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REVIEW ARTICLE

iPSC technology-based regenerative therapy for diabetes

Yasushi Kondo[1,2], Taro Toyoda[1], Nobuya Inagaki[2], Kenji Osafune[1]*

[1]Center for iPS Cell Research and Application (CiRA), Kyoto University, and [2]Department of Diabetes, Endocrinology and Nutrition, Kyoto University Graduate School of Medicine, Kyoto, Japan

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***Correspondence**
Kenji Osafune
Tel.: +81-75-366-7058
Fax: +81-75-366-7077
E-mail address:
osafu@cira.kyoto-u.ac.jp

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ABSTRACT

The directed differentiation of human pluripotent stem cells, such as embryonic stem cells (hESCs) and induced pluripotent stem cells (hiPSCs), into pancreatic endocrine lineages has been vigorously examined by reproducing the *in vivo* developmental processes of the pancreas. Recent advances in this research field have enabled the generation from hESCs/iPSCs of functionally mature β-like cells *in vitro* that show glucose-responsive insulin secretion ability. The therapeutic potentials of hESC/iPSC-derived pancreatic cells have been evaluated using diabetic animal models, and transplantation methods including immunoprotective devices that prevent immune responses from hosts to the implanted pancreatic cells have been investigated towards the development of regenerative therapies against diabetes. These efforts led to the start of a clinical trial that involves the implantation of hESC-derived pancreatic progenitors into type 1 diabetes patients. In addition, patient-derived iPSCs have been generated from diabetes-related disorders towards the creation of novel *in vitro* disease models and drug discovery, although few reports so far have analyzed the disease mechanisms. Considering recent advances in differentiation methods that generate pancreatic endocrine lineages, we will see the development of novel cell therapies and therapeutic drugs against diabetes based on iPSC technology-based research in the next decade.

INTRODUCTION

Type 1 diabetes is an autoimmune disease characterized by β-cell destruction in the pancreas, insulin deficiency and persistent high blood glucose levels. Although the supplementation of exogenous insulin is a life-saving treatment, it is still difficult to obtain physiological control of blood glucose levels. The transplantation of pancreatic β-cells as islet tissues or the entire pancreas is an alternative curative treatment for type 1 diabetes[1–3]. However, transplantation therapies are hampered by a serious donor shortage and the potential risk of tissue rejection. One possible solution to the donor shortage is the generation of β-cells or islet tissues from human pluripotent stem cells, such as embryonic stem cells (hESCs)[4] and induced pluripotent stem cells (hiPSCs)[5,6]. The directed differentiation of pancreatic lineage cells from hESCs/iPSCs has been vigorously studied towards regenerative therapy for type 1 diabetes, as well as basic pancreatic research[7–22].

Substantial progress in this research field has been made in recent years. In the USA, phase 1/2 clinical trials for type 1 diabetes patients have already been started with the use of hESC-derived pancreatic progenitors. In contrast, at the time of writing the present review, there have been no clinical trials carried out using the transplantation of hiPSC-derived pancreatic cells. Because of the potential advantages of hiPSCs over hESCs, however, such as the practicability of autologous cell transplantation and fewer ethical problems, research in hiPSC-based therapy is expected to lead to such therapy in the future. In the present review, we summarize recent advances in research on pancreatic regeneration and disease modeling using pluripotent stem cells, and also outline future perspectives of the clinical application for diabetes.

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NEW CELL SOURCES

Pancreatic β-cells secrete insulin, a hormone that plays a crucial role in the regulation of blood glucose levels. In type 1 diabetes, pancreatic β-cells are destroyed, which results in absolute deficiency of insulin. If patients' blood glucose levels are not properly controlled, a variety of serious complications take place. Although the risk of complications in type 1 diabetes patients has declined with recent advances in medical care, type 1 diabetes patients must always take care to control

their blood glucose levels by multiple daily insulin injections, which causes a great burden to the patients, to prevent complications.

In type 1 diabetes, the supplementation of pancreatic β-cells is considered one of the most potentially effective treatments for diabetes. One supplementation approach is the transplantation of islets obtained from brain dead or cardiac arrest donors through the portal vein. Although islet transplantation requires a sufficient number of islets from multiple donors, its safety and efficacy for the treatment of type 1 diabetes have been shown, enabling patients to withdraw from insulin injection therapy[1–3]. Islet transplantation is becoming approved as standard treatment around the world. This therapy has benefited from recent advances in isolation methods that stably obtain islet tissues of sufficient quality at the clinical level. However, various problems, such as a serious shortage of donors and side-effects of immunosuppressive agents, still remain to be solved, preventing islet transplantation from becoming a universal treatment for diabetes.

In order to solve the problem of donor islet shortage, a new cell source of human pancreatic β-cells is required. Pluripotent stem cells, such as ESCs or iPSCs, which have the capability to indefinitely self-renew and differentiate into any cell type of the body, are appealing candidates. Pancreatic β-cells generated from these stem cells are expected as a new cell source for transplantation therapy against diabetes. Since the report by Assady *et al.*[23], which for the first time generated insulin-producing cells from hESCs by spontaneous differentiation using embryoid body formation, an efficient generation of pancreatic β-like cells has been vigorously studied by various approaches[7–22]. Such efforts have made the clinical application of transplantation therapy using pancreatic cells derived from pluripotent stem cells closer to realization.

DIRECTED DIFFERENTIATION INTO PANCREATIC β-CELLS

In order to induce the differentiation of hESCs/iPSCs into pancreatic lineage cells, a strategy has been adopted to mimic and reproduce the normal developmental stages of the pancreas *in vitro* by using the expression of key transcription factors involved in pancreas development as an index. As shown in Figure 1, a fertilized egg differentiates through multiple developmental stages, such as definitive endoderm, primitive gut tube, posterior foregut, pancreatic endoderm and endocrine precursors, into insulin-expressing β-cells. In the case of hESC/iPSC differentiation, similar developmental stages are induced using a combination of growth factors or chemical compounds including Nodal-activin, Wnt, retinoic acid, hedgehog, fibroblast growth factor, epidermal growth factor, bone morphogenetic protein and Notch to activate or inhibit key signal pathways[7–22].

Until recently, most investigators have generated pancreatic β-like cells that produce and secrete insulin in response to stimuli, such as potassium chloride[7,17]. However, these cells do not secrete suitable amounts of insulin in response to changes in blood glucose levels, making them inferior to adult β-cells. In addition, the generated pancreatic β-like cells co-express other hormones, such as glucagon and somatostatin. Gene expression analysis has further shown that β-like cells induced from hESCs/iPSCs do not accurately represent features of mature adult β-cells and more closely resemble embryonic β-cells[24]. In contrast, several groups including ours have reported the generation of hESC/iPSC-derived embryonic pancreatic endoderm cells that have the capacity to differentiate into all pancreatic lineages including endocrine cells. These cells can differentiate and mature *in vivo* into adult β-cells with the capacity of glucose-stimulated insulin secretion 3–4 months after implantation into immunodeficient mice (Figure 2)[9,18,22].

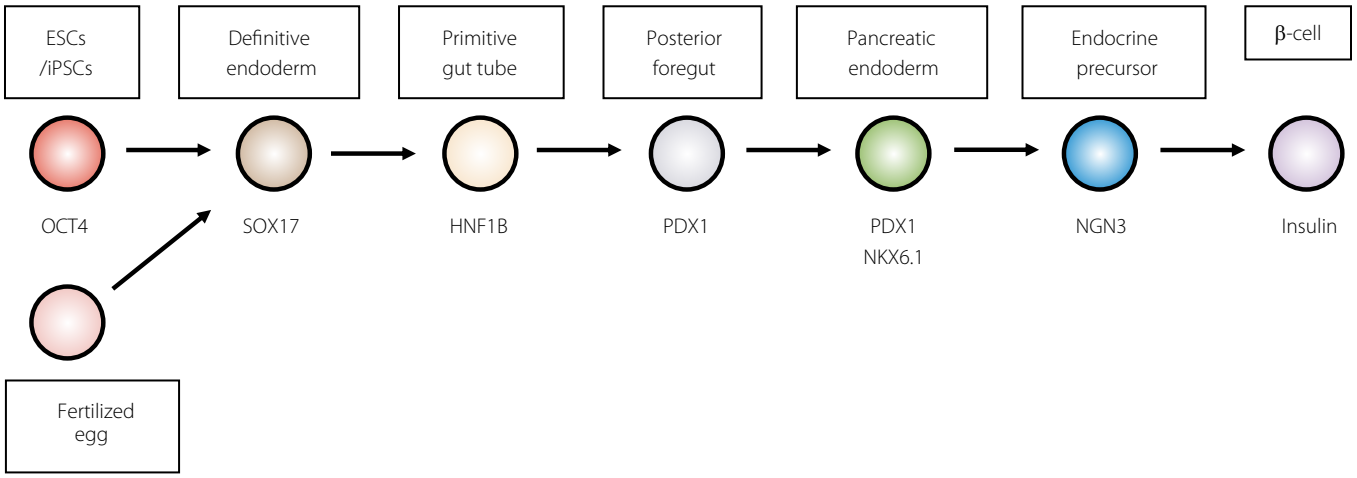

Figure 1 | Schematic diagram of the differentiation strategy to produce pancreatic endocrine lineages from such as human embryonic stem cells and induced pluripotent stem cells (hESCs/iPSCs) by mimicking *in vivo* development. The developmental stages and their corresponding marker genes are shown.

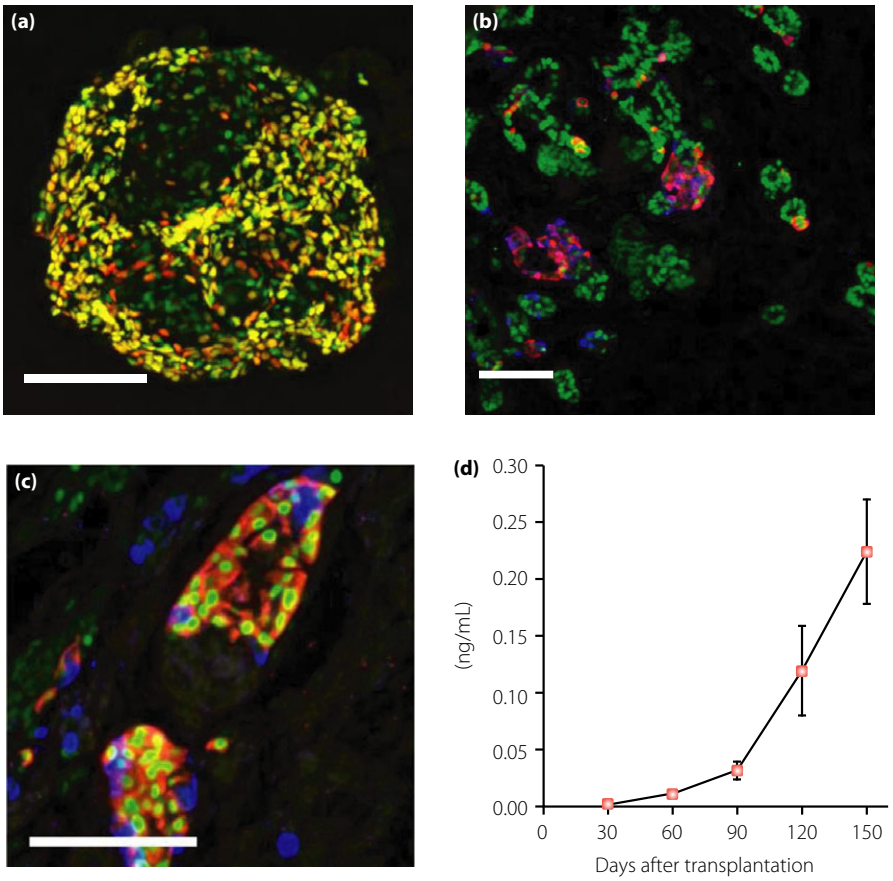

Figure 2 | Pancreatic endoderm cells differentiated from human embryonic stem cells (hESCs) mature into β-cells *in vivo*. (a) Section immunostaining images of hESC-derived pancreatic endoderm cells for PDX1 (green) and NKX6.1 (red), (b) human pancreatic tissues generated 30 days after implantation of hESC-derived pancreatic endoderm into immunodeficient mice for PDX1 (green), INSULIN (red) and GLUCAGON (blue), and (c) human islet-like structures generated 210 days after implantation. (d) Plasma human C-peptide levels in host immunodeficient mice. Scale bars, 100 μm. Adapted from Toyoda *et al.*[22] with permission (licensed under Creative Commons Attribution).

Our group also showed that high-cell density cultures, such as adherent cultures at higher-cell density and cell aggregation cultures, favored differentiation into pancreatic endoderm cells at the pancreatic bud stage[22]. These findings suggest that hESC/iPSC-derived pancreatic endoderm cells retain the potential to differentiate into pancreatic endocrine cells that are functionally comparable with adult β-cells.

More recently, two breakthrough papers have succeeded in the generation of functionally mature β-like cells from hESCs/iPSCs, although details on the maturation mechanism remain to be elucidated. Rezania *et al.*[20] optimized their differentiation protocol by adding factors, such as vitamin C, protein kinase C pathway activators, transforming growth factor-β receptor inhibitors and thyroid hormones, to generate insulin-producing cells at an induction rate of approximately 50%. Furthermore, they identified R428, a selective small-molecule inhibitor of tyrosine kinase receptor AXL, as a crucial factor for the maturation of β-cells. When the aforementioned insulin-producing cells were treated with the factors and R428, the expression level of *MAFA* messenger ribonucleic acid became higher than that of human pancreatic islets, and the cells acquired a responsiveness to changes in glucose levels, although less potently than that of adult human islets. After implantation into diabetes mouse models, the engrafted cells improved the blood glucose levels of host mice after 16 days, suggesting that insulin-producing cells suitable for cell therapy against diabetes could be produced from hESCs *in vitro*.

Pagliuca *et al.*[19] optimized their differentiation method for pancreatic β-cells by examining more than 150 combination treatments of >70 kinds of compounds. The group established a 5-week long differentiation method of hESCs/iPSCs to β-cells at an induction efficiency of >30%. The induced cells showed insulin secretion and intracellular Ca^{2+} handling in response to sequential changes in glucose concentrations. Furthermore, the cells were similar to human islets in terms of stimulation index, which is a ratio of insulin secretion levels between low- and high-glucose conditions, amounts of insulin secretion, and intracellular insulin content, suggesting they very closely resembled adult β-cells. When the induced cells were implanted into the renal subcapsules of immunodeficient mice, the engrafted

cells started to secrete insulin in response to changes in blood glucose levels as early as 2 weeks after implantation. After the implantation into diabetes mouse models, the cells improved blood glucose levels of the host mice for >18 weeks. Compared with previously reported implantations of hESC/iPSC-derived pancreatic endoderm cells, in which it took 3–4 months after implantation for the cells to mature, recent advances in the generation of β-cells *in vitro* substantially shorten the waiting time to acquire therapeutic effects after implantation.

After these two reports, other groups have reported success in the production of pancreatic β-like cells with the ability of glucose-responsive insulin secretion by strictly regulating the timing of the cell fate decision from pancreatic endoderm to endocrine cells[25–27]. Although several issues, such as the stability and cost of the differentiation, still remain to be improved before clinical application, recent advances in the generation of functionally matured β-like cells from hESCs/iPSCs *in vitro* are expected to overcome the major obstacles for regenerative medicine against diabetes.

Unbiased screenings have been used to identify small molecules that induce the differentiation of hESC/iPSCs. Small molecules with unique biological activities enable new biological studies, and might further lead to the development of new treatments by significantly lowering the cost[28]. Two reports have identified chemical compounds that act as inducers of pancreatic lineage cells, indolactam V for PDX1$^+$ pancreatic progenitor cells[12] and vesicular monoamine transporter 2 inhibitors for INSULIN$^+$ cells[29].

GENERATION OF WHOLE PANCREAS BY BLASTOCYST COMPLEMENTATION

Blastocyst complementation is an experimental strategy to generate whole organs in chimeric animals[30]. With this strategy, ESCs/iPSCs are injected into blastocysts of animals in which essential genes for the development of certain organs are knocked out. Dr Hiromitsu Nakauchi and his research team are leaders in this field. They carried out blastocyst complementation by injecting wild-type mouse ESCs/iPSCs into Pdx1−/− pancreatogenesis-disabled mice to generate a whole pancreas[31]. Pancreatic islets removed from the generated whole pancreas showed therapeutic effects when implanted into diabetes mouse models. The group also showed that whole rat pancreas can be generated by blastocyst complementation using the injection of rat iPSCs into Pdx1−/− mice, although the generated pancreas was mouse size[31]. Very recently, the same group carried out the reverse experiment, generating rat-sized pancreas consisting of mouse ESCs/iPSCs by injecting them into Pdx1−/− rat blastocysts. Mouse islets from the generated pancreas maintained blood glucose levels of diabetic mice induced by streptozotocin over 370 days after implantation without immunosuppression[32]. These findings show that whole human pancreas could be interspecifically generated from hESCs/iPSCs by blastocyst complementation using other animals, such as pigs. Towards this goal, the same group and their colleagues showed that this strategy can be carried out using pancreatogenesis-disabled pigs, in which *Hes1* gene was overexpressed under the guide of the Pdx1 promoter[33]. Whole pig pancreas was generated by injecting the blastocyst cells of other pigs into Pdx1-Hes1 transgenic pigs.

However, the generation of human pancreas using hESCs/iPSCs and other animals by blastocyst complementation might raise ethical concerns that the injected hESCs/iPSCs contribute to other undesired organs in the generated chimeric animals, such as the brain or reproductive organs. In an attempt to mitigate this concern, ESCs/iPSCs induced to commit into the targeted organ lineage were used to prevent differentiation into untargeted cell types in chimeric animals. It was reported that the overexpression of a mesendoderm marker, *Mixl1*, induced mouse ESCs to differentiate into endoderm, but not mesoderm, *in vitro*. Based on this finding, the Nakauchi group used mouse ESCs overexpressing the *Mixl1* gene for blastocyst complementation and succeeded in restricting the localization of mouse ESC derivatives into endodermal organs, such as the intestine[34]. Additionally, a previous study reported that the more developmentally advanced cell types than ESCs undergo apoptosis and fail to contribute to chimera when injected into pre-implantation stage blastocysts[35]. The Nakauchi group, however, have since succeeded in having these cells to contribute to chimera by blastocyst complementation[36]. They injected mouse ESC-derived Sox17$^+$ endoderm progenitor cells overexpressing the anti-apoptotic gene *BCL2* into blastocysts. This approach caused endoderm cells to integrate into the gut tissues of chimeric mice. Although there are still hurdles to overcome before generating human pancreas in animal bodies, including ethical issues, blastocyst complementation is an appealing strategy to generate functional pancreas tissues from ESCs/iPSCs.

TRANSPLANTATION METHODS

In parallel with the generation of pancreas tissues from pluripotent stem cells, the clinical application of induced pancreatic cells has been explored. In general, transplantation strategies are classified into two: (i) a method in which induced pancreas tissues are directly implanted into the patient body; and (ii) another in which a device that contains pancreatic tissues is implanted (Figure 3). In the first method, pretreatment to induce angiogenesis in the implantation sites is used to promote the engraftment and long-term survival of the implanted cells. One recent study reported that an embedded nylon catheter into the subcutaneous tissues of host mice for 1 month before cell implantation generated vascularized space[37]. Here, the embedded biomaterial induces the formation of vascular networks in the implantation site, and pancreatic cells are implanted after inflammatory reactions diminish, which makes a less intolerant environment for the implanted cells.

In contrast, Szot *et al.*[38] reported that the blockage of T-cell costimulatory pathways by the administration of CTLA4Ig, which suppresses T-cell activation, and an anti-CD154 antibody prevented the rejection of xenogeneic implantation of hESC-derived pancreatic endoderm cells into non-diabetic

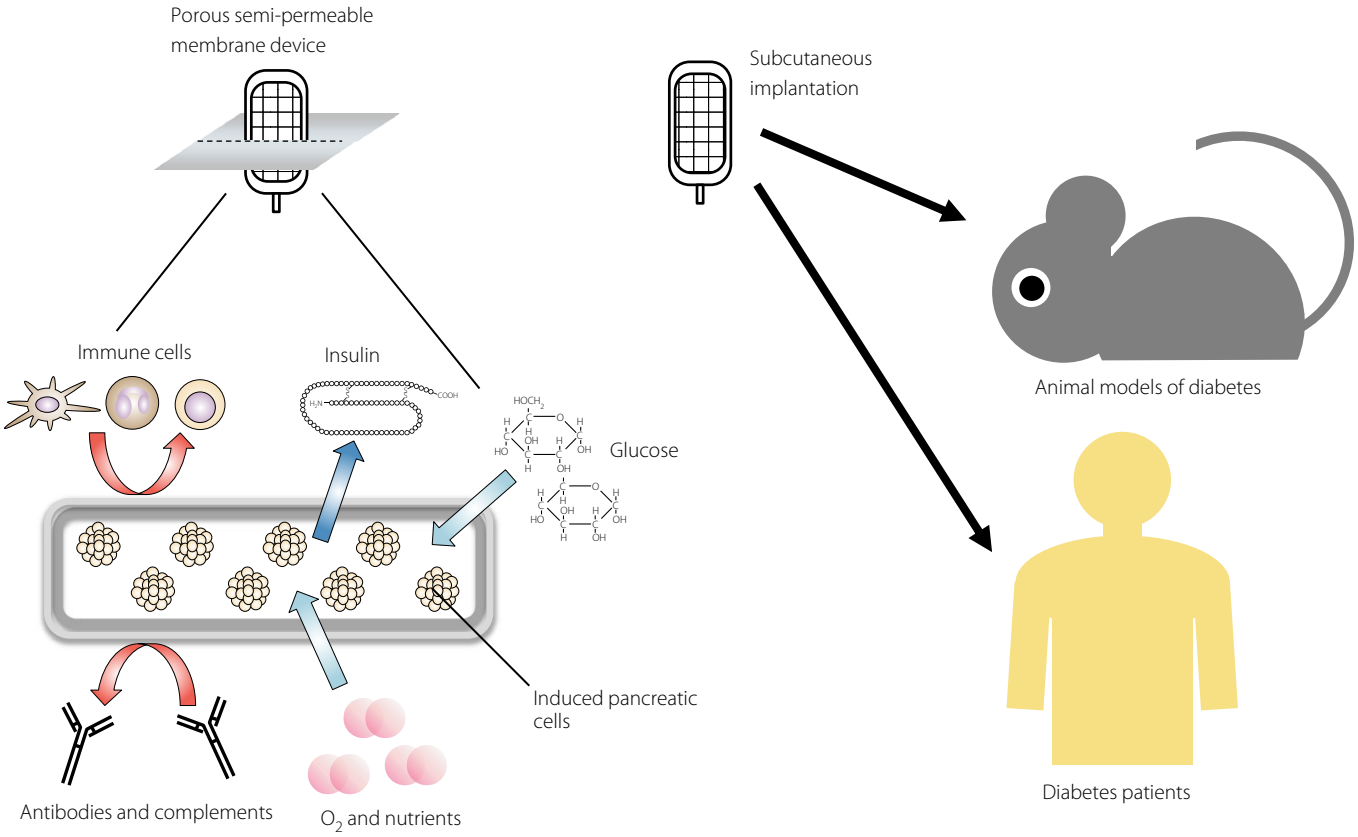

Figure 3 | Device-based methods for implanting human embryonic stem cells and induced pluripotent stem cells (hESCs/iPSCs)-derived pancreatic cells. hESC/iPSC-derived pancreatic cells encapsulated with immunoprotective devices are implanted into the bodies of diabetes animal models or diabetes patients. Oxygen, nutrients, insulin and glucose can pass through the porous membrane of the device to promote the survival, differentiation, maturation and glucose-responsive insulin secretion of encapsulated pancreatic cells. In contrast, immune cells or molecules, such as antibodies and complements, cannot pass, which prevents immune rejection or autoimmune responses against the cells.

immunocompetent mice for >90 days. In that report, the implantation of xenogeneic pancreatic endoderm cells differentiated from hESCs into subcutaneous tissues of host diabetes mouse models exerted therapeutic effects on diabetes without rejection.

In the second category of transplantation methods, pancreatic cells are encapsulated by a bioengineered device that includes semipermeable membranes. Oxygen and nutrients can pass to promote cell survival, differentiation and maturation, whereas immune molecules or cells cannot (Figure 3). It was reported that hESC-derived pancreatic cells encapsulated by these semipermeable membrane devices could further differentiate into pancreatic β-cells even after implantation into host mice[39]. In addition, because of vasculogenesis around the devices, the differentiated β-cells can secrete insulin in response to changes in glucose concentrations. Another report found that semipermeable membrane devices protected the rat islet grafts from immune systems of wild-type host mice for at least 180 days after transplantation[40]. A recent study reported the implantation of hESC-derived β-like cells encapsulated with an alginate derivative, triazole-thiomorpholine dioxide alginate. This device mitigated foreign body responses and implant fibrosis, and induced glycemic correction without immunosuppression for >170 days in immunocompetent mice[41].

These device-based implantation methods are expected to reduce or eliminate the need for immunosuppressive agents. Furthermore, these methods potentially have the advantage of removing the implanted cells with the device from the patient's body when adverse events, such as tumorigenesis or dysfunction, occur.

In 2014, ViaCyte Inc. started clinical trials for the treatment of type 1 diabetes patients using a semipermeable membrane capsule device that carried pancreatic progenitor cells differentiated from hESCs. This trial has attracted attention worldwide, as it represents an initial and important step for the development of new stem cell therapies for diabetes.

DISEASE MODELING AND DRUG DISCOVERY

iPSC technology enables the creation of novel *in vitro* disease models. Because iPSCs harbor the genetic information of patients from which they are generated, the disease-affected cell types differentiated from patient-derived iPSCs might reproduce disease phenotypes *in vitro*. These approaches are especially advantageous when reliable animal models are unavailable.

Table 1 | Summary of reports on iPSCs derived from patients with diabetes-related disorders (as of February 2017)

Type of diabetes	Mutation	Findings	References
Nondiabetic mutation carrier	PDX1(C18R)	Derivation of iPSCs from patients' somatic cells	69
Nondiabetic mutation carrier	PDX1(P33T)	Derivation of iPSCs from patients' somatic cells	68
Diabetic foot ulcer	NA	Derivation of iPSCs from patients' somatic cells	67
T1D	NA	Efficient differentiation of patient-derived iPSCs into glucose-responsive insulin-producing cells	66
T1D	NA	Differentiation of patient-derived iPSCs into β cells *in vitro*	65
T1D and T2D	NA	Assessed safety of transplanting pancreatic progenitors from patient-derived iPSCs	64
Patients with insulin receptor mutations	Exon 14 (nonsense; A897X), Exon 1 (missense; A2G), Exon 3 (missense; L233P), Exon 2 (nonsense; E124X)	Patient-derived iPSCs showed mitochondrial dysfunction with reduced mitochondrial size, oxidative activity, and energy production	63
MODY5	HNF1B (S148L)	Pancreatic progenitors from patient-derived iPSCs show compensatory mechanisms in the pancreatic transcription factor network	62
Congenital generalized lipodystrophy	BSCL2/SEIPIN (E189X and R275X)	Adipogenic differentiation of patient-derived iPSCs exhibited reduction of lipid droplet formation	61
T1D	NA	Differentiation of patient-derived iPSCs into early vascular cells and formation of 3D vascular network assembly *in vitro*	60
Longstanding T1D with severe or absent to mild complications	NA	Analyses using patient-derived iPSCs revealed that miR200-regulated DNA damage checkpoint pathway protects against complications in T1D	59
MODY5	HNF1B (R177X)	Patient-derived iPSCs showed mutant transcripts destroyed by nonsense-mediated mRNA decay	58
MODY3	HNF1A	Differentiation of patient-derived iPSCs into insulin-expressing cells	57
T1D	NA	Differentiation of patient-derived iPSCs into functional cardiomyocytes with well-regulated glucose utilization	56
T2D with cardiovascular disease	NA	Creation of diabetic cardiomyopathy models from patient-derived iPSCs that were used for evaluating candidate drug compounds	55
Patients with insulin receptor mutations	NA	Patient-derived iPSCs showed altered gene expression and reduced proliferation	54
T1D	NA	Patient-derived iPSCs generated with synthetic mRNAs encoding OCT4, SOX2, KLF4, c-MYC, and LIN28 upregulates pancreas-specific microRNAs	53
Wolfram syndrome	WFS1	Pancreatic β-like cells from patient-derived iPSCs showed increased ER stress led to insulin secretion failure	52
MODY2	GCK	GCK mutant β cells required higher glucose levels to stimulate insulin secretion	51
MODY1, 2, 3, 5 and 8	MODY1: HNF4A, MODY2: GCK, MODY3: HNF1A, MODY5: HNF1B, MODY8: CEL	Derivation of iPSCs from patients' somatic cells	50
T1D	NA	Multiple iPSC lines from individual patients showed intrapatient variations in differentiation propensity to insulin-producing cells	49

Table 1 (*Continued*)

Type of diabetes	Mutation	Findings	References
T2D	NA	Marked hyperglycemia disrupted anesthetic preconditioning-mediated protection in cadiomyocytes from patient-derived iPSCs	48
T1D and T2D	NA	Derivation of transgene-free iPSCs from patients using Sendai viral vectors	47
T1D	NA	Derivation of iPSCs from patients' somatic cells	46
Diabetes with mitochondrial DNA (mtDNA) mutation	mtDNA (A3243G)	Patient-derived iPSCs showed a bimodal degree of mutation heteroplasmy; mutation-free and -rich iPSC clones	45
T2D	NA	Derivation of iPSCs from elderly T2D patients and differentiation into insulin-producing islet-like progeny	44
T1D	NA	Differentiation of patient-derived iPSCs into insulin-producing cells *in vitro*	11
T1D	NA	Derivation of iPSCs from patients' somatic cells	43

MODY, maturity-onset diabetes of the young; T1D, type 1 diabetes; T2D, type 2 diabetes; GCK, Glucokinase; NA, not applicable.

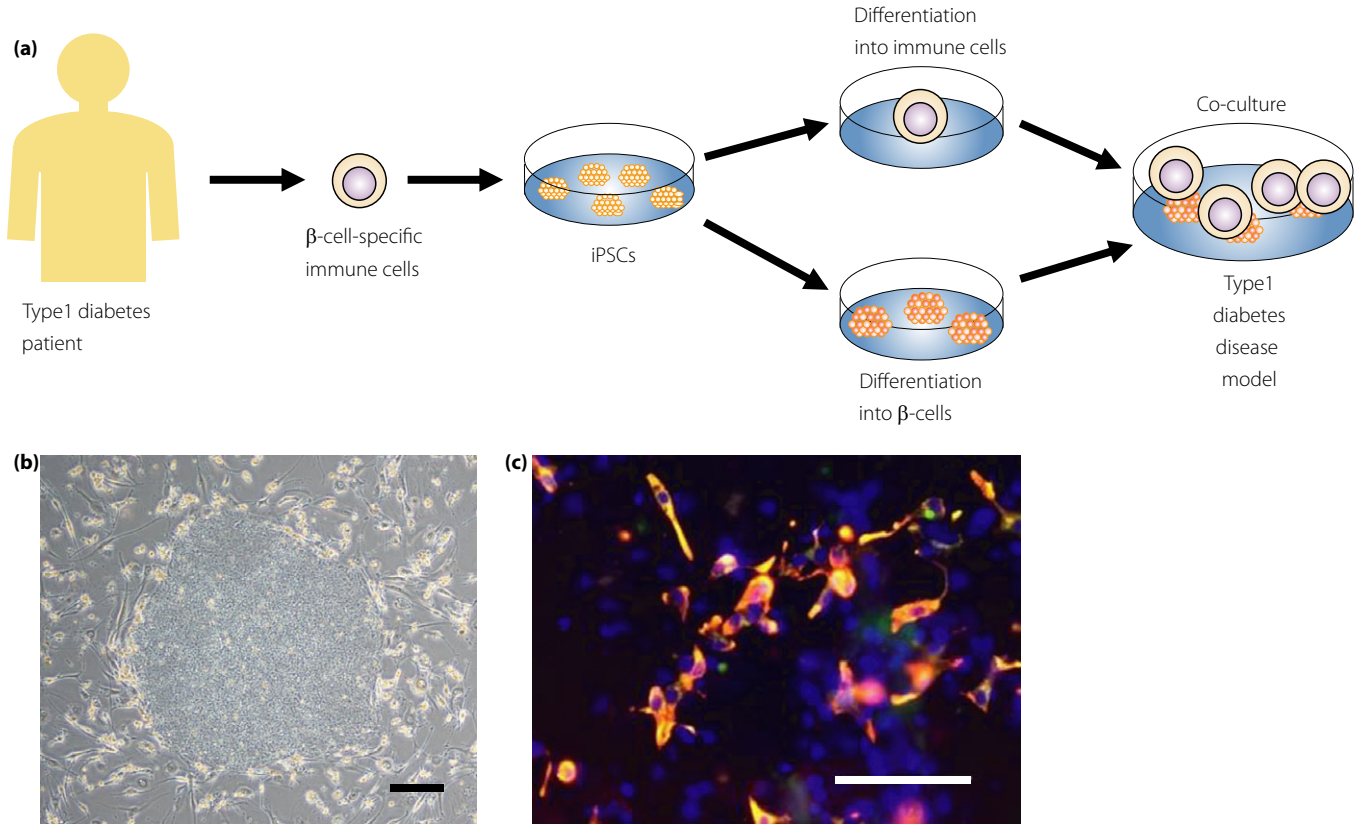

Figure 4 | Disease modeling using patient-derived induced pluripotent stem cells (iPSCs). (a) *In vitro* type 1 diabetes disease models using the differentiation of patient-derived iPSCs into pancreatic β- and immune cells. (b) iPSCs derived from a type 1 diabetes patient and (c) insulin-secreting cells differentiated from the iPSCs. Scale bars, 300 μm in (b) and 100 μm in (c). Figures (b) and (c) were provided by Drs Yoshiya Hosokawa, Akihisa Imagawa and Iichiro Shimomura, Department of Metabolic Medicine, Osaka University Graduate School of Medicine.

Substantial efforts have already been made to analyze disease mechanisms and develop novel therapeutic drugs using iPSC disease models[42].

As summarized in Table 1, multiple reports describe the generation of iPSCs from patients with diabetes-related disorders, including types 1 and 2 diabetes mellitus; maturity-onset

diabetes of the young types 1, 2, 3, 5 and 8, in which a single gene mutation causes diabetes; and mitochondria diabetes[11,43–69]. A report analyzing maturity-onset diabetes of the young type 2 showed that iPSC models can reproduce the disease phenotype caused by mutations in a glucokinase gene that cause a decrease in insulin secretion by glucose stimulation[51]. However, although many of these reports have shown the generation of patient-derived iPSCs and their *in vitro* differentiation into β-like insulin-producing cells, they have not reported the recapitulation of the disease phenotypes. The reason is that the β-like cells generated from patient-derived iPSCs are insufficient to recapitulate the phenotypes of diseases that are caused by a complex cellular environment including multiple cell types, such as immune cells. As described above, cells that functionally resemble adult β-cells can now be produced from human pluripotent stem cells *in vitro*. Combining multiple cell types *in vitro* with iPSC-derived β-like cells could create novel diabetes disease models that better elucidate the disease mechanisms and facilitate the discovery of novel therapeutics drugs (Figure 4).

CONCLUSION

Basic research on cell therapy strategies for diabetes using stem cells has advanced considerably in the past decade. The generation of functional pancreatic tissues from human pluripotent stem cells has become possible, and these cells could replace the donor islets used in islet transplantation. In the next decade, it is expected that many experimental diabetes treatments will be confirmed for therapeutic efficacy and safety.

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DISCLOSURE

KO is a founder and member without salary of the scientific advisory boards of iPS Portal, Japan. The other authors declare no conflict of interest.

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