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 perfusion 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

© 2019 by the American Diabetes Association. Readers may use this article as long as the work is properly cited, the use is educational and not for profit, and the work is not altered. More information is available at http://www.diabetesjournals.org/content/license.

See accompanying article, p. 953.
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...

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.
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 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.

**Funding.** M.R.R. is supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Public Health Services Research Grant R01 DK091331.

**Duality of Interest.** No potential conflicts of interest relevant to this article were reported.

**References**

1. Foster NC, Beck RW, Miller KM, et al. State of type 1 diabetes management and outcomes from the T1D Exchange in 2016–2018. Diabetes Technol Ther 2019;21:66–72
2. Rickels MR. Recovery of endocrine function after islet and pancreas transplantation. Curr Diab Rep 2012;12:587–596
3. Jennings RE, Berry AA, Strutt JP, Gerrard DT, Hanley NA. Human pancreas development. Development 2015;142:3126–3137
4. Odorico J, Markmann J, Melton D, et al. Report of the Key Opinion Leaders Meeting on stem cell-derived beta cells. Transplantation 2018;102:1223–1229
5. Kroon E, Martinson LA, Kadoya K, et al. Pancreatic endoderm derived from human embryonic stem cells generates glucose-responsive insulin-secreting cells in vivo. Nat Biotechnol 2008;26:443–452
6. Rezania A, Bruin JE, Riedel MJ, et al. Maturation of human embryonic stem cell-derived pancreatic progenitors into functional islets capable of treating pre-existing diabetes in mice. Diabetes 2012;61:2016–2029
7. Rezania A, Bruin JE, Arora P, et al. Reversal of diabetes with insulin-producing cells derived in vitro from human pluripotent stem cells. Nat Biotechnol 2014;32:1121–1133
8. Pagliuca FW, Millman JR, Gürler M, et al. Generation of functional human pancreatic β cells in vitro. Cell 2014;159:428–439
9. Pepper AR, Bruni A, Pawlick R, et al. Posttransplant characterization of long-term functional hESC-derived pancreatic endoderm grafts. Diabetes 2019;68:953–962
10. Kearns M, Ahmad NA. Diagnosis and management of pancreatic cystic neoplasms. Curr Treat Options Gastroenterol 2017;15:587–602
11. Pepper AR, Galiano-Bruin R, Pavlick R, et al. A prevascularized subcutaneous device-less site for islet and cellular transplantation. Nat Biotechnol 2015;33:518–523
12. Basta G, Montanucci P, Luca G, et al. Long-term metabolic and immunologic follow-up of nonimmunosuppressed patients with type 1 diabetes treated with microencapsulated islet allografts: four cases. Diabetes Care 2011;34:2406–2409
13. Carlsson PO, Espes D, Sedigh A, et al. Transplantation of macroencapsulated human islets within the bioartificial pancreas βAir to patients with type 1 diabetes mellitus. Am J Transplant 2018;18:1735–1744
14. Ludwig B, Reichel A, Steffen A, et al. Transplantation of human islets without immunosuppression. Proc Natl Acad Sci U S A 2013;110:19054–19058
15. Pullen LC. Stem cell–derived pancreatic progenitor cells have now been transplanted into patients: report from IPITA 2018. Am J Transplant 2018;18:1581–1582
16. Pepper AR, Pawlick R, Bruni A, et al. Transplantation of human pancreatic endoderm cells reverses diabetes post transplantation in a prevascularized subcutaneous site. Stem Cell Reports 2017;8:1689–1700
17. Motté E, Szepessy E, Suensens K, et al.; Beta Cell Therapy Consortium EU-FP7. Composition and function of macroencapsulated human embryonic stem cell–derived implants: comparison with clinical human islet cell grafts. Am J Physiol Endocrinol Metab 2014;307:E838–E846
18. Rickels MR, Robertson RP. Pancreatic islet transplantation in humans: recent progress and future directions. Endocr Rev 2019;40:631–668
19. Meier JJ, Hong-McAtee I, Galasso R, et al. Intrahepatic transplanted islets in humans secrete insulin in a coordinate pulsatile manner directly into the liver. Diabetes 2006;55:2324–2332
20. Rickels MR, Kong SM, Fuller C, et al. Improvement in insulin sensitivity after human islet transplantation for type 1 diabetes. J Clin Endocrinol Metab 2013;98:E1780–E1785
21. Rickels MR, Fuller C, Dalton-Bakes C, et al. Restoration of glucose counterregulation by islet transplantation in long-standing type 1 diabetes. Diabetes 2015;64:1713–1718