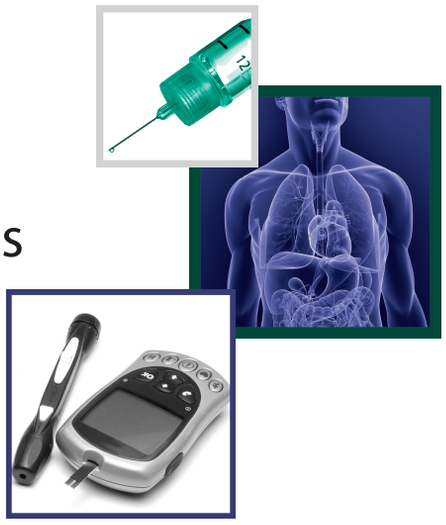
REVIEW

Pancreatic islet transplantation to the liver: how can vascularization problems be resolved?



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Practice Points

- Pancreatic islet transplantation intraportally to the liver is a feasible strategy to cure selected patients with Type 1 diabetes mellitus.
- However, several problems have been identified with this procedure, which increase the number of islets needed and restrict long-term insulin independence. Such problems include early islet cell death due to hypoxia and inflammatory events and late failure due to poor vascular ingrowth.
- Change of implantation site to striated muscle provides a possibility to restore the islet vascular network following transplantation, but other problems may arise such as overload in tissues with exaggerated early islet cell death.
- Modifications of islets for transplantation during culture by mere incubation or by bioengineering the tissue with cells and molecules may be used to improve vascular engraftment, also at the intraportal site.

SUMMARY Pancreatic islet transplantation to the liver provides the possibility of restoring glucose homeostasis in patients with Type 1 diabetes. However, several hurdles remain to be solved in order to optimize this treatment, including developing the means to restrict early islet cell death by hypoxia and inflammatory events and to facilitate the engraftment of islets by improvements in revascularization. Intraportally transplanted islets are poorly revascularized in contrast to islets implanted into muscle or the pancreas. Changing the implantation organ may therefore be a possibility for improving results in clinical islet transplantation, but other hurdles may then arise, including the issue of overload and excessive islet cell death when implanting islets in clusters. New strategies to improve revascularization, also at the intraportal site, by proangiogenic factors such as VEGF, and by inhibition of angiostatic factors such as thrombospondin-1 present in islets, or by cell therapy using mesenchymal stem cells or endothelial progenitor cells bound to the islet surface, provide possible solutions.

During the 1990s and before, the clinical outcome of allogeneic islet transplantation was poor, with insulin independence 1 year post-transplantation standing at less than 10% [1]. However, in more recent years, the transplantation of pancreatic islets from deceased donors has become a curative treatment for selected patients suffering from Type 1 diabetes [2]. Many problems still remain, including the need, in most cases, for at least two donor pancreases to restore glucose homeostasis, which is far more than the alleged 10–20% of the total

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islet volume suggested to be enough to maintain normoglycemia in humans [3]. Indeed, the functional capacity of the transplanted islets has been demonstrated to only correspond to approximately 20% of that found in a nondiabetic person [4]. There also seems to be a progressive decline in the function of islet transplantations, and very few patients remain insulin independent at 5 years post-transplantation [4]. These results markedly differ from those obtained for whole-pancreas transplantation. Since the histocompatibility barrier, the underlying autoimmune disease and the immunosuppressive agents used are the same for whole-pancreas transplants as for islet cell transplants, it is most likely that issues related to the isolation of islets and/or adaptation of the implanted islets to their new microenvironment play an important role in this context.

Change of implantation site to improve results in clinical islet transplantation?

Over the years, clinical islet transplantations have been almost exclusively performed through the intraportal route, and only in recent years has clinical islet transplantation to the liver been seriously questioned based on reports of extensive early islet cell death [5] and problems with long-term islet graft failure. The intravascular injection of pancreatic islets has been demonstrated to elicit an instant blood-mediated inflammatory response triggered by tissue factor and cytokines/chemokines expressed by pancreatic islets in the whole blood environment of the portal vein [6,7]. The liver *per se* also provides several immediate site-specific challenges, for instance owing to its inherently low oxygenation [8,9] and vast presence of resident macrophages [10,11], as well as possible high concentrations of immunosuppressive drugs the islet cells can be exposed to immediately after intraportal transplantation following the intestinal uptake of the drugs. Long-term site-specific challenges include lipotoxicity to the cells [12], insufficient vascular engraftment [13,14] and amyloid deposition [15]. There are also reports of site-specific alterations in islet function after intraportal islet transplantation, such as defective glucagon response to hypoglycemia [16,17] caused by an increased glucose flux and increased glucose levels within the liver secondary to increased glycogenolysis caused by systemic hypoglycemia [18] and substantial changes in the β-cell gene expression and function [19,20].

Experimentally, several sites other than the liver have been tested with variable success, including the subcutis, muscle, the intraperitoneal site, the renal subcapsular site, the spleen, bone marrow, the pancreas and the omental pouch. Some of these sites have also been tested clinically, and the forearm muscle has been shown to be feasible, at least for the autotransplantation of pancreatic islets, with a high and stable c-peptide level obtained as a result [21]. Moreover, we have recently been able to show that pancreatic islets experimentally implanted to striated muscle establish a vascular system similar to that of native islets, and that diabetic mice that have received intramuscularly implanted islets perform much better in a glucose tolerance test than mice that have undergone intraportal transplantation of similar numbers of islets [22]. Autotransplanted islets grafted to forearm muscle in patients also seem very well revascularized [22]; however, many issues still remain to be resolved in the clinical situation, including how to promote early survival of implanted islets, and prevent excessive fibrosis and overload of islets in muscle, especially when considering the number of islets to implant. Experimentally, when implanting clusters of islets, graft function at the intramuscular site mainly seems to be restricted by early islet cell death and concomitant excessive fibrosis, which is likely caused by high focal oxygen consumption and restricted oxygenation of the tissue, although later vascular ingrowth almost restores oxygen tension (pO_2) levels to that in native islets [23].

Similar to the intramuscular site, islets experimentally implanted in the pancreas become very well revascularized [24] and also function much better than intraportally transplanted islets [20]. However, although it is the most physiological environment of islets, the pancreas has rarely been considered as a potential implantation organ. Surgical interventions and injections are difficult to conduct in the pancreas and there is a high risk of complications due to leakage of enzymes from the exocrine cells, which causes tissue damage and inflammation. New techniques for implantation need to be developed if the pancreas is to become a realistic choice of implantation organ in clinical islet transplantation.

As an implantation organ, the liver has the benefit of being well characterized, and several interventions are being tested to counteract

identified problems with this implantation site (e.g., an instant blood-mediated inflammatory response), including by the development of less β-cell-toxic immunosuppressive regimens. For long-term success at this site, the means to enhance revascularization are probably pivotal. For a summary of the advantages and problems with the different discussed implantation sites, see **Box 1**.

Box 1. Pros and cons of the liver and some other implantation sites for islet transplantation.

Liver
- Advantages
  - Clinically well-established site for pancreatic islet transplantation
  - Curative treatment for selected patients suffering from Type 1 diabetes
  - Portal delivery of insulin
- Disadvantages
  - Instant blood-mediated inflammatory reaction
  - Low oxygenation
  - Resident macrophages
  - High local concentrations of immunosuppressive drugs
  - Lipotoxicity
  - Insufficient vascular engraftment
  - Amyloid deposition
  - Defective glucagon response to hypoglycemia
  - Substantial changes in the β-cell gene expression and function

Striated muscle
- Advantages
  - Clinical autotransplantation of islets results in high and stable c-peptide levels
  - Very well-revascularised islets
- Disadvantages
  - Early cell death
  - Excessive fibrosis
  - Overload of islets in muscle

Pancreas
- Advantages
  - Most physiological environment
  - Very well-revascularised islets
  - Minor changes in the β-cell gene expression and function
- Disadvantages
  - Risk of pancreatitis
  - Lack of techniques for surgical interventions and injections of islets in the pancreas

Role of normal islet vasculature

Native pancreatic islets are richly vascularized by tortuous capillaries that form a glomerular-like system, thereby ensuring that no islet cell is more than one cell away from arterial blood [25]. The blood perfusion of the islet vascular system is tightly regulated at the arteriolar level by nervous, endocrine and metabolic mechanisms [26–28]. The high blood perfusion of pancreatic islets is important for β-cells to maintain their oxygen-dependent nutrient metabolism and to release insulin at different demands [27,29]. The rich vascularization and high blood perfusion also facilitate glucose sensing by the β-cells and the distribution of islet hormones to their target organs [30]. Indeed, an intact islet microcirculation seems crucial to preventing sequestration of islet amyloid polypeptide as amyloid to the islet matrix in human islets; excessive amyloid can be observed to be rapidly formed during both human islet culture and after transplantation of microencapsulated islets [31,32]. Recent studies also show an important role for paracrine support of islet endothelial cells for β-cell proliferation and function [33–35].

Islet revascularization at the intrahepatic site

The isolation of pancreatic islets disrupts all vascular connections and following transplantation, the tissue needs to be fully revascularized to regain proper function. The revascularization process has been demonstrated to be initiated within 2–3 days and to be concluded within 10–14 days [36,37]. Engraftment of pancreatic islets in the liver has been difficult to investigate, since the islets virtually disappear out of sight and the resolution of imaging techniques is too poor for their study. However, vascular and functional experimental studies have revealed that despite being implanted intraportally, islets are revascularized by branches of the hepatic artery [37,38]. When microspheres were injected through either the hepatic artery or portal vein, only microspheres injected through the hepatic artery could later be found in the transplanted islets [37]. Likewise, only secretagogs (glucose and/or arginine) distributed through the hepatic artery, but not through the portal vein, elicited insulin release from the intraportally implanted islets [38]. Quantification of blood vessel numbers in mouse and human islets that were intraportally transplanted to the liver shows a substantially decreased vascular density when compared with those of native islets at 1-month follow-up [13,14]. Instead, rich vascularization can be seen in the immediate vicinity of the transplanted islets. Studies of pancreatic islets experimentally implanted to the renal subcapsular site show that donor islet endothelial cells may extensively contribute to islet graft revascularization and become incorporated in the new vascular

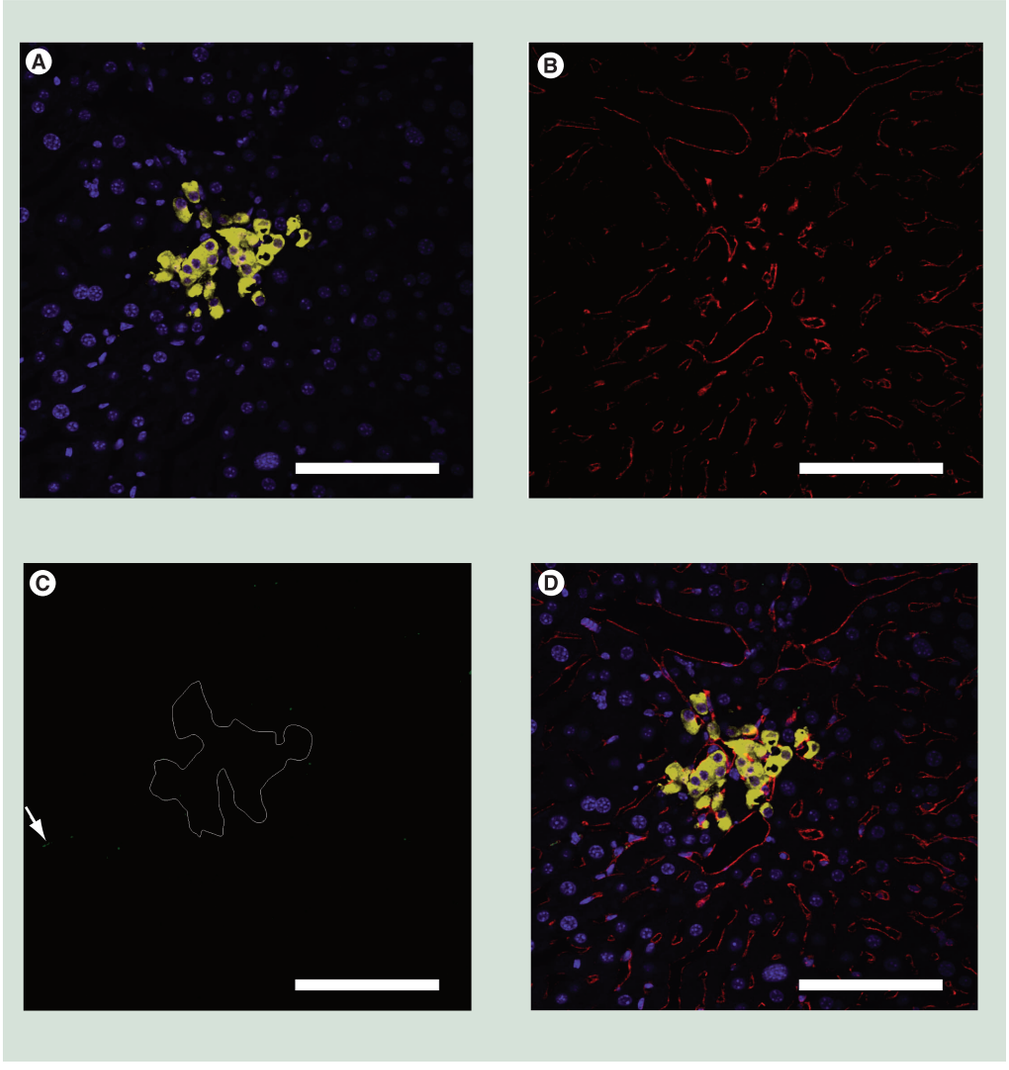

Figure 1. Images of an intrahepatically transplanted Tie-2 green fluorescent protein islet at 1 month post-transplantation. (A) Nuclei (4′-6-diamidino-2-phenylindole, blue) and insulin (yellow) staining; **(B)** CD31 staining (red); and **(C)** the presence of Tie-2 green fluorescent protein cells (green, arrow) reporting donor endothelial cells (Tie-2 is a tyrosine kinase selectively expressed in the endothelium). **(D)** Overlay image of **(A–C)**. Scale bars are 100 µm.

system [39–41]. However, formed blood vessels in intrahepatic islets seem to be mainly derived from the recipient, whereas very few donor endothelial cells can be seen to be incorporated into the new islet vasculature (Figure 1) [Henriksnäs J, Lau J, Carlsson P-O, Unpublished Data]. Interestingly, however, whereas islets still intact at 1 month post-transplantation are poorly revascularized, transplanted islets with disrupted integrity support vascular ingrowth [Henriksnäs J, Lau J, Carlsson P-O, Unpublished Data].

Our early studies using Clark microelectrodes indicated that islets implanted beneath the liver capsule have a chronically decreased pO_2 when compared with native islets [8]. More recent studies using a tracer technique based on the oxygen-dependent bioreductive metabolism of 2-nitroimidazoles show incorporation of the 2-nitroimidazole pimonidazole in 60–70% of intraportally implanted islets, both at 1 day and 1 month post-transplantation [42]. This marker accumulates in islet cells at pO_2 levels of less than 7.5–10 mmHg [42]. Moreover, the blood perfusion of the intrahepatic islet grafts as a mean seems to be only 5% of that of native islets 1 month post-transplantation, as measured with a microsphere technique [Henriksnäs J, Lau J, Carlsson P-O, Unpublished Data]. The more

substantial decrease in blood perfusion as compared with vascular density may perhaps be explained by dysfunctional blood flow regulation, as was previously described for islets implanted at the renal subcapsular site [43,44]. In contrast to native human islets, islets experimentally transplanted into the liver of nude mice rapidly form amyloid [45]. That formation of islet amyloid may also occur in clinical cases after intraportal transplantation was recently shown at an autopsy of a patient who died from a myocardial infarction [15].

Means to improve revascularization & function of intrahepatically transplanted islets

Revascularization of pancreatic islets seems, to a large extent, to be site dependent, in that mouse and human islets implanted in kidney, liver or spleen are poorly revascularized whereas islets implanted in pancreas or striated muscle are almost or fully revascularized [13,14,22–24,46]. The reasons for these differences are unclear, but indicate that interventions are needed to optimize vascular engraftment in the liver if this site remains the first alternative for transplantation of pancreatic islets. Most studies on islet revascularization so far have, for simplicity, used the renal subcapsular site as a model system in rodents. An advantage of this site is that the revascularization pattern is seemingly similar to that of islets in liver with regard to vascular density in implanted islets, with a vivid vascularization in the immediate surroundings of the islets [13,14,46]. However, whether successful interventions to improve islet revascularization at the renal subcapsular site in general are applicable to the liver remains to be determined. More studies of revascularization of intrahepatically transplanted islets are clearly needed and could perhaps be performed in experimental animals by temporarily clamping the left portal vein during the infusion of islets, resulting in selective transplantation to the right liver lobe. In this way, less liver tissue needs to be morphologically evaluated following histological sectioning than following conventional intraportal islet transplantation to whole liver.

■ Influence of islet culture

We have previously shown better vascular engraftment and function in islets implanted beneath the renal capsule if transplanted without prior culture [47]. Both vascular density and the oxygenation of implanted islets were better in the transplanted freshly isolated islets. These grafts also cured diabetic recipients to a larger extent than islet grafts composed of cultured islets. Recently, we also investigated differences in the vascular engraftment of intraportally transplanted freshly isolated and cultured islets. At 1-month follow-up, islets transplanted after overnight incubation had several-fold higher blood perfusion than islets cultured for 4 days prior to transplantation, where only a corresponding tendency to increased blood vessel numbers existed [Henriksnäs J, Lau J, Carlsson P-O, Unpublished Data]. This indicates that some of the vascular structures in the islet grafts composed of cultured islets lacked perfusion.

■ Induction of proangiogenic factors

Angiogenic factors, predominantly VEGF, have been investigated in many studies for their capability to improve islet graft revascularization and function. VEGF is of major importance for the early formation and maintenance of the vascular network in native islets [48,49], and has also been demonstrated to be crucial for islet graft revascularization [49]. Overexpression of VEGF in pancreatic islets and β-cells by different techniques also improves both islet graft vascular density and function at the renal subcapsular site [49–51]. A recent a study also showed that *in vivo* gene delivery of VEGF in plasmids improved revascularization and restoration of euglycemia after human islet transplantation into mouse liver [52]. Such a technique using ultrasound-targeted microbubble destruction for gene delivery has the advantage of being nonviral, but may nevertheless be restricted in the clinical setting by the size and depth of the human liver. An interesting alternative approach may be the anchoring of VEGF to the surface of pancreatic islets for transplantation [53] – a technique that remains to be tested *in vivo*. Pretreatment of islets for transplantation with the iron chelator desferrioxamine (DFO) to induce VEGF expression may be another approach, but effects could be limited by the transient effects on VEGF levels for only 72 h [54]. DFO treatment, or VEGF overexpression *per se*, may have other beneficial effects for transplanted islets besides induction of revascularization, since DFO also improves graft

function of encapsulated human islets [55] and VEGF may act as a survival factor and promote islet viability in cultured human islets [56].

Inhibition of angiostatic factors

The presence and endogenous upregulation of several mitogens for endothelial cells within the islets after transplantation, such as VEGF and FGF-2 [57], seems to attract recipient blood vessels to the islets; however, intraislet vascular formation at several implantation sites, including the liver, is low [13]. In a recently established *in vitro* model, liver endothelium was shown to be able to migrate and proliferate in response to islet stimuli, mainly VEGF. However, intraislet vascular growth, as investigated in the islet endothelium, did not occur in response to endogenous levels of VEGF, but only through blocking endogenous levels of either of the angiostatic factors endostatin, thrombospondin-1 (TSP-1) or α-1 antitrypsin present in islets [58]. At least TSP-1 also seems to restrict revascularization of islets after transplantation, as investigated at the renal subcapsular site. Both genetically TSP-1-deficient islets and TSP-1 siRNA-transfected islet cells demonstrated an increased vascular density, blood perfusion and oxygenation at 1 month post-transplantation [59]. The increased revascularization of islet grafts composed of TSP-1 siRNA-transfected islet cells correlated with increments in both their first and second phase of glucose-stimulated insulin secretion. Interestingly, a similar decrease in TSP-1 levels in mouse and human islets may be achieved by the mere incubation of islets for transplantation with prolactin [60]. Such pretreatment has been demonstrated to also improve revascularization, blood perfusion, oxygenation and function after experimental transplantation to the renal subcapsular site [60]. Prolactin supplementation to the medium during culture also improves β-cell survival in culture [61] and early post-transplantation [60]. The protocol with pretreatment of islets *ex vivo* could minimize the risk of side effects when used in the clinical setting, but the use of prolactin is limited at the present time by the costs for commercially available recombinant human prolactin.

Box 2. Overview of possible strategies to improve revascularization of islets transplanted intraportally into the liver.

- Use of freshly isolated islets
- Induction of pro-angiogenic factors VEGF, HGF, FGF and MMP-9
- Inhibition of angiostatic factors TIMP, α-1-anti-trypsin, endostatin and thrombospondin-1
- Coating of islets with endothelial cells, endothelial progenitor cells and mesenchymal stem cells

Use of cell therapy

Cell therapy to modify engraftment and the immune response to transplanted islets provides a means to affect islet transplant outcome by a multitude of effects and not just via single molecules. Administration of granulocyte-macrophage colony-stimulating factor to mobilize endothelial progenitor cells (EPCs) and other bone marrow cells was tested and found to improve syngeneic islet graft revascularization and function at the intrahepatic site [62]. Later, cotransplantation of bone marrow cells with islets at the renal subcapsular site was also tested and shown to improve their vascularization and function [63]. The specific role of EPCs in islet engraftment has recently been reported and shows improved revascularization and function of murine islets cotransplanted with EPCs [64]. Furthermore, mesenchymal stem cells (MSCs) improve islet revascularization in an *in vitro* model system [65] and promote graft revascularization and function *in vivo*, partially through effects mediated by VEGF secretion [39,66]. However, MSCs secrete a multitude of proangiogenic and antiapoptotic factors that are beneficial for islet engraftment, which also become upregulated by hypoxia [40,41]. Model systems where endothelial cells, EPCs, MSCs or other cells are bound to the surface of islets prior to islet transplantation may prove useful for application of these novel strategies in intraportal islet transplantation [65,67,68].

For a summary of different intervention strategies to improve islet revascularization at the intraportal site, see **Box 2**.

Conclusion & future perspective

Pancreatic islet transplantation may restore glucose homeostasis in patients with Type 1 diabetes mellitus, but many hurdles remain to optimize protocols to preserve the long-term function and mass of the transplanted endocrine tissue. Crucial for proper engraftment of implanted islets are the means to stimulate vascular ingrowth in order to restore the islet vascular network. This can be obtained by change of implantation site, but other problems may then arise. Alternatively, the better characterized intraportal site for clinical islet transplantation can be used, but islets for transplantation then need to be pretreated or bioengineered to facilitate revascularization.

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No writing assistance was utilized in the production of this manuscript.

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