# Toward Engineering a Novel Transplantation Site for Human Pancreatic Islets

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Islet transplantation is a promising therapy for treatment of type 1 diabetes. A real breakthrough was reported when the Edmonton protocol was introduced in 2000. This protocol induced insulin independence in diabetic patients for 1 year (1). Although these clinical islet transplantations demonstrated the application of the technique, the long-term function of the islet grafts was not that successful. After 2 years, less than 50% of the patients remained insulin independent. Five years following transplantation, this declined to just 10% (1). In recent years, some groups demonstrated remarkable progress in islet transplantation outcomes (2), and experienced groups have been able to produce insulin independence after transplantation of islets from a single donor by controlling all known parameters for optimal islet donation (3).

Recently, a number of groups have focused on the identification of factors determining success or failure of islet grafts. Various signs point to the transplantation site as a major factor in graft failure. The majority of islet transplantation is currently accomplished by the infusion of islets into the liver via the portal vein. Several alternative sites were investigated in animals and humans for efficacy as transplantation sites for islets, but none adequately accommodated islet engraftment. A rather novel approach that has been investigated recently is the engineering of an artificial site by using biopolymeric scaffolds. These scaffolds facilitate revascularization and allow adequate glucose sensing and insulin release. Recent developments in this area are reviewed in this article because of their potential clinical application.

## CAUSES OF GRAFT FAILURE OF TRANSPLANTED ISLETS

The exact causes of islet graft failure remain to be identified, but high metabolic pressure, recurring autoimmunity, and alloimmunity may be the basis of declining graft function over the long term (2,3). However, a key issue that cannot be explained by these responses is that the vast majority of islets are lost in the period shortly after transplantation (4). Limitations of the liver as a transplantation site have therefore received considerable attention during the past decade. The potential inadequacies of the liver as a transplantation site for human islets are discussed below because of their essential role in islet grafts.

**Adequacy of vascularization.** Ideally, the transplantation site should allow rapid vascularization of the tissue in order to keep the ischemia period between transplantation and revascularization as short as possible. Studies in rodents suggest that the liver meets this requirement because rodent islets in the liver allow rapid restoration of normoglycemia and adequate control of metabolism (4). There is, however, a key difference between humans and rodents that might make extrapolation of these data challenging (Fig. 1). Rodent islets are relatively large in proportion to liver veins. The size of rodent islets varies between 100 and 350 µm, while the average liver vein is 500 µm in diameter. Vascularization is proposed to occur by islets, causing local thrombosis with ischemia and fast tissue repair responses. Revascularization and integration in the liver parenchyma are consequences of these responses (4), which take several days. In humans, the situation is very different. Islets are 100–200 µm in size while the average liver vessel is 10 mm. This implies that the islets are small relative to the vessels. In order to be caught in the liver, the relatively small human islets have to adhere to blood clots that adhere to the walls of the liver veins. The resulting process of remodeling and vascularization occurs much slower in humans than in rodents (4). A significant loss of islets due to ischemia may be a consequence.

**Low oxygen tension in the liver.** The ischemia-induced loss of viable β-cells may be enhanced by low oxygen tension in the liver parenchyma (Fig. 2) (5). Even in rats where the process of vascularization is fast, 50% of the islets are lost due to hypoxia during the posttransplantation avascular period (6). Another hypoxia-associated threat is that a microenvironment of hypoxia triggers the innate immune system, resulting in the release of inflammatory cytokines such as interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), and interleukin (IL)-1β. In turn, these damage the islet grafts (7). And, last but certainly not least, recent insight suggests that human β-cells under hypoxic conditions may dedifferentiate and undergo an epithelial-to-mesenchymal transition under the influence of hypoxia-mediated activation of hypoxia-inducible factor-1α (HIF-1α) (8). HIF-1α mediates Twist expression, which contributes to the development of progressive fibrosis (8). All these processes may impair adequate engraftment in the liver, thereby reducing long-term graft success.

**Instant blood-mediated inflammatory reaction, alloreactivity, and autoimmunity in the liver.** Many studies suggest that the instant blood-mediated inflammatory reaction (IBMIR) is responsible for the loss of islets in the immediate posttransplantation period (9). IBMIR is an immediate thrombotic reaction that occurs after direct contact between islets and ABO-compatible blood. This reaction is triggered by islet-derived chemokines and tissue factor (9). IBMIR involves activation of coagulation and the complement system, and induces rapid binding of platelets to the islet surface (Fig. 3). The activated platelets generate a fibrin
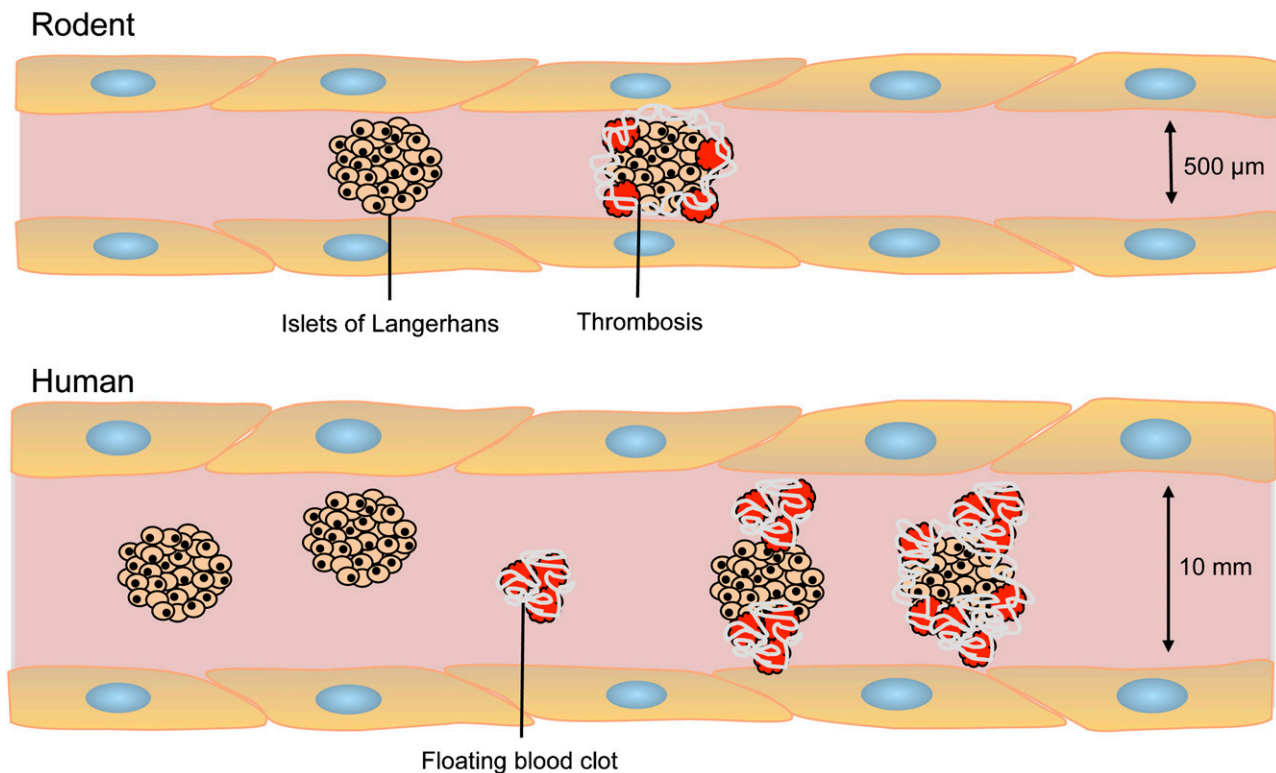
FIG. 1. The principal difference between human and rodent intraportal islet infusion. Rodent islets are relatively large in comparison with the liver veins. Therefore, these islets are instantly trapped in liver veins and cause local thrombosis, which results in vascularization. Human islets are relatively small in proportion to the veins. In order to be trapped and induce vascularization, these islets adhere to blood clots that adhere to the walls of the liver veins.

capsule around the islets that results in islet entrapment in blood clots. Although this may be beneficial for vascularization, there is also a deleterious process that may be activated. Activated platelets upregulate P-selectin expression to attract and interact with neutrophils and monocytes (10). Thrombin, tissue factor, fibrin, and fibrinogen may directly affect macrophages. All these interactions catalyze the inflammatory reaction (10). Unstressed islets secrete lower levels of tissue factor and inflammatory mediators. The use of unstressed islets can therefore mitigate the IBMIR. Further, the coating of islets with anticoagulation factors such as heparin, nicotinamide, or statins can circumvent this reaction (10). However, this may not be enough to prevent an immune response.

A recent study (11) with freshly isolated human islets demonstrated expression of a large panel of genes reminiscent of cells undergoing a marked nuclear factor-κB (NF-κB)-dependent proinflammatory response. Islets expressed matrix metallopeptidase 1 (MMP1) and fibronectin 1 (FN1) (Fig. 4). These factors are involved in tissue remodeling, adhesion, and cell migration. Furthermore, the proinflammatory cytokines IL-1β and IL-8, as well as the chemokines CXCL2, CCL2, CXCL12, CXCL1, CXCL6, and CCL28 that induce neutrophil and monocyte recruitment, were expressed. Expression of these genes was maintained after implantation in mice. Other studies have shown expression and production of proinflammatory mediators post–islet transplantation. These include macrophage migration inhibitory factor, TNF, and IFN-γ (12). This may have long-term consequences for islet survival because there is a correlation between monocyte chemoattractant protein-1 (MCP-1) expression and human posttransplant outcomes (13). In addition, islets express

ATF3, a transcription factor involved in β-cell apoptosis (11). Together, these data demonstrate substantial involvement of the innate immune system in islet graft failure.

Many of the islet-derived cytokines and chemokines also have chemotactic effects on the innate immunity in the liver. Natural killer T (NKT) cells, monocytes, macrophages, granulocytes, and dendritic cells (Kupffer cells) can migrate to injured islets and contribute to their destruction (14). Although injured islets do not necessarily contribute to the development of alloreactivity (14), it has been shown that both humeral and cellular adaptive allogenic immune responses in the liver are involved in human islet graft rejection.

An increased frequency of antidonor-specific cytotoxic T-cell precursors as well as pretransplant HLA sensitization and donor-specific antibodies are associated with accelerated graft failure (3) (Fig. 5). Bosi et al. (15) demonstrate a correlation between newly formed autoantibodies and accelerated risk of graft failure. However, islet graft failure can also occur in the absence of increased autoantibody, suggesting that the autoimmune humeral response is not a crucial factor in islet graft failure. The presence of the pretransplant autoreactive cells are considered to be more important. When the activity of T-cells specific for islet autoantigens such as insulinoma-associated protein 2 (IA2) and glutamate decarboxylase (GAD) (16) is high, grafts are more likely to fail. If immunosuppression is effective, recipients will develop an allograft-specific cytokine profile skewed toward a T-helper 2 or regulatory phenotype.

Maintaining a regulatory phenotype in the liver might be complex because the liver contains abundant NKT cells
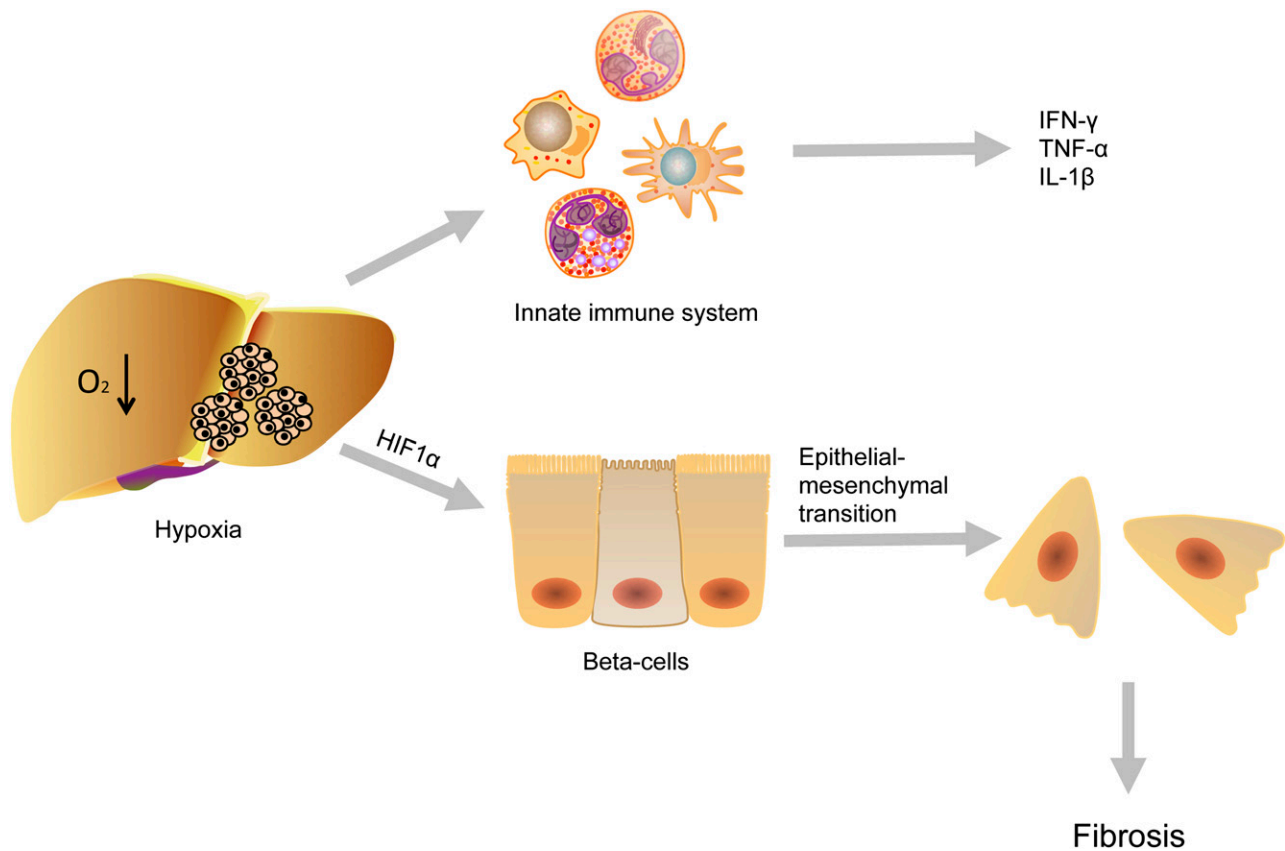
**FIG. 2. Islet loss by hypoxia.** The low oxygen tension of the liver parenchyma enhances hypoxia (4). Hypoxia triggers the innate immune system resulting in the release of the cytokines IFN-γ, TNF-α, and IL-1β, which are harmful for islets (7). Furthermore, β-cells undergo an epithelial-to-mesenchymal transition under hypoxic conditions. This transition is provoked by HIF-1α activation, which mediates Twist expression (8). Twist expression contributes to the development of fibrosis.

(17). A special feature of the NKT cell subset is that it coexpresses invariant T-cell receptors and NK cell–related surface markers. Activated NKT cells produce large amounts of cytokines such as IL-4, IL-10, IL-5, IFN-γ and TNF-β. These cytokines activate CD4 T-helper 1 cells, CD8 cytotoxic T-cells, and cells of the innate immune system (17). This immune response may be responsible for significant damage to the islets, as well as promoting

efficient immune recognition and even allograft islet rejection (17).

**High concentrations of immunosuppressive drugs.** Islets are exposed to relatively high concentrations of immunosuppressive drugs in the liver (3,14). However, it is not clear whether this high concentration is beneficial or detrimental to islet survival. The high concentrations of immunosuppressive drugs could be toxic and may interfere
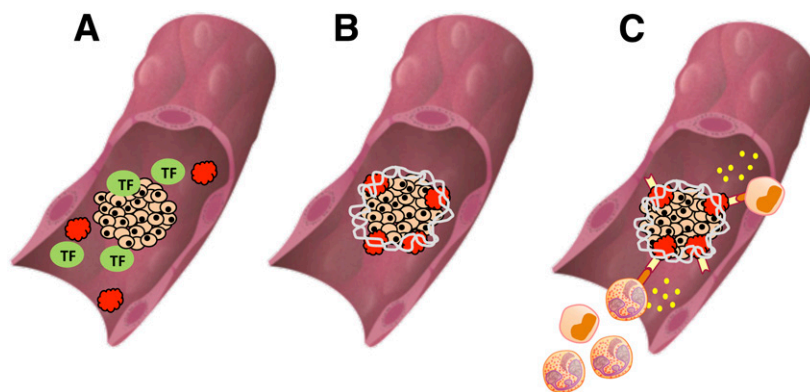


**FIG. 3. IBMIR.** This inflammatory reaction is elicited by direct contact between islets and blood (9). *A*: Islets express tissue factor (TF), which activates a thrombotic reaction. *B*: Thrombin forms fibrin capsules around the islets and activates platelets, which bind to the islets. *C*: Activated platelets upregulate P-selectin markers resulting in the infiltration of neutrophils and monocytes. Also, thrombin stimulates the activation of granulocytes and monocytes. In addition, platelet-activating factor produced by portal endothelial cells and complement products serve as chemoattractants for more neutrophils and macrophages. Tissue factor, fibrin, and fibrinogen may directly affect macrophages.
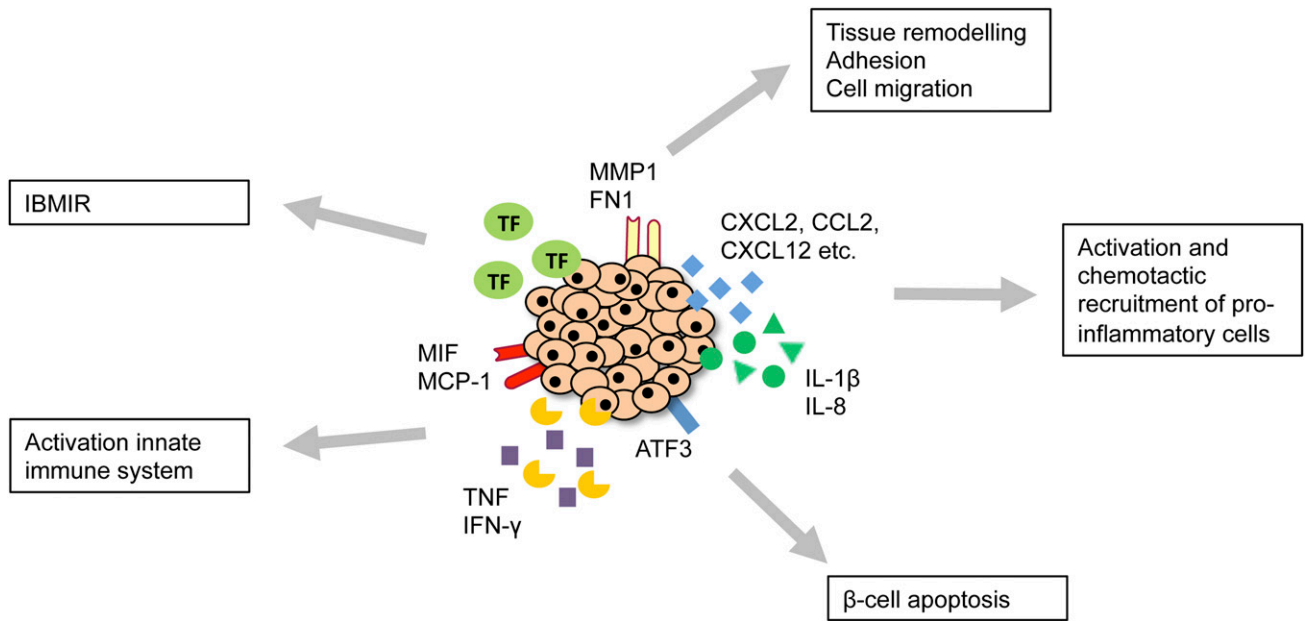
FIG. 4. Nonspecific, innate, and adaptive immune responses against human islet allografts in the liver. Nonspecific immunological responses such as IBMIR have been well-characterized but human islets provoke more responses in the immediate posttransplant period. After isolation, human islets express MMP1 and FN1, which are involved in tissue remodeling, adhesion, and cell migration (11). Also the proinflammatory cytokines IL-1β and IL-8 are expressed as well as the chemotactic chemokines CXCL2, CCL2, CXCL12, CXCL1, CXCL6, and CCL28 that induce neutrophil and monocyte recruitment (11). These—but also other proinflammatory mediators such as MIF, TNF, IFN-γ, and MCP-1—have been shown to influence graft survival by activating the innate immune system (13,14). In addition, human islets express ATF3, a transcription factor that plays a role in β-cell apoptosis (14).

with angiogenesis and impair β-cell proliferation (3,14). On the other hand, the high levels could more effectively protect the islets from undesired responses from the immune system (14). More studies are needed to determine whether the high concentrations are beneficial or detrimental to functional islet survival. Besides immunosuppressive drugs, islets are also exposed to other circulating toxins, which are detoxified by the liver and could have a detrimental effect.
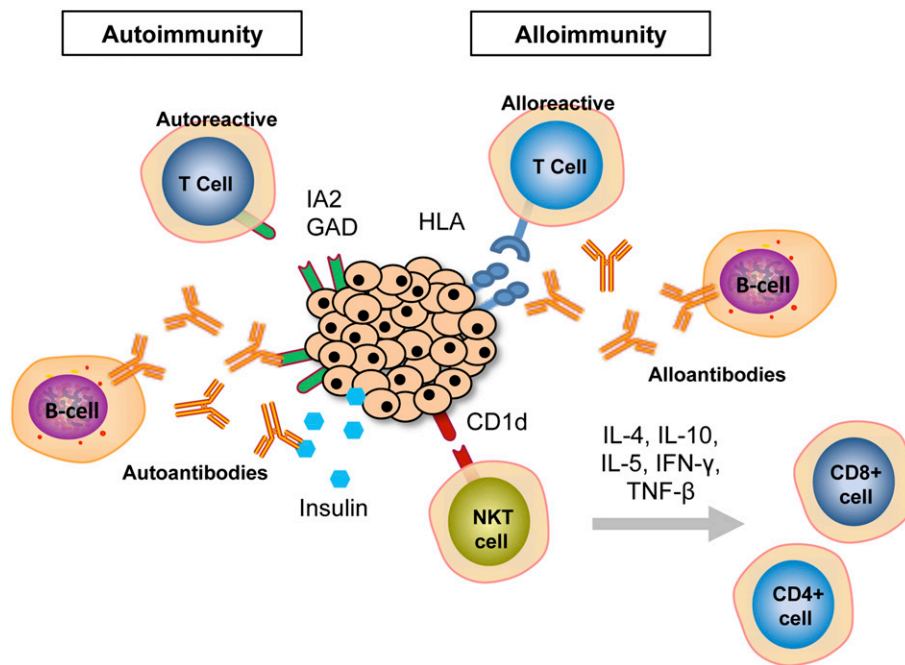


FIG. 5. Adaptive responses against human islet allografts in the liver. Pretransplant autoantibodies and autoreactive T-cells are triggered by antigens such as IA2, GAD, and insulin (15,16). HLA is recognized by alloreactive cytotoxic T-cell precursors and antibodies (3). Furthermore, the high amount of NKT cells in the liver with alloreactivity can expedite the allograft rejection process via nonclassical MHC molecules such as CD1d. Secretion of IL-4, IL-10, IL-5, IFN-γ and TNF-β activates the CD4 T helper 1 cells, CD8 cytotoxic T-cells leading to graft failure (17). Notably, NK and NKT cells are not suppressed by the currently applied immunosuppressants.

## OTHER TRANSPLANTATION SITES

The human liver may not be the most optimal site for islet transplantation. Although other sites have been studied to avoid liver-associated problems, many of these are not acceptable alternatives. Due to IBMIR, several extravascular sites (such as the pancreas [18], gastric submucosa [19], striated muscle [20], peritoneum [21], omentum [22], bone marrow [23], kidney capsule [24], lymph node [25], spleen [26]) and a few immunoprivileged sites (27) (like the anterior eye chamber, the testis, and the thymus) have been considered as potential islet transplantation sites. These sites are discussed below in terms of their potential clinical application.

Theoretically, the pancreas is an attractive site for islet transplantation because it provides optimal oxygen tension and allows insulin secretion in physiologically relevant areas (18). Rodent and canine studies show adequate glucose metabolism and minimal inflammatory and fibrotic responses after intrapancreatic islet transplantation (18). However, islet transplantation into the pancreas is an invasive procedure that carries a high risk of pancreatitis and recurrence of autoimmunity (27). Therefore, the pancreas is not likely to be an acceptable alternative to the human liver.

Animal experiments also show adequate islet function after grafting in the gastric submucosa (19). The submucosal space provides rapid revascularization because of its dense vascular network. It also provides the advantage of easy access by endoscopy, and it simulates physiologic glucose sensing (19). Outcomes of animal models however are not yet translated to the clinic. Although it might be an attractive site, human studies are needed.

Striated muscle provides high vascularization, easy accessibility, monitoring with routine biopsies, and minimal surgical complications. This site has been tested in the clinic for islet autotransplantation after chronic pancreatitis (28). Studies of intramuscular islet transplantation show improved revascularization compared with liver transplantation as well as sufficient oxygen tension (20). However, central necrosis and extensive fibrosis occurred that ultimately resulted in graft failure (20).

The greater omentum seems an ideal transplantation site because of its adequate arterial supply, portal drainage, easy accessibility, and large vascular network. Both transplantation in the peritoneum and the omental pouch reversed hyperglycemia in animal models, but high numbers of islets were needed to achieve these results because of the considerable loss of islets (21,22). This makes clinical transplantation in the peritoneum or omentum, where scarce donors are applied, unrealistic.

The bone marrow has received much attention during recent years. The bone marrow is relatively easily accessible and well vascularized. Rodent studies showed efficient and safe islet transplantation into bone marrow (23). No human data are available, but a clinical pilot is ongoing in Italy (27).

Islet transplantation under the kidney capsule of animals results in normoglycemia despite the relatively poor blood and oxygen supply (24). However, the kidney capsule remains clinically irrelevant as a transplantation site due to the required invasive surgery, the limited space for the transplant mass needed for reversion of diabetes, and the possible induction of diabetic nephropathy (27).

Recently, Komori et al. (25) showed in mice that the lymph node might be an efficacious site for islet transplantation. This site is easily accessible with minimal invasive techniques. The dense vasculature of lymph nodes provides direct access to nutrients and oxygen. However, the lymph node is the center of the immune system and contains large numbers of immune cells such as T and B cells. These packed lymphocytes could therefore create hurdles for clinical application.

Islet transplantation into the spleen has been shown to reverse diabetes in animal models (26). The spleen provides adequate vascularization and insulin distribution, but the spleen also contains large numbers of lymphocytes and is also associated with a high risk of bleeding. These factors imply barriers for clinical application.

To prevent graft rejection induced by the immune system, several immunoprivileged sites have been investigated (27). Successful islet transplantation is possible in the anterior eye chamber, testis, and thymus. However, these sites are not suitable in clinical settings due to the vitality and small size of the organs (27).

Despite successful animal experiments and the promising futures for some clinical trials with other transplantation sites, an optimal islet transplantation site has yet to be defined. There are multiple requirements for such an optimal site: *1*) minimal activation of the immune system, *2*) rapid revascularization, *3*) mimicking of physiological glucose sensing and facilitation of insulin release, and *4*) easy accessibility for minimal invasive surgery and follow-up of the transplant (27). A site with these characteristics will provide an optimal environment for islet survival and functioning.

**An engineered transplantation site for pancreatic islets.** The human body does not contain an environment that meets the ideal requirements for islet transplantation. There are two strategies that can therefore be pursued: *1*) modify an existing organ such that it meets the requirements or *2*) engineer an alternative transplantation site. Modifying an organ is not without risks because it might interfere with normal functioning. The other approach—engineering a transplantation site for pancreatic islets—was proposed as early as 1996 (29). Islets can be implanted under the skin or in the peritoneal cavity with solid support devices. These devices contain a collagen IV network together with acidic fibroblast growth factor or an osmotic pump containing vascular endothelial growth factor. This was effective in inducing capillary ingrowth for rapid islet revascularization. Isogenic transplanted islets have been shown to survive and function for prolonged periods of time in the devices. However, when compared with islets in the naïve pancreas, glucose-induced insulin responses were reduced, which was associated with the loss of islets in the immediate period after implantation (30).

In the past few years a new concept has been introduced to minimize the loss of islets by ischemia or undesired immune responses. This concept involves "seeding" individual islets onto three-dimensional scaffolds. The scaffolds are made of biopolymer fibers that provide a three-dimensional support structure for the islets and mimic the pancreatic microenvironment (31) (Fig. 6). Islets engraft more effectively in three-dimensional structures than in two-dimensional structures, and the scaffold improves viability by promoting cell adherence and nutrient diffusion thereby increasing islet survival immediately after transplantation. This scaffolding also allows for local administration of immunosuppression to suppress the specific immune responses directed against the islets. In addition, a polymer scaffold prevents direct exposure to blood in the first few weeks after transplantation thereby attenuating an inflammatory response but not creating an immune barrier (31). These islet scaffolds have been shown

to be efficacious in animal studies. Diabetic rodents became normoglycemic shortly after transplantation with an islet-containing polymer device (31). However, these results have not yet been translated into clinical practice. **Essence of an extracellular matrix.** Surprisingly, the immediate microenvironment–the role of the extracellular matrix (ECM)–has not received much attention in the selection of an adequate islet transplantation site. However, many studies illustrate the necessity of an adequate ECM for optimal survival of islets (32). The composition of the pancreatic ECM is very specific and probably serves as a reservoir for mandatory growth factors that potentiate tissue repair and homeostasis (32). Islet survival increases when islets are cultured in ECM proteins such as fibronectin, collagen IV, or laminin (33). Also the synthetic RGD sequence (argenin-glycin-aspartic), which is present in many ECM molecules, enhances β-cell survival by decreasing the sensitivity to apoptosis (33). Combining this with collagen IV, an ECM component abundantly present in blood vessels, may at the same time facilitate revascularization (29). A functional ECM might contribute to faster recovery from the isolation stress because many ECM components function as anchors for growth factors that contribute to tissue recovery. However, this cannot be done in too high quantities because collagen IV not only enhances islets survival, it can also decrease islet functionality by diminishing glucose-induced insulin responses (34). Many ECM-derived peptide fragments (peptide fragments located on laminin α-1 chain: RGD, IKLLI, and IKVAV; laminin γ-1 chain: LRE; laminin β-1 chain located: PDSGR and YIGSR; and type 1 collagen α 1(I)-CB3 fragment: DGEA) and growth factors have been shown to enhance β-cell survival and function (35) and might even lead to a significant reduction in the amount of islets and donors required to cure diabetes. Such reductions require further consideration because they might be important for advancing the islet transplant field. The overall shortage of donor organs remains a major challenge, particularly for the widespread use of islet transplantation. Multiple donors are required, and the islet yield and success rate are determined by factors such as the health status of the donor, the condition of the pancreas, the mode of transport, and the quality of the isolation procedure (36). Due to the low number of donors, only a small number of carefully selected patients are eligible for islet transplantation. This may change if we are able to engineer a site that not only meets the requirements described above, but also is more efficacious than conventional sites because it reduces the number of required islets. We recently showed that growth hormone–releasing agonists may be one such factor (37).

## CHALLENGES IN ENGINEERING AN ARTIFICIAL TRANSPLANTATION SITE FOR PANCREATIC ISLETS

Although the principal applicability of an artificial transplantation site for pancreatic islets has been shown (38),
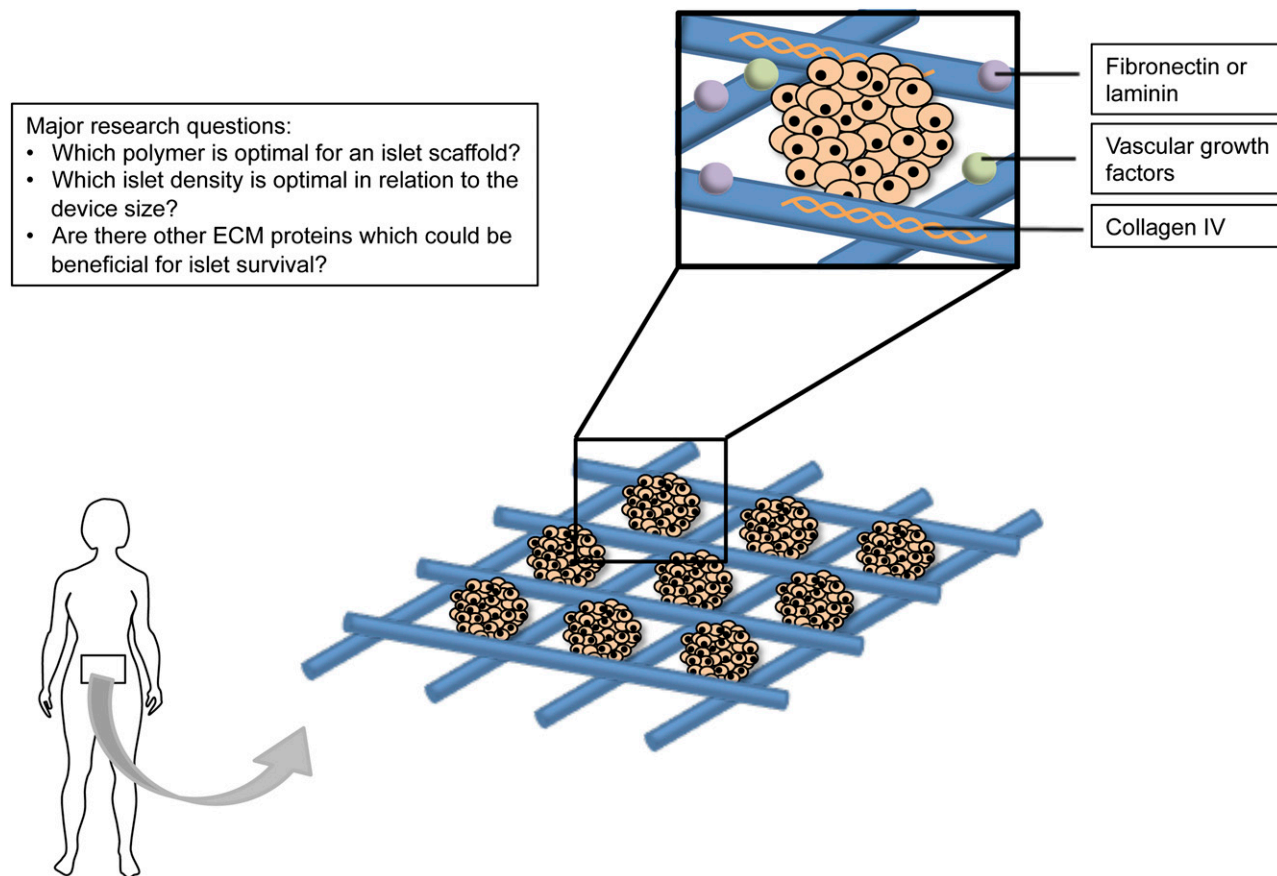


FIG. 6. A three-dimensional biopolymeric scaffold for islet transplantation. This polymer device can be placed in the peritoneal cavity. The device provides a three-dimensional support structure for the islets and mimics the pancreatic microenvironment. Furthermore, by adding ECM proteins such as collagen IV, fibronectin or laminin, or specific components of these ECM components, islet survival can be stimulated (31,33). The ECM also serves as depot for angiogenic and growth factors such as VEGF, which supports revascularization of the islets.

there are a number of critical items that remain to be solved before application in humans can be considered. In previous studies from our group, we used polytetrafluoroethylene as a scaffold (30). Although inert, it was associated with strong fibrotic responses. This was solved by implanting the devices 4 weeks before the introduction of the islets. However, the introduction of the islets required puncturing and damaging of the device and immune activation. In order to meet the requirement of minimal activation of the immune system, new scaffolds should be developed. The success of any polymer as scaffold material depends on several properties including induction of severe inflammation and foreign body responses, permeability, and revascularization. The polymers should allow nonlaborious and easy engineering into different geometries, and ideally, should already be approved for human application.

A number of polymers have favorable properties to serve as scaffolding material for an artificially engineered islet transplantation site. The polymer poly(DL-lactide-cocaprolactone), commercially available as Neurolac, is one example (39). Neurolac is already U.S. Food and Drug Administration (FDA)/CE (European conformity) approved for the repair of small peripheral nerve defects (39). It degrades over 24 months by hydrolysis, and the degradation products are nonacidic (40). However, some animal and clinical studies reported a foreign body response (39,40), which should be tested for its compatibility with the functional survival of islets.

Polyactive is another candidate. This is a copolymer of poly(ethylene oxide terephtalate) (PEOT) and poly(butylene terephtalate) (PBT) and is FDA/CE approved for orthopedic medical devices (41). Polyactive is a thermoplastic polymer (42) that has mechanical and degradation properties that can be controlled by the PEOT/PBT weight ratio (42). The degradation products after hydrolysis are nonacidic and do not induce a systemic immune response (43). In animal studies, Polyactive was shown to be surrounded by capillary-rich granulation suggesting that the polymer itself contributes to vascularization (43). Although Polyactive triggered the influx of immune cells at the implantation site in animal studies (43), application of the copolymer in the clinic was not associated with any side effects, and no inflammatory or abnormal cellular response was found 1 year after implantation (44).

Polysulfone is also a promising candidate polymer that has been shown to be compatible with the survival of islets in animal models (45). This polymer is FDA approved as dialyzer, is histocompatible, associated with low cytotoxicity (46), and provides a matrix for cell growth and cell attachment. Histological analysis revealed that many blood vessels were formed on the surface of Polysulfone. However, a foreign body response was also observed (45). Whether this interferes with islet function has yet to be determined.

The responses against biomaterials are highly dependent on the site of implantation (47). Most studies have been performed in the subcutaneous tissue and the peritoneal cavity. Both sites qualify as transplantation sites for islets because they are easily accessible. A device can be placed and fixed on the abdominal wall or be fixed in the skin. In both rodents and pigs, the use of devices has shown some success (48,49), but whether this can be translated to humans depends in part on the size of the scaffold. The average islet diameter is 150 μm. Although the whole islet graft is only 1 inch in size, it should probably be seeded with more space between the islets in a three-dimensional polymeric device to allow rapid ingrowth of vessels and to avoid ischemia during revascularization. Exact calculations of the ideal size are not yet available. A three-dimensional scaffold is expected to facilitate revascularization to improve oxygenation, nutrient access, glucose sensing, and insulin release shortly after islet transplantation. As outlined above, the scaffold should contain ECM components and growth factors to enhance the efficacy of the site. What this matrix should look like has yet to be determined.

Notably, an artificial transplantation site for pancreatic islets may be applicable not only for free islets but also for islets encapsulated in immunoprotective membranes (50). Encapsulated islets are usually transplanted as free-floating grafts in the peritoneal cavity (29). This interferes with the ability to retrieve the graft and to predict the distance of the islets to the blood vessels. Artificial sites may solve both problems and may also contribute to prolongation of graft longevity which, at present, is a major obstacle for clinical application of immunoisolated islets.

## CONCLUSION

A biopolymeric scaffold allows the creation of a novel ectopic transplantation site for islets. Such a novel transplantation site is needed because the liver does not provide an optimal environment for islet engraftment, and alternative sites also seem to be insufficient. This novel engineered site should meet all the requirements for an optimal transplantation site–minimal induction of the immune system, rapid revascularization, adequate glucose sensing and insulin release, and easy accessibility. This approach may lead to a significant reduction of the loss of islets because direct contact with blood and islets is prevented. Furthermore, because the site is easily accessible, it may allow for the application of local, tailored immunosuppression, which has recently been suggested as a necessary tool to manage the complex and individual differences in immune responses (14). However, the characteristics of several biopolymers should be investigated further to ensure that the polymer does not interfere with islet function or induce toxicity for islets. Engineering of a novel transplantation site is immensely important for the successful treatment of type 1 diabetic patients.

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