Accelerating Regenerative Medicine Approaches to Type 1 Diabetes Through Direct Cell Reprogramming

Current treatment of type 1 diabetes mellitus (T1DM) depends on the replacement of endogenous insulin by regular subcutaneous injections of exogenous insulin. Unfortunately, exogenous insulin therapy is associated with patient compliance issues and life-threatening hypoglycaemic events. Alternatively, recent convergences of biomaterial and regenerative medicine advances suggest transplantation of stem cell-derived beta cells as an "off-the-shelf" cell therapy treatment approach to T1DM, potentially providing long-term therapeutic benefits to patients, with minimal adverse effects. However, derivation of mature beta cells from stem cells is a lengthy and expensive process requiring multiple cell purification steps. Direct cell reprogramming of source stem cells to target beta cells, guided by analysis of gene regulatory networks using bioinformatic algorithms, offers a potential solution. This article will explore regenerative approaches to resolve T1DM with a focus on how direct cell reprogramming could influence the field.

T1DM is an autoimmune condition that targets insulin-secreting beta cells of the pancreas, resulting in their dysfunction and destruction, and currently affects an estimated 1.4 million adults in the USA[1]. This loss of endogenous insulin underlies the life-threatening state of chronic hyperglycaemia that classically defines diabetes mellitus. The current gold-standard treatment of T1DM is to replace lost endogenous insulin with exogenous insulin administration through regular subcutaneous injections. However, this treatment regime relies on patients regularly monitoring their blood glucose levels and self-administering doses of insulin, which can yield dangerous hypoglycaemic episodes[2]. Cell-based therapies represent an alternative solution, by restoring endogenous insulin production and secretion.

An early iteration of this cell-based approach is cadaveric islet transplantation (Edmonton protocol), which offers proof of principle of the therapeutic effects of restoring endogenous insulin in T1DM through evidence of sustained efficacy in clinical trials[3]. Cadaveric islet transplantation is not currently a viable mainstream T1DM treatment, as healthy cadaveric islet grafts are in short supply, embed variably, and require long-term immunosuppression of recipients. These key limitations of scalability, variable efficacy and immunorejection can be circumvented by deriving islet grafts from expandable sources such as human embryonic stem cell (hESC) and human induced pluripotent stem cell lines, combined with cell encapsulation or *in vivo* beta cell regeneration (Table 1).

Melligen Cells

Human hepatocytes express key molecular elements of the glucose-sensing apparatus present in pancreatic beta cells, including the glucose transporter GLUT2, and the enzyme glucokinase (GCK). By driving ectopic insulin (INS) and GCK expression in a hepatic cell line, HUH7, through transfection, Lawandi *et al.* were able to produce an insulin-secreting cell line, dubbed 'Melligen cells', with physiological glucose-sensing properties. When these hybrid cells were transplanted into mouse models of T1DM, normoglycaemia was restored without inducing bouts of hypoglycaemia[4]. Whilst these preclinical results are promising and support the clinical potential of an expandable line of pseudo-beta cells, there are safety concerns associated with Melligen cells. These cells are derived from a hepatocyte-derived carcinoma, therefore teratoma formation is possible if implanted into humans without encapsulation, and long-term efficacy has yet to be established.

Stem Cell-derived Grafts

Two main strategies exist for deriving functional islet grafts from human pluripotent stem cells (hPSCs). One method involves production of pancreatic endoderm cells (PECs) which are subsequently transplanted into patients. *In vivo*, these PECs differentiate into mature, functional islets. The other strategy involves derivation of functional mature islets from hPSCs *in vitro* prior to transplantation.

Pancreatic Endoderm Cell Grafts

PEC generation from hESCs involves a stepwise differentiation protocol of ~12 days in total, comprised of four short stages (Figure 1A). This protocol mimics the embryonic development of the pancreas *in vivo*, requiring recapitulation of the stem cell niches present at each step of differentiation through media supplementation with specific growth factors and small molecules. Encapsulation of PECs within a biocompatible transplant shields the PECs from immune cell intervention, to allow for subcutaneous transplantation of PECs. In preclinical models, these PECs mature into islets structures with associated endocrine cell types (e.g. beta cells, alpha cells and delta cells), which restore normoglycaemia in diabetic mouse models within 50–70 days, and are maintained long-term (>100 days)[5].

Given that PECs can be generated in a short period from expandable suspension cultures of hESCs, cryopreserved without detrimental effect, and protected from the host immune system, PEC transplantation has great potential as a scalable off-the-shelf cell therapy for T1DM. Despite this, key barriers to PEC use as a mainstream T1DM treatment remain. Firstly, although PECs mature into functional islets *in vivo*, the makeup of these islets is highly variable between individuals, with 50–100% of the grafted cells maturing into endocrine cells[6]. This variability has potential implications for the efficacy of the

Cell therapy	Time to generate graft	Estimated teratoma risk	Estimated production cost	Estimated scalability	Clinical trial status
Melligen cells	Minimal (established cell line)	High (modified cancer cell line) Low (if encapsulated)	Low	Very high	Preclinical
Pancreatic endoderm cells (PECs)	12 days	Moderate Low (if encapsulated)	High	High	Phase 1/2
Stem cell derived islets (SC-islets)	1 month	Moderate Low (if encapsulated)	High	Moderate	Preclinical
In vivo beta cell regeneration	N/A	Unknown (see text)	Low	Very high	N/A

Table 1: Summary of key properties associated with potential T1DM cell therapies.

graft and dose of PECs required to restore normoglycaemia. The non-endocrine contingent could also cause adverse effects, impeding graft viability, and residual non-pancreatic impurities in the PEC graft increase the risk of teratoma formation[8]. Encapsulation can help to minimise these risks by confining the graft to the transplant device and enabling easy removal. Cost is another concern, as although PEC generation is straightforward and quick, the media supplements used to maintain hESCs and generate PECs are extremely expensive[7], and therefore limit the potential of PECs as a scalable diabetes treatment.

Stem Cell-derived Islet Grafts

Generation of terminally differentiated stem cell-derived islets (SC-islets) requires a six-stage protocol of about a month (Figure 1B). The first four stages of the protocol largely overlap with that of PEC generation, with an additional two stages required to produce mature endocrine cells[8,9]. Mature SC-islets can then be encapsulated and administered subcutaneously, enabling off-the-shelf use in a similar manner to PECs.

Although SC-islets require a longer amount of time and greater expense in terms of media supplements to generate than PECs, the extra steps also allow for endocrine and beta cell enrichment of up to 80%, reduce non-endocrine contaminant, and enable SC-islet size determination to avoid large islets, which graft poorly[9,10]. These quality-control steps reduce the variability in SC-islet grafts and create a more defined cell therapy compared with PEC grafts. SC-islet grafts, however, still contain some non-endocrine impurities and other cell types, including up to 5% enterochromaffin cells[9].

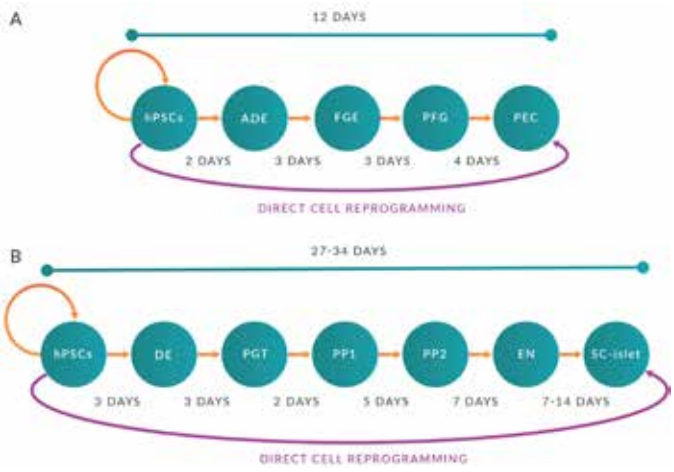

Figure 1: Schematic detailing current protocols for (A) pancreatic endoderm cell (PEC) and (B) stem cell-derived islet (SC-islet) generation from hPSCs. Green lines represent potential single direct cell reprogramming steps. Anterior definitive endoderm (ADE), foregut endoderm (FGE), posterior foregut (PFG), definitive endoderm (DE), primitive gut tube (PGT), pancreatic precursor 1 (PP1), pancreatic precursor (PP2), endocrine progenitors (EN).

An Omics-based Approach to Direct Cell Reprogramming for T1DM Therapy

Predicting Reprogramming Factors

Rather than using trial-and-error approaches to identify key reprogramming factors, the advent of omics data analysis enables the prediction of key reprogramming factors using computer algorithms. These can be used to predict subsets of transcription factors (TFs) required for direct cell reprogramming by identifying genes differentially expressed in source and target (or closely related) cell types. The relative expression levels of target cell-specific TFs can then be individually weighted by their regulatory influence over target cell-specific genes, and ranked accordingly. TFs from these rankings can then be combined to maximize coverage of the target cell transcriptomic network when expressed.[11].

Enhancing *In Vitro* Transplant Production

Currently used protocols for producing transplantable PECs and SC-islets from hPSCs depend on multistage, expensive, and in the case of SC-islets, long differentiation protocols[5,9]. These shortcomings could be mitigated by the development of direct cell reprogramming protocols which accelerate the graft production using a reduced number of steps, or potentially even a single step. Not only would this consolidation of the production protocol enable grafts to be produced at a faster rate, but it would also reduce associated costs by diminishing requirements for expensive media supplements. Furthermore, a direct cell conversion approach may reduce the non-endocrine cell contamination and batch variability currently seen in grafted tissue.

Using an algorithm to establish these novel, TF-driven direct cell-reprogramming protocols for T1DM treatment would require transcriptomic data obtained by RNA-sequencing source and target cell samples. Ideally, this would be carried out using homogenous cell populations to improve the accuracy of the predicted conversion factors. Producing pure beta cell grafts would constitute a more defined cell therapy product than undifferentiated PECs or heterogenous SC-islets, simplifying cell dosing and reducing variability between individual grafts. However, at present, it is unclear whether pure beta cell grafts can function physiologically outside of the islet niche, with clear implications for clinical relevance[12].

***In Vivo* Beta Cell Regeneration**

Direct cell reprogramming strategies could also be employed as an alternative off-the-shelf strategy to treat T1DM. Taking an *in vivo* regenerative approach would avoid the substantial costs associated with generating grafts derived from hPSCs *in vitro*. Additionally, cells converted *in vivo* avoid allogeneic immune rejection, and don't require costly encapsulation like allogeneic *in vitro* grafts. A range of source cell types and tissues, including alpha cells, hepatocytes, gallbladder, and intestinal epithelium, have been successfully converted *in vivo* into insulin-producing (INS+) cells through genetic reprogramming[13]. However, key challenges must be overcome to produce an efficacious, safe and marketable *in vivo* cell therapy for T1DM. These include the selection of an accessible source tissue, achieving targeted transgene delivery within minimal off-target effects, and avoiding autoimmune rejection.

To date, successful attempts at producing INS+ cells by direct cell reprogramming have utilised source cells that are often difficult to access, and are developmentally related to beta cells constitutively expressing key genes associated with beta cell function (e.g. hepatocytes, expressing GLUT2 and GCK)[4,13]. However, most of these INS+ cells do not truly resemble beta cells, and can retain functional expression of source cell genes potentially limiting their therapeutic potential or causing adverse effects. For example, alpha cell-derived INS+ cells can maintain glucagon production, a key alpha cell function absent in canonical beta cells[14]. Taking a big data approach, the potential use of more accessible but uninvestigated source cell types (e.g. adipocytes) for *in vivo* regeneration of canonical beta cells could be examined using algorithms.

Potential transgene delivery systems include integrating lentiviral vectors driving constitutive expression, or an adenovirus or Sendai virus, yielding transient expression[15]. It remains to be explored whether transient conversion factor expression is sufficient to drive beta cell regeneration, or whether constitutive expression is required, and this will determine the delivery system used. Transient expression could also be achieved through small molecule administration to drive direct cell reprogramming, indeed sustained exposure to the neurotransmitter GABA converts alpha cells to beta-like cells *in vivo*[16]. Off-target effects are a major safety concern

with transgene delivery, particularly with integrative vectors, as they could generate tumours. To alleviate these concerns, transient or conditional expression systems could be used to ensure transgenes are expressed only briefly, or specifically in source cells. Furthermore, inducible suicide genes, such as *CASP9*, could be used to selectively kill converted cells if adverse off-target effects are observed post-treatment[17].

Although *in vivo* regeneration of beta cells has the potential to normalise glucose tolerance in T1DM patients, it does not address the underlying autoimmune disease which could target regenerated beta cells and limit their therapeutic capacity. Aside from lifelong co-administration of immunosuppressants, autoimmune rejection could be circumvented by selecting immune-privileged source tissue or through genetic engineering. Candidate immune-privileged source tissues include the gut epithelium. For example, INS+ cells produced by conversion of K-cells (a subtype of gut hormone-secreting cells) were unaffected by autoimmune responses in non-obese diabetic mice[18]. Converted cells could also be rendered hypoimmunogenic by overexpression of immunoregulatory factors such as PD-L1, HLA-G and CD47, and/or targeted knockout of HLA class Ia & II molecules[19,20].

A New Era for Diabetes Treatment?

Over the last couple of decades, significant advances have been made in deriving off-the-shelf functional grafts from hPSCs to restore T1DM in animal models, and in the development of encapsulation devices for minimally invasive administration. Although some of these devices have entered clinical trials, the complex multi-step differentiation protocols used to produce these functional grafts limit scalability and increase costs, thereby limiting the potential for widespread T1DM treatment. Alternatively, beta cell regeneration could take place *in vivo*, avoiding the need for graft production, encapsulation and implantation, but requiring careful selection of an appropriate conversion factor delivery system and source tissue to avoid dangerous off-target effects. Moreover, regenerated beta cells would need to evade autoimmune rejection, perhaps through genetic engineering. Moving forward, direct cell reprogramming, guided by big data approaches, could facilitate and refine development of scalable and efficacious T1DM cell therapies. By combining improvements such as these with the pioneering scientific efforts detailed in this article, regenerative approaches could bring an end to the almost century-long dominance of exogenous insulin as the gold standard of T1DM treatment.

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