β -cell regeneration and differentiation: how close are we to the ‘holy grail’?

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Abstract

Diabetes can be managed by careful monitoring of blood glucose and timely delivery of exogenous insulin. However, even with fastidious compliance, people with diabetes can suffer from numerous complications including atherosclerosis, retinopathy, neuropathy, and kidney disease. This is because delivery of exogenous insulin coupled with glucose monitoring cannot provide the fine level of glucose control normally provided by endogenous β -cells in the context of intact islets. Moreover, a subset of people with diabetes lack awareness of hypoglycemic events; a status that can have grave consequences. Therefore, much effort has been focused on replacing lost or dysfunctional β -cells with cells derived from other sources. The advent of stem cell biology and cellular reprogramming strategies have provided impetus to this work and raised hopes that a β -cell replacement therapy is on the horizon. In this review, we look at two components that will be required for successful β -cell replacement therapy: a reliable and safe source of β -cells and a mechanism by which such cells can be delivered and protected from host immune destruction. Particular attention is paid to insulin-producing cells derived from pluripotent stem cells because this platform addresses the issue of scale, one of the more significant hurdles associated with potential cell-based therapies. We also review methods for encapsulating transplanted cells, a technique that allows grafts to evade immune attack and survive for a long term in the absence of ongoing immunosuppression. In surveying the literature, we conclude that there are still several substantial hurdles that need to be cleared before a stem cell-based β -cell replacement therapy for diabetes becomes a reality.

Key Words

- development
- diabetes (all)
- islet cells
- insulin

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Introduction

Diabetes mellitus encompasses a group of metabolic disorders that affect the ability to regulate blood glucose levels and can be classified into two main groups, type 1 and type 2. Previously known as juvenile-onset diabetes, Type 1 diabetes is thought to result from T-cell-mediated

autoimmune destruction of insulin-producing β -cells and is believed to have a genetic component (reviewed in Concannon *et al.* (2009)), although, recently, both these contentions have been challenged (Skog *et al.* 2013). Type 2 diabetes is characterized by insulin resistance in

peripheral tissues and is sometimes associated with β -cell dysfunction; both features resulting from prolonged exposure to elevated blood glucose levels (Tuomilehto *et al.* 2001).

Before the discovery of insulin, type 1 diabetes was almost always fatal. With the advent of insulin, diabetes was transformed into a chronic condition managed by careful monitoring of diet and blood glucose levels, in conjunction with insulin replacement therapy via s.c. injections or through an insulin pump (Tamborlane *et al.* 1979, Weissberg-Benchell *et al.* 2003, Renard *et al.* 2010). However, this system is imperfect as it fails to provide the fine control over blood glucose afforded by properly functioning islets. Moreover, inappropriate balance between insulin and glucose intake can cause severe hypoglycemia, a situation that can be exacerbated during acute illnesses (Krinsley *et al.* 2011). Critically, patients who are insulin dependent are sometimes unaware of hypoglycemic episodes, presenting the risk of loss of consciousness and the inability to be awakened from sleep (Cryer 2005).

At times where patients are at a high risk of severe hypoglycemia, replacement of insulin-producing β -cells through transplantation is considered. Originally, whole-organ pancreas transplantation was the only option. Successful pancreas transplants could restore normoglycemia and reduce, and sometimes reverse, secondary diabetic complications such as diabetic neuropathy (Bohman *et al.* 1985, Fioretto *et al.* 1998). Despite these benefits, similar to insulin therapy, pancreatic transplants are not without cost, with potential risks related to surgical complications, lifelong immunosuppression, and graft rejection. In order to avoid major invasive surgery, treatments involving the transplantation of cadaveric derived islets were developed. Successful transplantation improved glycemic control and often protected patients from hypoglycemia compared with pre-transplantation (Shapiro *et al.* 2000, 2006, Street *et al.* 2004). However, as in the case of pancreatic transplants, patients still require ongoing immunosuppression. Perhaps more importantly, in common with all transplantation-based therapies, the dearth of organ donors meant that this treatment option would only ever be available to a select few.

Against this backdrop, research has focused on other potential sources of β -cells that could be used in place of donor-derived pancreatic tissue. Of particular interest are pluripotent stem cells, immortal stable cell lines that can be differentiated into any cell type found in the body, including insulin-producing β -cells. This review briefly discusses the use of this stem cell type as a source of β -cells, examines how such cells might be delivered, and clarifies

what issues will need to be addressed before a stem cell-based therapy might become reality.

Human pluripotent stem cells

Pluripotent stem cells (PSCs) are immortal cells that can be differentiated into any cell type found in the body. Owing to this, much interest has been focused on the possibility of deriving insulin-producing β -cells from PSCs for the treatment of diabetes. There are two types of PSCs: Embryonic stem cells (ESCs) derived from blastocyst-stage human embryos and induced PSCs (iPSCs) (Fig. 1), derived by reprogramming somatic cells to an ESC-like state. Human ESCs were first cultured by Thomson *et al.* (1998) and paved the way for the subsequent reprogramming of somatic cells by Takahashi & Yamanaka (2006) and shortly thereafter by Thomson *et al.* (Yu *et al.* 2007), to generate iPSCs that grow under the same conditions as ESCs and display similar properties. Researchers using both mouse and human somatic cells found that the introduction of four ESC-associated factors, OCT3/4, SOX2, c-MYC, and KLF4 (Takahashi & Yamanaka 2006, Takahashi *et al.* 2007, Yu *et al.* 2009), or OCT4, SOX2, NANOG, and LIN28 (Yu *et al.* 2007), induced pluripotency. Subsequently, further research found that other combinations of factors, small molecules, and RNAs can be used to achieve an equivalent outcome. The differentiation potential, proliferative capacity, morphology, and gene expression profiles of iPSCs are highly similar to those of ESCs, but use of the former avoids the ethical complications of deriving ESCs from human blastocysts (Yu *et al.* 2007, Rao & Condic 2008).

In spite of their similarities, iPSCs hold slightly different promise than ESCs, namely the use of iPSCs for patient-specific therapy. Human iPSCs are a genetic match to the person from whom they were generated, theoretically circumventing the issue of immune rejection of iPSC derivatives. However, in the case of type 1 diabetes, it would not be unreasonable to suppose that iPSC-derived β -cells would be rejected by the same autoimmune mechanism that led to the disease in the first place. Therefore, at least for type 1 diabetes, it is questionable whether iPSCs would offer any advantages over ESCs as a source of new β -cells for transplantation therapies. Finally, the amount of work and cost required to derive, validate, and ensure safety for any given cell line means that individualized iPSCs may not be economically practical as a source of new β -cells. In this sense, a generalizable 'off-the-shelf' stem cell-derived product is likely to be more viable, particularly in the short term. This could either take the form of an ESC-derived product, or potentially

Figure 1

Morphology of human embryonic stem cells (ESCs) and human induced pluripotent stem cells (iPSCs) grown in monolayer cultures (left panels) and as single colonies (right panels) on mouse embryonic feeder cells (photographs taken using a $\times 5$ objective lens).

cells differentiated from HLA-matched iPSCs sourced from pre-typed iPSC banks (Inoue *et al.* 2014). In either case, some form of immunoprotection for the graft or immunosuppression will be required.

Development of the pancreas

In order to differentiate PSCs *in vitro* toward a pancreatic cell fate, it is helpful to understand the process of pancreatic organogenesis. Extensive studies involving mouse models have led to a better understanding of the processes underlying early development of the embryo and the steps leading to the formation of complex tissues such as the pancreas. Using these processes as a roadmap, researchers have been able to guide PSCs through analogous stages of development toward the formation of desired cell types (Fig. 2). The pancreas is a derivative of definitive endoderm, one of the three primary germ layers generated during the process of gastrulation. Once generated, definitive endoderm folds into a primitive gut tube, which is then further regionalized into subdomains, a process that occurs under the influence of growth factors secreted by juxtaposed tissues. The pancreas arises from two patches of epithelium that evaginate dorsally and ventrally from the foregut endoderm, situated between the

stomach and duodenum (Lammert *et al.* 2001, Field *et al.* 2003). The dorsal bud receives signals from the notochord and dorsal aorta and establishes a permissive environment for dorsal pancreatic specification within the gut endoderm (Hebrok *et al.* 1998). Conversely, the ventral bud is induced by a different set of signals originating from the adjacent cardiac mesenchyme and lateral plate mesoderm (Kumar *et al.* 2003). Following budding, the pancreatic primordia undergo considerable growth and branching, culminating in fusion of the two buds into a single organ (Villasenor *et al.* 2010). Over this period, expansion of the pancreatic epithelium is principally driven by mesenchymal cells, which secrete proliferative growth factors such as FGF10 (Bhushan *et al.* 2001). In mice, pancreatic development is accompanied by the branching of epithelial tubules; a stage sometimes referred to as the 'secondary transition' during which mono-hormonal insulin-expressing cells begin to emerge. During this time, acini form and begin to differentiate while mesenchymal derived growth factors continue to drive epithelial growth and new acinar formation (Landsman *et al.* 2011). Concomitant with this process, endocrine progenitors delaminate from the epithelium and aggregate to form islets (Bouwens & De Blay 1996). Maturation of endocrine cells within these islets generates glucagon, insulin, somatostatin, and pancreatic

Figure 2

Timeline of pancreatic development *in vivo* and during PSC differentiation. Top line briefly summarizes the key cell types generated during pancreas development, while genes present or absent, which define these stages, are shown directly below. Days after conception indicate the number of

days of embryonic/fetal development corresponding to each stage.

Differentiation day gives an approximate estimate of the number of days required to reach these stages *in vitro*. Timelines have been based on studies by Piper *et al.* (2004), Riedel *et al.* (2012) and Jennings *et al.* (2013).

polypeptide-producing cells. Studies on mice suggest that endocrine precursors continue to differentiate throughout fetal development and for up to 3 weeks after birth. After this stage, endocrine tissue is maintained through a low frequency of replication (Dor *et al.* 2004, Brennand *et al.* 2007).

Although the process of human pancreatic development is thought to resemble that documented for mice, there are a number of points of difference that are pertinent to any discussion of PSC differentiation toward pancreatic lineages. First, it is arguable whether the developmental stages of the human pancreas can be precisely equated with those documented for mice (Sarkar *et al.* 2008). A key issue relating to the similarities of mouse and human pancreatic development centers on the appearance and function of cells expressing multiple hormones. Experiments conducted on mice suggest that insulin- and glucagon-expressing cells have distinct developmental origins (Herrera 2000). As such, it is unclear as to how and/or whether cells, which express multiple hormones during early mouse development (Teitelman *et al.* 1996), contribute to the adult mouse endocrine system. In human development, cells expressing multiple hormones range from 5 to 20% of hormone-positive cells, a fraction that remains relatively stable between weeks 10 and 20 of fetal life (Jeon *et al.* 2009). At this stage, it is still unclear whether such cells ever give rise to fully functional β-cells or whether they represent a fetal cell type that makes no contribution to the adult endocrine organ (Bocian-Sobkowska *et al.* 1999). Importantly, polyhormonal cells are a common feature of many PSCs to pancreatic differentiation protocols described to date (e.g., see D'Amour *et al.* (2006)) and, therefore, the potential of this cell type is an issue that needs to be urgently addressed.

Differentiation of pluripotent stem cells into pancreatic cells

Although numerous groups have published protocols for the differentiation of PSCs toward either pancreatic progenitors or endocrine cells, the most influential methods come from the biotechnology company, Viacyte, Inc. (<http://viacyte.com>). In a protocol developed by D'Amour *et al.*, combinations of growth factors involved in pancreatic development were used in a stage-specific manner to guide undifferentiated PSCs to insulin-expressing cells through a series of obligatory intermediate cell types identified through developmental studies (D'Amour *et al.* 2005, 2006, Kroon *et al.* 2008, Schulz *et al.* 2012). Since 2006, many other laboratories have published their own modifications of these pancreatic differentiation protocols (e.g., Jiang *et al.* 2007, Xu *et al.* 2011, Rezanian *et al.* 2012).

Although there are a large number of elements that vary between pancreatic differentiation protocols, some common themes are evident. All methods are based on the ontogenetic framework described above that is used to rationalize the use of particular factors/treatments at specific stages of differentiation. To successfully differentiate PSCs to a pancreatic fate, it is recognized that multiple crucial developmental steps need to be accurately modeled (Fig. 2). These include the induction of definitive endoderm, the patterning and specification of endoderm to a pancreatic fate, and the generation of endocrine/exocrine cells (Biemar *et al.* 2001, Field *et al.* 2003, Nostro & Keller 2012). The first step invariably employs activin A to induce definitive endoderm. Activin A is a transforming growth factor beta (TGFβ) family member that mimics the action of Nodal, the ligand used by the embryo to drive development of the mesoderm and definitive endoderm (Osada & Wright 1999,

Lowe *et al.* 2001, Kubo *et al.* 2004, Jiang *et al.* 2007, Kroon *et al.* 2008). Following this, definitive endoderm cells are treated with retinoic acid to induce foregut, from which pancreatic endoderm derives. This treatment was initially used in mouse ESCs for pancreatic differentiation (Micallef *et al.* 2005) and was rapidly adopted for human PSC systems (D'Amour *et al.* 2006). For both activin A (Nodal) and retinoic acid, key experiments defining the developmental role of these factors were conducted in model organisms such as xenopus, zebrafish, and mice (Conlon *et al.* 1994, Esni *et al.* 2001, Stafford & Prince 2002, Chen *et al.* 2004). Following the emergence of pancreatic endoderm, many methods include a treatment with the bone morphogenetic protein antagonist, noggin, or the small molecule analogs dorsomorphin or dorsomorphin homolog 1, because these factors promote the development of pancreatic progenitors defined by the expression of PDX1 (Jiang *et al.* 2007, Kroon *et al.* 2008, Bose *et al.* 2012). The final steps that are necessary to convert pancreatic endoderm to functional endocrine cells remain unclear. For this reason, many researchers transplant cells into immunocompromised mice at the pancreatic progenitor stage, allowing the final steps of differentiation and maturation to occur *in vivo* (Shim *et al.* 2007, Kroon *et al.* 2008, Rezanian *et al.* 2012, Schulz *et al.* 2012, Rezanian *et al.* 2013, Kirk *et al.* 2014). In instances where the final stages of differentiation were attempted *in vitro*, factors such as nicotinamide, insulin-like growth factor 1 (IGF1), and hepatocyte growth factor (HGF) were used to induce β -cell maturation, generating insulin- and glucagon-expressing cells (D'Amour *et al.* 2006, Jiang *et al.* 2007, Mfopou *et al.* 2010). However, unlike the cells that differentiate and mature *in vivo*, endocrine cells derived from a wholly *in vitro* differentiation approach frequently express more than one hormone and display an immature non-glucose-responsive phenotype (D'Amour *et al.* 2006, Basford *et al.* 2012, Micallef *et al.* 2012). Indeed, a direct comparison between insulin-expressing cells generated *in vitro* and those isolated from endogenous sources showed that, at a transcriptional level, PSC-derived β -cells most closely resembled fetal β -cells (Hrvatin *et al.* 2014).

In contrast to the success of generating pancreatic progenitors from PSCs, the overall yield of end-stage differentiated insulin-expressing β -cells remains low. For this reason, it is probable that if PSC-derived cells are used therapeutically, it is very likely that pancreatic progenitors – which retain both proliferative and differentiative capacities – will be the first choice for clinical trials.

Delivery of stem cell-derived products

Before any cellular based therapy can be used for the treatment of diabetes, it is necessary to consider how such cells would be delivered and what potential pitfalls may be encountered. In the case of type 2 diabetes, it would be theoretically possible to use patient-specific iPSC-derived pancreatic progenitors or β -cells without the need for immunosuppression. However, for type 1 diabetes, the impact of immune-mediated destruction of the transplanted islet cells will still need to be addressed, even if patient iPSCs are the source. Moreover, in either case, the question of safety of the transplanted cells also needs to be considered. In particular, as undifferentiated PSCs have the potential to form teratomas in xenotransplants, there is an ongoing concern that PSC-based cellular therapies may pose a safety risk (Hentze *et al.* 2009).

As most differentiation protocols enrich rather than purify cell types of interest, the presence of other cell types could represent a safety hazard, particularly if these off-target cells retained substantial proliferative potential. Furthermore, if differentiation is not 100% efficient, undifferentiated human PSCs with teratoma-forming potential may persist. Indeed, a number of studies reported that when differentiated cultures were transplanted into animal models, teratoma formation was sometimes observed (Sipione *et al.* 2004, Fujikawa *et al.* 2005, Kroon *et al.* 2008, Stadtfeld *et al.* 2008). These tumors are similar to spontaneous human teratomas that contain derivatives of each germ layer, endoderm, mesoderm, and ectoderm.

There are a number of ways this safety issue could be addressed. First, improvements in differentiation efficiency could reduce the frequency of unwanted cell types to negligible levels (Hentze *et al.* 2009). Secondly, procedures for purifying the desired cell types or for selecting against unwanted cell types could be developed. Having said this, any positive selection strategy that uses physical purification methods, such as cell selection using antibodies, is likely to be too expensive to apply on a large scale. Therefore, if purification methods are to be employed, it is likely that such methods would use drug-based selection against specific unwanted contaminant cell types. Lastly, as an additional safety precaution, cells could be encapsulated in a device that restricted their dispersion and facilitated their retrieval in the event of unwanted growth or differentiation. This approach also has the advantage that such a device could also serve to shield the cells from the immune system.

Bio-artificial pancreas and encapsulation technologies

One attractive aspect of using PSC-derived pancreatic cells for the treatment of diabetes is that a number of strategies for introducing exogenous β -cells into patients have been developed over many years. These strategies were originally developed for β -cells derived from other sources, such as cadaveric derived islets, pig islets, or human fetal pancreatic tissue. In this context, it is worth briefly reviewing what strategies are available and how these could be incorporated into a stem cell-based treatment of diabetes.

In its most basic form, the artificial pancreas is a device in which insulin is delivered from a portable pump guided by a s.c. glucose sensor (Steil *et al.* 2003, Weinzimer *et al.* 2008, Renard *et al.* 2010). Although much progress has been made toward this goal, it is questionable whether any electronic device will ever provide a level of glucose control that approaches that delivered by functional β -cells. In this context, the latest embodiment of the artificial pancreas is likely to include insulin-producing β -cells – derived from either organ donors or newly developed stem cell sources – which act as both a sensor of glucose concentration and a source of insulin. Irrespective of the source, such a bioartificial pancreas would comprise two components: insulin-producing cells and a barrier that would hide or protect these cells from the immune system. In essence, this means that the cells need to be encapsulated in a device or substance that will enable nutrients and factors to diffuse in but prevent immune cells from gaining direct access to the insulin-producing cells.

Currently, there are two main approaches to encapsulation, microencapsulation, and macroencapsulation. Microcapsules were originally designed to accommodate only a few islets within a semi-permeable bead, normally constructed of alginate or polyethylene glycol. The spherical configuration of these structures results in a higher surface area-to-volume ratio leading to a higher diffusion rate (Fritschy *et al.* 1991, Omer *et al.* 2003, Dufrane & Gianello 2012). Moreover, microcapsules can be injected in large numbers, are durable, and are difficult to disrupt mechanically. Nevertheless, a potential concern is that, in some cases, microcapsules with or without islets have been found to elicit an inflammatory response, which eventually results in fibrosis that cover the capsules (Robitaille *et al.* 2005; reviewed in de Vos *et al.* (1999)). Under these conditions, islets progressively deteriorate due to the lack of oxygen and nutrients, ultimately causing the cells to die (de Groot *et al.* 2004).

Studies in which alginate-encapsulated islets were transplanted into mouse models of diabetes demonstrated that glucose and insulin transport steadily increased over time, lasting anywhere from 28 days to 2–3 weeks (Pareta *et al.* 2013, Park *et al.* 2013). Furthermore, Park *et al.* (2013) reported a linear relationship between the number of capsules and insulin production, providing a framework to calculate the number of capsules required to fully restore blood glucose control in animals and potentially humans.

Although alginate has some desirable attributes as a capsule material, there are additional challenges related to the need to carefully balance pore size such that cells obtain the nutrients they require while remaining completely isolated from the immune system (Pareta *et al.* 2013, Park *et al.* 2013). Islets require relatively high concentrations of oxygen and studies have demonstrated that hypoxia has a deleterious effect on their survival and function (Dionne *et al.* 1993). When transplanted, islets initially depend on diffusion of oxygen from the surrounding tissue until revascularization, a process that takes ~7–10 days (Menger *et al.* 1992). However, microencapsulated islets do not revascularize, thus subjecting islet grafts to extended periods of hypoxia, which is detrimental to their long-term survival and function (de Groot *et al.* 2004).

Despite the considerations mentioned above, encapsulated islets are most often transplanted intraperitoneally, a site that allows ample room for multiple microcapsules (reviewed in Calafiore (2003)). However, this site has many disadvantages, including the lack of vascularization and the potential immune response from resident intraperitoneal T cells and macrophages, the latter increasing the likelihood of fibrotic growth over the encapsulated islets (de Vos *et al.* 2003). Therefore, alternative sites are currently being investigated to ensure that the most adequate site is chosen to solve each of these complications (reviewed in de Vos *et al.* (2010)).

Macrodevices involve a flat diffusion chamber, made of materials including alginate, nitrocellulose, and agarose (reviewed in Suzuki *et al.* (1998) and O'Sullivan *et al.* (2011); Fig. 3). These planar devices could have the potential to house a large number of islets. Owing to their size, it is envisaged that such containers would be readily retrievable and/or reloadable; unlike microcapsules that would be difficult to locate once transplanted. Compared with microcapsules, macrodevices have a greater mechanical strength and can be implanted with minimal surgery. However, earlier versions suffered from similar drawbacks as microcapsules, namely, fibrotic

Figure 3

Promises and pitfalls of macroencapsulation devices for the delivery and protection of endogenously produced β -cells. (A) Idealized scenario in which the device allows free diffusion of glucose, nutrients, and insulin but excludes harmful immune cells. (B) Possible problems that may occur with

macroencapsulation devices, including fibrosis covering the device and the entry of harmful anti- β -cell antibodies or immunoinflammatory molecules or cytokines.

overgrowth and the subsequent necrosis of the transplanted islets (de Vos & Marchetti 2002).

The potential drawbacks of macroencapsulation devices have been partly addressed in the construction of the Theracyte device. This device ranges in length from ~ 2 to 4 cm, with a rectangular planar shape. The device contains a loading dock and is made of a biocompatible bi-layered polytetrafluoroethylene membrane. Studies have demonstrated that when the device containing neonatal pig cells positive for insulin and glucagon was transplanted into monkeys, the cells remained viable for up to 8 weeks with no inflammatory reaction (Elliott *et al.* 2005). Since these initial studies, a number of groups have shown that such a device can support the growth and differentiation of fetal or PSC-derived pancreatic progenitors and that this composite bioartificial pancreas can restore glucose control in mouse models of experimentally induced diabetes (Lee *et al.* 2009, Ludwig *et al.* 2012, Bruin *et al.* 2013, Rezaei *et al.* 2013, Kirk *et al.* 2014). Currently, the US biotechnology company, ViaCyte, Inc., is applying to conduct clinical trials for a combination product that incorporates PSC-derived pancreatic progenitors and a two-dimensional planar device (Encaptra), which has shared the characteristics of the Theracyte device.

A potential issue with devices of this nature concerns their carrying capacity in relation to size. Kirk *et al.* (2014) have recently reported poor results when PSC-derived pancreatic progenitors were incorporated into a 5 μ l device compared with the 20 μ l device. The larger device, $\sim 2 \times 1$ cm, demonstrated a sufficient capacity to control glycemia after alloxan treatment of severe combined immunodeficiency/beige mice. Owing to the planar nature of the device, increasing the capacity requires a linear increase in the total area, that is, for a 1 cm wide device, increasing the volume from 20 to 40 μ l requires a doubling in length. If a proportionately larger device is required for humans, then an average human weighing 70 kg might require a device 46 m long! Clearly, further innovations in the structure of these devices may be required in order for them to be ready for clinical application.

An alternative approach would be to devise a treatment in which β -cells were replenished from endogenous sources – either through the activation of endogenous pancreatic stem cells or by the *in vivo* reprogramming of non-endocrine cells toward a β -cell phenotype (Zhou *et al.* 2008, Smukler *et al.* 2011). However, in the case of type 1 diabetes, any regenerative treatment may still require the issue of the immune system

to be addressed – and, currently, it is unclear how activated endogenous pancreatic stem cells would fare if the ongoing immune assault could not be attenuated or eliminated. Similarly, neo- β -cells brought into existence through reprogramming approaches or growth factor treatment would still face a hostile immune system that would need to be addressed.

One potential solution to immune-mediated destruction of a cellular therapeutic product would be to engineer glucose-responsive non- β -cells that could avoid immune surveillance. However, this might not be possible if the proteins required for regulated insulin release are the target of autoimmunity. In particular, a recent work demonstrating that pro-insulin itself could be a key auto-antigen indicates the potential difficulties in avoiding ongoing immunological assault (Pathiraja *et al.* 2014).

Similarly, in type 2 diabetes, it seems difficult to envisage a scenario in which newly derived β -cells would not fall victim to the same adverse conditions that precipitated the decline in β -cell function often associated with this disease (Potter *et al.* 2014). As such, for both types of diabetes, it might transpire that exogenously produced β -cells will represent the best hope of achieving normoglycemia for patients with either type 1 or type 2 diabetes.

The quest to find a new treatment for diabetes has been punctuated with obstacles that reflect the various complexities of the disease. The need to find a new source of insulin-producing β -cells has driven research into the potential use of stem cells, which, in turn, has benefited from decades of assiduous developmental studies. In the case of type 1 diabetes, the need to deal with sustained attack from the immune system has prompted the development of encapsulation strategies that have relied heavily on materials science and transplantation biology. If a workable solution is to be achieved, it will probably require the coming together of several streams of research that span different fields of scientific endeavor. Optimistically, this quest, based on scientific insight, will prove to be more fruitful than that of the mythical quest for the holy grail.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the review.

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