Functional islets and where to find them

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CHAPTER 2. CURRENT PERSPECTIVES IN CLINICAL ISLET ISOLATION AND TRANSPLANTATION

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Abstract

Pancreatic islet isolation and subsequent transplantation have evolved from an experimental technique to an established procedure for complicated type 1 diabetes. Although logistically and financially demanding, high yields of transplantable islets can be achieved as more knowledge is gained about the suitability of donor pancreases and new technologies enable better preservation and isolation. Subsequent intraportal transplantation of the islets leads to reconstitution of endogenous insulin production, and sometimes complete independence from exogenous insulin treatment. Important issues, such as the need for potent immunosuppression, a lack of donor islet material, and an optimal islet engraftment site remain. Non-hepatic transplantation sites, often using a transplantation device, are under investigation. To ensure islet survival, an islet scaffold would require optimal vascularization. Enveloping the islets in a capsule could abrogate the need for immunosuppression. Such a device could also allow the use of islets from other sources, such as animal or stem cell derived islets. Of course, the ideal device would combine both optimal nutrient delivery and immunoevasion.

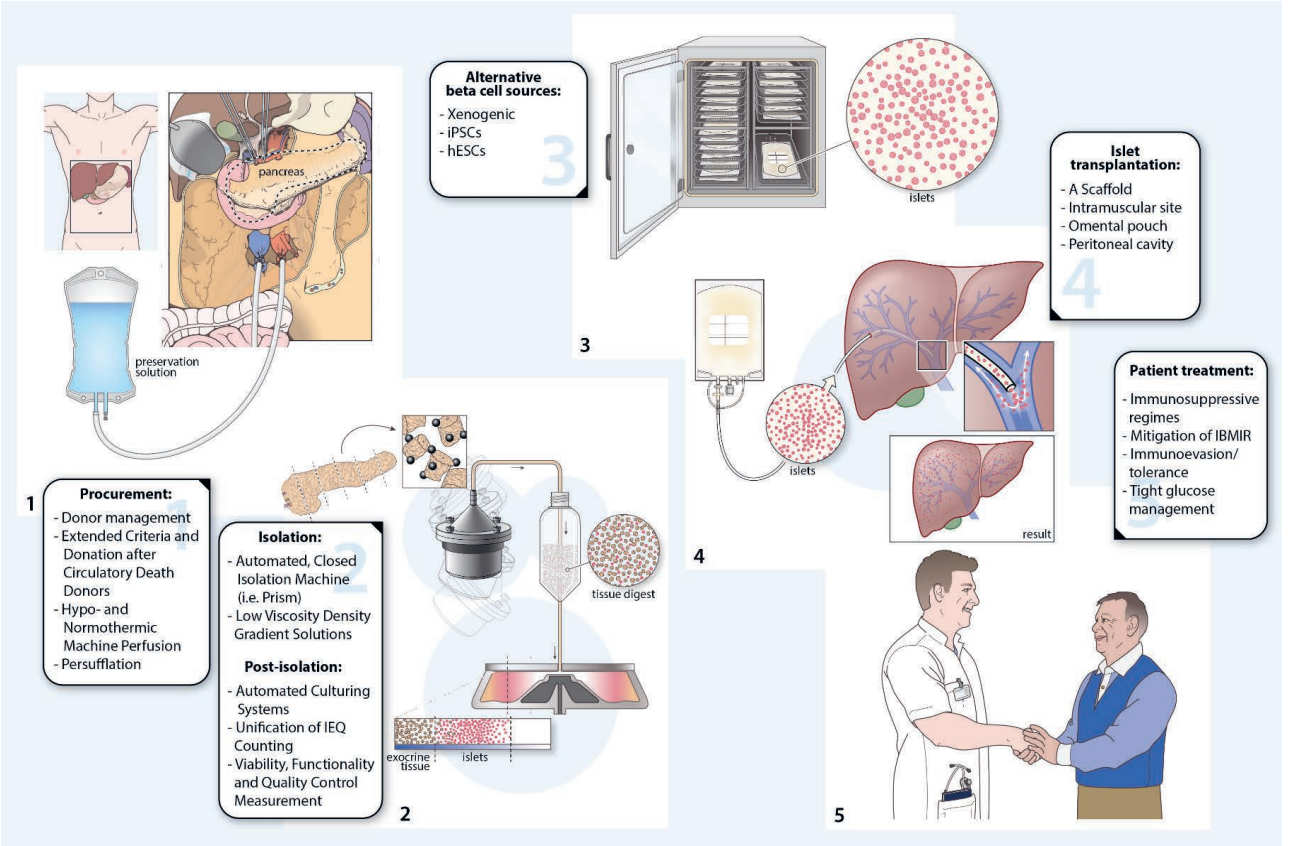

Figure 1: Current practices in clinical islet isolation and transplantation are depicted in five stages: 1) organ procurement, 2) islet isolation, 3) islet preservation and culturing, 4) islet transplantation, and 5) patient treatment. Promising research avenues discussed throughout this paper are highlighted in the text boxes.

Introduction

Type 1 diabetes (T1D) affects over half a million children and up to 40 million adults worldwide[1, 2]. T1D is characterized by insulin deficiency caused by autoimmune destruction of the insulin-producing beta cells in the islets of Langerhans of the pancreas. When untreated the insulin deficiency leads to hyperglycemia, ketoacidosis, and ultimately death. Treatment with exogenous insulin is lifesaving but cannot achieve normoglycemia or prevent glycemic variability with a high risk of hypoglycemia. Due to this glycemic variability and risk of hypoglycemia, target glucose values are set higher than the physiological range in order to avoid these hypoglycemic events. This comes with the cost of long-term micro- and macrovascular complications such as retinopathy, nephropathy and cardiovascular disease. These factors contribute to a reduced life expectancy in patients with T1D[3-5].

The goal of beta cell replacement therapy is to achieve normoglycemia by reconstitution of endogenous insulin production through administration of functional beta cells. Several studies have shown that reconstitution (or retention) of endogenous insulin production leads to reduced glycemic variability and fewer long-term complications[8,9].Importantly, the clinical outcome resulting from the reconstituted insulin production is directly dependent on the number of functional beta cells that are present[6, 7].

Currently, beta cell replacement in clinical practice is established through either transplantation of a whole vascularized pancreas or through solitary islet transplantation. Whole pancreas transplantation has the advantage of transplanting the beta cells in their own biological environment, with optimal vascularization. If successful, this procedure often leads to optimal reconstitution of endogenous insulin production. However, the pancreas is a well-vascularized exocrine digestive organ in contact with the digestive tract; transplanting this organ requires major abdominal surgery and bears considerable risk of infection, thrombosis and bleeding[8]. Isolated islets can be transplanted, but the isolation leads to removal of the islets from its normal environment in the pancreatic parenchyma and to the destruction of the islet capillary network. Transplantation of the islets through an infusion into the portal system is a minimal invasive procedure with fewer procedural risks. However, reconstitution of endogenous insulin production after islet transplantation is usually substantially less than after whole pancreas transplantation. This suboptimal endogenous insulin production is due to factors such as ischemia (until revascularization of the islets has been reestablished), an acute inflammatory response in response to intraportal islet infusion, initial exposure to high

concentrations of immunosuppressants, and suboptimal grafting sites[9, 10]. Although clinical outcomes of islet transplantation are steadily improving, the procedure is only performed in a selected number of patients with T1D. These are generally patients with hypoglycemia unawareness and recurrent severe hypoglycemic episodes, or those with severe and progressive complications despite optimal medical management[11-13]. Important factors that preclude broad implementation of islet transplantation in patients with T1D are the lack of donor organ tissue and the necessity of immunosuppressant therapy, combined with suboptimal long term outcomes[9]. In this review, we will focus on the procedure of clinical islet isolation and transplantation, and address factors limiting implementation and outcomes that could potentially be addressed by the use of biomaterials to improve efficacy, broad application, and the need for immunosuppression.

Isolation

Donor Selection

Identification of a suitable organ donor is the first step in the clinical islet isolation and transplantation process. Several donor and pancreas procurement characteristics have been identified that predict islet yield and/or function after islet isolation such as age, BMI, cause of death, duration of hospital stay, vasopressor usage, blood glucose concentration, abdominal organ (kidney, liver and pancreas) damage markers, cold ischemia time (CIT) and expertise of the organ procurement team[14-23].

Risk scores have been developed to calculate the probability of a successful islet isolation based on (some of) these variables. These risk scores have been criticized due the incorporation of subjective parameters that are difficult to quantify (i.e. level of surgical expertise, stiffness of organ, or degree of edema) and varying definitions of what constitutes a successful isolation[18, 20, 21, 24 , 25]. Furthermore, these risk scores do not measure the functionality or viability of the islet preparation which may better predict the transplant function *in vivo*[26]. Reliance on risk scores can lead to counterintuitive decisions. For example, a study on cadaveric pancreases showed that obesity correlates with an increase in the number of islets present in a pancreas,[27] but obesity is also associated with impaired islet function[28]. Another such factor is donor age. A younger donor age seems to correlate with a lower islet yield. This is partly because pancreas mass increases until one's 40's[29] (a higher pancreas mass correlates with a greater number of islets[30]), but also because of the difficulty

of freeing juvenile islets from the surrounding exocrine tissue using current enzyme mixes[31]. Evidence suggests that younger islets are superior to older islet once engrafted, which has led several groups to preferentially select pancreases from younger donors for isolation[31].

In the last several decades, donor characteristics have become less favorable resulting in a decrease of optimal pancreases for islet isolation[32, 33]. This has led several centers to explore the use of pancreases from donors with extended (suboptimal) donor criteria including higher age and donation after circulatory death (DCD) instead of donation after brain death (DBD). Organ retrieval from DCD donor can take place once death due to cardiac arrest has been established[34]. This leads to an inevitable period of warm ischemia in the donor organ[35]. In 2019 DCD had increased to 58.8% of all organ donation procedures in the Netherlands, and to 34.0% of procedures in Belgium[36, 37]. Although islet yield from DCD pancreases is on average lower, these islets appear to be as functional *in vitro* and *in vivo* as islets from DBD pancreases[38].

Isolation technique – Enzymatic Distention

After the arrival of the pancreas in the islet isolation facility, the pancreas is prepared for perfusion with digestive enzymes. Often, the spleen and duodenum are removed first, as well as peripancreatic tissue such as fat and blood vessels, for better visualization during the perfusion process. Tissue glue or clamps can be used to prevent leakage.[39] The current standard technique of perfusion of digestive enzymes is through a cannulated pancreatic duct[40]. This can be done in a retrograde fashion through the orifice of Wirsung in the head of the pancreas after removal of the duodenum, or in a retrograde and antegrade fashion from the body of the pancreas after a midsection incision[41]. The pancreas is kept at a low temperature to minimalize autolytic digestion during this phase[40, 42].

A blend of digestive enzymes is used prepared for infusion. Several studies have shown that the batch and type of enzyme used in the isolation procedure affect islet yield[16, 43-48]. The most important enzyme, collagenase, is obtained from Clostridium histolyticum bacteria. The ratio between certain classes of collagenases (class I and class II) are essential for optimal pancreas digestion[49]. Dependent on the production methodology, different classes of collagenase are produced and purified in each batch. To minimize the influence of this variability supplementary enzymes are added to the blend such as neutral protease proteases, thermolysin (derived from Bacillus thermoproteolyticus rokko bacteria), and clostripain (also derived from Clostridium histolyticum[50, 51]. The enzymatic activity is often expressed in

Wünsch units or trypsin-like activity[44]. These measurements are unfortunately not highly predictive of successful isolations[44].

Next, the enzyme blend is administered to the pancreatic tissue, either manually with a syringe, or with a recirculating pump (which can also be pressure controlled)[39, 52]. Also, a thin tube can be inserted through the pancreatic duct to the tail to allow the enzymes to be perfused starting at the tail[53]. This intraductal perfusion aims to uniformly distribute the enzymes throughout the pancreas and allow for binding to extracellular matrix components[40]. The length of this perfusion differs amongst centers and can last 10 minutes[54] or up to 30 minutes[39]. Next, the pancreas is often cut into several pieces and loaded into a digestion chamber (also known as a Ricordi chamber). This cylindrical chamber contains several marbles, and is closed off with a metal mesh (generally 400 µm pore)[55]. The chamber is then warmed to 37°C and agitated to start the mechanically-assisted enzymatic digestion process[56]. After digestion, the tissue is collected, cooled, centrifuged, washed and pooled. Often, the tissue is then resuspended in UW solution, which raises the density difference between exocrine tissue and islets[57].

Attempts have been made to isolate islets using other systems than enzymatic digestion, for example: (differential) sensitivity to freezing,[58] anti-acinar cell monoclonal antibodies,[59] cryo-isolation,[60] dielectrophoresis,[61] hypoosmotic exposure,[62, 63] (differential) sensitivity to water permeability,[64] magnetic retraction,[65, 66] quadrupole magnetic sorting,[67] and selective osmotic shock,[68] but these have not resulted in implementation in clinical human islet isolations.

Isolation technique – Purification

During pancreatic islet transplantation, the amount of tissue that can safely be infused in the recipient's liver is limited due the risk of raising the portal venous pressure[69]. It has been hypothesized that this risk increases dramatically when more than 10 ml of tissue is infused[70]. Consequently, it is necessary to reduce the total tissue volume to be transplanted, while retaining as many islets as possible. Human islets do not have a uniform size or other easily exploitable distinct physical difference to exocrine tissue other than their difference in density (specific gravity). Therefore, isopycnic centrifugation (density gradient separation) is the preferred method to purify islets[71]. Large scale isopycnic centrifugation became possible after the implementation of the COBE 2991 cell processor and is now ubiquitous in islet isolation

laboratories[72]. Estimates of islet loss after its use range between 15-51%[73]. After purification, the tissue volume is reduced from approximately 40 mL to 2-6 mL[74].

A range of media have been employed to create density gradients for islet purification, and are still used worldwide. Ficoll and Biocoll are synthetic sucrose based solutions which can be purchased with differing densities and have been used to make continuous gradient in many centers[75, 76 , 77-79, 80 , 81]. UW solution has also been shown to be an excellent alternative to the lower density component of the density gradient[82]. Recently, CT contrast agents have been proposed as suitable solutions for the heavier component in creating a density gradient, such as iodixanol or iohexol. They are potentially advantageous to other solutions due to their very high density, low viscosity and high osmolality[83 , 84-86].

After density purification isopycnic centrifugation, assessment of the total mass of islets (or beta cells[87]) is crucial to determine the amount of potential endogenous insulin production for the transplant recipient, and also to determine factors that improve the isolation process and to compare isolation results among centers[88]. The predominant method to estimate the total islet volume, is by determining the number of islet equivalents (IEQ)[89]. A sample (generally a 1:1000 sample) of each fractions after purification is stained with dithizone that colors the islets red. Then, each islet is counted and placed into a category based on its average diameter. Each category encompasses a range of 50 μm, starting at 50 μm diameter and ending at 400 μm diameter, and has a conversion factor to relate to an “standard” islet size of 150μm diameter[90]. Evaluations of the reproducibility of this type of manual assessment of total islet quantification show variation in the sampling technique and estimation islet sizes[91]. Even when using still images of islet preparations, the average percent coefficient of variation can be over 20% in experienced hands[92, 93]. It has been proposed to use an estimation of the volume and purity of islet fractions to obtain an IEQ[93, 94]. Digital analysis for IEQ quantification has been shown to reduce user islet size estimation variability in several studies[88, 95-99]. No one system has been adopted by all isolation centers, as software costs and differing approaches to the imaging of islets persist[100]. A newly developed free webpage, named Isletnet, hopes to be able to accurately determine IEQ based on an artificial intelligence logarithm[101]. However measured, recent studies have shown that there is not a perfect correlation between IEQ and *in vivo* functionality. The total volume of the islet product seems to predict this more accurately[102]. This has led some centers to adopt a system of quantifying IEQ as a product of the volume and purity of an islet preparation[93].

Advances in islet isolation methodology

In recent years, several technological advances have appeared in the field of islet isolation. The focus of a number of these studies has been on pancreas preservation. Although hypothermic machine perfusion (HMP) of the pancreas and subsequent islet isolation has been documented since the late 1970's,[103, 104] the systems were too cumbersome and pancreases tended to become edematous[105]. Decades later, as machinery miniatured, as more marginal pancreases were being procured, and as promising results from HMP of other transplanted organs were being achieved, new reports using lower perfusion pressures demonstrated little edema and proper islet yields after isolation[106-108]. Also, it was shown that the ATP content of a DCD pancreas can be increased during HMP to the level of a DBD pancreas[109].

Moreover, other modes of pancreas preservation have been explored using normothermic (37°C) conditions. Normothermic regional perfusion (NRP) was developed to quickly reestablish in situ perfusion in the organs awaiting procurement in DCD donors[110]. One case of a successful islet isolation using NRP has been reported[111]. Similar to HMP, normothermic machine perfusion (NMP) perfuses a pancreas ex vivo, but the organ is metabolically active, requiring oxygenation and nutrient administration. The first attempts to perform NMP, on a series of four pancreases, showed proper insulin release and flow, but also signs of edema and necrosis[112].

Oxygenation has been hypothesized as the most important actor in organ regeneration during (machine) perfusion[113]. To this end, it has been proposed that administration of oxygen in gaseous form (persufflation) is sufficient to prevent energy depletion prior to islet isolation[114, 115]. Furthermore, persufflation can reduce inflammatory responses while allowing for a longer preservation time without noticeable impaired islet functionality or viability[116].

Since the publication of the ubiquitous semi-automated method of islet isolation,[55] the islet isolation protocol has persisted essentially intact, with only minor revisions broadly incorporated[117]. The processed has remained an open, manual, time-consuming protocol requiring at least three operators to complete. Accordingly, the PRISM (Pancreatic Islet Separation Method)[117] was developed with a continuous flow centrifuge at its core, which enables collection, washing, concentration, and density gradient purification of pancreatic digest[118, 119]. The protocol was developed with a continuous flow centrifuge at its core, which enables collection, washing, concentration, and density gradient purification of pancreatic

digest[118]. This closed method was further automated by creating a machine with software controlling each step through a touchscreen panel[119]. Promising results show the ability to consistently yield high numbers of functional, viable islets, but have yet to be confirmed in other centers[118, 119].

Islet culture

The first major clinical success of islet transplantation (published as the Edmonton Protocol), required islets to be transfused within 4 hours after isolation[11]. This direct transplantation approach may allow for a greater number of islets to survive *in vivo* than if islets are maintained in culture prior to transplantion[120]. However, culturing islets for several one or more days before transplantation may offer several potential benefits[120]. Firstly, patients requiring a transplantation, but who live afar, can use this time to arrive at the transplantation facility[121]. This also provides time to start immunosuppressant induction treatment and achieve therapeutic levels of immunosuppressants before transplantation[73]. Furthermore, this period permits the islets to recuperate from the challenging isolation process[122]. Measuring the consumption of oxygen during this time may be a suitable method to quantify the islets' recovery[123]. In fact, the percentage of islets lost during culture can be used to indicate the quality of the tissue to be transplanted[39]. Residual digestive enzymatic proteins may also be further diluted and washed out after subsequent medium changes[124].

Generally, medium is refreshed within 24 hours after isolation, and thereafter within 48 hours[65]. Evidence suggests, however, that more islets survive when medium is refreshed within 6 hours of isolation[125, 126]. To this end, an automatic culture system, such as the one developed by Macopharma, continuously refreshes medium. However, it does not allow for multiple fractions to be cultured simultaneously[127]. This system is no longer in production. Another system which was developed to culture islets, utilized rotation to keep islets in a continuous fall[128].

Quality control

In contrast to other forms of allogeneic transplantation, an islet product can be (and in many countries must be) assessed prior to being infused[129]. At a minimum, these release criteria ensure the recipients safety and determine the expected in vivo functionality for the recipient[130]. Each islet production center must adhere to its legislative body's interpretation of judicial guidelines and regulations, which designates the reasoning and values of release

criteria[74]. Generally, release criteria for islet products include: IEQ, product volume, islet purity, islet viability, islet functionality (*in vitro* responsiveness to glucose by a glucose stimulation), microbial infection, and morphology among others[39, 131, 132].

An islet product is most often tested for functionality by their *in vitro* responsiveness to glucose by a glucose stimulated insulin secretion test (GSIS). The standard GSIS test involves incubating islets in solution with a sub-physiological glucose concentration (1.0-3.3 mM), followed by a solution with a supra-physiological glucose concentration (16.7-25mM)[85, 133, 134]. This can also be performed in a dynamic fashion in which islets reside in a small chamber that is continuously perifused with solutions changing in glucose concentration (usually a low-high-low glucose concentration)[89].

Islet viability is important to assess in order to determine the amount of apoptotic/necrotic cells in a transplantation product. Generally, the average viability of the islet cells in the product must be at least 70%[129]. A fluorescence staining assay is most often used because of its ease of use. This assay utilizes fluorescein diacetate (FDA) and propidium iodide (PI, FDA/PI) to label live and dead cells respectively by testing membrane integrity[135]. Other types of viability stainings, such as SYTO-13/ethidium bromide, calcein AM/ethidium homodimer are more sensitive, and under consideration by some centers[136]. Infections in islet products are often determined through Gram staining, endotoxin assays and (an)aerobe cultures of the culture and transplant medium[137].

Measurements of oxygen consumption rate (OCR), which is related to mitochondrial function, have been shown to assess viability and health of islets in several studies[138-141]. OCR assays can be performed in microchambers, which also allows for the possibility to perifuse these chambers with differing glucose concentrations[142, 143]. By combining functionality with viability, these measurements correlate well with transplantation outcomes[144]. The best predictor of clinical transplantation outcome is the functionality of transplanted islets under the kidney capsule of immunodeficient mice[145]. However, as vascularization and functionality assessment takes at least several days this test cannot be used in practice as a release criterium[146].

If the islets have met the release criteria, the preparation is pelleted and resuspended in a balanced salt solution, often supplemented with human serum[147]. Early experiences with islet transplantation led on occasion to complications arising from an increased portal pressure during infusion[148]. It was hypothesized that this was due to aggregation of islets in the

syringes used for intraportal infusion. Therefore, flexible transplantation bags were introduced, allowing for manual homogenization of the preparation[149].

Transplantation

Islet transplantation leads to reconstitution of endogenous insulin production in patients with complicated T1D, but requires potent immunosuppression. The current transplant site is the liver, but this site suffers from low oxygen availability, increased local inflammation, and elevated glucose and immunosuppressant concentrations. More patients with T1D would be able to benefit from this procedure, if immunoevasion (for example through encapsulation) could be achieved. Improved islet graft survival through optimal vascularization would benefit short and long term outcomes and allow for the use of novel beta cell sources.

Islet transplantation procedure

Islet transplantation is currently performed through an infusion into the portal vein[13]. Commonly, a percutaneous transhepatic approach under local anesthesia is used, in which the portal vein is visualized through ultrasonography and/or fluoroscopy[150]. In some centers, access is gained through a mini-laparotomy or laparoscopy, which can also be used as a fallback method[151, 152]. After gaining access to the portal vein a catheter is placed midway between the portal bifurcation and the splenic vein. Angiography is often employed to verify positioning of the catheter tip and portal vein patency. Through the catheter the islets are slowly infused under gravitational force and gentle agitation of the transplantation bag. Portal pressure is monitored before, during and after the procedure. Lower tissue volumes are generally not associated with a rise in portal pressure,[153] but if portal pressure rises excessively the procedure should be aborted, or a portion of the islet product may be placed in the peritoneal cavity or other non-hepatic sites[154].

Most centers use heparin during the islet transplantation, starting with an intraportal injection before the product is infused[153]. After this, either continuous intravenous heparin or low-molecular weight heparin is administered[12, 153]. This is to reduce the risk of portal vein thrombosis, and to facilitate islet grafting by reducing the coagulation and inflammatory components of the instant blood-mediated immune response (IBMIR)[155, 156]. After the procedure, tight glycemic control is maintained, preferably through intravenous insulin therapy, to facilitate optimal islet grafting[155]. Important procedure-related complications to

look for in the first 48 hours include bleeding from the puncture site, infection, and portal thrombosis. Often, bleeding episodes can be treated with supportive care only. In extreme cases, a radiological or surgical intervention may be required. Portal thrombosis can be diagnosed through ultrasonography and is treated by anticoagulation. Antibiotics should be administered to prevent and/or treat procedure-related infections, based on local microbe susceptibility and presence[12, 13, 24, 153, 157].

Immunosuppression

One of the major drawbacks of (allogeneic) islet transplantation is the need for potent immunosuppressant therapy[158]. This is an important reason why islet-alone transplantation (islet transplantation without a previous or concurrent other organ transplantation) is often only performed in patients with T1D that is complicated by severe hypoglycemic events or extreme glycemic lability. In islet-after-kidney transplantation (islet transplantation in patients that already have a kidney transplantation), the threshold to transplant is lower because these patients use chronic immunosuppression already, and the procedure can be viewed as an alternative to simultaneous pancreas-kidney or pancreas-after-kidney transplantation[12, 157].

Immunosuppressant therapy in patients that will undergo an islet transplantation consists of induction and maintenance therapy. Historically, interleukin-2 (IL-2) receptor blockade was used as induction therapy[11]. Blockage of the IL-2 receptor prevents activation and proliferation of T lymphocytes[159]. Some centers still use IL-2 receptor blockade as primary induction therapy, but many have switched to induction therapy with T-lymphocyte depletion to provide more potent immunosuppression[13]. For optimal T-lymphocyte depletion, either anti-thymocyte globulin (ATG) or anti-CD52 (alemtuzumab) is used[12, 160-163]. Both potently and rapidly deplete the T-lymphocyte reservoir. For follow-up islet infusions, IL-2 receptor blockade is still preferred, to prevent over immunosuppression[13, 24, 157].

An important aspect of intraportal islet infusion is IBMIR. Islets that are introduced directly into the portal blood stream elicit a potent inflammatory response that is characterized by activation of complement, coagulatory pathways and a cytokine response. The major loss of islets shortly after transplantation is in part attributed to the IBMIR[155, 156]. To mitigate IBMIR, heparin is administered, and many centers also employ anti-inflammatory agents such as etanercept (anti-TNF alpha) and anakinra (anti-IL1) during islet transplantation[13, 163]. Given the important islet loss attributed to IBMIR, many new treatments are being

investigated. One such compound is low molecular dextran sulfate, which has recently shown similar efficacy to intravenous heparin[164]. Other compounds under investigation include α-1 antitrypsin, liraglutide, reparixin and NF-κB inhibitors[165-168].

Maintenance immunosuppressant therapy is generally life long, and is meant to prevent islet allograft rejection. Among the most potent immunosuppressant agents used are the calcineurin inhibitors (CNIs), such as tacrolimus and ciclosporin[169]. CNIs act by impeding T-cell activation through the inhibition of calcineurin[169, 170]. Important side effects include beta cell toxicity and chronic kidney damage. Tacrolimus has a more profound toxic effect on beta cells than ciclosporin which makes this compound a double-edged sword: potent immunosuppression is necessary to maintain islet allograft function, but the immunosuppressor itself is toxic to the islets. Corticosteroids are potent as well but are also associated with a higher risk of diabetes and beta cell dysfunction[158, 169]. Effective alternative immunosuppressant treatment regimens with less side effects are clearly needed. Many centers use antimetabolites (such as mycophenolate mophetil or azathioprine) or mammalian target of rapamycin (mTOR) inhibitors (such as sirolimus or everolimus), but these do not appear to be potent enough on their own to maintain islet allograft function[158, 169, 171]. For these reasons, the most commonly used maintenance regimen is dual therapy with tacrolimus and mycophenolate[13]. Other treatment strategies include co-stimulation blockade or addition of low-dose steroids to lower the dose of CNIs[12, 160, 172].

Immunosuppression is associated with considerable side effects and complications. Foremost, suppression of the immune system leads to an increased risk of infection. This pertains to both opportunistic and common infections. In the long term, immunosuppressant therapy is associated with an increased risk of malignancy. This is most notable for skin malignancies, but also described for solid and hematologic neoplasms[9, 153, 158, 161, 163, 169].

Engrafting sites

As transplantation site the liver has several advantages, such as easy accessibility, size, regenerative capacity, the availability of an afferent vein, a well-characterized safety profile, extensive clinical experience and a physiological insulin secretory site[11, 173, 174]. However, the liver's microenvironment is also cited as a cause of the poor survival of islets after transplantation. This may in part be attributed to the IBMIR associated with the infusion of islets directly into the blood stream (the portal vein), and may even be enhanced in the liver due to local immunologically active macrophages (Kupffer cells)[173, 175, 176]. Still, other factors

may play a role as well, such as low oxygen content of the portal vein (pO2 10-15 mmHg versus 40 mmHg in arterial blood)[177], relative hyperglycemia of portal venous blood (due to drainage of the digestive tract and local gluconeogenesis), and higher local concentrations of immunosuppressants (due to first pass effect)[173].

Given the possible contribution of the liver site to the poor long term islet allograft survival, many alternative graft sites have been investigated. Ideally, this site would be safe, easily accessible, and would allow for optimal islet grafting, vascularization and survival, and physiologic release of insulin.

The bone marrow is a site that has been under thorough investigation[173, 174]. It is easily accessible and may offer a protected microenvironment as compared to the liver, although a recent study does not support this hypothesis[178]. Important downsides include the low oxygen tension and the non-physiological release of insulin[174]Still, a pilot study in humans demonstrated restoration of endogenous insulin production in patients with an autologous islet transplant in the bone marrow, but this effect was not replicated in an allogeneic transplant setting[179, 180]. In fact, the allogeneic transplant trial did not show any evidence for sustained islet allograft function in six of seven recipients after four months, possibly due to recurrent autoimmunity[180].

Another transplant site of interest is the omental pouch[173, 174]. This is a richly vascularized organ with portal venous drainage. In an autologous setting, it has been shown to lead to comparable outcomes as intraportal islet transplantation in a small case series[181]. No human studies with allogeneic islet transplantation in the omental pouch have been published thus far, but a phase 1/2 trial is under way to test this method[182]. This site is also a typical site where the option for scaffolded islet transplantation is explored. Even though the omentum is well vascularized, additional vascularization may lead to improved islet survival. A possible way to achieve this prevascularization is by using a vascularized device. An interesting study showed the feasibility of such a device, a nonbiodegradable knitted polymer pouch with large ports, in rats. This device had a subcutaneous delivery port so that islets could be introduced after the device had been vascularized in the host's omentum. Seven out of ten diabetic rats had long term normal blood glucose levels with this device[183]. This approach is called macroencapsulation. Another option is microencapsulation, where only one or a few islets are protected by a biomaterial layer. In this case vascularization is provided per islet microcapsule, but the capsule itself protects from immune activation. Pareta et al.[184] showed

the feasibility of this approach by transplanting islets in 300–400 µm microcapsules consisting of a double alginate layer in the omentum. When diabetic rats were transplanted with a marginal mass of these encapsulated islets, a significant reduction in blood glucose levels was observed.

The final site of interest is the muscle, which is already used in clinical practice as a site for autotransplantation of parathyroid tissue[185]. The muscle is an easily accessible site with rich vascularization and less activity of the innate immune system as compared to the liver[173]. It is also ideally situated to obtain tissue biopsies and has a large capacity. A downside is the systemic release of insulin[174]. Again, this approach has already shown some efficacy in the setting of autologous islet transplantation[186, 187]. A somewhat controversial human trial with human fetal islets has shown the potential of this procedure, although islet graft function in the long term was poor[188]. In a small case series a similar result was reported: islet graft function was poor or absent[189]. As with the omentum, research on the muscle as islet transplant site focuses on the use of scaffolds or devices to provide optimal prevascularization[190]. An interesting novel approach is to produce a biological scaffold from the donor's parathyroid tissue. With this approach, islets from the donor are transplanted into the receiver's muscle tissue in a scaffold made of parathyroid tissue. This approach has shown to improved vascularization and engraftment of co-transplanted islets *in vitro*[191]. Many other devices are being tested, as in omental islet transplantation. Interestingly, the muscle is also typically targeted as a graft site in islet xenotransplantation[192].

The skin is targeted in the same way as the muscle, but suffers from poorer vascularization and a more active innate immune system[193, 194]. Studies are ongoing, but currently in the preclinical phase[195]. Other sites, such as the spleen and the kidney capsule, have been found to be unsuitable. The spleen offers a similar profile to the liver, but is less accessible and less safe. The potential advantage, absence of portal hypertension, does not appear to outweigh these problems[173]. The kidney capsule is the graft site of choice in the murine islet transplant model. Transplanting islets in the kidney capsule lead to poor results in larger mammals, probably due to poor vascularization and a tight capsule[173 174, 196, 197].

Patient results

Outcomes of islet transplantation have been steadily improving, owing in part to improved isolation techniques, immunosuppressant regimens and patient management[13]. Almost all the major centers have reported their outcomes in several specified subgroups, such as islet-alone

transplant recipients with complicated hypoglycemia, islet transplantation recipients who have received a previous transplantation (mostly kidney, but also pancreas, lung, and even liver), or simultaneous islet and kidney recipients[11, 12, 24, 157, 198-201]. Islet graft function-related outcome measures of importance include insulin independence, graft failure, severe hypoglycemic events, HbA1c (with targets ranging from <48 to <53 mmol/mol Hb (6.5 – 7%)), insulin requirement, fasting glucose concentrations, fasting or stimulated C-peptide concentrations, or combined scores of these parameters (such as the beta score, beta-2 score and Igls score)[202-204]. Patient reported outcomes are generally focused on general and diabetes-related quality of life, and fear of hypoglycemia[157, 205]. Important complications that are frequently reported comprise diabetes-related complications (i.e. retinopathy, nephropathy), kidney function, infection, procedure-related complications (i.e. bleeding, thrombosis) and malignancy.

Two landmark trials have recently been published describing these outcomes in the two major groups of islet transplant recipients, islet-alone and islet-after-kidney patients[24, 157]. Hering et al.[24] published the outcomes of islet transplantation in a group of patients with T1D complicated by severe hypoglycemia. In this trial, 48 patients received an average of two islet infusions. The primary outcome of freedom of severe hypoglycemic events with an HbA1c of <53 mmol/mol Hb was achieved by 87.5% of the patients after one year, and 70% after two years. 52.1% of patients were insulin independent after one year, but this percentage had halved at two year follow-up. Diabetes-related quality of life improved, and general quality of life was stabilized[205]. Lablanche et al.[157] reported the outcomes of a randomized trial of intensive medical treatment versus islet transplantation in patients who had received a previous kidney transplantation. In this randomized trial 26 patients were assigned to islet transplantation, and 24 to intensive medical management (and after initial trial follow-up islet transplantation as well). Recipients received 1–3 islet infusions. HbA1c after six months was reduced to 38 mmol/mol Hb (5.6%), while it remained stable around 66 mmol/mol Hb (8.2%) in the medical management group. 84% of patients in the islet transplant group had an HbA1c of <53 mmol/mol Hb (7%), as compared to 0% in the medical management group. 92% of patients in the islet transplant group were free from severe hypoglycemic events, whereas in the medical management group 36% of patients were free from severe hypoglycemia. Total insulin independence at one year was 59%. Quality of life improved in the islet transplantation group, but not in the medical management group. One patient died on the

waiting list, due to severe hypoglycemia. Seven islet infusions in six recipients were complicated by hemorrhage, and one portal vein thrombosis was reported.

So, with current protocols, over half of the islet recipients achieve insulin independence and over 85% achieve treatment success. Both these favorable outcomes diminish over time. This phenomenon is attributed to several factors. An important role may be played by immunosuppressants such as tacrolimus, sirolimus and prednisolone, which have diabetogenic properties[158]. Another important factor is chronic rejection, which is seen in any type of allogeneic transplantation[158]. A final factor is the liver, which is a suboptimal grafting site due to local inflammatory conditions, low oxygen tension and local exposure to high concentrations of glucose and immunosuppressants[173, 175, 176].

Novel beta cell sources

An important limitation of clinical islet transplantation is the lack of donor organ tissue. One or more donor pancreases are still required per islet transplantation[13]. Since organ donation rates vary between 1 and 35 per million per year and the incidence of T1D lies around 200 per million per year (in Europe), organ donation will not be able to provide a suitable supply of islet tissue even if the problem of immunosuppression is solved[206, 207]. Two major sources of islet tissue are currently explored. The first is utilizing islets from an animal source such as pigs: xenotransplation. The second is generating beta cells (or beta-like cells) from pluripotent stem cells.

Xenotransplantation, almost always with porcine islets, bears its own important challenges[208]. First of all, animal islets elicit a greater immune response in humans than human islets do. This is accounted for by the IBMIR but also by increased recognition of cytotoxic T-cells through the CD40 ligand. To solve this, more potent immunosuppression could be employed, with of course a greater risk of side effects[209]. Another strategy is genetic modification of the donor pigs[210, 211]. Both these strategies do not solve some other important problems with xenotransplantation, such as the presence of infectious animal-specific pathogens (such as porcine endogenous retroviruses - PERVs), ethical issues, and social acceptance. Interestingly, certain pig strains have been developed that do not carry PERVs[174, 208, 212]. At this moment, xenotransplantation has been extensively investigated in pig to non-human primate models with promising results, and the first positive safety results of trials with encapsulated porcine islets have been reported[213].

Insulin producing cells could also be differentiated from human pluripotent stem cells. Early on, embryonic stem cells were used. Currently, research is mostly focused on generating beta(-like) cells from induced pluripotent stem cells[214]. Already, insulin producing human cells have been generated and tested with positive results [215, 216]. Problems associated with stem-cell based beta(-like) cell transplantation include dedifferentiation of the insulin producing cells and formation of neoplasms[217]. For the first problem, refinement of differentiation protocols is an ongoing process. Because of the risk of neoplasm formation, these cells are introduced into the patients in a scaffold. Several of these pilot trials are currently underway[218].

Use of biomaterials in islet transplantation

Both xeno-islets and stem-cell derived islets or islet-like cells have the important downside of an uncertain safety profile[174]. Xenotransplants are associated with animal-specific viral infections, and stem-cell derived islets could be prone to form neoplasms[219]. These problems may be solved by encapsulation. In islet encapsulation, a semipermeable barrier permits exchange of nutrients and insulin, but prevents an immune response. Both microencapsulation (encapsaluting a single or a few islets) and macroencapsulation (encapsulating an entire islet graft) may be viable options[194, 220]. The first human trial with immunoevasive macroencapsulated islets has shown poor islet graft function. There may be several reasons for this poor function, such as limited diffusion of insulin over the capsule membrane, foreign body response to the capsule, and lack of oxygenation[221]. Several case series have been published with microencapsulated islets, demonstrating safety and prolonged C-peptide positivity in these patients, although complete glycemic control is not achieved yet[222].

In islet transplantation, an optimal transplant site has not yet been identified. Biomaterials may offer the opportunity to create a prevascularized and preferably retrievable graft site to improve initial islet survival. Such a scaffold could be placed in minimally invasive sites such as skin and muscle, or in sites that have ideal local circumstances such as the omentum. Avoidance of direct infusion into the blood stream would lead to reduced acute inflammation, further improving islet survival. Optimal vascularization may also benefit long term islet survival.

The ideal device would allow for optimal delivery of nutrients to the islets, but also provide immunoevasion. Whether such a device is realistic is uncertain, since encapsulation in

essence prevents vascularization. Devices could also focus on different aspects of islet transplantation. One such aspect is optimal (pre)vascularization, leading to optimal survival and graft function. This device would still necessitate immunosuppression but would offer the chance of curation for a patient, off-setting the downsides of immunosuppression in patients with complicated T1D. Also, several studies have shown that marginal beta cell mass reduces glycemic variability, complication risk, and hypoglycemia burden[6, 7]. In this respect, any biological device with beta(-like) cells in it that is immunoevasive and offers some endogenous insulin production would be beneficial for a large group of patients with T1D.

In conclusion

The field of clinical islet isolation and transplantation offers many potential areas of research. Current islet isolation techniques yield enough islets to be able to transplant in some cases, but the procedure is still time-consuming, expensive, and lacking in efficiency. Intraportal islet transplantation reconstitutes endogenous insulin production, sometimes leading to insulin independence, and stabilizes glycemic control, but islet graft function deteriorates in the long term. Furthermore, the need for immunosuppressant therapy and the lack of sufficient donor tissue limit broad implementation. Application of biomaterials may improve many aspects in clinical islet isolation and transplantation.

The struggle to supply enough transplantable tissue in the current reality of decreasing quality donor characteristics is ongoing. Concurrently, research is revealing which pancreases are suitable not only for isolation, but also for subsequent transplantation. As the process of islet isolation has come to maturity, more consistent results are achieved, yet have not shown significant improvement. Technological advances may drive efficiency and push back logistic restraints which have obstructed wider implementation of isolation centers into more institutions. Likewise, progress in quality control parameters is being made to better correlate *in vitro* measurements of allogeneic islets to transplantation outcomes and should be considered when an alternative source of insulin producing cells become available.

In islet transplantation, prevascularized grafts could be created with biomaterials. This would improve islet survival and allow for retrieval of transplanted islets, specifically those from novel sources such as xeno-islets or stem cell-derived islets. Alternative engraftment sites such as muscle and omentum could also be exploited in this way. Furthermore, islet micro- or macroencapsulation may offer a way to abrogate the need for immunosuppression and its associated problems.

In conclusion, even though much progress has been made in clinical islet isolation and transplantation, several problems need to be addressed before this treatment could be implemented in general T1D care. Biomaterials may offer solutions to many of these problems.

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