

# Islet Cell Transplantation

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**Abstract:** Islet cell transplantation is an attractive alternative therapy to conventional insulin treatment or vascularized whole pancreas transplantation for type 1 diabetic patients. It represents a successful example of somatic cell therapy in humans based on complex procedures for islet isolation from whole pancreas. The islets, that are only 1% of the total pancreas tissue, are isolated by two steps method starting with collagenase digestion that operates a rapid dissociation of the stromal component of the gland, while preserving islet anatomical integrity. After digestion, islets are then separated from exocrine tissue by centrifugation in density gradients.

Transplantation consists of a simple injection of few milliliter-purified tissue in the portal vein through a percutaneous trans-hepatic approach performed in local anesthesia.

Several studies have now demonstrated that islet transplant can replace pancreatic endocrine function without major side effects and with liver viability preservation in selected patients affected by long-term type 1 diabetes. It can restore endogenous insulin secretion, achieve insulin independence in more than 80% of patients, and recover the metabolism of glucose, protein and lipids. Improved control of glycated HbA1c, reduced risk of recurrent hypoglycemia and of diabetic complications are also seen as important benefits of islet cell transplantation, irrespective of the status of insulin independence.

Many protocols are now on going for reduction of immunosuppression therapy in recipients, induction of tolerance, and prolongation of graft function.

**Keywords:** Type 1 diabetes mellitus, brittle diabetes, diabetic complications, islet cell transplantation, engraftment, immunosuppression, autoimmunity, glucotoxicity.

Type 1 diabetes mellitus is a syndrome characterized by an abnormal glucose metabolism secondary to the loss of endogenous insulin secretion, mainly affecting young people. Chronic administration of exogenous insulin prevents the acute and fatal consequence of endogenous deficiency of this hormone, but does not hinder the development of long-term complications regarding kidney, eye, vascular and nervous systems. Combined transplantation of vascularized pancreas and kidney, has been proposed to delay the progression of diabetes complications and to avoid the side effects of conventional insulin therapy [1]. However, this is a major surgical procedure burdened by a high rate of complications. Since late in the sixties, different groups have explored the possibility of extracting from the pancreas only the islets of Langerhans that are responsible for insulin secretion.

## THE PROCEDURES

### Islet Isolation

Islets of Langerhans that produce insulin and glucagon, are a complex micro-organ with a proper

inner vascularization and three-dimensional cytoarchitecture, deeply incorporated within the exocrine tissue. Main requirements for successful islet isolation from the whole pancreas are therefore, the complete desegregation of exocrine tissue and in the meantime, the maintenance of islet morphology integrity. The simultaneous achievement of these aims is a difficult challenge [2].

Procedures for islet isolation are being progressively developed. Ricordi created the first method suitable for large-scale human islet isolation [3]. It consisted of the enzymatic-mechanical digestion of pancreas achieved after the injection of collagenase solution into the main pancreatic duct. The gland is subsequently placed in a warmed digestion chamber, where collagenase progressively destroys exocrine tissue favored by mechanical digestion obtained by continuous shaking. This method, although with some modification [2], remains nowadays the most efficient and commonly used.

There are many variables affecting isolation outcome starting from the characteristic of donors and their clinical history, to the experience of isolation teams [4-5]. The quality of pancreas is one of the important determinants of islet yield and quality. Recently, the technique for pancreas preservation for isolation purpose has been changed: organs are commonly transported in

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perfluorocarbons + Belzer solution (Two layer methods, [6]) that has a very high capacity for dissolving oxygen, and a negligible oxygen-binding constant. This solution releases oxygen into the surrounding tissue, improves pancreas preservation, pancreas graft survival after transplantation, and increases islet yields after isolation [7].

The choice of collagenase is also critical for isolation success. The collagenase commonly used for isolation purpose is extracted from *Clostridium Hystolyticum* batches, purified by HPLC method with low endotoxin content [8-9]. The activity of the final enzymatic blend is variable between batches and nowadays, there are not standardized parameters predictive of batch efficiency [5, 10]. It was shown that collagenase should be used at conditions that render it highly active [5]. This can be achieved by selecting only enzyme blends with the highest collagenase activity, or may be achieved by increasing the amount of enzyme used. Over-digestion is undesirable, since many fragmented islets are produced. This appears to result from both a delayed interruption of digestion and a relatively long exposure of free islets to collagenase such as when a long digestion time is required for isolation.

Collagenase is injected into the main duct by peristaltic pump. The team of University of Alberta reported successful results achieved by an automated device for collagenase injection into the pancreas, both controlling temperature of solution and pump pressure [11]. This equipment represented a real progress toward the complete automatization and standardization of procedures.

Isolated islets are then purified from exocrine tissue on the bases of their different densities. Digested tissue are centrifuged in density gradient and the islets, characterized by a lower density, migrate in the upper layer whilst the exocrine tissue in the lower one. Various isopycnic continuous and discontinuous gradients have been reported [12]. Until now, technique using dialyzed Ficoll in Euro-Collins solution has been the most employed and it was considerably improved from use of Cobe 2991 cell separator, which allows large scale purification and guarantees sterility [13].

### Islet Quality Test

Preparations are considered adequate for transplantation according to the following criteria: (1) number of transplanted islets (usually >5,000 per kg of recipient body weight); (2) purity (usually >20%, assessed by dithizone-staining determination of islet/total mass); and (3) islet sterility [14].

Islet "viability" has long been regarded as one of the most important prerequisites and several tests, such as vital assays, morphological assessments, and *in vitro* secretion studies, have been proposed to assess this property before transplantation [15]. Insulin secretion in response to glucose stimulation was initially proposed as the most reliable marker of

islet viability, but no correlation with graft function was eventually found [5, 16]. The most common method for assessing islet viability is based on vital dye that evaluates the integrity of the membrane (i.e. propidium iodide, fluoresceine diacetate) [17]. Recently, a new method was proposed that allows the simultaneous assessment of islet cell viability/apoptosis and islet cell composition [18].

Islet preparation for transplantation has to be sterile in order to avoid microbiological transmission to recipients under immunosuppressive therapy. Microbiological characterization of islet preparation for transplant is problematic. Definitive response from microbiological assessment on supernatants is usually available not before than 5 days, because islets are usually transplanted not earlier than after 3 days of culture. It is well known that human islets suffer when maintained in culture for many days. Strategies to prevent microbiological transmission to recipients include the isolation processing under strict sterile conditions and in laboratories under stringent environmental control and precise standard operative procedures regulated by quality standard system.

### Islet Cell Transplantation

Transplant procedure consists of a simple injection of islets into the portal vein under local anesthesia with minor surgical risks for the patient. Islets are initially injected into the portal vein by a manual syringe. Some years ago, the Miami University proposed a closed gravity fed bag system for islet infusion [19]. The use of gravity allows for a control rate of infusion as well as providing a safety mechanism through natural reduction of flow that parallels any increase in portal pressure, therefore allowing the operator to prevent precipitous pressure rises.

Complications associated with the procedure include portal vein thrombosis, hemorrhages, and transient increase of transaminase values [20]. Few cases of massive portal vein thrombosis were reported [21-22]. They are related with the volume of transplanted tissue and therefore, with a poor purification of the preparation. Generally, it is now prevented by limiting up to 10 ml the volume of transplanted preparation, by the addition of heparin to the transplant solution and by an accurate monitoring of the portal vein pressure. Hemorrhages through the point of catheter insertion frequently appear few hours after transplantation, but seldom it is so prolonged that a significant decrease in hemochrome occurs that should be corrected by blood transfusion. Various techniques have been used to reduce the risk of tract bleeding, including gelfoam, intravascular coils, and cautery [23]. D-Stat, a collagen/thrombin paste injected into the peripheral tract appeared to be the most efficient method to simplify tract closure and to reduce bleeding complications in islet cell transplantation [24].

In islet recipient under new immunosuppressive therapies (including sirolimus, tacrolimus and daclizumab), nearly to 50% of recipients showed a transient increase of transaminase values [25]. Enzyme changes were correlated with islet release of pro inflammatory factor, MCP-1 and tissue factor, and not with the volume of injected tissue, thus suggesting that the damage of liver might be a consequence of an inflammatory reaction more than a consequence of micro-thrombosis [26]. It is interesting that this reaction does not occur in patients treated with cyclosporine and micofenolate that might reduce the consequence of inflammation.

## CLINICAL RESULTS

The main indications for islet cell transplantation are: type I diabetic patients already under immunosuppressive therapy for other transplantations (kidney, liver); patients with brittle diabetes, characterized by unstable glycemia, unawareness hypoglycemia, or progressive chronic complications despite intensive insulin treatment.

The first report of successful islet allotransplantation in humans was in 1990 [27]. It was achieved by an infusion of a single islet preparation in a type 1 diabetic patient already under immunosuppressive therapy for previous kidney transplantation. Currently, about 1000 islet cell transplantations have been performed worldwide.

The success rate has progressively improved in recent years. The International Islet Cell Transplantation Registry showed for a long period, an overall insulin independence of 11% at 1 year after the transplant in type 1 diabetic patients after kidney transplantation [28]. In 2000, the successful experience of the Edmonton group that achieved insulin independence in 7 consecutives brittle type 1 diabetic recipients, deeply changed the expectancy toward this procedure [29]. Changes introduced by the Edmonton group including a new immunosuppressive therapy, based on anti IL2 receptor monoclonal antibodies, sirolimus and low dosage tacrolimus, the absence of pre transplant culture period with a mean minimum number of transplanted islets (10000/kg of recipient body weight, often isolated from more than one pancreas). Their successful results were largely replicated in other experienced centers. Additional requirements for the Edmonton protocol for successful islet cell transplantation appeared to be a well-trained laboratory for islet production and a clinical team experienced in management of immunosuppression therapy [30]. The percentage of insulin independent recipients increased to nearly 80 % one year after islet cell transplantation [31]. Recently, it was reported that a combination of maximized viable islet yield, pre-transplant islet culture, and preemptive immunosuppression can result in successful single-donor islet transplants [32].

The new immunosuppression therapy based on tacrolimus and sirolimus also improves the overall

success rate in islet-kidney-transplanted patients, with 80% of insulin independence: the switch of immunosuppressive protocol prior to islet cell transplantation does not impair the function of transplanted kidney [33].

After successful islet cell transplantation, the overall glucose homeostasis is greatly improved as it is suggested by the optimization of glycosylated hemoglobin values, of mean amplitude of glycemic excursions (a measure of fluctuations in blood glucose concentrations), of insulin resistance [14, 20] and by a decrease of hepatic glucose production [34]. Interestingly a positive impact on glucose homeostasis is also observed in recipients with a partial function of the graft that is in those patients with only a partial reduction in their insulin requirement and with c-peptide values higher than 0.5ng/ml. Similarly, their amino acid and lipid metabolism were restored after transplantation [35] and the appearance and progression of diabetic complications progressively decreased [36-39]. Cardiovascular system appeared to take great advantage from islet cell transplantation, as it was shown by an overall improvement in some parameters of cardiac function such as ejection fraction, peak filling rate in end-diastolic volume (EDV), and QT dispersion [39]. More critical is the impact of islet graft on renal function. In islet after kidney recipients, better kidney graft survival rates were reported with a decrease in microalbuminuria and natriuresis, and an increase in Na(+)/K(+)-ATPase immunoreactivity compared to only kidney transplanted patients [37]. However, data in recipients under an immunosuppression therapy based on the Edmonton protocol are different. In fact, in these patients, an impairment of kidney function was observed in particular, in the presence of a pre-transplant initial nephropathy, probably due to the nephrotoxicity of tacrolimus [40-42]. This is the reason because a normal renal function is nowadays considered a pre-requirement for islet transplants and this is one of the inclusion criteria for the waiting list.

A progressive decline in graft function occurs few years after transplantation. In fact, 5-year follow-up reveals that about 80% have C-peptide present post-islet transplant, but only 10% maintain insulin independence [42]. The median duration of insulin independence was 15 months. The transplantation of additional islet preparations might overcome this problem. Strategies to prolong graft function remain matter of study.

## THE PROBLEMS

### Rate of Complications: The Role of Immunosuppressant

Islet cell transplantation is not an invasive procedure characterized by low rate of complications. These include side effects of the transhepatic catheter insertion (see transplant procedures) and

those of the immunosuppressive therapy, the most common being mouth ulcers, hypercholesterolemia, diarrhea, anemia and leucopenia [42]. Few patients with microalbuminuria developed macroproteinuria. A general problem of all transplantation requiring immunosuppression therapy is the increased risk of malignancy in the long-term follow up. An increased risk for neoplasm should not be excluded for islet cell transplantation even considering that the immunosuppressive therapy currently used for islets has been shown to be safer than those of the past.

The rate of rejection is not known: it is often difficult to distinguish when the graft function is acutely loss for acute rejection or for primary non-function, and when the progressive decrease of islet function is due to chronic rejection or to functional exhaustion. Studies on HLA mismatches and islet allograft function are anecdotal. In islet-after-kidney recipients, it was reported that subsequent islet implantations can reduce alloreactivity for repeated HLA mismatches. Recently, the usefulness of monitoring T-cell reactivity was demonstrated against islet allograft with the aim to correlate immune function with graft survival and to identify conditions for preservation of beta-cell function [43]. The value of these observations should be further confirmed on large number of patients.

On the contrary, the monitoring of recurrence of autoimmunity is well recognized and standardized by auto-antibodies evaluation. An increased autoantibody concentration in serum of recipients after transplantation was associated with a loss of graft function [44-45].

### Islet Engraftment

There are an estimated 1 million islets in a healthy pancreas. Data on islet auto-grafts in nondiabetic patients who have undergone pancreatectomy indicate that the presence of as few as 300,000 islets should lead to insulin independence for more than two years in approximately 75 percent of recipients [46]. The use of at least 10,000 islets for Kg (70Kg = 700,000 Islet) in the Edmonton protocol, together with the variable engraftment and success seen in patients receiving islets after kidney transplants, remain barriers to a wider application of islet cell transplantation to cure type 1 diabetes. Multiple donors would be needed to replace normal islet numbers, which is firstly, scarcely practical given the current restricted supply of donors, and secondly, complicates the procedure and therapy. Among the factors determining the clinical outcome, islet engraftment has been considered one of the principal obstacles for subsequent graft function. Only part of the transplanted beta cell mass survives after the infusion [47]. Non-specific inflammatory damage to the islets may occur during transplantation and this has been demonstrated in experimental animal models. Macrophages infiltrate freshly transplanted islet and secrete a variety of beta-cell toxic metabolites [48]. Intraperitoneally,

transplanted islets would lodge in the presinusoidal space, which are lined by Kupffer cells, and hepatic macrophages, which may play a role in the early nonspecific inflammatory destruction of transplanted islet tissue. Islets produce MCP-1, one of the most powerful chemokine for macrophage and it was reported that islet preparations producing high amount of MCP-1 showed a progressive loss of their *in vivo* function when transplanted in recipients [49]. Islets were recently shown to produce and release also tissue factor, and it was suggested that this could activate the coagulatory cascade in islet recipients [50]. It remains the case that to improve human islet cell transplantation will be necessary to devise strategies to overcome the damage caused by nonspecific inflammation before islets can engraft and start producing insulin.

### Islet Exhaustion

Islet recipients usually lose their insulin independence during the first 2-3 years of follow-up. Sometimes, graft loss is progressive in some months. First phase of insulin response to glucose and to arginine, together with a decreased ratio between insulin and proinsulin is the first sign showing that islets are losing their secretory reserve and that beta mass is decreasing [14, 20].

In some patients, auto- and alloreactivities against transplanted islets cannot be blamed as causes of this phenomenon, as indicated by the fact that ICA, GAD65, and I-A2 auto-antibodies remained negative for the entire follow-up. An important cause of continuous  $\beta$ -cell loss may be the diabetogenicity of the immunosuppressive drugs. Different immunosuppressants have a direct negative effect on human  $\beta$ -cells [51]. Proliferation and differentiation of ductal epithelium cells participate in the regeneration of new  $\beta$ -cells after partial pancreatectomy and other insults [52]. Islet grafts are generally deprived of duct cell tissue and this might preclude the capability of the graft to counteract *in vivo*  $\beta$ -cell loss *via* this pathway of  $\beta$ -cell regeneration. Finally, the occurrence of glucose values partially higher than the normal could induce glucotoxicity and accelerated graft loss [53].

### PERSPECTIVES

Great advances in islet allotransplantation are expected in the next few years.

Several strategies have been proposed to reduce the inflammation and therefore, the number of islets required to restore normoglycemia in recipients. These included modifications of isolation procedures and of culture conditions [54-57], identification of other site for transplantation [58-59] and pharmacological treatments of recipients with anti-inflammatory and anticoagulant drugs [60-61]. One of the most interesting approaches is beta cell engineering with the aim of increasing their resistance to stress of engraftment and to oxidative damage [62].

New pharmacological strategies are characterized by a reduced drug toxicity and/or are aimed at achieving tolerance toward islet graft by the use of lymphocyte-depleting antibodies (anti-thymocyte globulin, campath-1H, hOKT3gamma1 (ala,ala)), or costimulatory blockade (anti-CD154 mAbs, CTLA4-Ig) [63-64]. Alternatively, islets should be protected from recipient immune surveillance by their encapsulation in microcapsule [65].

Recently, a role in islet mass remodeling of mesenchymal and endothelial progenitor stem cells together with ductal cells was postulated [66-67]. Future perspectives include the possibility to co-transplant islet cells together with their precursors in order to prolong graft function in islet recipients.

## IN CONCLUSION

Human islet cell transplantation now represents a feasible clinical option for diabetologists to select patients affected by type 1 diabetes mellitus. There are many positive effects in the overall glucose metabolism in recipients as well as in the prevention of diabetic complications that lead to an improvement of the quality of life.

The results, though promising, still point to the need for further progress in the availability of transplantable islets, improving islet engraftment, extending islet function, and reducing toxic immunosuppression [68]. In addition, alternative islet cell sources are required to expand the number of possible recipients. Manipulation of stem cells and xenotransplantation may yet yield sufficient islets to overcome the problem of donor shortage. These are strong bases to further successes of this procedure.

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