# Stem Cell–Derived Islets: Next Steps for Histologic and Functional Assessment During Development as a Cellular Therapy for the Treatment of Diabetes

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Type 1 diabetes (T1D) results from autoimmune destruction of the insulin-producing β-cells in the endocrine pancreatic islets of Langerhans. Patients are thus dependent on exogenous insulin therapy delivered by multiple daily injections or continuous subcutaneous infusion pumps to control elevated glucose levels and prevent the development of life-threatening ketoacidosis. Due to the pharmacokinetic and pharmacodynamic limitations and complexity of subcutaneous insulin delivery, most patients living today with T1D cannot achieve levels of glucose control recommended for the prevention of diabetes complications (1). Therefore, biologic insulin therapy delivered by β-cell replacement has long been hoped to supplant exogenous insulin therapy for T1D but has been realized only in the limited context of pancreas or isolated islet transplantation using deceased donor organs (2). Recent progress in the generation of functional islet β-cells from human stem cell sources (3) has reinvigorated hope for a one day limitless supply of islets for transplantation therapy (4).

Human embryonic stem cells (hESCs) differentiated to a pancreatic endoderm progenitor stage in vitro have the potential to further differentiate into functional pancreatic islets in vivo (5,6). Further differentiation to a pancreatic islet stage in vitro can generate cell clusters with the capacity for glucose-dependent insulin secretion before transplantation (7,8). While both approaches are capable of reversing streptozotocin-induced diabetes in immunodeficient mouse models, the use of pancreatic endoderm cells (PECs) has previously been accompanied by the sporadic growth of mesodermal cells reminiscent of the formation of teratomas. The use of pancreatic islet stage cells is hoped to minimize off-target differentiation; however, these later stage endocrine cells still undergo further in vivo differentiation and so may not fully eliminate the potential risks associated with transplanting immature stem cell–derived tissue.

In this issue of *Diabetes*, Pepper et al. (9) provide a long-term functional and histologic characterization of hESC-derived PECs transplanted in an immunodeficient mouse model with streptozotocin-induced diabetes using a subcutaneous "device-less" site. All PEC recipient mice established normoglycemia by 200 days, consistent with the in vivo differentiation and functional maturation of the pancreatic islet graft and which was maintained for over 500 days until graft removal. Functionally, the matured grafts demonstrated glucose-responsive insulin secretion assessed both in vivo by measurement of human C-peptide and ex vivo by measurement of insulin secretion during dynamic perifusion following explant. Histologically, the PEC differentiated to mostly islet tissue; however, all PEC recipient grafts also developed small palpable cysts by 200 days that constituted metaplastic pancreatic ductal mucinous hyperplasia. While the low proliferation evidenced by Ki-67 staining that did not change by the over 500 days of observation supports a benign classification for these lesions, cystic structures originating from native pancreatic ductal tissue that produce mucus can become neoplastic (10). The PEC grafts demonstrated no evidence of teratoma formation, suggesting that the risk for mesodermal differentiation may be eliminated. Whether further in vitro differentiation to pancreatic islet stage tissue prior to transplantation might eliminate the development of unwanted ductal cysts requires additional long-term studies (Fig. 1).

Importantly, the long-term functional regulation of glucose homeostasis was achieved by transplantation of the PEC graft in a subcutaneous "device-less" site that is readily accessible to monitoring, including by biopsy and by retrieval. The device-less site has previously been described by Pepper et al. (11) where a vascular catheter is placed subcutaneously to generate a foreign-body response that includes neovascularized collagen; removal of the

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**See accompanying article, p. 953.**



COMMENTARY

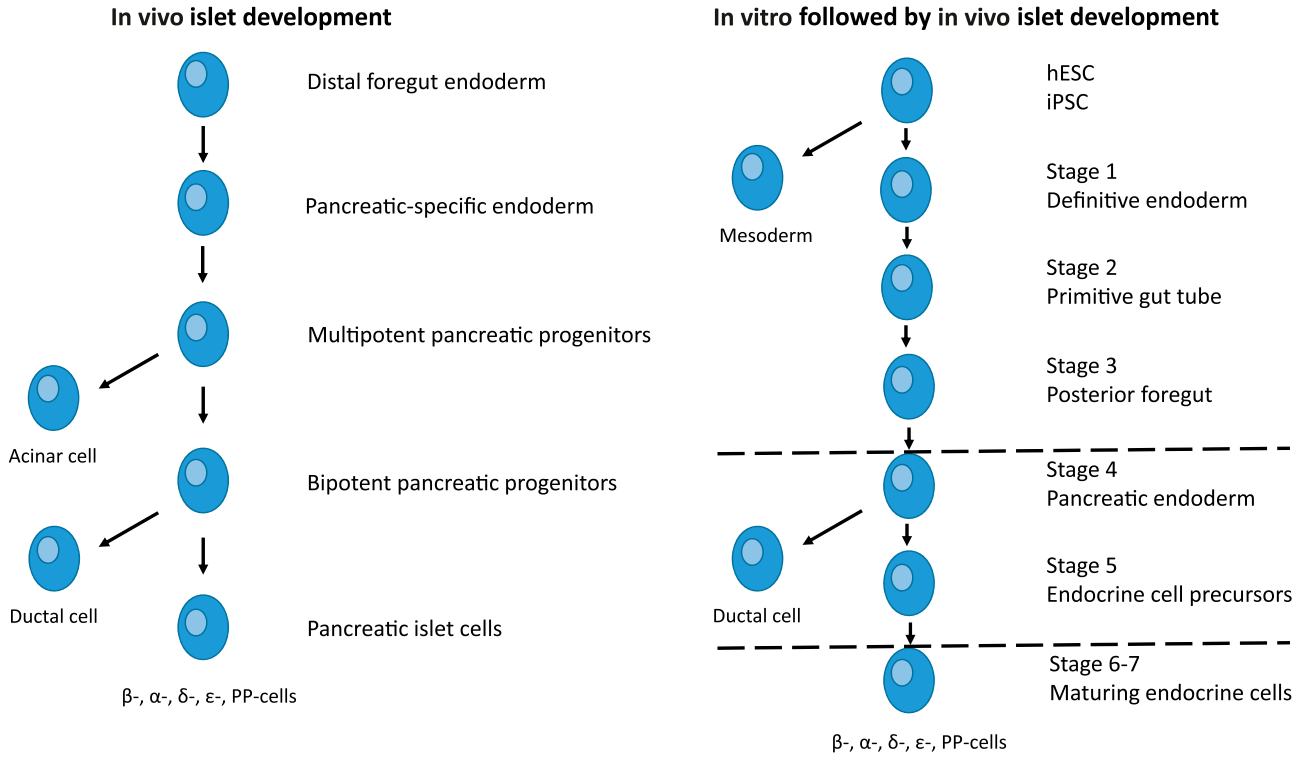


**Figure 1**—Left: In vivo islet differentiation during normal embryonic and fetal development. Right: In vitro islet development of hESCs or inducible pluripotent stem cells (iPSC) designed to mimic normal differentiation (adapted from Jennings et al. [3]). In the report by Pepper et al. (9), no mesodermal or acinar cell tissue was identified following in vivo maturation of transplanted stage 4 PECs into functional islet grafts; however, all grafts contained mucinous ductal tissue organized in cystic structures by 200 days. Whether further in vitro differentiation and transplantation of, for example, stage 7 pancreatic islet cells may eliminate the development of ductal tissue during in vivo maturation requires additional long-term studies.

catheter after 1 month terminates the foreign-body response and leaves a pocket of vascularized matrix that provides a bio-scaffold to support engraftment of transplanted cells. This prevascularization of the compartment prior to cellular transplantation likely enhances oxygen delivery during engraftment and revascularization of the graft that is critical to support the high metabolic activity of islet tissue and physiologic glucose sensing and hormone secretion that is otherwise impaired when encapsulating islets in devices. Encapsulation devices elicit an ongoing foreign-body response resulting in surrounding fibrosis that further prevents oxygen delivery to contained islets and represents a major barrier to the promise that islet encapsulation will provide a path for immunosuppression-free transplantation. Clinical studies have failed to show efficacy of micro- (12) or macroencapsulated (13) human islets except when coupled to a refillable oxygen chamber contained within the device (14). The ViaCyte phase I/II clinical trial transplanted PEC grafts subcutaneously in an Encaptra macroencapsulation device that contains a cell impermeable inner membrane and permeable outer membrane that does allow vascularization in rodent models (ClinicalTrials.gov identifier NCT02239354). Preliminary results in humans indicated cell survival was significantly limited by a foreign-body response with fibrosis that impeded vascularization of the cell permeable outer membrane (15). Thus, the device-less site reported here may be an attractive alternative for transplantation of stem cell–derived islets, albeit with the requirement in humans for immunosuppression as for deceased donor islet transplantation.

In addition to β-cells, human islets also contain glucagon-producing α-cells and smaller proportions of somatostatin-producing δ-cells, ghrelin-producing ε-cells, and pancreatic polypeptide–producing F cells (PP-cells), as well as neurovascular elements that include both the sympathetic and parasympathetic nervous systems that modulate islet activity and hormone secretion. PEC grafts have shown endocrine differentiation to β-, α-, δ-, ε-, and PP-cells, resembling the make-up of mature human islets (16). In human T1D, the loss of functional β-cells from within the native islet disrupts paracrine regulation of α-cell function. As a result, during the development of low blood glucose, α-cells fail to release glucagon that is necessary to increase hepatic glucose production and prevent or correct hypoglycemia. Therefore, cellular therapy for treatment of T1D requires replacement of intact islets with normally functioning α-cells in addition to β-cells. To that end, it is important that the differentiated PEC grafts described here contain both β- and α-cells, as is also the case for stem cells further differentiated to a pancreatic islet stage in vitro prior to transplantation (7,8). Still required is more complete assessment of the

responsiveness of matured PEC and other stem cell–derived islet graft β-cells to turn off insulin secretion and the α-cells to turn on glucagon secretion appropriately to defend against the development of low blood glucose (17).

To date, the liver is the only site that has enabled sufficient survival of transplanted islets to consistently reverse diabetes and achieve insulin independence in large animal models and humans (18). This may be explained by the extensive surface area of the bio-scaffold provided by the hepatic sinusoids to support intrahepatic engraftment, and by the portal vein providing limited, but critical, oxygenation until the islets become revascularized by the hepatic arterial system. Normally, insulin and glucagon secreted from islets enter the portal circulation where insulin suppresses, and glucagon activates, hepatic glucose production. In addition, the insulin exposed to the liver is secreted in coordinate pulses, the amplitude of which is dependent on the functional islet β-cell mass and contributes to insulin action on the liver. When transplanted in patients with T1D, intrahepatic islets reestablish coordinate pulsatile insulin secretion (19), normalize hepatic insulin sensitivity (20), and restore appropriate glucagon secretion in response to insulin-induced hypoglycemia that normalizes glucose counterregulation (21). Whether the subcutaneous device-less site can be scaled and provide physiologic function sufficient for curative cellular therapy in large-animal models of diabetes requires consideration as part of preclinical development of stem cell–derived islets for the treatment of diabetes.

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