REVIEW

Beta-cell function and human islet transplantation: can we improve?

Jennifer Chen[1] and **Jenny E Gunton**[1,2,3,4]

[1]The Westmead Institute for Medical Research, The University of Sydney Westmead Hospital, New South Wales, Australia
[2]Department of Endocrinology and Diabetes, Westmead Hospital, Sydney, New South Wales, Australia
[3]Garvan Institute of Medical Research, Sydney, New South Wales, Australia
[4]St Vincent's Clinical School, University of New South Wales, Sydney, New South Wales, Australia

Correspondence should be addressed to J E Gunton: jenny.gunton@sydney.edu.au

Abstract

Islet transplantation, a therapeutic option to treat type 1 diabetes, is not yet as successful as whole-pancreas transplantation as a treatment for diabetes. Mouse models are commonly used for islet research. However, it is clear disparities exist between islet transplantation outcomes in mice and humans. Given the shortage of transplant-grade islets, it is crucial that we further our understanding of factors that determine long-term islet survival and function post-transplantation. In turn, this may lead to new therapeutic targets and strategies that will improve transplant outcomes. Here, we summarise the current landscape in clinical transplantation, highlight underlying similarities and differences between mouse and human islets, and review interventions that are being considered to create a new pool of β-cells for clinical application.

Key Words
- β-cells
- islet transplantation
- human
- diabetes

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Introduction

The pancreas lies posterior to the stomach, extending from the spleen to the duodenum. It contains cells which perform exocrine and endocrine functions. Endocrine cells are found in clusters called the islets of Langerhans or pancreatic islets. Although they consist of only 1–2% of the adult pancreas, islets are critical for regulating glucose homeostasis. The hormone insulin is secreted solely by islet β-cells. When blood glucose levels rise, insulin is released. Diabetes occurs when β-cell dysfunction or loss leads to an inability to release enough insulin to control glucose. Other islet endocrine cell types include glucagon-secreting α-cells, δ-, pancreatic polypeptide (PP), and ε-cells.

Insulin reduces blood glucose by initiating peripheral glucose uptake particularly in muscle and adipose tissue, and repressing glucagon activity. Glucose transporters GLUT1, GLUT2 and GLUT3 (predominantly GLUT1) facilitate glucose uptake in human β-cells. In mouse β-cells, GLUT2 is the primary glucose transporter. Glucokinase phosphorylates glucose into glucose-6-phosphate, preventing the diffusion of glucose back into circulation; it is the major β-cell glucose-sensor for both organisms (De Vos *et al.* 1995). Glucose-6-phosphate is catabolised through glycolysis and the Kreb's cycle to produce ATP from adenosine diphosphate (ADP). Increased cytoplasmic ATP:ADP ratio is detected by ATP-sensing potassium channels which then close, depolarising the cell membrane. This opens voltage-gated Ca^{2+} channels, triggering insulin granule exocytosis.

The key function of differentiated β-cells is glucose-stimulated insulin secretion (GSIS). GSIS normally occurs over two phases. First-phase GSIS occurs rapidly, by definition, within 10 min of a glucose stimulus. This is thought to be mediated by the release of mature secretory insulin granules already close or docked to the β-cell plasma membrane. Then, there is a gradual second

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phase, beginning 10–20 min after glucose stimulus. In humans, the activating threshold for GSIS is 3 mmol/L glucose in the 'lowest' glucose-sensing cells, with a range of response thresholds recruiting more β-cells as glucose increases (Henquin *et al.* 2006). In rodents, the threshold is 5 mmol/L.

In type 1 (T1D) diabetes, β-cells are destroyed by the immune system (Atkinson & Eisenbarth 2001, Sherry *et al.* 2005), and in type 2 (T2D) diabetes, they have impaired function (van Haeften 2002, Gunton *et al.* 2005). In the early stages of T1D, there is preferential β-cell loss but later there is often 'by-stander' damage and loss of the other islet cell types as a consequence of the immune attack. In human T2D, there is differential β-cell loss, and insulin degranulation identified using electron microscopy.

Insulin immunohistochemistry is the most commonly used technique for labelling β-cells. However, negative immunostaining may be due to severe degranulation rather than loss of β-cell mass (Butler *et al.* 2003, Marselli *et al.* 2014, Cinti *et al.* 2016). However, some T2D patients have comparable granule density in nonhormone-expressing endocrine cells to nondiabetic controls which questions the degranulation model. The presence of these nonhormone-expressing endocrine cells may be attributed to β-cell dedifferentiation or formation of immature new endocrine cells (Butler *et al.* 2016). Supporting reversion to a nonmature state is the elevated expression of gastrin, a foetal pancreas biomarker, found in diabetic mouse β-cells and T2D human β- and δ-cells (Dahan *et al.* 2017).

Islet transplantation is a promising treatment for adults (>18 years) with T1D for a duration of 5 or more years and experiencing severe hypoglycemic unawareness events (Transplantation Society of Australia and New Zealand). The procedure aims to restore insulin independence, defined by the Collaborative Islet Transplant Registry (CITR) as the absence of the need for exogenous insulin administration for more than 14 consecutive days. However, clinical application is hampered by donor availability, and in the early transplant patients, poor rates of long-term graft function. Approximately half of all transplant recipients remained insulin independent 12 months after the last islet infusion; most experienced a gradual decline in function. While an overall reduction of exogenous insulin requirement and improved glucose handling was observed, only 16% of recipients were insulin independent across all follow-up times. However, importantly, severe hypoglycemia was eliminated in 90% of recipients over 5 years of follow-up. Variables affecting graft outcomes included recipient age, induction and maintenance immunosuppression, and total islet equivalents (IEQs) infused (CITR). Long-term graft function is hindered by numerous factors including the toxicologic profile of immunosuppressives, limited graft β-cell proliferation, and substantial islet necrosis and apoptosis (Shapiro *et al.* 2017).

Research efforts to study the human endocrine pancreas is limited by lack of access to tissue in living people, technical challenges in measuring β-cell function, and inability to measure β-cell mass. Few studies have attempted to take pancreas tissue from a living person: two Japanese studies described pancreatic punch biopsies as a safe procedure with minimal complications, but the Diabetes Virus Detection study (DiViD) was discontinued due to a high rate of complications (Imagawa *et al.* 2001, Krogvold *et al.* 2014). Thus the safety of obtaining pancreatic biopsies is not clear, and if it is not safe, then it is arguably unethical. Furthermore, islets are not homogenously distributed in the human pancreas, thus a single biopsy may not be representative.

In studying isolated islets, they are obtained only from organ donors. Availability is low, and there are challenges in isolating good quality human islets (Ricordi *et al.* 2016). In addition, islets de-differentiate soon after isolation in culture, losing their specialised phenotype (Negi *et al.* 2012, Spijker *et al.* 2013). Indeed, after 7 days, few islets retain GSIS. Consequently, many studies have looked at characterising islet physiology to further our understanding of mechanisms and pathways that contribute to graft failure post-transplantation (Omori *et al.* 2016, Cross *et al.* 2017, Gan *et al.* 2018). Others have focused on developing strategies to stimulate β-cell proliferation or providing an alternative source of β-cells by inducing cell transdifferentiation.

A limitation of human β-cell lines is that very few display GSIS (Halvorsen *et al.* 2000, Ravassard *et al.* 2011). In addition, *in vitro* models do not encapsulate the complex interactions between physiological systems in whole organisms that can affect graft outcomes. Thus, a commonly used model organism for studying β-cell function is the laboratory mouse, where advantages include its relative genetic similarity of humans, shorter lifespan and readily manipulable genome.

The drugs alloxan and streptozotocin are used to induce diabetes in mice (Kodama *et al.* 2005). They are taken up by the cells as glucose analogues and cause free-radical induced DNA damage. However, these drugs are one of the few examples where human islets are more robust than the animal models; human β-cells are resistant, possibly due to rapid export of the toxins (Yang & Wright 2002). This feature is useful when testing

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human islet transplantation, as recipient mice can be given diabetes-inducing doses of the β-cell toxins shortly before transplantation without damaging the human islets (Eizirik *et al.* 1994, Stokes *et al.* 2017). Other notable differences between mouse and human islets include the spatial arrangement of endocrine cell types, especially in large human islets, and differences in islet vasculature and autonomous nervous system (ANS) innervation. In this review, we will first provide a brief overview of the current islet transplantation landscape and then discuss the similarities and differences between mouse and human islet biology that could contribute to differential graft success.

Current state of islet transplantation

Deceased donors are the primary source of human islets for transplantation. In most cases, they are brain-dead but heart beating, although some countries use non-heart-beating donors. Islet isolation yield and success rate is generally higher in heart-beating donors, and obese and younger donors (<47 years) although there are conflicting reports (Matsumoto *et al.* 2004, Niclauss *et al.* 2011). Graft function of islets from donors >40–45 years old perform worse, possibly due to defects in intracellular ATP production (Ihm *et al.* 2006, Niclauss *et al.* 2011).

In humans, restoration of normoglycemia and insulin independence in many patients requires ~12,000 IEQ/kg body weight (Shapiro *et al.* 2000). This usually is obtained from 2 to 3 donors. Considering the substantial number, it is critical to find strategies to minimise the number of islets that are lost during isolation and post-transplantation (Davalli *et al.* 1995). In more recent years, increasing numbers of people become insulin independent with islets from 2, or sometimes only 1 donor. Activation of the recipient's immune response to transplanted islets remains a barrier yet to be completely overcome (Bennet *et al.* 2000, Barra & Tse 2018).

The longevity of graft function has clearly progressed since the initial successes with the Edmonton protocol immunosuppression regimen (Brennan *et al.* 2016). This glucocorticoid-free protocol with low dose tacrolimus (a calcineurin inhibitor (CNI)), sirolimus (a mTOR (mechanistic target of rapamycin, previously called the mammalian target of rapamycin) inhibitor) and an interleukin-2 receptor antagonist (Daclizumab) saw improvements over the modest success initially seen with the drug combinations used for whole organ transplantation. The 'Edmonton protocol' has evolved since then; post-2007 saw a shift towards T-cell depleting agents (e.g. Alemtuzumab, hOKT3y-1-ala-ala) and TNF alpha (TNFα) inhibitors with or without interleukin-2 receptor-antagonists for immunosuppression induction (CITR). These changes aim to control early inflammatory events that may limit short-term graft survival. Benefits of TNFα antagonists requires T-cell depletion at induction (Froud *et al.* 2005, Williams *et al.* 2018).

Similarly, there has been a shift in maintenance immunosuppression therapy away from mTOR inhibitors to a combination of CNI and inosine-5′-monophosphate dehydrogenase (IMPDH) inhibitors (mycophenolate mofetil or mycophenolic acid) between 2007 and 2015 (CITR). The side effect profile of mTOR inhibitors may explain this change, yet pre-treatment with mTOR inhibitors before transplant can enhance insulin sensitivity (Benedini *et al.* 2018). In line with this, the well-documented adverse outcomes of long-term CNI use has prompted investigation into alternate primary immunosuppressants. CNI-free protocols involving co-stimulation blockade-based agents (Belatacept, Abatacept) or a combination of mTOR and IMPDH inhibitor maintenance have been trialled with up to 40% of patients retaining insulin independence for at least 3 years (Maffi *et al.* 2014).

Transplant sites

It is clear that mouse islets usually provide superior transplant outcomes to human islets, even with comparable or larger mass of human islets (Stokes *et al.* 2017). Most studies in mouse recipients use the kidney capsule as the graft site as the surgical protocol is relatively easy and gives superior outcomes. However, the human kidney does not readily allow creation of an anatomical space under the capsule to place the islets. In addition, there are valid concerns about potential damage to the kidney in people with diabetes. Moreover, some of the immunosuppressive medications used in islet transplant recipients can cause renal impairment.

The current clinical site for human islet grafts, the portal vein, leads to marked loss of islets in the short term. This is estimated to be over 50%, and many patients experience 'dumping' shortly after transplantation where they have hypoglycaemia due to release of large quantities of insulin from dying β-cells. An ideal implantation site would minimise clotting, especially the immediate blood mediated immune reaction (IBMIR), have high blood vessel density for adequate islet blood supply and oxygenation and be readily accessible and safe surgically.

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Notably, Sertoli cells in the testes and parenchymal cells of the eye anterior chamber both possess site-specific immunosuppressive mechanisms to circumvent clotting and IBMIR (Selawry & Cameron 1993, Griffith *et al.* 1995). This could act to reduce or eliminate the need for chronic systemic immunosuppression. Sertoli cells have the capacity to induce β-cell regeneration and augment graft survival in mice (Luca *et al.* 2010, Li *et al.* 2013). In the eye, there are a high concentration of autonomic nerves and blood vessels. Moreover, a relatively low number of islets are needed for glycemic control without compromising visual acuity. One syngeneic mouse model found that islets engrafted into the eye were susceptible to immune attack but this may be due to variations in islet preparation and surgical protocol (Mojibian *et al.* 2013). Establishing the safety and efficacy of this approach is the subject of active clinical trials (NCT02916680, NCT02846571, NCT04198350). We speculate that the clinical acceptability of ocular transplants will vary between patients.

Other alternate engraftment sites being evaluated in preclinical models and early clinical trials include muscle, bone marrow, and omentum (Christoffersson *et al.* 2010, Stokes *et al.* 2017, Stice *et al.* 2018). Revascularisation of islets before transplantation is also being considered to prevent graft loss, although hypervascularisation may facilitate cytokine immune attack (Bowers *et al.* 2019).

Agents to improve islet viability in culture

Most centres have adopted variations of the Edmonton protocol for isolating and transplanting human islets. Islets are cultured short-term for quality control studies including assessment of purity, viability, and lack of infection (Hering *et al.* 2004, Froud *et al.* 2005, O'Connell *et al.* 2013). There is some controversy regarding whether culturing islets pre-transplantation results in better islet engraftment outcomes (Noguchi *et al.* 2012). Nevertheless, the culturing period provides a window where islets may be treated with agents that promote regeneration of vasculature and extracellular matrix (ECM) fractured during isolation, or counteract localised coagulation cascade response. After intraportal islet infusion, islets are exposed to the portal venous oxygen tension, which is relatively hypoxic (Carlsson *et al.* 2001). Data from a computational oxygen simulation support the hypothesis that the decrease of oxygen tension is a primary cause of central necrosis in cultured islets (Komatsu *et al.* 2017). To maximise transplantation success, it is important to re-establish oxygenated blood flow post-transplantation either by minimising destruction of the original islet vascular network or stimulating re-vascularisation in the implantation organ (reviewed in (Jansson & Carlsson 2002)).

The drug deferoxamine is an iron chelator which inhibits degradation of the hypoxia inducible factor 1α (HIF-1α) protein. Overnight culturing of human islets in deferoxamine using normal therapeutic concentrations had a protective effect on islets transplanted into mice and led to greater CD31/PECAM1 immunostaining, a biomarker for blood vessels. This suggests there was greater angiogenesis in the human islets, although the study period was limited to 28 days. Islet cell apoptosis was lower in deferoxamine treated islets. Glucose outcomes for human islets transplanted into mice were superior for islets cultured with deferoxamine pre-transplantation (Stokes *et al.* 2013).

Other studies have focused on reducing the rapid production of monocyte chemoattractant protein-1 (MCP-1/CCL2) and tissue factor. These proteins induce inflammatory and apoptotic responses through the recruitment of clotting factors (Johansson *et al.* 2005). Nicotinamide, a vitamin B3 isotype (Moberg *et al.* 2003) has been proposed to treat this. This compound has also been implicated in the prevention of macrophage mediated destruction of rodent islets (Kolb *et al.* 1990). In a syngeneic islet transplant mouse model, pre-culturing islets (300 IEQ) in nicotinamide allowed a faster return to normal glucose in addition to prolonging graft survival (Jung *et al.* 2009). In a mouse model of ischemic injury, increasing NAD (NAD+) levels stimulated neovascularisation and restored vascular density in muscle (Das *et al.* 2018). Phase 1 clinical trials examining agents which may augment isolated islet survival include the synthetic peptide PKX-001 (NCT03073577), thought to be cytoprotective against immunosuppressant toxicity and BMX-010 (NCT02457858), an antioxidant which has been shown to be efficacious in mouse islet engraftment outcomes.

Another strategy currently being explored to increase islet viability targets the intra-islet and peripheral islet ECM and endothelium that is damaged during enzymatic dissociation of islets. Mesenchymal stem cells (MSC), implicated in tissue repair and maintenance of cellular homeostasis, secrete factors with anti-inflammatory, proangiogenic and regenerative properties (Kuljanin *et al.* 2019). MSCs can be isolated from many sources such as adipose tissue and bone marrow. Moreover, MSCs isolated from patients with chronic pancreatitis have comparable effectiveness in protecting human islets exposed to

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hypoxia as those isolated from a healthy donor (Wang *et al.* 2019). Islets precultured with adipose-derived MSCs reversed hyperglycemia in a syngeneic mouse transplant model. Notably, recovered MSC-treated islets infused at the intraportal site had double the average islet area compared to untreated islets (Rackham *et al.* 2014). Subsequent investigations suggested that one mechanism may be mitochondrial transfer from the MSCs to islets but it is unclear if this effect can be replicated in human islets (Rackham *et al.* 2020). Co-culture of MSCs with a mouse islet microvascular endothelium cell line (MS-1) may have beneficial effects on islet endothelial cell function through activation of the Wnt signalling pathway (Wang *et al.* 2017). While these findings collectively show that MSCs have protective effects, timely expansion of MSC populations *in vitro* and protocol standardisation may be clinically difficult (Rackham *et al.* 2018). This has prompted research into islet preculture with 'cell-free' exogenous MSC secretory factors cocktails. However, this achieved only modest success in restoring normal glycemic control in diabetic rodents (Rackham *et al.* 2018).

Islets – differences between human and mouse

Human islets are distributed for research use when isolated islets do not meet clinical transplant requirements and the donor had consented for research in this event. As previously noted, mouse-to-mouse islet transplants have a greater rate of success compared to human-to-mouse transplants. There are distinct differences between mouse and human islets which may underlie this disparity; we will discuss some of these similarities and differences below.

Islet architecture

β-cells are the predominant cell type in mouse islets, averaging about 70% of islet volume. They are arranged in a largely homotypic core, meaning the majority of β-cells neighbour other β-cells. This core is surrounded by a 'halo' of α-cells, in addition to smaller numbers of δ-, PP, and ε-cells (Fig. 1). Mouse islets are usually round or oval in shape, and in normal animals, there is little divergence from the norm.

During the developmental stage, β-cell arrangement in humans is similar to mice (Jeon *et al.* 2009). In the adult, some human islets also share a similar cellular arrangement to mouse islets, perhaps dependent on islet size (Bonner-Weir *et al.* 2015). However, in other islets, β-cells are arranged in streams, interspersed between α- and δ-cells (Fig. 1). Human islets also come in a wider range of sizes and shapes than normal mouse islets. In an obese but normal glucose tolerant person, human islets can be over 500 µm in size, whereas in normal mice, islets are usually 150 µm. For small human islets, β-cells constitute the majority cell type, but large islets possess a similar proportion of α- and β-cells (Kim *et al.* 2009). In a person with normal glucose tolerance, the proportion of β-cells in human islets is lower than that in mice, being 48–59% (Cabrera *et al.* 2006).

It is likely that despite having more β-cells, very large islets are dis-advantageous for transplantation. When islets are isolated, they are removed from their original blood supply, limiting the diffusion of oxygen and nutrient supplied from the outside surface. Thus, hypoxia provides a great challenge for islet survival. Larger islets by definition have a greater distance from the surface to the centre, possibly impairing survival of the islet core (Komatsu *et al.* 2017). Very large islets are frequently isolated from overweight or obese human donors, but these often undergo central or complete necrosis or apoptosis (Burke *et al.* 2016). We suggest that larger islets are likely to contribute to the relatively lower tolerance of human islets to the isolation and transplant process. Isolated islets intended for transplantation are categorised using the islet equivalent number (IEQ) scale where one IEQ represents an islet sphere of 150 µm diameter. Taking these factors into account, we propose that larger islets should be assigned a lesser weighting on the IEQ scale in consideration of their predicted smaller contribution

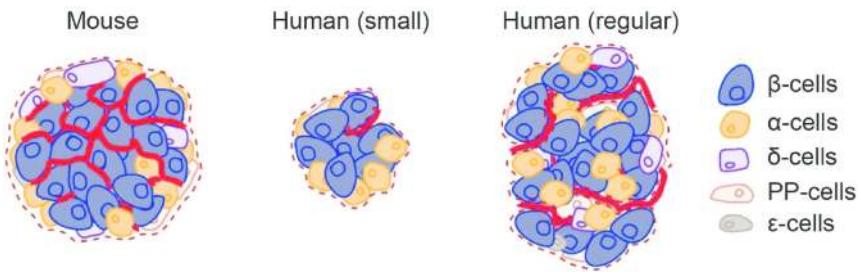

Figure 1
Schematic of endocrine cells arrangement in mouse, and small and regular-sized human islets of Langerhans. The islet vasculature network is depicted in red. Broken lines represent the basement membrane.

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to recipient glycemic control. The total IEQ isolated per kilogram of recipient weight is a major factor in the decision of whether islets will be transplanted.

Islet vasculature and basement membrane

Mouse pancreatic islets possess high capillary density and have a surrounding outer capsule and basement membrane that is in contact with individual β-cells. Possibly formed by endothelial cells, the basement membrane acts as an ECM and is thought to play a role in insulin production and release, and cell proliferation. In humans, blood vessels in islets are surrounded by a double layer basement membrane but there is no outer-islet capsule (Kragl & Lammert 2010). This may make them more susceptible to fragmentation. The proteins in the murine islet extracellular matrix have been characterised, but less is known about these proteins in human islets (Llacua *et al.* 2018).

ECM components such as collagen or fibronectin are important for angiogenesis. Arterial blood flow is up to 20 times greater (by weight) in islets compared to the exocrine pancreas. In rats, this amounts to about 10–15% of total blood flow in the pancreas (Jansson & Hellerström 1983). This network is disrupted during isolation whereby islets are in an avascular state post transplantation (Irving-Rodgers *et al.* 2014). Thus, core hypoxia, leading to cell death becomes a major issue, as inadequate oxygen and nutrients diffuses from the periphery to the core. This issue permeates the steps of the isolation, culture, and transplantation process, contributing to graft failure. Revascularisation commences approximately 3 days after engraftment and can take up to one month to complete (Gibly *et al.* 2011). Increased necrosis and apoptosis in avascular islets led to a substantial loss in islet mass by 72h after transplant (Biarnés *et al.* 2002). Presently, there is no proangiogenic drug approved by the Food and Drug Administration (FDA). Preculturing without supplementation appears to inhibit vascular engraftment (Olsson & Carlsson 2005). Transplantation of MSC-endothelial cell islet composites or the engineering of supportive synthetic scaffolds may stimulate angiogenesis (Takahashi *et al.* 2018, Bowers *et al.* 2019).

Human islets exhibit a five-fold less dense vasculature per islet area (Brissova *et al.* 2015), making them even more likely to succumb to these problems. Indeed, there was a 64% reduction in human islet vascular fraction 8 weeks post transplantation into the anterior chamber of the mouse eye (Cohrs *et al.* 2017). Interestingly, blood flow direction also differs between the two species, influenced by islet cell organisation. In humans, blood flows either in a polarised manner, from one side of the islet to the other, or travels outward from the islet core to the mantle from a primary feeding arteriole. (Christoffersson *et al.* 2010, Tang *et al.* 2018). It is possible that variations in blood flow directionality is linked to islet size, dependent upon the variability in human islet cytoarchitecture seen between small and large islets, concentrating around β-cell clusters to support their high oxygen needs (Bonner-Weir *et al.* 2015). Corroborating this notion is the reported polarisation of β-cells, where the majority of insulin granules are located at a distinct basal region adjacent to the islet blood vessels.

In rodents, three different blood flow patterns have been described in medium to large islets (160–500 µm): the arteriole feeds the inner islet core, and blood then flows outwards to arrive at non-β-cells, other endocrine cells are first supplied at the periphery before travelling towards the β-cell core, or from one pole of the islet to another. In general, core to peripheral cell blood flow is the most common pattern, perhaps also reflective of the spatial arrangement of mouse endocrine cells. Polar or outer-to inner directional flow was found in 40% of islets (Nyman *et al.* 2008). In mice, there are high levels of contact between endocrine cells and vasculature (>90%) which could allow greater flexibility. In both species, islet size does not influence vascular fraction. Post transplantation, the revascularisation pattern may be influenced by blood flow direction and by donor age (Cohrs *et al.* 2017).

Islets isolated from T2D patients exhibit greater vasculature density than non-diabetic controls. A higher frequency of vessel fragmentation accounts for this observation which in turn may contribute to β-cell dysfunction and death (Brissova *et al.* 2015). This change is also observed in T1D islet vessels (Canzano *et al.* 2019). Loss of β-cell mass may also bring the vasculature closer together.

Islet innervation

Innervation in islets also differs between mice and humans. The murine islet core of β-cells is largely innervated by parasympathetic fibres, and the outer part of the islet containing α- and other cell types are densely supplied by both sympathetic and parasympathetic nerves (Rodriguez-Diaz *et al.* 2011*a*, Tang *et al.* 2014). Interestingly, young ob/ob and db/db mice, often used as a model for T2D were found to have 49% greater sympathetic nerve density than their lean littermate counterparts. Islets undergoing chronic sympathetic stress are postulated to accelerate β-cell degeneration (Chien *et al.* 2016). In contrast, human islets have comparatively sparse innervation and some islets have no detectable

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innervation of endocrine cells. Instead, the sympathetic axons are in contact with pericytes and smooth muscle cells of the vasculature; this enables the stimulation of contractile cells of the islet vascular network to control of the rate of blood flow in the islet vasculature by changing blood vessel diameter (Almaça *et al.* 2018). Thus, input from the ANS may play an important role in human islet function by driving endocrine signalling to cells downstream of release sites in order to modulate overall insulin secretion (Rodriguez-Diaz *et al.* 2011*b*). Similarly, diabetic human islets are also more densely innervated as seen in diabetic mouse models (Tang *et al.* 2018).

Overall, there is a lack of studies analysing reinnervation of islets after transplantation. The rate of innervation is difficult to assess in humans post transplantation. A wide range of tracers and probes intended for *in vivo* β-cell imaging using magnetic resonance imaging (MRI), optical imaging, positron emission tomography (PET), and single-photon emission computed tomography (SPECT) are under evaluation in preclinical and clinical settings (Wei *et al.* 2019). In extracted tissue samples, the development of optical clearing techniques used with confocal and light sheet microscopy allows for mapping of graft reinnervation. Islet grafts obtained in mice at different time points revealed that there was projection of sympathetic nerves to α-cells 3 weeks post-transplantation under the kidney capsule. After 6 weeks, sympathetic nerve density in diabetic mouse recipients only reached 60% of that of pancreatic islets measured in situ (Juang *et al.* 2014). Optical clearing methods have also challenged the previous notion that there is minimal projection of sympathetic and parasympathetic nerve projection into the human islet core (Tang *et al.* 2018).

In mouse transplants, the transplantation site affects the rate of innervation (Korsgren *et al.* 1993). Extrapolating this data, reinnervation efficiency may contribute to the differences in transplant success that vary by organ location. It is interesting to note that the reinnervation of islets is mostly derived from sympathetic nervous system in the liver or spleen, but a mixture in the kidney capsule which may contribute to the greater transplant success rates observed at this site (Stokes *et al.* 2017).

Alternative strategies of sourcing β-cells

Stimulating beta-cell proliferation *in vivo*

The difficulty in obtaining human islets suitable for transplantation has directed considerable research focus into sourcing an adjunct pool of islets suitable for transplantation or replenishing β-cells lost in diabetes pathogenesis. Inducing endogenous β-cell proliferation in diabetic subjects to counteract dysglycemia may circumvent the long wait for a suitable donor.

In humans, after the neonatal period, there is a sharp decrease in β-cell proliferation, with this being notably muted in adults at under 0.5% (Meier *et al.* 2008, Gregg *et al.* 2012). A decline of β-cell proliferation is also observed in mice (Krishnamurthy *et al.* 2006). There is also little evidence of β-cell regeneration capacity in the adult human pancreas. However, a small number of studies show that under certain circumstances, human β-cells do display ability to proliferate (Ogilvie 1933, Van Assche *et al.* 1978, Butler *et al.* 2010). In contrast, mice show excellent regeneration capacity, at least when young.

In humans, compensatory β-cell hyperplasia correlates with weight gain in the absence of diabetes (Rahier *et al.* 2008). Overweight and obese people who maintain normal glucose tolerance have increased β-cell mass compared to lean normal people, suggesting that they have had increased β-cell proliferation over time (Ogilvie 1933). Conversely, their weight matched counterparts with T2D have decreased β-cell mass accompanied by increased β-cell loss by apoptosis. Donors with T2D have markedly greater adipocyte infiltration in the whole pancreas compared to the overweight and obese with normal glucose tolerance (Tang *et al.* 2018). By contrast, diabetic mice fed a high fat diet with significant weight gain have increased β-cell mass and proliferation compared to mice that consumed a normal diet (Peyot *et al.* 2010).

β-cell mass increases during pregnancy, mostly mediated by prolactin and placental lactogen. This is more prominent in mice, compared to humans (Van Assche *et al.* 1978, Parsons *et al.* 1992, Butler *et al.* 2010). Normal pregnancy is a state of insulin resistance, particularly in the third trimester when some women develop gestational diabetes. Women who maintain normal glucose tolerance in pregnancy do so by increasing their β-cell function. Total β-cell function is determined by β-cell mass and the function of individual β-cells. There are only small autopsy studies looking at β-cell mass in pregnant women, but it appears that there is a significant increase in β-cell mass in normal pregnancy (Van Assche *et al.* 1978, Butler *et al.* 2010). Interestingly, the earlier study involving a larger number of younger women had an increase in β-cell number, whereas in the second study where there were a higher number of older pregnant women, there was a greater increase in β-cell area rather than number. This suggests the possibility of an age-related decline in capacity of β-cells to proliferate in response to

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pregnancy. It is consistent with maternal age being a major risk factor for gestational diabetes (Freinkel 1980).

The mechanism via which prolactin acts is at least partly through the inhibition of menin, a transcriptional regulator (Karnik *et al.* 2007). Numerous other mitogenic stimuli or pathways have been shown in mice, and ex vivo and grafted human islets to have the potential to promote β-cell proliferation. These include glucose itself (Levitt *et al.* 2011), glycogen synthase kinase 3 beta (GSK3B) inhibition (Shen *et al.* 2015), phosphorylated AKT (Hughes & Huang 2011), and gamma-aminobutyric acid (GABA) (Soltani *et al.* 2011) (Fig. 2). However, the lack of uniform donor characteristics and recipients used to examine the efficacy of these mitogens may contribute to the conflicting findings reported. For example, grafted human islets exposed to chronic hyperglycemia in mice did not have a higher proliferation rate than those in normoglycemic mice (Dai *et al.* 2016). A possible explanation could be that islets used in Dai *et al.* (2016) were from healthy, non-diabetic human donors whereas the study by Levitt *et al.* (2011) who found that glucose infusion did indeed induce human β-cell proliferation in grafts also observed that this was positively correlated with increasing donor body mass index (BMI). As mentioned earlier, in non-diabetic humans, as BMI increases, so does β-cell mass. Another example of this is the ectopic expression of the transcription factor paired box 4 (PAX4). Mouse PAX4 stimulates β-cell proliferation in human islets, yet human PAX4 does not (Brun *et al.* 2004).

Mitogens that do stimulate β-cell proliferation could pose as novel treatment strategies, but a common pattern is that the increase in proliferation is often small (up to 2–4% proliferation rate from ≤1%). This suggests stimulating β-cell proliferation may be a complementary, but not a frontline therapy for treating diabetes.

Alpha to beta cell transdifferentiation

Another area that has gained attention is the induction of α-cell transdifferentiation into β-cells. During development, pancreatic ductal, endocrine and exocrine cells arise from a common multipotent progenitor. Transdifferentiation is a related but different process when already differentiated cells are converted into a different cell type (Fig. 3). While β-cells are terminally differentiated, in rodents, adult endocrine and exocrine cells possess remarkable plasticity, giving a potential avenue for sourcing β-cells. This has been shown in mouse models of profound β-cell loss and lineage tracing experiments. Some studies report bihormonal expression of insulin and glucagon in mouse α-cells after near total β-cell destruction, while others have observed age-dependent spontaneous reprogramming of mouse δ-cells into insulin producing cells (Thorel *et al.* 2010, Chera *et al.* 2014). Other groups have successfully

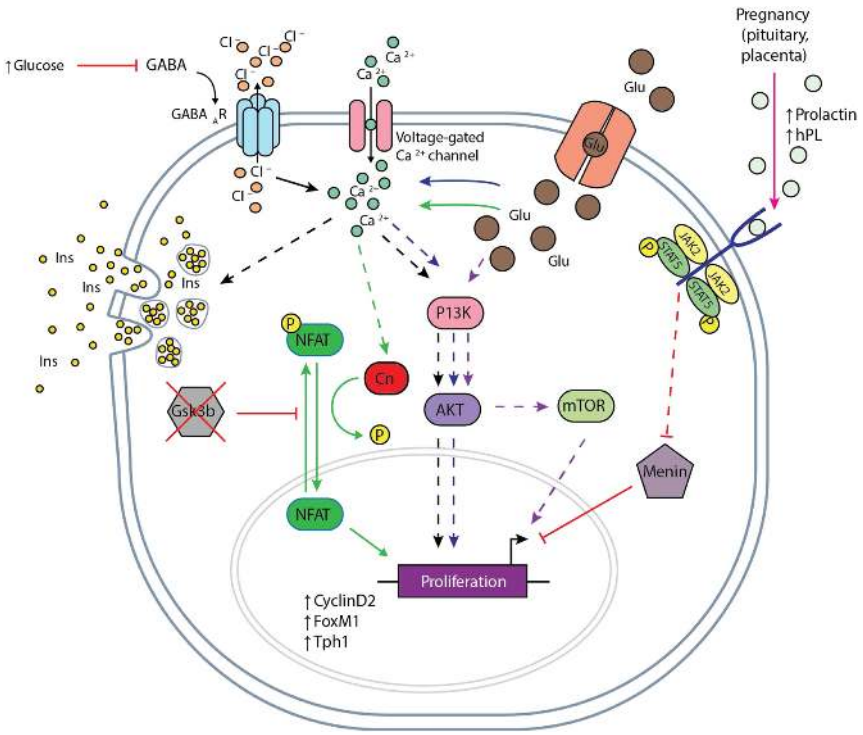

Figure 2
A number of mitogens have been implicated in promoting β-cell proliferation in mice and humans. These are summarised in this figure. Influx of glucose into the cell either activates PI3K/AKT/mTOR signalling (purple arrow), or the resulting Ca^{2+} influx activates PI3K/AKT/mTOR signalling (blue arrow) or calcineurin which dephosphorylates NFAT (green arrow) to stimulate β-cell proliferation. Activation of $GABA_AR$ Cl^- channel depolarises the membrane to allow the entry of Ca^{2+} into the cell (black arrow) which signals through the PI3K/AKT/mTOR pathway. Hormones released during pregnancy activate the JAK2/STAT5 pathway which inhibits the anti-proliferative effects of menin (pink arrow). AKT, protein kinase B; Cn, calcineurin; FoxM1, forkhead box protein M1; $GABA_AR$, GABA type A receptor; hPL, human placental lactogen; JAK2, Janus kinase 2; mTOR, mechanistic target of rapamycin; NFAT, nuclear factor of activated T-cells;PI3K, phosphoinositide 3-kinase; STAT5, signal transducer and activator of transcription 5, Tph1, tryptophan hydroxylase 1.

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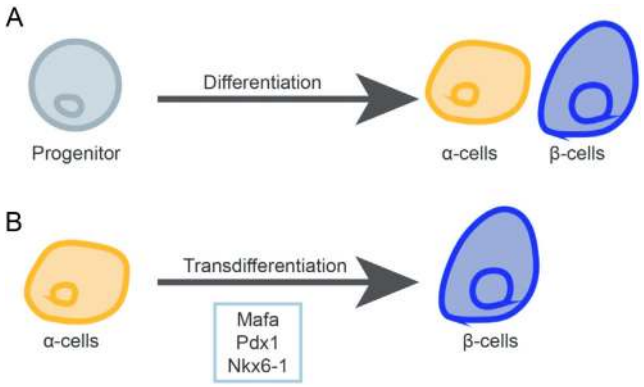

Figure 3
Endocrine cell (A) differentiation and (B) transdifferentiation. Transdifferentiation of α-cells into β-cells can be induced through the ectopic expression of transcription factors important in β-cell development and maturation.

induced endocrine cell transdifferentiation in mouse α-cells, largely through the overexpression of transcription factors required for β-cell development such as PDX1 (pancreatic and duodenal homeobox 1), or maturation such as MAFA (MAF bZIP transcription factor A, important for murine maturation) (Matsuoka *et al.* 2017, Xiao *et al.* 2018). There is also a field of research on ductal and acinar cells reprogramming into β-cells (Bonner-Weir *et al.* 1993, Desai *et al.* 2007). This is covered in more detail in the following reviews (Wei & Hong 2016, Aguayo-Mazzucato & Bonner-Weir 2018).

During the early developmental stages, human glucagon-positive cells co-express insulin (9–21 weeks old foetus) (Jeon *et al.* 2009). However, the capacity for adult human endocrine cells to transdifferentiate is questioned. Examination of pancreata and islets isolated from T1D patients found no evidence of α- to β-cell differentiation (Brissova *et al.* 2018). On the other hand, bihormonal expression in endocrine cells during pregnancy or in T2D pancreas has been reported (Butler *et al.* 2013, Yoneda *et al.* 2013). Recent advances in genome sequencing techniques have allowed the mapping of endocrine cell gene expression profile. Chromatin profiling using ATAC-seq (Assay for Transposase Accessible Chromatin with high-throughput sequencing) of human α- and β-cells lends support to the notion of α-cell epigenomic plasticity. Sorted human α-cells shared a similar open chromatin profile for genes that were found to be specific to β-cells, indicative of a fluid cellular identity (Ackermann *et al.* 2016). Further support for transdifferentiation in human pancreatic cells was recently demonstrated by Furuyama *et al.* (2019) using human islets. Murine β-cell transcription factors PDX1, MAFA and NKX6-1 (NK6 homeobox 1) were transduced into purified human α-cells using a bicistronic adenoviral vector prior to transplantation into diabetic immunodeficient mice. Achieving approximately a 38% success rate with the combination of PDX1 and MAFA, the transplanted cells were functional with diabetic mice demonstrating normoglycemia for up to 6 months. It is unclear why ~62% of cells did not undergo transdifferentiation. Perhaps success was influenced by transduction efficiency, or that the successful groups of transdifferentiated β-cells shared a particular baseline phenotype (Furuyama *et al.* 2019). Cellular heterogeneity in β-cells is well documented (Benninger & Hodson 2018).

In mice, a low α-cell count is sufficient to prevent dysregulation and maintain the counter-regulatory action of glucagon in both normal and diabetic mice (Shiota *et al.* 2013). The situation is less clear in humans; our ability to measure α-cell function *in vivo* is less exact than measuring β-cell function. Similar to β-cell mass, we cannot accurately measure α-cell mass in a living person. Human islets have a greater number of α-cells, and some studies suggest α-cell mass expansion in T1D and T2D diabetic humans (Klöppel *et al.* 1985, Cinti *et al.* 2016). However, a recent study refutes this in the context of T1D (Brissova *et al.* 2018). Evaluation of T1D α-cells found a decline in functional activity. The α-cells also had altered expression of transcription factors important in preserving α- and β-cell identity (Brissova *et al.* 2018). Another study reports no change in α-cell mass in T2D (Marselli *et al.* 2014). Overall, transdifferentiation may also be a limited strategy for treating diabetes.

Conclusion

Human islet transplantation is a therapeutic option to treat diabetes, although there is still varied success in clinical outcomes. There is considerable interest in alternative sources of islets such as stimulating endocrine cell proliferation and transdifferentiation. However, these strategies are still in early stages. While using murine models allows for controlled studies that target specific pathways or genes, there are often challenges in translating findings, perhaps due to the fundamental differences in islet physiology between the mouse and human.

These differences can be partly overcome by using a 'humanised model' where human islets are transplanted in the diabetic mouse (or other animal), allowing study of human islets after transplantation. Better understanding of factors influencing human islet survival will help to

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optimise human transplant outcomes. In summary, islet transplantation remains a promising treatment, with the outcomes steadily improving over time, but further refinements are still needed to achieve the very high rates of success seen with whole pancreas transplants.

Declaration of interest

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

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