

Thrombosis and Inflammation in Intraportal Islet Transplantation: A Review of Pathophysiology and Emerging Therapeutics

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Abstract

With the inception of the Edmonton Protocol, intraportal islet transplantation (IPIT) has re-emerged as a promising cell-based therapy for type 1 diabetes. However, current clinical islet transplantation remains limited, in part, by the need to transplant islets from 2–4 donor organs, often through several separate infusions, to reverse diabetes in a single patient. Results from clinical islet transplantation and experimental animal models now indicate that the majority of transplanted islets are destroyed in the immediate post-transplant period, a process largely facilitated by deleterious inflammatory responses triggered by islet-derived procoagulant and proinflammatory mediators. Herein, mechanisms that underlie the pathophysiology of thrombosis and inflammation in IPIT are reviewed, and emerging approaches to improve islet engraftment through attenuation of inflammatory responses are discussed.

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Introduction

Islet Transplantation Has Emerged as a Promising Treatment for Type 1 Diabetes

Islet transplantation has long been conceived as a promising treatment for type 1 diabetes.¹⁻⁵ Despite advantages over whole pancreas transplantation,⁶⁻¹² more than half of the islet allografts performed between 1990 and 1998 failed within two months and only 8% of patients remained insulin independent beyond one year.¹³ In 2000, Shapiro and colleagues introduced the Edmonton Protocol, which combined transplantation of freshly isolated islets with a steroid-free immunosuppressive regimen.¹⁴

During this procedure, islets are infused percutaneously into the hepatic portal vein (intraportally) where they travel to and ultimately lodge within the liver sinusoids (**Figure 1**). In their seminal report, 7 of 7 patients remained insulin independent one year post-transplantation.¹⁴ This success has reinvigorated widespread interest in islet allotransplantation, and since 2000 more than 500 patients worldwide have received islet transplantation using the Edmonton Protocol and slight modifications

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Abbreviations: (APC) activated protein C, (EC) endothelial cell, (FasL) Fas ligand, (IEQ) islet equivalents, (IFN) interferon, (IL) interleukin, (iNOS) inducible nitric oxide synthase, (IPIT) intraportal islet transplantation, (MCP-1) monocyte chemoattractant protein-1, (NO) nitric oxide, (PEG) poly(ethylene glycol), (TAT) thrombin-antithrombin complex, (TF) tissue factor, (TM) thrombomodulin

Keywords: anticoagulant, anti-inflammatory, cell surface modification, conformal coating, instantaneous blood-mediated inflammatory reaction, intraportal islet transplantation, islet encapsulation, poly(ethylene glycol), type 1 diabetes mellitus

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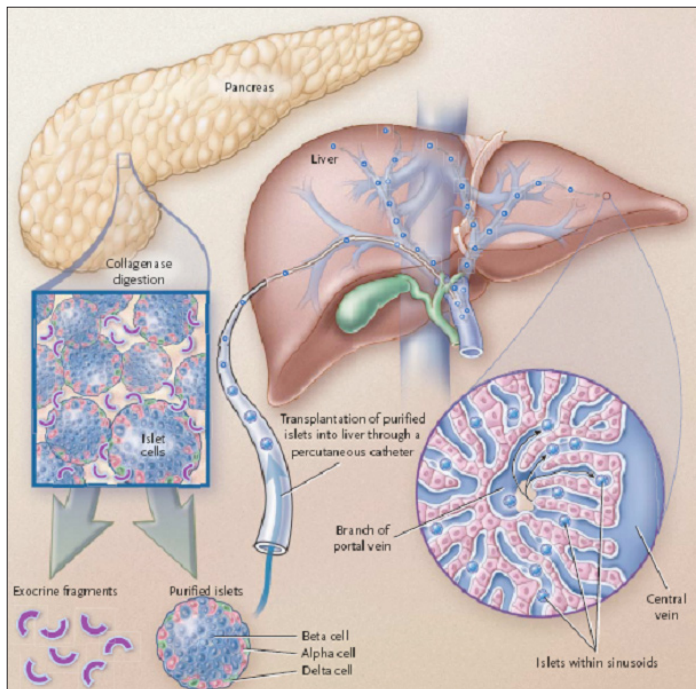


Figure 1. Intraportal islet transplantation. Under the Edmonton Protocol, islets are isolated from donor pancreases, purified, and infused percutaneously into the portal vein of the liver, where they travel to and ultimately lodge within the distal portal sinusoids. Consequently, islets come into direct contact with blood cells and coagulation proteins and are in immediate proximity to portal vein endothelial cells, resident macrophages of the liver (Kupffer cells), and hepatocytes (Reproduced with permission from the Massachusetts Medical Society.⁶)

thereof.¹³ Importantly, at the three leading islet transplant centers, 90% of patients receiving islet transplants remained insulin independent at one year¹⁵ and Shapiro and colleagues have reported 60% insulin independence at three years,¹³ rates comparable to, albeit lower than, those observed in whole pancreas transplantation.¹²

Islet Transplantation Is Compromised by Early Islet Destruction and Primary Non-function

Despite marked improvements, islet transplantation remains limited, in part, by the need to transplant islets from 2–4 donor organs, often in separate infusions, to reverse diabetes in a single patient,^{8,14,16–19} further burdening a limited donor islet source,²⁰ increasing health care costs,¹² and the incidence of procedural complications. Though single-donor islet transplantation has been reported,²¹ in a recent international trial of the Edmonton Protocol, 44% of patients required three islet infusions, and less than half of them remained insulin independent at one year.²² It has been estimated that a normal human pancreas contains approximately 500,000 islet equivalents (IEQ),¹⁹ only 10–20% of which appear to be necessary to maintain euglycemia.²³ Currently,

patients receive ~10,000–12,000 IEQ/kg (~700,000–850,000 IEQ for a 70 kg person), nearly twice the number in a normal pancreas and substantially more than should be required to maintain insulin independence.^{14,19,23} This discrepancy suggests that transplanted islets are functionally impaired and/or fail to engraft. Indeed, metabolic challenges after transplantation indicate that the functional capacity of transplanted islets is only 20–40% of that of a non-diabetic person even in insulin-independent islet recipients,²⁴ and it has been estimated that as few as 10–20% of infused islets survive clinical transplantation.¹⁹ This is supported by animal models, in which 50–70% of transplanted islets are lost in the immediate post-transplant period.^{25–27} Importantly, rates of insulin independence drop to ~10% five years post-transplant,²⁸ and it has been suggested that early islet destruction results in engraftment of a limited islet mass that becomes exhausted with long-term metabolic demands.^{12,27,28}

Early Islet Destruction and Primary Non-function Are Mediated by Inmate Inflammatory Responses

Despite being transplanted across identical auto- and allo-immune barriers, the extent of graft destruction is significantly greater in islet transplantation than in whole pancreas transplantation. This is perhaps most clearly illustrated in experimental models of *syngeneic* islet transplantation into *non-autoimmune* diabetic mice.^{27,29,30} Even under such ideal transplantation conditions, islet insulin content and function are significantly compromised,^{30,31} and an estimated 60% of transplanted islet tissue is lost within 3 days post transplantation²⁷ by both necrotic and apoptotic mechanisms,^{27,29} demonstrating that *early* islet destruction is not allo- or autoantigen-specific. In contrast, in the absence of immunosuppression, allografted islets that survive such initial inflammatory insults are destroyed by *specific* immune responses ~7–22 days later (i.e., *allorejection*).^{32–36} While a number of factors likely contribute to early islet destruction in the immediate post-transplant period, including delayed and insufficient revascularization of the graft,³⁷ ischemia-reperfusion injury,³⁸ and glucose and lipotoxicity,^{39,40} compelling evidence has emerged that early islet destruction is largely mediated by innate inflammatory responses. Animal models of islet transplantation have demonstrated significant inflammation at the graft site, characterized by activation of portal vein endothelial cells (ECs),⁴¹ intense infiltration of leukocytes into and around islets,^{33,41–43} and elevated levels of proinflammatory mediators^{41,43–46} that adversely effect β -cell viability and function.^{45,47,48} Unlike conventional implantable materials, which are

largely passive bystanders of inflammatory responses and subsequent device failure,⁴⁹ islets directly contribute to their own destruction via expression and secretion of bioactive mediators that initiate and propagate inflammatory and procoagulant pathways. This is perhaps best illustrated by Bottino *et al.* who demonstrated that intraportal infusion of islets, but not equivalently sized glass microspheres, triggered increased cytokine production in the immediate post transplant period.⁴⁴ Therefore, while attenuation of immune responses to transplanted islets remains a critical area of investigation, outcomes of islet transplantation may be dramatically improved through prevention of inflammatory responses largely responsible for early islet destruction and primary non-function. Herein, we describe possible mechanisms through which islets trigger inflammatory responses after IPIT and discuss current areas of investigation that hold particular promise for abrogating such responses.

Pathophysiology of Thrombosis and Inflammation in Intraportal Islet Transplantation

Islets Initiate Activation of Coagulation Cascades

Recent evidence indicates that deleterious inflammatory responses may be generated, in large part, by an instantaneous blood-mediated inflammatory reaction triggered by islets in direct contact with blood (**Figure 2**).⁵⁰⁻⁵³ Korsgren and colleagues have demonstrated that tissue factor (TF), the primary physiological initiator of the coagulation system,⁵⁴ is expressed by and released from β and α cells of isolated islets.⁵¹ TF initiates the extrinsic arm of the coagulation pathway by interacting with factor VIIa, catalyzing the conversion of factor X to its active form, fXa, resulting in conversion of prothrombin to thrombin. Indeed, islets incubated in non-anticoagulated blood *in vitro* induced a significant thrombotic response, as evidenced by fibrin clots surrounding islets and increased levels of thrombin-antithrombin complex (TAT), prothrombin fragments 1 and 2, and fXIa-antithrombin complex.^{50,51} Platelets were also activated, as evidenced by reduced platelet counts and release of β -thromboglobulin from alpha granules,⁵¹ further amplifying thrombin generation and promoting aggregation of platelets on the islet surface, presumably through interactions between platelet adhesion molecules and islet-derived extracellular matrix proteins.⁵⁵ Interestingly, Lamblin *et al.* observed, in a porcine allograft model, elevated TAT upon IPIT, but found no such effect when a similar volume of polystyrene beads was infused, demonstrating the cell-specific nature of the thrombotic response.⁵⁶ Perhaps more compelling, in nine patients undergoing clinical

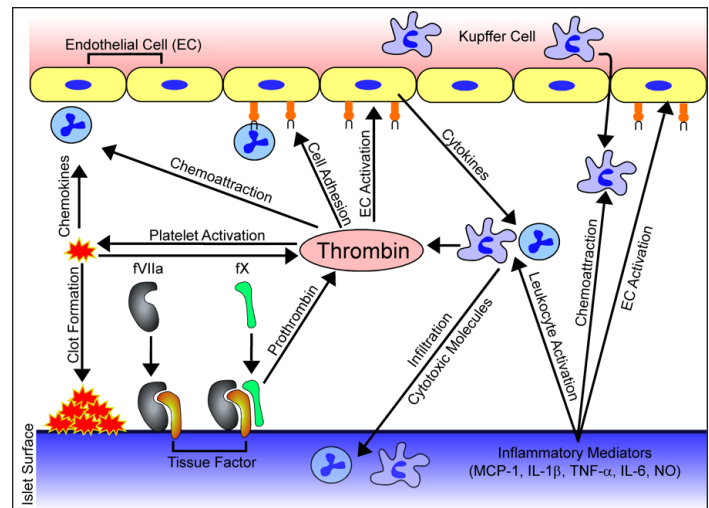


Figure 2. Mechanisms of thrombosis and inflammation in intraportal islet transplantation. Tissue factor expressed on islets interacts with factor VIIa (fVIIa) resulting in activation of factor X (fX), which converts prothrombin to thrombin, a key mediator of thrombotic and inflammatory events. Local thrombin generation triggers platelet activation and adhesion, further amplifying coagulation cascades, and ultimately entrapping islets within fibrin clots. Furthermore, thrombin acts as a chemoattractant and can trigger expression of endothelial cell adhesion molecules, promoting migration of neutrophils, monocytes, and Kupffer cells to the portal bed. Additionally, islets release a number of inflammatory mediators including MCP-1, IL-1 β , TNF- α , IL-6, and NO, which may trigger or exacerbate thrombotic and inflammatory responses post-transplantation through activation of endothelial cells and attraction and activation of leukocytes.

IPIT, serum levels of prothrombotic markers (TAT, fVIIa-antithrombin, and D-dimer) were significantly elevated 15 minutes to 24 hours post-transplantation,⁵³ and patient serum levels of cross-linked fibrin degradation products have been shown to correlate with pre-transplant levels of TF expression by islets.⁵⁷

Islet-initiated Coagulation Contributes to Inflammatory Responses

Though perhaps better known for its role in coagulation, thrombin also acts as a conductor of cellular responses during inflammation (**Figure 2**).⁵⁸ Thrombin can trigger expression of EC adhesion molecules,⁵⁸⁻⁶⁰ and stimulate EC production of the proinflammatory cytokines interleukin (IL)-6 and IL-8, as well as platelet-activating factor, a potent neutrophil activator.⁵⁹ Furthermore, thrombin acts as a chemoattractant⁶¹ and directly triggers platelet activation, resulting in the release of alpha-granule chemokines and the expression of P-selectin, thereby attracting neutrophils and monocytes to the portal bed and promoting their arrest and activation.^{59,62,63}

In accord with the known effector functions of thrombin, EC activation (expression of intracellular adhesion

molecule-1, E-selectin, and P-selectin), neutrophil infiltration, and increased production of cytokines and inflammatory mediators [IL-1 β , tumor necrosis factor- α , IL-6, interferon (IFN)- γ , nitric oxide (NO)] are observed 6–12 hours after transplantation in syngeneic animal models of IPIT, resulting in significant islet apoptosis within 24 hours.^{41,42,64} Though monocytes, Kupffer cells, portal vein ECs, and hepatocytes likely participate in generation of this cytotoxic inflammatory milieu,⁴⁸ evidence is emerging that neutrophilic granulocytes act as the principle effector cell in early islet destruction.^{42,65} Yasunami *et al.* have recently demonstrated that IFN- γ produced by neutrophils plays a crucial role in early islet destruction and that injection of antibodies against neutrophil surface markers Gr-1 and CD11b dramatically attenuates this effect.⁴² Interestingly, despite use of a simplified *in vitro* model of islet-blood contact, Moberg *et al.* have demonstrated that neutrophils begin to infiltrate islets within 15 minutes, and are the predominant cellular infiltrate.⁶⁵ Significantly, addition of melagatran, a low molecular weight thrombin inhibitor, has been shown to reduce neutrophil infiltration while preserving islet morphology.⁶⁶ Hence, islet-initiated thrombin generation appears to contribute significantly to the initiation and/or elaboration of inflammatory responses implicated in islet destruction and primary non-function.

Islet-derived Inflammatory Mediators Contribute to Thrombotic and Inflammatory Responses

While blood-mediated responses play a critical role in islet destruction, evidence of inflammation and islet death in syngeneic animal models of islet transplantation into the kidney capsule suggest that direct islet-blood contact is not a prerequisite for initiation of inflammatory responses.^{27,29,43,45,66} As a result of the metabolic and mechanical stress associated with isolation and culture, isolated islets express and/or release an array of inflammatory mediators^{32,48,67–79} that may trigger or exacerbate thrombotic and inflammatory responses post-transplantation (**Figure 2**). Indeed, an inverse correlation between pre-transplant expression levels of inflammatory mediators and islet engraftment has been observed in both animal models^{72,76} as well as clinical islet transplantation.^{57,77} Significantly, Piemonti *et al.* have demonstrated increased rates of insulin independence and significant reduction in insulin requirements in patients who received islet grafts expressing low levels of monocyte chemoattractant protein-1 (MCP-1)⁷⁷; similar results have been reported in syngeneic murine models.⁷⁶ Soluble factors released from islets have been shown to activate portal vein ECs^{41,80} and Kupffer cells,^{48,81} further contributing to the elaboration of inflammatory

responses. Indeed, in an animal model of IPIT, transient inhibition of Kupffer cells has been found to reduce levels of proinflammatory mediators (TNF- α , IL- β , NO) 3–6 hours post transplantation, resulting in improved islet engraftment.⁴⁴ While the contribution of islet-derived inflammatory mediators in early islet destruction has yet to be fully elucidated, particularly in IPIT where coagulation-mediated inflammatory events are presumed to dominate, their role in potentiating the inflammatory response must be considered. Moreover, consideration has recently been given to islet transplantation to alternative sites^{82–86} or pre-vascularized supports,^{87–91} and islet-derived inflammatory mediators will likely play a significant role in the fate of these grafts as well.

Emerging Strategies to Inhibit Thrombotic and Inflammatory Responses

Pre-transplant Manipulation of Islet Inflammatory Pathways

Through appropriate culture conditions and additives, cell signaling processes may be manipulated to downregulate expression of islet-derived prothrombotic and inflammatory mediators.^{73,75,92–95} Use of specially formulated culture media⁹³ or supplementation with the vitamin nicotinamide⁹² has been shown to downregulate TF and MCP-1 production by islets. Matsuda *et al.* have recently demonstrated that incubation of islets with the p38 pathway inhibitor SB203580 for one hour prior to transplantation suppressed IL-1 β , TNF- α , and inducible nitric oxide synthase (iNOS) expression by islets, markedly increasing the diabetes reversal rate after transplantation of a marginal islet mass.⁷³ Additionally, signaling pathways may be modulated to reduce islet susceptibility to cytokine or nitric oxide mediated damage.^{32,96–99} Pre-transplant overnight culture with the anti-inflammatory agent lisofylline has been shown to reduce proinflammatory cytokine-induced islet apoptosis, thereby allowing insulin independence to be achieved using 30% fewer islets.⁹⁸ As islet culture and shipping are being used more frequently in clinical islet transplantation,^{8,17,21,100} supplementation of media with modulators of inflammatory pathways should provide a facile approach for abrogating islet-initiated thrombosis and inflammation.

Systemic Administration of Anticoagulant and Anti-inflammatory Agents

While the immunosuppressive agents administered under the Edmonton Protocol are effective T- and B cell inhibitors,^{14,101} they appear to have minimal impact on *innate* inflammatory responses against islets mediated

principally by neutrophils and macrophages. Therefore, adjunctive administration of anticoagulant and/or anti-inflammatory agents presents a rational strategy for improving islet engraftment. **Table 1** summarizes notable systemic anticoagulant and anti-inflammatory therapies which have improved early outcomes in animal models of islet transplantation. Renal subcapsule transplantations have also been included as the efficacy of such therapies may translate to intraportal transplantation, despite potential differences in the pathophysiology of early graft destruction. For example, pravastatin (Pravachol)^{102,103} and 15-deoxyspergualin^{104,105} have proven effective in both

kidney and intraportal transplant models. Nonetheless, a need exists to evaluate the efficacy of anti-inflammatory agents in the proper clinical context.

While anticoagulants such as melagatran,⁶⁶ heparin,⁵⁰ and N-acetyl-L-cysteine¹⁰⁶ have demonstrated efficacy *in vitro*, few investigations have adequately explored the efficacy of systemic anticoagulant therapies *in vivo*.^{41,107,108} Contreras *et al.* have recently demonstrated that intravenous administration of activated protein C (APC) dramatically inhibits intrahepatic fibrin deposition, portal vein EC activation, cytokine production, and leukocyte

Table 1.
Anti-inflammatory and Anticoagulant Agents for Improving Islet Engraftment *in Vivo*

Therapeutic agent	Animal model	Transplant site	Treatment regimen	Proposed mechanism(s)	Ref.
Anti-IFN- γ mAb	Mouse iso & allo	Liver	IP (d 0,2,4)	IFN- γ blockade	46
Anti-IL-1 β mAb	Mouse iso & allo	Liver	IP (d 0,2,4)	IL-1 β blockade	46
Anti-TNF- α mAb	Mouse iso & allo	Liver	IP (d 0,2,4)	TNF- α blockade	46
Acetylsalicylic acid	Rat to mouse	Kidney	Oral (daily)	Inhibition of COX-2 and NF- κ B, \uparrow anti-inflammatory cytokine production	160
IL-1ra (Anakinra)	Rat to mouse	Kidney	IP (d -1, 0, 4h)	Inhibition of IL-1 action	160
Activated protein C	Mouse iso	Liver	IV (-1 h)	Inactivation of fVa and fVIIIa, fibrinolysis, anti-apoptotic, NF- κ B inhibition	41
Pravastatin (Pravachol)	Canine auto	Liver	Oral (d -2 to 13)	Inhibition of Ras production, suppression of macrophages, neutrophils, NK cells	110
Pravastatin (Pravachol)	Mouse iso	Kidney	Oral (d 0-14)	Inhibition of Ras production, suppression of macrophages, neutrophils, NK cells	103
Low MW dextran sulfate	Porcine to mouse ^a	Liver	IV (-10 m, d 1-6)	Inhibition of complement and coagulation	107
α 1-antitrypsin	Mouse allo	Kidney	IP (d -1, 1x/3 d)	Serine protease inhibition, inhibition of neutrophil elastase, inhibition of cytokines	32
S-methyl-isothiourea	Porcine to rat ^a	Liver	SC (7 d continuous)	Inhibition of iNOS, hepatic NO generation	165
S-(2-aminoethyl)-isourea	Porcine to rat	Liver	SC (7 d continuous)	Inhibition of iNOS, hepatic NO generation	165
4-phenylbutyrate	Mouse iso	Kidney	Oral (d -2 to 7, 2x/d)	Inhibition of IL-1 β production	166
α -galactosylceramide	Mouse iso	Liver	IP (d -15, -11, -7)	Inhibition of IFN- γ by NKT cells	42
Anti-tissue factor mAb	Primate allo	Liver	IV (-10 to 20 m)	Tissue factor blockade	117
Nicotinamide	Rat iso	Liver	IP (daily)	Inhibition of NO-mediated toxicity	167
15-deoxyspergualin	Mouse iso	Kidney	IP (d 0-4)	Inhibition of macrophage function, inhibition of NF- κ B dependent cytokine production	104
15-deoxyspergualin	Primate allo	Liver	IV (-4 h to d 14)	Inhibition of macrophage function, inhibition of NF- κ B dependent cytokine production	105

iso, isograft; allo, allograft; IP, intraperitoneal; IV, intravenous; Auto, autograft; SC, subcutaneous.
^aathymic animals

infiltration, consequently reducing the incidence of islet apoptosis and increasing the rate of conversion to euglycemia after transplantation of a marginal islet mass.⁴¹ Interestingly, single-dose administration of APC one hour prior to transplantation dramatically attenuated inflammatory events 6–12 hours later. This is particularly compelling given the relatively short half-life of APC (10–20 minutes),¹⁰⁹ suggesting that the portal bed may be “primed” to receive islets. Yasunami *et al.* have demonstrated this phenomenon through repeated administration of the glycolipid α -galactosylceramide prior to transplantation, a process that dramatically reduces early islet loss through inhibition of V α 14 natural killer T cell-dependent IFN- γ production by neutrophils.⁴²

In contrast to immunosuppression,¹⁰¹ effective inhibition of deleterious early inflammatory responses may be achieved with short-course therapy. In a murine model of IPIT, Satoh *et al.* have recently shown that islet dose may be reduced four-fold through simultaneous blockade of IL-1 β , TNF- α , and IFN- γ in the four days post-transplant.⁴⁶ Similarly, short-course oral administration of pravastatin, a cholesterol-lowering drug approved by the Food and Drug Administration, has been shown to reduce the number of islets required to reverse diabetes in a canine autograft model of IPIT.¹¹⁰ While single dose or short-term therapy holds considerable promise for improving the outcome of IPIT, challenges remain in finding therapeutics and treatment regimens that minimize adverse complications.

Localized Protection of Islets Through Re-engineering the Islet-host Interface

As adverse side effects of systemic anticoagulant and anti-inflammatory therapy may limit their potential therapeutic impact, recent efforts have focused on developing strategies to *locally* attenuate thrombosis and inflammation (Figure 3). Under normal physiological conditions, ECs lining the extensive microvasculature of pancreatic islets provide both physical and biochemical barriers to thrombosis and inflammation.⁶⁸ During islet isolation and culture, however, this barrier is disrupted,^{68,111} exposing procoagulant and inflammatory mediators while simultaneously stripping away EC-derived regulators of inflammation. In this regard, the native endothelium has emerged as a structural and biochemical model for re-engineering the islet-host interface.^{112–114} Indeed, Johansson *et al.* have recently lined human islets with aortic endothelial cells via co-culture, and found that the presence of a peripheral EC layer prolonged clotting times, increased platelet counts, and decreased TAT levels *in vitro*.¹¹⁵

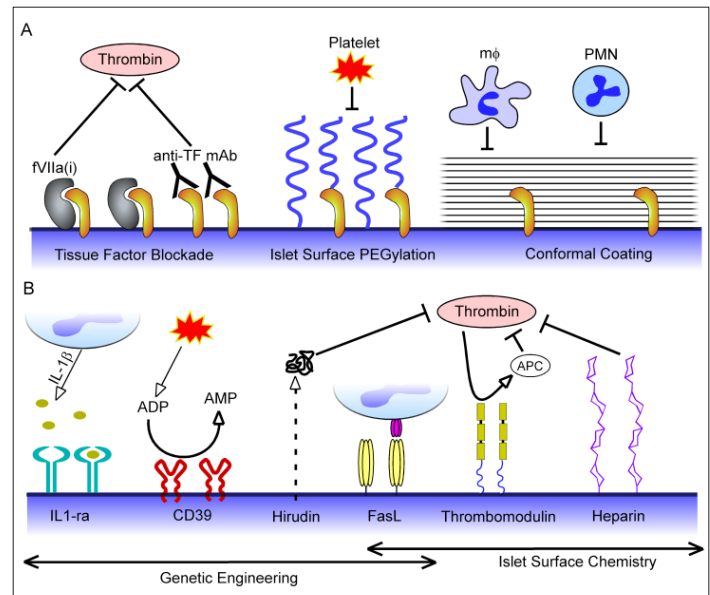


Figure 3. Emerging strategies to locally attenuate thrombosis and inflammation through re-engineering of the islet-host interface. (A) A physical barrier to thrombosis and inflammation may be achieved through blockade of tissue factor (TF) expressed on the islet surface by incubating islets with site inactivated factor VIIa (fVIIa(i)) or anti-TF antibodies prior to transplantation. Though more commonly explored as strategies for protecting islets from host immune responses, islet surface PEGylation and conformal coatings may also provide physical barriers to thrombosis and inflammation by masking tissue factor and/or inhibiting platelet adhesion and infiltration of leukocytes (monocytes [m ϕ] and polymorphonuclear leukocytes [PMNs]). (B) Genetic engineering and tissue-targeted chemistry may be used to actively modulate the islet surface to display or express anticoagulant and anti-inflammatory molecules. Expression of IL-1 receptor antagonist (IL1-ra) may sequester cytotoxic IL-1 β produced by activated leukocytes, while the ectonucleotidase CD39 degrades proinflammatory and procoagulant adenine nucleotides (ATP and ADP) released from activated endothelium and platelets to AMP. Thrombin, a key mediator of thrombosis and inflammation in islet transplantation, may be locally inhibited through genetic engineering of islets to secrete hirudin and by chemical modification of islet surfaces with heparin and thrombomodulin. Though presentation of Fas ligand (FasL) on islet surfaces through genetic engineering and tissue-targeted chemistry has been primarily explored to confer local immunoprotection to islet grafts, such an approach might also improve early islet engraftment by induction of neutrophil and macrophage apoptosis via the Fas-FasL pathway.

Alternatively, several investigators have begun to explore biomaterial-based or genetic engineering approaches to mimic the native endothelium. Within the intact pancreas, ECs provide a physical barrier between islet-expressed TF and coagulation factors and also prevent adhesion and/or infiltration of platelets and leukocytes.¹¹⁶ In this regard, generation of barriers between islets and the intraportal environment offers a rational approach for passively inhibiting islet-mediated thrombotic and inflammatory events (Figure 3A). Indeed, blockade of TF through pre-incubation of islets with site-inactivated fVIIa⁵¹ or anti-TF antibody^{51,117} has been shown to inhibit

thrombotic responses and improve islet survival both *in vitro*⁵¹ and *in vivo*.¹¹⁷ Microencapsulation devices prevent cell-cell contact and dramatically impede diffusion of antibodies and other macromolecules to their respective targets on the islet surface,¹¹⁸⁻¹²¹ and, therefore, may provide a strategy to shield islet-associated TF and prevent leukocyte infiltration into the graft. However, most microcapsules developed for islet encapsulation (**Figure 4A**) are not suitable for transplantation into the liver microvasculature due to their large diameter (400–800 μm).¹²²⁻¹²⁴ Therefore, several investigators have deposited coatings of defined thickness that conform to the islet surface, thereby reducing void volume while retaining the presence of a polymer barrier¹²⁵⁻¹³⁰ (**Figure 4B–D**). An emerging approach to generating conformal barriers has been through immobilization of poly(ethylene glycol) (PEG) to the islet surface, creating a steric barrier to prevent molecular recognition between cell surface receptors and soluble ligands.^{9,131-137} *In vitro*, covalent coupling of PEG to islets has been shown to inhibit islet-mediated activation of lymphocytes in culture,¹³⁸ and protect islets from complement¹³⁷ and TNF- α .¹³⁹ Moreover, PEG has been shown to inhibit graft infiltration by host immune cells in models of renal subcapsular transplantation,¹⁴⁰ a protective mechanism that may be operative in intraportal transplantation as well. Importantly, PEGylation of islets has been shown to improve early islet engraftment in a xenogenic model of IPIT.⁹ This effect was attributed to shielding of islets from complement and xenoreactive antibodies,^{9,137} however it is conceivable that TF expressed on the islet surface was also masked. Indeed, Chen *et al.* have reported similar results in an allograft model.¹⁴¹ Despite promising preliminary results, the efficacy of islet PEGylation may be limited by dependence on purely steric barriers. Therefore, several groups have begun to explore the possibility of constructing permselective membranes of nanoscale thickness directly on the surface of individual islets.¹⁴²⁻¹⁴⁴ Krol *et al.*¹⁴² and Miura *et al.*¹⁴³ have reported the coating of islets with polyelectrolyte multilayer films, while Teramura *et al.* have recently coated islets with a multilayered poly(vinyl alcohol) film covalently assembled via thiol/disulfide exchange.¹⁴⁴ While the efficacy of these films has yet to be evaluated *in vivo*, they offer promising approaches for generating conformal barriers for IPIT.

Arguably more critical than establishment of a physical barrier, however, is the ability of the native endothelium to actively regulate coagulation and inflammation (**Figure 3B**). Heparin, an EC surface glycosaminoglycan, provides one such biochemical barrier through its ability to enhance the capacity of cofactor II and antithrombin

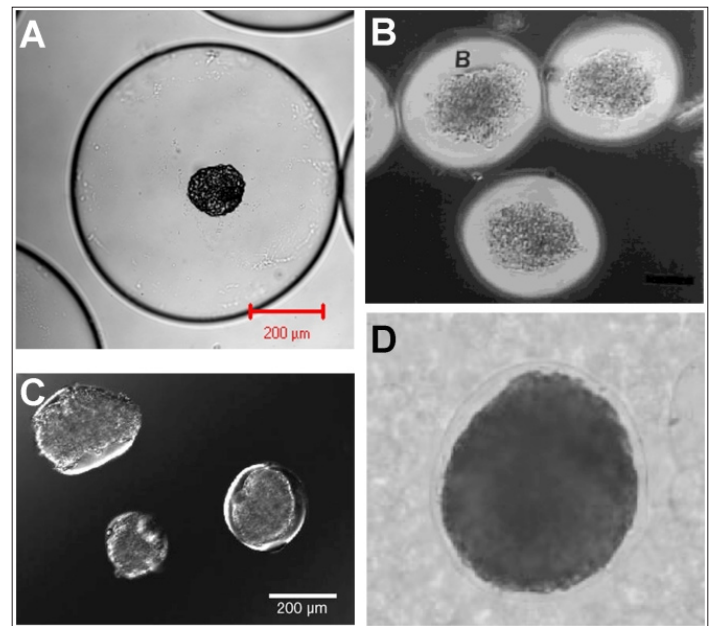


Figure 4. Conformal coatings significantly reduce void volume relative to conventional microcapsules and offer a potential approach to protecting islets during intraportal islet transplantation. (A) An islet encapsulated in a conventional alginate microcapsule. (B) Conformal PEG hydrogel coatings fabricated on porcine islets via interfacial polymerization. (Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.¹³⁰) (C) Islets coated with PEG hydrogel via selective withdrawal, (Adapted with permission from Wyman JL *et al.*¹²⁸) (D) An alginate hydrogel coating formed on a HEK 293 cell spheroid using an aqueous two phase emulsion. (Reprinted with permission from Wiley-Liss, Inc., a subsidiary of John Wiley & Sons, Inc.¹⁶⁸)

to inactivate thrombin. Moreover, heparin can inhibit the formation of NO through its capacity to bind superoxide dismutase¹⁴⁵ and has been shown to limit complement activity.¹⁴⁶⁻¹⁴⁸ Korsgren and colleagues have recently employed biotin/avidin interactions to immobilize macromolecular heparin complexes to the surface of islets.¹⁴⁹ Significantly, surface heparinization of intraportal islet grafts reduced TAT production and early islet damage in an allogeneic porcine model. In light of the significant thrombotic response observed after clinical islet transplantation,^{51,53,57} where heparin is delivered *systemically* during islet infusion,^{14,21} these findings potentially illustrate the increased therapeutic efficacy achieved through local delivery of anticoagulants to the portal bed. Direct comparison between delivery of islet-grafted and systemic heparin will be necessary to unequivocally demonstrate this concept.

Perhaps of greater physiological significance than heparin, thrombomodulin (TM), expressed constitutively by endothelial cells, binds thrombin, sequestering it from participating in thrombotic and inflammatory processes, while simultaneously redirecting its catalytic activity

towards the generation of the potent anticoagulant and anti-inflammatory APC.^{150,151} Our lab has recently developed a novel two-step process for covalently conjugating recombinant human TM to the islet surface in a chemo- and bio-orthogonal manner (**Figure 5**).¹⁵² Using a biosynthetic approach, we have generated a TM construct containing a C-terminal azido (N_3) group, which can be covalently tethered to the islet surface via Staudinger ligation¹⁵³ through the use of a heterobifunctional *N*-Hydroxysuccinimide-PEG-triphenylphosphine linker (**Figure 5**). Conjugation of TM to islets does not influence islet viability or function and increases APC production with an attendant inhibition of islet-mediated coagulation. Moreover, local presentation of TM may act in synergy with the physical barrier provided by surface-grafted PEG chains.

In addition to thrombin generation, local release of adenine nucleotides, including adenosine triphosphate (ATP) and adenosine diphosphate (ADP), from activated endothelium and platelets further potentiate proinflammatory and prothrombotic events. CD39, a transmembrane protein expressed on endothelial cells, regulates these events through its capacity to catalyze the degradation of ATP and ADP to adenosine monophosphate (AMP).^{154,155} Dwyer *et al.* have recently generated transgenic mice that express human CD39 on pancreatic islets. These islets were found to have increased ATPase activity compared to wild-type controls and a consequent capacity to inhibit islet-mediated coagulation.¹⁵⁶ Similarly, genetic engineering approaches have been used to induce expression of the potent anticoagulant hirudin¹⁵⁷ as well as the anti-inflammatory IL-1ra,^{158,159} an inhibitor of IL-1 β action that has improved

islet engraftment when administered systemically.¹⁶⁰ Both genetic engineering¹⁶¹ and cell surface chemistry approaches¹⁶² have been used to display Fas ligand (FasL) on the islet surface, a strategy that could improve the outcome of IPIT by local induction of neutrophil and macrophage apoptosis via the Fas-FasL pathway.^{163,164} Hence, resurfacing the biochemical landscape of islet surfaces through genetic engineering and tissue-targeted chemistry holds considerable promise for locally attenuating thrombotic and inflammatory responses in IPIT.

Conclusions

Concomitant with continuing advancements in islet isolation and immunosuppressive therapy is an emerging need to address the innate inflammatory responses that underlie early islet destruction and primary non-function. Mitigation of thrombotic and inflammatory responses through pre-transplant downregulation of inflammatory mediators expressed by islet grafts, systemic administration of anti-inflammatory and anticoagulant agents, and re-engineering of the islet surface holds considerable promise for improving islet engraftment. While recent investigations, many of which have been described herein, have provided a framework for understanding mechanisms of thrombosis and inflammation in IPIT, continued exploration of this process, particularly in larger animal models, will be necessary to further identify key therapeutic targets. Use of adjunctive anti-inflammatory therapy may also attenuate immune responses through inhibition of inflammatory cell recruitment and antigen presentation, potentially facilitating use of modified immunosuppressive therapies

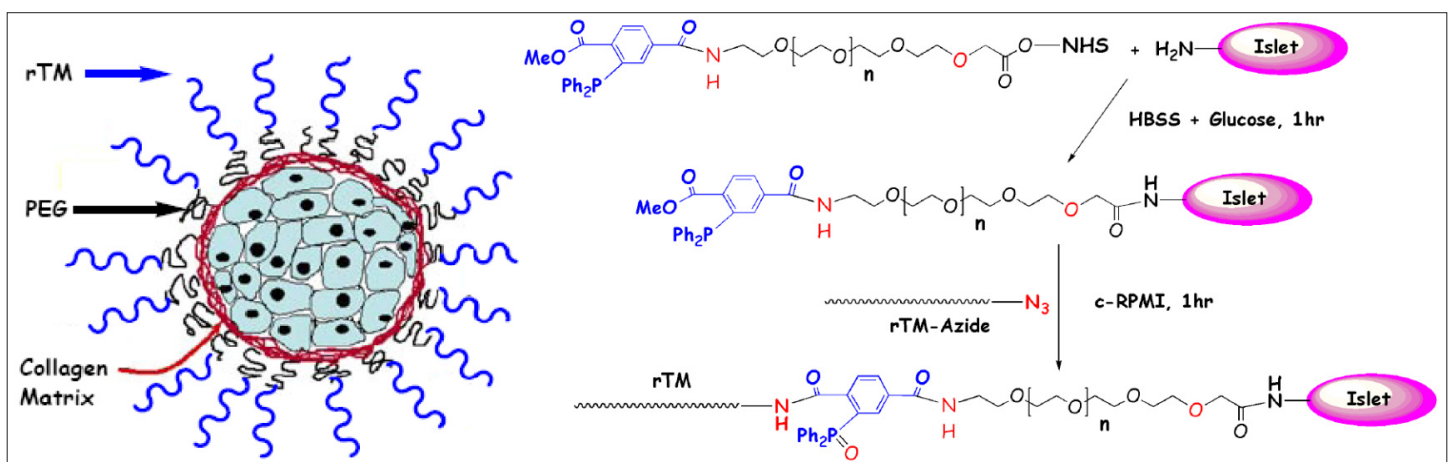


Figure 5. Site-specific immobilization of thrombomodulin (TM) on pancreatic islets through use of Staudinger ligation. Using a biosynthetic approach, human TM was genetically engineered to contain a C-terminal azido (N_3) group (rTM-azide), which can react chemoselectively with phosphine via Staudinger ligation. Phosphine groups were generated on the islet surface via active ester coupling between cell surface amines and a heterobifunctional phosphine-poly(ethylene glycol)-*N*-Hydroxysuccinimide linker, thereby allowing TM to be covalently tethered to islets in a chemo- and bio-orthogonal manner (Reprinted with permission from the American Chemical Society.¹⁵²).

with important clinical benefits. Furthermore, even as xenogenic tissue and insulin producing cell lines emerge as viable alternatives to allogeneic islet grafts, pernicious inflammatory responses are likely to persist and, in this regard, anti-inflammatory therapeutics will play an important role in the efficacy of the next generation of cell-based therapies for diabetes.

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