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REVIEW

Bioengineered stem cells as an alternative for islet cell transplantation

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Abstract

Type 1 diabetes is an autoimmune and increasingly prevalent condition caused by immunological destruction of beta cells. Insulin remains the mainstay of therapy. Endeavours in islet transplantation have clearly demonstrated that type 1 diabetes is treatable by cellular replacement. Many challenges remain with this approach. The opportunity to use bioengineered embryonic or adult pluripotential stem cells, or islets derived from porcine xenograft sources could address future demands, but are still associated with considerable challenges. This detailed review outlines current progress in clinical islet transplantation, and places this in perspective for the remarkable scientific advances now occurring in stem cell and regenerative medicine approaches in the treatment of future curative treatment of diabetes.

Key words: Islet transplantation; Hypoxia; Stem cell; Diabetes

Core tip: This paper gives a historical overview of the use of islet transplantation for the treatment of type 1 diabetes mellitus. Islet cell transplantation has seen enormous development over the years; however, this has not been without its limitations. The aim of this paper is to provide an overview of the feasibility of an alternative cell source for clinical islet transplantation.

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INTRODUCTION

Type 1 diabetes mellitus (T1DM) is an autoimmune disease characterised by impairment of pancreatic beta cells resulting in complete insulin deficiency. Current treatment requires multiple insulin injections and dietary restriction. However, even with strict management and blood glucose level monitoring, episodes of hypoglycaemia and chronic diabetic complications (such as nephropathy, retinopathy, and neuropathy) still occur[1]. Islet transplantation offers an alternative treatment option through restoration of the physiological response to changes in blood glucose levels. However due to ongoing clinical challenges, this modality is only offered to a select group of patients.

The Edmonton group was the first to demonstrate sustained long-term insulin-independence in 2000[2] through islet transplantation and from this success the "Edmonton Protocol" was established. Islet transplantation is a relatively non-invasive procedure that involves infusion of islets containing the insulin-secreting beta cells derived from cadaveric donors, into the recipient's portal vein. Despite high rates of insulin independence one-year post-transplant, patient follow-up has demonstrated islet graft attrition with time such that insulin independence rates significantly decline 5-year post-transplant with patients being restarted on small to modest amounts of insulin[3,4].

A major caveat to the current protocol is that a subset of patients will require repeat islet transplantation. One reason for this is due to poor initial engraftment[5] resulting in a reduced initial beta cell mass. The current limitations to engraftment are multiple and include variance in the islet isolation process[6], site of transplantation[7,8], and instant blood-mediated inflammatory reactions[9,10]. The outcomes of transplanting islets into alternative transplant sites have been well studied over the past two decades, but no site has received as much attention as the subcutaneous site[11-16]. This is in large part related to its potential for less invasive retrievability, which may translate into increased safety. It should be pointed out however, that despite the obvious limitation of the intraportal hepatic site, no patient has yet been rendered insulin-free by cellular transplantation in a site other than the liver. The other reason for requiring a subsequent transplant is that islet cells undergo progressive graft failure[17,18] largely related to auto- and alloimmunity. Lifelong immunosuppression has played a central role in the success of the current islet transplantation protocol. Despite ongoing development of immunosuppression agents and optimised regimens, progressive graft loss is still an enduring issue. This is further exacerbated by the diabetogenicity of many of the immunosuppression drugs implemented in clinical practice[19]. Furthermore lifelong immunosuppression regimens are also related with significant morbidity to the patient[20,21]. As an alternative to immunosuppression, the utility of immune isolating devices is currently being explored[22].

A review of the current islet transplantation protocol indicates well-recognised limitations. Herein, we discuss the potential of using bioengineered stem cells as an alternative cell source to address the acute organ donor shortage and meet potential future need in the ever-expanding diabetes population. A historical summary will discuss the roadblocks that were overcome in developing the "Edmonton Protocol", with a highlight on the research that has evolved since describing the pathophysiology behind its current limitations. The use of immunosuppression-free regimens and the use of the subcutaneous site will be reviewed. Predicted outcomes of synergising these research areas with bioengineered stem cells will be discussed. Focus will be on the feasibility and limitations of translating this idea into clinical practice.

RESEARCH STRATEGY

Studies were identified through Medical Subject Headings in PubMed. The following text words were used: (1) ["Islets of Langerhans Transplantation" (Mesh)] AND ["Neovascularization, Pathologic" (Mesh)]; (2) ["Islets of Langerhans Transplantation/methods" (Mesh)] AND ["Subcutaneous Tissue" (Mesh)]; (3) ["Islets of Langerhans Transplantation" (Mesh)] AND ["Vasculature" (Mesh)]; (4) ["Islets of Langerhans" (Mesh)] AND ["Stem Cells" (Mesh)]; and (5) ["Islets of Langerhans" (Mesh)]) AND ["Immunosuppression" (Mesh)]. In addition, reference lists of all relevant articles were examined for further pertinent studies. Inclusion criteria included articles published in peer-reviewed journals and animal studies. Exclusion criteria included gray literature, novel lab techniques, and articles that lacked an abstract. The search was limited by the ability to access articles. Primary authors and experts in the field were not contacted to identify additional published, unpublished, or "in-progress" studies. Information was last accessed in June 2014.

DISCUSSION

Historical vignettes

Insulin was first discovered through the efforts of Nobel Prize winners Banting and Best in the 1920s[23,24]. As a result of their efforts, exogenous insulin replacement therapy is and remains the mainstay treatment for T1DM[24]. However, even with strict regulation, there is a small subset of patients with "brittle diabetes" who are unable to achieve normoglycemia and suffer from life-threatening hypoglycaemic unawareness[1]. It is this group of patients who will benefit the most from cellular replacement therapy. Interestingly, attempts at cellular replacement actually preceded Banting and

Best's discovery of insulin by twenty years, whereby efforts were made to treat a 13-year-old diabetic child with fragments of sheep pancreata[25].

Given the clinical difficulties in managing patient complications through insulin replacement therapy alone, attention turned to transplantation with the hopes of offering a cure to diabetes. Kelly and Lillehei at the University of Minnesota were the first to attempt whole pancreas transplantation in 1966[26] carried out as a simultaneous pancreatic kidney (SPK) transplantation. Over the past several decades the surgical techniques have been refined, with most attention being directed towards exocrine drainage of the pancreas into the recipient intestine[27]. Since its introduction, over 35000 transplants have been carried out worldwide, mostly as SPK, with proven success in reversing diabetes and achieving insulin independence[28]. However, this involves major abdominal surgery with procedural techniques that are still undergoing refinement. Subsequently, it is primarily reserved for patients with end-stage renal disease associated with T1DM for whom dialysis and insulin independence can be achieved simultaneously.

An alternative to whole pancreas transplantation is islet transplantation. The ability to isolate islets evolved from the work of Best and Banting in their endeavours to isolate insulin[23]. The first to isolate islets was Polish Professor, Stanislaw Moskalewski, who prepared pancreatic islets in 1965 from a guinea pig for physiological study[29]. In 1972, Paul Lacy from Washington University was the first to demonstrate the ability to reverse diabetes through islet transplantation in an induced diabetic animal model[30]. Further advances in islet transplant research came from Kemp *et al*[31] who completed a major animal study demonstrating the superiority liver implantation *via* the portal vein compared to other sites, such as the renal capsule and subcutaneous space, in the size of the required cell mass to reduce hyperglycaemia. The first clinical attempt to translate these findings in a patient with type 1 diabetes led to one month of insulin independence, followed by cellular rejection attributed to inadequate immunosuppression[30]. Ricordi *et al*[30] at Pittsburgh University improved on these findings considerably with several clinical cases of prolonged diabetes reversal in 1990. Their use of the newly introduced FK-506 (tacrolimus) agent and steroid avoidance protocols together with cluster transplants after abdominal exenteration for abdominal malignancies (in the absence of T1DM), led to considerable success[30]. An essential contribution to clinical translation was the introduction of the "automated method" for islet extraction and the Ricordi chamber developed by Camillo Ricordi, which remains the mainstay technique for clinical islet isolation currently[32-34].

The first human trials aimed at treating autoimmune T1DM through islet allotransplantation began in 1974 under the direction of Sutherland *et al*[35] at the University of Minnesota. The included patients had all undergone previous kidney transplants and were already on immunosuppression regimens. This trial demonstrated the ability to reduce insulin requirements, but usually failed to achieve sustained insulin independence[35]. The inability to achieve sustained insulin independence also hampered subsequent clinical trials up until 2000. In 2000, Shapiro *et al*[2] at the University of Alberta demonstrated the ability to sustain insulin independence out to one year post-transplant in all seven of their initial patients. This is now considered one of the major milestones in the history of islet transplantation. This success allowed for large improvements in the current islet transplant protocol and led to international recognition of islet transplantation, with numerous new programs being developed worldwide demonstrating both reproducibility and further refinement and improvement of these results[18].

Longer-term follow-up of patients transplanted using the "Edmonton Protocol" demonstrates ongoing limitations of islet transplantation durability. An initial 5-year patient follow-up demonstrated graft loss all but 15% of patients in the program[17]. Furthermore, around 25% of patients required a second transplant after two to three years[36] in order to achieve sustained graft function. Even with these shortcomings, the "Edmonton Protocol" offered a benchmark for subsequent islet transplantation research. With redefined immunosuppression therapy, islet transplantation is now able to match the results of pancreas-alone transplantation, with 5-year insulin independence rates of 50% now being observed[37,38].

Current protocol

The current islet transplant protocol begins with isolation of donor islet cells. Ideally, when a donor pancreas organ becomes available, the islets should be procured within 6-8 h. The isolation of islets involves both mechanical and enzymatic digestion. After digestion, the isolated islets must undergo purification in order to collect as much islet mass as possible (minimal requirement is $\geqslant$ 5000 islet equivalents per kilogram for initial transplants). Isolated cells are then kept in 250 cc of transplant media culture for 24-72 h, and must meet set product release criteria prior to being used for transplantation.

Islets are transplanted by gravity infusion into the portal vein. Percutaneous access is performed by an interventional radiologist under local anaesthesia, ultrasound and fluoroscopic guidance. The isolated islets [still in transplant media and now loaded with heparin (70 units/kg recipient weight)] are then subsequently infused[39]. A successive rinse solution is then given. As the catheter is removed, the created tract is sealed with radio-opaque thrombostatic material to prevent the risk of post procedural bleeding[40,41].

Review of the isolation process and subsequent transplantation has demonstrated a negative relationship on the ability of the cells to survive post-transplantation.

This is partially because the isolation process strips the islets of their inner vascular network[6]. Unlike whole organ transplantation, islets initially are not directly anastomosed to a blood supply and as such, remain markedly hypoxic within the portal venous terminal branches until they are able to establish a direct connection to a blood supply through p. This initial process may take up to 10-14 d to begin, and vascular remodelling ensues over several months thereafter. Although the portal vein does allow for diffusion of nutrients, including oxygen, into the islets, the lower oxygen tension of the liver compared to the pancreas places the islets in a relatively hypoxic environment. Chronic hypoxia then occurs due to a delay in engraftment, which ultimately leads to a large proportion of dead cells. The delay in engraftment is highly dependent upon stress-cell signalling between islet and surrounding hepatic arterial vasculature for stimulation of angiogenesis and remodelling[42,43].

It is quite remarkable that the entire metabolic regulation provided by the transplanted islets comes from just a small fraction (perhaps 30%-40%) of islets that eventually revascularize over time[5]. Another caveat, is that, even if the cells are able to engraft, their inner vascular density is not as robust as native islets[44,45], which may contribute to progressive graft failure due to ongoing relative hypoxia[46] (Table 1).

Immunosuppression

Allogeneic transplantation faces the challenges of allo-immunity. Immunological mechanisms underlying allo-immunity are complex and are related to both T-[47] and B-cell[48] mediated immune reactions. Without appropriate immunosuppression, this results in acute rejection and subsequent irreversible destruction of the donated tissue. While the risk of acute rejection may be lessened to a small degree through close tissue matching[49], long term graft rejection will occur if the immune system is not appropriately suppressed.

The autoimmune pathogenicity of T1DM poses a unique challenge to immunosuppression regimens. The destruction of pancreatic beta cells occurs in genetically susceptible individuals as a result of the formation of autoantibodies (anti-insulin, anti-GAD, and anti-IA-2)[50,51]. Theoretically, when pancreatic islet cells have been completely abolished, these autoantibody titres should decrease, but the autoreactive B cells that produce anti-islet antibodies remain quiescent. With the re-introduction of islet cells *via* transplantation, these autoreactive B cells undergo clonal expansion, such that the graft is exposed to a primed and more chronic immunological attack. This is supported by liver biopsies from patients undergoing transplantation under the "Edmonton Protocol" where beta-cells have been specifically destroyed[52].

The armamentarium of immunosuppressive drugs has expanded since the early days of transplantation. Initial drugs included high dose corticosteroid therapy and anti-metabolite compounds such as 6-mercaptopurine and azathioprine. The introduction of calcineurin inhibitors (cyclosporine and subsequently tacrolimus) in 1983 was a major turning point, as these agents are more selectively targeted to immune suppression with less off-target impact[19]. These are not without side effects and are known to increase the risk of developing *de novo* cancers, hypertension, dyslipidemia, diabetes and opportunistic infections[20,21]. Islet transplantation is a life-enhancing rather than life-saving therapy, and therefore these side effects remain of particular concern as they contribute significant morbidity with chronic use. In addition, many of the available immunosuppression drugs are toxic to the islets and interfere with islet function. While graft failure is likely multifactorial in its pathogenesis, exposure to diabetogenic immuno-suppressants (corticosteroids and calcineurin inhibitors) plays a negative role.

Current immunosuppression used in Edmonton and many other international sites for islet transplantation consists of a combination of induction therapy, anti-inflammatory therapy and maintenance therapy. Induction therapy is designed to deplete T-cells prior to transplantation and in clinical trials, has demonstrated superior long term results[53]. Following transplantation (up to post-transplant day 10), anti-inflammatory agents are given and include anti-TNF (etanercept) and anti-interleukin 1 receptor antagonist (anakinra). Patients are then placed on maintenance therapy. Currently the Edmonton group uses a combination of tacrolimus and MMF for maintenance[38]. Optimisation of maintenance therapy poses significant challenges as detailed above, including beta-cell toxicity and diabetogenicity[19]. However, large improvements have been observed with these redefined immuno-suppression regimens, with 5-year insulin independence rates of 50% being achieved[17,37,38].

Rationale for the use of porcine xenografts and human embryonic stem cells

The current limitations of islet transplantation place a tremendous burden on the system to obtain the needed donor cell populations. As detailed above poor survival post transplantation as well as progressive graft failure even with optimised immunosuppression regimens means that some patients will go on to require a subsequent transplantation. If islet transplantation is to be a sustained treatment option for all type 1 diabetic patients, alternative cells sources will be required. Currently two options are being explored as potential alternative cell sources. These include xenografts and bioengineered human embryonic stem cells.

The use of xenografts for islet transplantation has been studied extensively as an alternative cell source. As a result of this research, the international xenotransplantation association was established[54]. This association has been instrumental in developing consensus guidelines for the use of porcine xenografts

Table 1 Oxygen tension of alternative transplant sites and the ability to support islet transplantation

Site	Oxygen tension of native tissue (mmHg)	Oxygen tension of transplanted islets (mmHg)	Percent to pancreas	Vascular density of transplanted islets (vessel/mm^2) (perfusion rate)	Ref.
Pancreas	Approximately 40	n/a	n/a	1074 ± 174 (6-7 mL/min per gram)	[44-46,61,75]
Portal vein	Approximately 40	Approximately 5	12.50%	< 100 TPU	
Spleen	No data	Approximately 5	CBD	> 100 TPU	
Kidney capsule	15	Approximately 5	12.50%	> 100 TPU	
Peritoneal lining	Approximately 50	No data	CBD	No data	[76]
Intramuscular space	15	25	63%	1162 ± 120	[77,78]
Subcutaneous site	8	No data	CBD	No data	[79]

CBD: Cannot be determined.

in all aspects of transplantation including islets. The rationale for porcine islets stems from the historical use of porcine insulin to treat T1DM, prior to the use of biosynthesised recombinant insulin[55]. Given the compatibility between porcine and human insulin, it is hoped is that similar compatibility will be seen with islets. However, transplantation of xenogenic tissue may represent a nearly insurmountable immunological barrier in humans. It has been possible to obtain sustained islet graft function in monkeys receiving human islets, but heavy (and risky) inductive and maintenance immunosuppression with agents usually considered too aggressive for routine clinical use, are required to achieve such function. Currently, two clinical trials are ongoing in New Zealand (DiaBCell) and in Russia. No subjects to our knowledge have been rendered insulin free with such approaches to date, and for these trials porcine islets have been encapsulated in alginate-based capsules as a mechanical barrier to immune cell engagement.

There have been several identified advantages of using xenografts as an alternative cell source. Firstly, pig islets represent a potential unlimited, on-demand source of islets. This would mean that patients could achieve insulin independence from one transplant as substantial islet mass could be infused at one time. Secondly, given that the islets can be harvested from young, healthy, living pigs with limited exposure to environmental hazards, theoretically, the quality of these islets would be superior to those harvested from deceased human donors. And thirdly, there is the potential to eliminate the requirement for immunosuppression by genetically modifying the source pigs[54].

However, safety concerns over using xenografts also need to be considered. One of the major concerns is the potential for zoonosis, which not only applies to be the recipient, but also to the population at large. Even with regulations to develop designated pathogen-free pig sources, long term follow-up of patients receiving xenografts still needs to be carried out to identify potentially yet unidentified pathogens[54]. The major issue with xenografts is that they carry a much higher immunological risk resulting in a more vigorous rejection reaction[56]. One reason for this is that humans have pre-formed anti-Gal antibodies [Gal (galactose-α1,3-galactose) is an oligosaccharide expressed of pig endothelium]. This results in immediate complement activation as anti-Gal antibodies bind to the surface of the transplanted xenografts[56]. Another reason is that xenografts activate a more robust instant blood-mediated inflammatory reaction (IBMIR)[56]. Following transplantation platelets cause macroscopic coagulation of the islets leading to the recruitment of complement components as a secondary response. The resulting inflammatory response contributes to large islet losses. This taken together would mean that patients would have to be placed on intensive immunosuppressive regimens in order for xenograft survival. However, due to the associated morbidity of immunosuppression agents, this is far from an ideal option.

The other option for an alternative cell source is pancreatic endoderm derived human stem cells. Stem cell research has seen large innovations for cellular replacement therapy over the last few years. Two unique properties that stem cells possess are the ability to renew (proliferative) and the potential to differentiate into any tissue type (pluripotency). To date, *in vitro* propagation of pancreatic endoderm tissue from these pluripotent cells has been achieved successfully[57,58].

There are several advantages to using stem cells. Firstly, these cells (unlike human islets and porcine islets) do not have to be isolated from a whole organ. This has a two-fold advantage. One, this removes the requirement for specialized isolation centres and offers an "on-demand" reproducible and controlled cell source. And secondly, the bioengineered stem cells possess much higher tolerance for hypoxia and ability to neovascularize over time. As detailed above, the isolation process leads to a delay in engraftment as islet cells regenerate their inner vasculature. Theoretically, this means that these cells would be able to engraft more rapidly. Secondly, from a safety perspective, these cells are human derived and would therefore not carry the same pathogen risks or immunological barrier as xenografts.

Stem cell transplantation, however, is not without limitations. One of the current difficulties that stem cell

researchers face is the inability to fully mature the cells into functional insulin-secreting cells *in vitro*[57,58]. When these cells are transplanted, they do mature *in vivo*, but this maturation is delayed, and difficult to predict or control. Currently, the shortest time for *in vivo* maturation is eight weeks post-transplantation[58]. The delay in maturation presents an issue with monitoring since these stem cells, similarly to deceased donor islet transplantation, face the risk of early silent rejection at a time prior to functional maturation. However, this limitation is also seen in the current protocol, where direct monitoring of islet function post-transplantation is not yet possible. The current indirect methods of monitoring islet function through blood sugar levels and secreted C-peptide can be used to monitor for maturation of insulin-secreting stem cells.

Safety is the other major concern with use of embryonic derived stem cells for cellular replacement therapy. Teratoma development is the most well recognized risk. Classic teratomas are unique tumors that originate from stem cell populations and demonstrate tissue types from all three cell lines. They are usually detected when they cause morbidity either through a mass effect or through the release of hormones from functional endocrine tissue. The development of these classic teratomas in immune-compromised animal models implanted with monodermal propagated cells indicates a limitation in the purification protocol[58]. While the teratoma histogenesis is not fully understood, the intrinsic properties of pluripotency and self-renewal are risk factors for tumor formation[59]. To improve the safety of using *in vitro* differentiated stem cells these properties would need to be silenced. Another tumor concern is the development of embryonic carcinomas. These are teratomatous-like tumors that are monodermal in histology. These represent a proliferation of a single cell line and are thought to arise from mutations that occur during the differentiation process[59]. Furthermore, although tumorigenesis is largely influenced by the intrinsic properties of the cells, there are extrinsic factors within the microenvironment that appear to influence their development. As of yet, these features are not fully understood, but may influence where the cells can be transplanted. The site for transplantation will also be limited by retrievability if they do go on to develop tumors. A major interest in developing new beta cells from inducible pluripotential stem cells (iPSc) from the patient's own cells could change this dynamic. These cells would be entirely biocompatible from an alloimmune perspective, and not being of embryonic source may potentially be much less susceptible to teratoma or malignant transformation. There would still be a major barrier from recurrent autoimmune attack, which would require strategies for control.

Limitations of the current transplant site

Under the current "Edmonton Protocol", the hepatic portal vasculature is used as the site for islet transplantation. The portal vein offers a rich vascular environment for the newly infused islets. However, a large proportion of cells are initially lost, indicating the hostility of the environment. Some of the well-recognised factors that contribute to the hostility of the liver environment include the lower oxygen tension of the portal vasculature (compared to the pancreas), high exposure to immunosuppression drugs and toxins, and immunological destruction by both the innate and adaptive immune responses. In addition, a large initial loss is attributable to IBMIR (described above). In addition to poor survival outcomes, once the islets have been infused into the liver, they are not readily retrievable. The intraportal hepatic site has demonstrated that islet transplantation is beyond a proof-of-concept therapy, however due to the aforementioned limitations, the portal vein may not be the most ideal site, and indeed, may not be appropriate for more novel transplant technologies such as embryonic or iPScs.

Other transplant sites have been explored for islet transplantation and include: pancreas, spleen, gastric submucosal site, intraperitoneal site/omental pouch, kidney capsule, striated muscle, as well as immunoprivileged sites, including bone marrow, thymus, brain and testis. Review of the practicality of these transplantation sites was recently published by Vériter *et al*[8] and highlighted important criteria to consider when selecting a site for islet cell transplantation. The criteria included: (1) space of the site and the volume of the transplanted tissue; (2) contact to an abundant blood supply with a good oxygen supply; (3) access to physiological blood glucose levels; (4) ease of access and the potential for rapid retrieval; and (5) minimal early inflammatory reaction and promotion of long-term survival[8]. Given these requirements it is understandable why finding an ideal site has been so challenging thus far for islet cell transplantation.

The emphasis for a site with good vascular access has been well researched. Islets in the native pancreas have a rich glomerular-like vascular system (flow rate = 5-7 mL/min per gram)[60] that allows them to readily respond to changes in blood glucose and maintain a high partial pressure oxygen tension (pO_2 = 40 mmHg)[44]. Perhaps evolved from this, islets do not intrinsically possess a system to deal with hypoxic stress, with much lower anti-oxidant levels than any other tissue type. As such, irrespective of where islets are transplanted, they will be exposed to hypoxia due to the destruction of their inner vasculature by the isolation process[7,44]. The ability of the islets to regenerate their vascular densities will impact on their survival and functional outcomes. Studies have shown that when islets are transplanted under the kidney capsule there is a marked decrease in vascular density with an associated decrease in blood flow of around 25%-50% of endogenous islets[44,61]. This

is also associated with a decreased partial oxygen pressure of 5 mmHg[44,61]. Furthermore, vascular distribution was altered in transplanted islets with a higher density of capillaries being observed in the stromal connective tissue compared to the endocrine tissue[44,61]. Limited studies on the vascular densities of transplanted islets into alternative sites are available; however, comparison of native oxygen tension at these sites compared to the pancreas may shed light on the suitability of these sites (Table 1).

The subcutaneous site is one of the most extensively studied alternative sites for islet transplantation. The best recognized advantage being that it is readily accessible allowing for a minimally invasive monitoring, imaging and for biopsy/retrieval. Conversely, historical use of the subcutaneous site in both animal models and humans demonstrated an inability to completely reverse diabetes[7,62] due to the poor vasculature and oxygen tension of the site. However, numerous studies have since demonstrated the ability to manipulate the site to increase vasculature and oxygen tension. These methods included (and are not limited to) the use of polymers[12], meshes[14] and encapsulation devices[13,16]. In addition, angiogenic stimulation has been achieved through co-transplantation with growth factors (*e.g.,* fibroblast growth factor[63], hepatocyte factor, and vascular endothelial growth factor) and mesenchymal stem cells[64] (Table 1). These methods revealed the potential to manipulate the transplant site in order to create the ideal microenvironment for the islets to survive. They also highlight that native oxygen tensions alone are not suitable in predicting survival outcomes.

However, although studies have shown the ability to create a microenvironment in the subcutaneous site to support islet transplantation and reverse diabetes in an animal model, there were observed limitations in functional outcomes. In particular, islets transplanted into the modified subcutaneous site demonstrated an apparent delay in responding to changes in blood glucose levels[14]. This could be related to inefficiency in transporting insulin from the subcutaneous site into the blood stream[14] and/or a deficiency in responding due to decreased inner vascular density[44,61]. Again, limited studies are available to discuss the internal vascular density outcomes at the alternative sites. While it has been shown that the microenvironment is important for sustaining the islets during the engraftment period, it is unclear how the microenvironment impacts of vascular density outcomes and whether or not this could be further improved.

Some of the methods detailed above, in addition to manipulating the subcutaneous site, demonstrated the ability to transplant islets without the use immuno-suppression agents[13,14,16]. Clinical translation of this concept is predicted to dramatically change islet transplant outcomes as both patient morbidity and drug-related islet cell damage would be decreased markedly. Prototypic macroencapsulation devices consist of a semi-permeable membrane that allows nutrient exchange and insulin release, and prevents the immune cells from accessing the transplanted cells within. The biomaterial of the device stimulates angiogenesis around the device through an inflammatory reaction, but fail to provide a direct connection to a blood supply as vessel ingrowth is blocked. As such, one of the limitations of using this device is that the inner islets are hypoxic[65,66], similar to observations of large islet masses transplanted into the portal vein. In response, studies emerged with the aim of improving internal oxygenation of the devices. The approaches included changes in the size and shape of the devices[67], the size of the islet clusters, the material used[68,69], and the use of an external oxygen supply[70]. In addition, other groups looked at improving local oxygenation at the device through the use of electrochemical processes[71] or local photosynthesis[72]. Another limitation of the immune isolating device is the stimulation of the foreign body reaction to the biomaterial[73,74]. This inflammatory reaction persists for the *in vivo* lifespan of the device and ultimately leads to the formation of an avascular capsule around the device thereby limiting its function.

Expected outcomes with human embryonic and adult inducible pluripotential stem cells

Human embryonic stem cells, as detailed above, are an attractive alternative cell source for islet transplantation. The possibility of using human embryonic stem cells for islet transplantation has only been a reality in the last few years[57,58] and as such there are limited outcome studies available to report. However, knowledge of the bioengineered stem cell properties can be used to extrapolate on the potential outcomes. In addition, the use of stem cells in the context of the current protocol, will help to identify how this cell type can address some of the ongoing challenges. In addition, current research innovations can be synergized with the use of stem cells to enhance their translational application.

As has been previously noted, deceased donor islets have a poor engraftment rate. This has been largely attributable to destruction of their inner vasculature during the isolation process. Therefore, the advantage of using stem cells is that they already have a well-established vasculature. This may allow them to engraft at a more rapid rate and with a higher survival rate compared to donor islets. It could also be predicated that these stem cells will have a more robust vasculature than transplanted islets and therefore might function at a higher efficiency.

With current research focusing on the subcutaneous site and the development of immune isolating devices, a more adaptive cell type is required in order to withstand the relatively hypoxic environment of these devices. One property of stem cells is their ability to proliferate, which should convey a survival advantage when stressed. However, at noted above, these stem cells must undergo maturation after implantation prior to being functional and it is unknown whether or not

the proliferating cell type would be at just one stage of maturation or multiple. This poses safety concern as these cells may go on to develop into embryonic tumors. However, if the cells were enclosed within a device, then this concern would be limited.

As noted above, these previously studied immune isolating devices stimulate a robust foreign body reaction. While they remove the requirement for immunosuppression to protect against immune rejection, the devices are constantly under attack for their *in vivo* lifespan. Some proposed mechanisms for overcoming this reaction is to provide patients with lifelong anti-inflammatories and/or anti-proliferative agents. However, the limitation with using anti-proliferative agents is that they would interfere with the expansion and function of the stem cells. Alternatively, given that stem cells offer a ubiquitous source for transplantation, the other possibility is replace these devices at set time intervals. This would be less attractive for patients, but clearly attractive from the cell manufacturer's perspective.

CONCLUSION

Islet transplantation has been associated with remarkable research output and innovation in the last two decades. The introduction of the "Edmonton Protocol" ignited the possibility of providing all patients suffering from T1DM with a cure. One of the largest problems for islet transplantation, and transplant in general, is the limited supply of donor tissue. Insulin-secreting stem cells offer a potential solution to this problem and may in fact address some of the limitations that require large donor cell populations.

While using stem cells as an alternative source is still a novel idea for islet transplantation, it has promising potentials for the future. In particular, it may synergize well with other current innovations such as immune isolating devices and may open the door for using the subcutaneous site as an alternative transplant site. Further research on clinical outcomes is required but current speculations on outcomes are positive for the utility of stem cells in islet transplantation.

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