGF+LDN193189+PdBU (TPPB)+retinoic acid+Sant1			
KGF LDN193189 (day 1) PdBU (days 1–2) Retinoic acid Sant1	7	Suspension	[10]
KGF LDN193189 Retinoic acid Sant1 TPPB	6	Monolayer	[46,47]
Ascorbic acid FGF7 LDN193189 Retinoic acid (days 2–5) Sant1 TPPB	5	Monolayer	[29]

Table 3. Continued

Chemicals	Duration (day)	Culture type	Reference
KGF+LDN193189+PdBU (TPPB)+ retinoic acid+Sant1+Y-27632 +activin A			
LDN193189 (day 1) KGF Sant1 PdBU (days 1–2) or PdBU (day 1) [50] Y-27632 or Y-27632 (days 3–7) [51] Retinoic acid Activin A (days 3–7) or activin A (days 2–6) [50]	7 6 [50]	Suspension	[14,49–51]
KGF LDN193189 (day 1) PdBU (day 1) Retinoic acid Sant1 Y-27632 (day 1) Activin A (days 2-6)	6	Suspension (day 1) →monolayer (days 2–6)	[48]
Ascorbic acid FGF7 LDN193189 Retinoic acid Sant1 TPPB	2	Monolayer	[15]
Activin A Ascorbic acid EGF FGF7 LDN193189 Nicotinamide Retinoic Acid ROCKi Sant1 TPPB	4	Aggregation	
Other chemicals			
bFGF (days 1–14) EGF Noggin	21	Aggregates	[53]
FBS 2% FGF4 Retinoic acid	9	Monolayer	[40]
Indolactam V	4	Monolayer	[32][a)]
EGF (days 3–4) KGF (day 4) TTNPB (days 1–3)	4	Suspension	[45]

FGF, fibroblast growth factor; bFGF, basic FGF; EGF, epidermal growth factor; KGF, keratinocyte growth factor; PdBU, Phorbol 12,13-dibutyrate; ROCKi, Rho kinase inhibitor.
[a)]Mouse embryonic stem cells.

gut tube, with a detailed comparison of chemicals and conditions [10,11,14,15,27,29–32,37,38,40–54]. The protocols for inducing posterior foregut or pancreatic progenitors can be cat-

egorized into several groups: (1) FGF10 with cyclopamine and retinoic acid, (2) cyclopamine with noggin and retinoic acid, (3) KGF with LDN, PdBU, retinoic acid, and Sant1, (4) KGF with LDN, PdBU, retinoic acid, Sant1, Y-27632, and activin A, and (5) others.

Table 4 presents the protocols for *in vitro* pancreatic endocrine differentiation from pancreatic progenitors, providing a detailed comparison of chemicals and conditions [10,11,14,15,27,29–31,37,42–54]. The protocols for inducing pancreatic endocrine cells can be broadly categorized into 4 groups: (1) exendin-4 with Nicotinamide and GF, (2) RA with T3, XXI, Alk5i II, Sant1, and betacellulin, (3) noggin with EGF and KGF, and (4) others.

4. Clinical trials

Recent advances in hPSCs differentiation have led to the development of a pancreatic endodermal cell product derived from hPSCs that matured into insulin-secreting endocrine cells over several months in animal models. For clinical trials in patients with type 1 diabetes, this hPSC-derived pancreatic product was used alongside a macroencapsulation device that prevented the cells from escaping. However, the trial was halted due to inadequate engraftment function of the product [12]. The device, designed to minimize immune responses, contained pancreatic endocrine cells; however, fibrosis around the device compromised its functionality. Further clinical trials employed a device with enhanced material transport, which secreted C-peptide in response to glucose. Despite this, the secretion levels were insufficient to demonstrate clinical benefits [13,71]. The pancreatic endocrine cells in these trials exhibited a higher proportion of α cells compared to β cells, and their function was again hindered by fibrosis. There remains an urgent need to develop hPSC-derived pancreatic β cells that function effectively and to advance transplantation technologies.

Future perspectives

1. Highly functional hPSC-derived pancreatic β cells

The above-described improvements led to transcriptional maturation of stem cell-derived pancreatic β cells, enhanced insulin secretion in response to glucose, properties akin to primary adult islets, and the development of necessary ion channels and exocytosis structures. Although the function of stem cell-derived β cells has improved, specific metabolic and transcriptomic differences remain, glucose metabolism is abnormal, and there is poor coupling to mitochondrial respiration [15]. This results in differences in glycolytic and mitochondrial glucose metabolism and significantly reduced activity of metabolic pathways. Compared to primary pancreatic islets, they are transcriptionally and metabolically immature, with correspondingly lower function. These deficiencies are mitigated after transplantation, which improves glucose responsiveness and insulin secretion. They also differentiate into other pancreatic cells and pancreatic endocrine cells, including pancreatic β cells [14]. There is a need to further develop technologies for differentiating stem cells that closely resemble adult pancreatic primary β cells and are capable of mass production. This development should utilize signaling small molecules and culture methods that efficiently improve pancreatic endocrine differentiation [15,65,72]. In recent years, several technologies have emerged. Single-cell transcriptional profiling is useful for categorizing differentiated cells [14] and comparing maturation levels [15]. In addition to recapitulating endocrine cells, clustering by isolating and reaggregating differentiated cells enriches pancreatic β cells [50,65]. Enhanced differentiation in planar culture alone is also a necessary technique [47]. Furthermore, it is helpful to precisely regulate Wnt, NODAL, and TGF-β signaling during definitive endoderm differentiation, and ALK5, BMP, SHH, retinoic acid, PKC, ROCK, T3, and Wnt signaling during pancreatic differentiation [7,72–75].

2. Transplantation and immune evasion

When highly functional stem cell-derived pancreatic β cells are clustered into groups similar in size to pancreatic islets and transplanted into patients with type 1 diabetes mellitus, they can regulate blood glucose by performing the functions of pancreatic β cells. As the functionality of these stem cell-derived pancreatic β cells improves, the number of cells needed for transplantation may decrease, simplifying the transplantation process. This approach offers several advantages, including minimal requirements for blood and nutrient supply at the transplant site and flexibility in choosing transplant sites [7].

Transplanted stem cell-derived pancreatic β cells are targeted by the host immune system, inducing a severe immune response in the recipient and resulting in graft rejection. This can even lead to the complete elimination of the cell therapy. The risk of immune rejection necessitates the continuous use of immunosuppressants, which are gradually improving. However, these can lead to a variety of side effects, including immunosuppressive infections [16,76]. If the side effects of immunosuppression outweigh the benefits of improved glycemic control or those of insulin administration, these therapies become less effective and more challenging to use, necessitating alternative therapeutic approaches to evade immune responses. Encapsulation of pancre-

Table 4. Protocols for pancreatic endocrine differentiation (pancreatic endoderm and endocrine precursors)

Chemicals	Duration (day)	Culture type	Reference
Exendin-4+nicotinamide+GF			
DAPT (stage 4)	2–3 [27]	Monolayer	[27,44]
Exendin-4	6 [44]		
Exendin-4 (stage 5) [27]	3 [27]		
IGF1	6 [44]		
HGF			
Nicotinamide	8	Aggregates	[53]
IGF2			
FGF	4	Monolayer	[43]
Indolactam V			
Nicotinamide (additional 8 d)			
bFGF	7–9	Monolayer	[54]
Nicotinamide			
Exendin-4			
BMP4			
FGF10	4	Monolayer	[42]
Compound E			
Exendin-4			
IGF1	7		
Exendin-4			
BMP4			
Nicotinamide			
Retinoic acid+T3+XXI+Alk5i II+Sant1+betacellulin			
Retinoic acid (days 1–7)	14–21	Suspension	[10]
T3			
XXI (days 1–7)			
Alk5i II			
Sant1 (days 1–7)			
Heparin (days 1–7)			
Betacellulin (day–7)			
Sant1	3	Air-liquid interface	[29]
Retinoic acid			
Alk5i II			
T3			
LDN193189			
Zinc sulfate			
Heparin			
Alk5i II (day 7)	7–15		
T3 (day 7)			
LDN193189 (day 7)			
GSiXX (day 7)			
Zinc sulfate (day 7)			
Heparin			
Alk5i II	7–15		
T3			
N-acetyl cysteine			
R428			
Trolox			
Zinc sulfate			
Heparin			
XXI	7	Suspension	[14,49–51]
Alk5i II			
T3			
Retinoic acid			
Sant1 (day 1–4) [49,51]			
Betacellulin			

Table 4. Continued

Chemicals	Duration (day)	Culture type	Reference
Alk5i II	7	Monolayer	[46–48]
Betacellulin [46,48]			
Retinoic acid			
Sant1			
T3			
XXI			
Latrunculin A (day 1)			
Sant1	4	Aggregation	[15]
Retinoic acid			
LDN193189			
Alk5i II			
GC1			
Betacellulin			
GSiXX			
LDN193189	7–8	Suspension	
Alk5i II			
GC1			
GSiXX			
ZM447439	0–42	Suspension	
T3			
N-acetyl cysteine			
Noggin+EGF+KGF			
Noggin	4	Suspension	[37]
KGF			
EGF			
Ascorbic acid	4	Monolayer	[31]
Noggin			
EGF			
Nicotinamide			
KGF	4	Monolayer	[52]
Noggin			
EGF			
Y-27632 or blebbistatin			
Other chemicals			
LDN-193189	5	Suspension	[45]
TPPB			
Alki II			
KGF			
Ascorbic acid (stage 4)	14	Monolayer	[30]
SB431542			
Noggin			
SB431542 (stage 5)			
Noggin			
L-685,458			
Noggin	3–4	Monolayer	[11]
Alk5i II			
TPPB (day 3)			
Noggin	1	Suspension	
Alk5i			

GF, growth factor; IGF, insulin-like growth factor; HGF, hepatocyte growth factor; FGF, fibroblast growth factor; bFGF, basic FGF; BMP4, bone morphogenetic protein 4; T3, triiodothyronine; XXI, γ-secretase inhibitor XXI; Alk5i, Alk5 receptor inhibitor; EGF, epidermal growth factor; GSiXX, γ-secretase inhibitor XX; KGF, keratinocyte growth factor; Alki II, Alk inhibitor II.

atic islets with biomaterials featuring finely tuned pores allows insulin secretion while preventing immune cell penetration, thus evading the immune response and reducing or eliminating the need for immunosuppressants. The use of biomaterials such as alginate hydrogels, known for their excellent biocompatibility, as encapsulating agents also prevents contact with host immune cells [77]. MHC matching can reduce immune rejection, and the production of gene-edited hypoimmune pancreatic β cells, along with the induction of immune tolerance through preconditioning or trained immunity, will also be beneficial.

Adequate cell numbers and transplantation location are also important factors in the transplantation process. Additionally, transplanted cells experience hypoxia until sufficient blood vessels are formed, leading to cell death, and approximately half of the transplanted β cells may be lost after transplantation [4]. Given the high oxygen demand of β cells, which makes them particularly susceptible to hypoxia, developing methods to protect these cell masses from hypoxia is essential. To ensure the safe administration of these new therapies, it is crucial to develop techniques that allow precise control over differentiated β cells, enabling them to resist immune evasion and hypoxia. Genetic manipulation aimed at reducing the stress response in pancreatic islets by promoting angiogenesis shortly after transplantation could be beneficial in helping them withstand stress.

3. Genome editing

Another approach to improve the quality of stem cell-derived pancreatic β cells is genome editing. Recent advances in genome editing technology have made it possible to introduce any genetic factor into the genome. This capability enables the modification of cells to incorporate functional genes, which can be further refined to either evade post-transplant immunity or enhance survival during the post-transplant angiogenesis period [16,18,68]. To functionally improve hPSC-derived β-cells and facilitate their purification, genes such as PDX1, INS promoter, SLC30A8, and ZNF148 can be targeted for editing. To reduce the risk of allograft rejection by the recipient's immune system, modifications can be made to B2M, CIITA, CD47, HLA, PD-L1, and CXCL10. Additionally, editing RNLS may offer protection against ER stress [18,68].

Pancreatic β cells differentiated from hPSCs are expected to exhibit increased functionality. These cells are being developed to improve immune evasion and post-transplant survival through the application of differentiation, transplantation, and genome editing technologies.

Notes

Conflict of interest

No potential conflict of interest relevant to this article was reported.

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Authors' contributions

Conceptualization: all authors; Funding acquisition: DHL; Investigation: HC, HYC, SWL; Project administration: DHL; Supervision: DHL; Visualization: HC; Writing–original draft: all authors; Writing–review & editing: all authors.

Data availability

Please reach out to the corresponding author to inquire about the availability of data.

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