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REVIEW

Recent progress in pancreatic islet transplantation

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Abstract

Diabetes mellitus remains a major burden. More than 200 million people are affected worldwide, which represents 6% of the world's population. Type 1 diabetes mellitus is an autoimmune disease, which induces the permanent destruction of the β-cells of the pancreatic islets of Langerhans. Although intensive insulin therapy has proven effective to delay and sometimes prevent the progression of complications such as nephropathy, neuropathy or retinopathy, it is difficult to achieve and maintain long term in most subjects. The successes achieved over the last few decades by the transplantation of whole pancreas and isolated islets suggest that diabetes can be cured by the replenishment of deficient β cells. However, islet transplantation efforts have various limitations, including the limited supply of donor pancreata, the paucity of experienced islet isolation teams, side effects of immunosuppressants and poor long term results. The purpose of this article is to review the recent progress in clinical islet transplantation for the treatment of diabetes and to describe the recent progress on pancreatic stem/progenitor cell research, which has opened up several possibilities for the development of new treatments for diabetes.

Key words: Type 1 diabetes; Pancreatic islet transplantation; Islet isolation; Pancreatic β-cells; Islet regeneration

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INTRODUCTION

Type 1 diabetes results from a cell-mediated autoimmune attack against insulin-producing β cells in the islets of Langerhans of the pancreas. At the time of clinical diagnosis, over 70% of the β cell mass has typically been destroyed. For patients with type 1 diabetes, exogenous insulin injection to control blood glucose is a lifesaving treatment, but it also has a negative impact on personal and social functioning, not least because of the daily risk of hypoglycemic episodes. In addition, normoglycemia cannot be achieved by exogenous insulin and secondary complications such as retinopathy, neuropathy, nephropathy and cardiovascular disease occur despite good glycemic control. Intensive insulin therapy can help prevent long term diabetic complications and the introduction of insulin pumps into clinical practice has raised the possibility of mimicking the basic endogenous insulin secretion pattern, which directly relates to better glycemic control[1-3]. For insulin-dependent diabetes, controlling the blood glucose level is sometimes difficult, even with intensive insulin therapy. Pancreatic islet transplantation has recently emerged as one of the most promising therapeutic approaches for improving glycometabolic control in type 1 diabetic patients. Although the first human islet allograft transplant was performed in 1974[4], clinical success rates were low until 2000[5]. At that time, dramatic improvement was achieved with the Edmonton

Protocol[6] (Table 1).

The "Edmonton Protocol" introduced several modifications to the transplantation procedure, such as the use of a steroid-free immunosuppression regimen and transplantation of a mean islet mass of 11 000 islet equivalents per kilogram of the patient's weight[6]. The Edmonton breakthrough produced sudden changes: approximately 35 centers began to perform islet isolations and the annual numbers of procedures increased to more than 100 transplants a year, the insulin-free status changed to about 85%, or approximately 60%-70% if all centers were counted[7]. However, islet transplantation efforts have limitations, including the limited supply of donor pancreata, the paucity of experienced islet isolation teams, the side effects of immunosuppressants and poor long term results[8]. Further improvements are necessary to make islet transplantation a routine and effective clinical treatment. This review describes the recent progress in clinical islet transplantation for the treatment of diabetes.

PANCREAS PROCUREMENT AND PRESERVATION

The procedure used for pancreas procurement has remained relatively unchanged since the first transplant and is often performed as part of a multi-organ procurement of the pancreas, liver and kidney[9]. Pancreata are procured using a standardized technique for whole pancreas transplantation to minimize warm ischemia. University of Wisconsin (UW) solution is used for *in situ* perfusion of the donor[10,11].

We recently reported the ductal injection of 1 mL/g pancreas weight of preservation solution before pancreas storage to improve islet yields[12,13]. Since UW solution has several disadvantages for islet isolation, including the inhibition of Liberase activity, we used a new preservation solution [modified Kyoto (MK) solution][14,15]. Compared with the UW solution, the MK solution exerted lower inhibition of collagenase digestion. Moreover, the MK solution significantly inhibited trypsin activity in the digestion step because the solution contains ulinastatin[16]. Ductal injection of the MK solution increased the ATP level in the pancreas tissue, reduced trypsin activity during the digestion step and prevented islet apoptosis[12]. This suggests that the ductal injection of the MK solution leads to improved outcomes for pancreatic islet transplantation.

The two-layer preservation method (TLM), which uses the concept of normobaric oxygenation comprising a cold organ preservation solution (UW solution) with a perfluorochemical (PFC) oxygen carrier solution, with the pancreas being suspended between the two immiscible layers, has been utilized for many clinical trials of islet transplantation[17-20]. However, two recent large-scale studies showed no beneficial effect of TLM, compared with UW storage, on human islet isolation and transplantation[21,22]. We reevaluated the effect of TLM using three different groups: group 1: UW simple storage; group 2: TLM performed by multiorgan procurement teams (not specialists in islet isolation); and group 3: TLM performed by specialists in islet isolation. There were no significant differences between groups 1 and 2 whereas islet yields were significantly higher for group 3 compared with either group 1 or 2. Our data suggest that performance of TLM by experts could improve the outcome of islet isolation and transplantation[17]. Interestingly, one of the groups which reported the lack of beneficial effects of TLM found that the PFC-based one-layer method improved islet yield[23] and the isolation index (fragmentation rate of islets, which is calculated as the ratio between islet equivalents and islet number), compared with TLM[24]. Their data clearly suggest the beneficial effects of pancreas oxygenation by PFC.

ISLET DIGESTION

Human islet isolation is conducted using the standard Ricordi technique with modifications introduced in the Edmonton protocol. The introduction of the semi-automated method for controlled pancreatic digestion using a dissociation chamber (Ricordi Chamber) has dramatically increased islet yields from human pancreata[25] and the general principles of this method still form the basis of current islet isolation technology[26-29]. The factors that influence the process include digestion time, digestion temperature, collagenase concentration and the route of administration of collagenase, which vary widely among protocols. Tissue dissociation enzymes are critical reagents that affect the yield and quality of human pancreatic islets required for islet transplantation. After the discontinuation of the manufacturing Liberase HI because of a small potential for prion disease transmission, the Serva NB-1 enzyme has been commonly used for human islet isolations. Recently, a new enzyme, Liberase mammalian tissue free (MTF) was developed, which is similar to Liberase HI with the exception that no mammalian tissue is used in the manufacture of the collagenase component. One group reported a comparison of the MTF enzyme with Serva NB-1 in clinical islet isolations[30]. The average islet mass after purification was similar between the two groups and there were no significant differences in the isolation success rates (> 400 000 IE) between the two groups, suggesting that MTF may be successfully used for high-yield human islet isolation and clinical transplantation and provides similar quality islets to those derived using NB-1.

ISLET PURIFICATION

Purification of islets from exocrine tissue is a critical step for maintaining high islet yields. Large-scale continuous purification using the COBE2991 cell processor, with Ficoll solutions, is the current gold standard method[27-32]. Two solutions with fixed density (low density; approximately 1.077 g/cm^3, high density; approximately 1.100 g/cm^3) are commonly used for the purification. However, the density of islets/acinar tissue depends on several condi-

Table 1 Criteria for clinical islet transplantation in Japan

Inclusion criteria
< 75 yr of age
Undetectable C-peptide levels (< 0.1 ng/mL)
Duration of type 1 diabetes mellitus for > 5 yr accompanied by recurrent neuroglycopenia
Reduced awareness of hypoglycemic episodes and/or severe glycemic lability despite intensive insulin therapy and glycemic monitoring
Exclusion criteria
Presence of uncorrectable coronary disease
A body mass index of > 25 kg/m^2
Inadequate renal reserve defined as serum creatinine level of > 1.5 mg/dL, creatinine clearance of < 80 mL/min per 1.73 m^2 of body surface area, or a urinary albumin level of > 300 mg/24 h
Negative serological findings for Epstein-Barr virus at the time of assessment

tions, such as warm ischemic time, cold ischemic time, pre-incubation time before purification and osmolality of both the pre-incubation solution and purification solution. The percentage of islets recovered from a standard Ficoll purification has been reported to range from 55%-65%[33,34]. We recently showed the effectiveness of controlled-density gradients using iodixanol[35]. According to the outcome of the density determination step, the density of the purification solutions was controlled (low density, 1.075 g/cm^3; high density, 1.085-1.110 g/cm^3) by changing the volumetric ratio of iodixanol and the purification solutions. The islet yield after purification and the post-purification recovery rate was significantly higher when the controlled density gradient purification method was used than when using the standard continuous gradient purification by Ficoll solutions. The percentage of islets recovered from the controlled-density solution was approximately 80%[35]. Another group also showed the effectiveness of controlled-density gradients using a continuous gradient for the density determination step[36].

Although a controlled-density gradient improves the islet recovery rate in human islet isolation, 20% of islets were still lost during the purification. Ichii *et al*[37] reported that an additional gradient purification method following regular purification could be of assistance in maximizing the number of islet preparations successfully used for transplantation. We also evaluated a combined continuous density/osmolality gradient for supplemental purification of human islets[38]. Low-density/osmolality (1.075-1.110 g/cm^3/400-410 mOsm/kg) and high-density/osmolality (1.090-1.125 g/cm^3/495-505 mOsm/kg) solutions were produced by changing the volumetric ratio of iodixanol, 10 × HBSS, and RP solutions. The addition of supplemental purification could contribute approximately 8% to islet recovery, with viability and potency comparable to that obtained by regular purification. These supplemental purifications following regular purification could maximize the islet yield and improve clinical islet transplantation.

ISLET CULTURE/PRESERVATION

Recently, many centers have introduced the culturing of human islets prior to transplantation[39-43] because it provides many benefits to clinical islet transplantation. *In vitro* culture may reduce islet immunogenicity by depletion of viable hematogenous and lymphoid cells[44] and reduce exocrine contamination of transplanted tissue. Culturing islets may also help them recover from the insult of collagenase digestion. Other benefits are additional quality control testing of isolated islets, initiating time-dependent immunosuppressive protocols, and culture also preserves the islets during travel time for recipients living far away from transplant centers. However, we and other groups have shown that freshly isolated islets are superior to cultured islets for islet transplantation[45-49]. We also reported that the clinical outcome in patients who received cultured islets was significantly lower than in patients who received fresh islets, although the number of subjects evaluated was not sufficient to draw definitive conclusions[50]. It is well documented that isolated islets deteriorate rapidly in culture[41,42,50,51]. Since barely half of the processed pancreata meet the criteria for clinical transplantation in most centers, islet loss during culture results in an even lower transplant rate.

We evaluated the optimal temperature for the culture/preservation of isolated human islets before transplantation. Isolated islets were cultured or preserved for 48 h in the following culture/preservation conditions: preservation at 4℃ in UW solution, and culture at 22℃ or 37℃ in culture medium. The 4℃ preservation of isolated islets prevents deterioration of islet equivalents (24% loss in 37℃ culture and 19% loss in 22℃ culture, but less than 5% loss in 4℃ preservation) and improves the outcome of islet transplantation, thus suggesting that 4℃ preservation is superior to 22℃ or 37℃ culture before human islet transplantation[52].

ISLET TRANSPLANTATION

The implantation site for human islet transplantation has not changed over the years. In more than 90% of cases, the portal vein is cannulated either as part of an operation or as an invasive radiology procedure. The Edmonton group has reported complications of bleeding (8%), portal venous thrombosis (3%) and bile leakage (1%) due to the implantation procedure[7]. Heparin has been added to the process in order to reduce clotting, termed the instant blood mediated inflammatory reaction[53,54]. Moreover, the use of heparin or anti-coagulative agents for several days following islet transplantation, together with intensive

insulin treatment for the first few weeks after transplantation, have recently been reported as critically important variables which improve the efficiency of initial islet engraftment.

IMMUNOSUPPRESSION

A steroid-free immunosuppressive protocol with a combination of sirolimus and tacrolimus, which the Edmonton group reported, has been utilized in many clinical islet transplant trials. However, even with this relatively safe immunosuppressive therapy, the islet graft function deteriorates within 5 years in 50%-80% of patients. Some immunosuppressive agents also have significant and harmful side effects for the transplanted islets[55].

It is currently difficult to sensitively monitor islet rejection, and improvements in such monitoring are mandatory. For example, Close *et al*[56] reported that polyclonal antibody induction may offer better results than daclizumab, according to registry data. Bellin *et al*[57] recently showed improved success with a modified immunosuppressive protocol and use of antithymocyte globulin plus etanercept as induction therapy. These results suggest that modifications of the immunosuppressive protocol are key to improving long term graft survival.

NEW SOURCES OF INSULIN-PRODUCING CELLS

The results obtained through human pancreatic islet transplantation have spurred the search for new sources of insulin-producing cells. Regeneration of β cells from stem and progenitor cells is an attractive method to restore the islet cell mass. Pancreatic stem/progenitor cells have been identified and the formation of new β cells from pancreatic duct, acinar and liver cells is an active area of investigation[58-62]. Embryonic stem (ES) cells and induced pluripotent stem (iPS) cells are also alternative sources for the treatment of diabetes[63,64]. D'Amour *et al*[65] recently developed a five-step protocol for differentiation of human ES cells to endocrine cells capable of synthesizing the pancreatic hormones insulin, glucagon, somatostatin, pancreatic polypeptide and ghrelin. Protocols for the *in vitro* differentiation of ES cells based on normal developmental processes have generated β-like cells that produce high levels of insulin, although at low efficiency and without full responsiveness to extracellular levels of glucose.

On the other hand, a significant number of problems remain unsolved in terms of the clinical application of these strategies, such as the ethical issues and need for immunosuppression after transplantation. The technical breakthrough of iPS cells has significant implications for overcoming the ethical issues associated with ES cell derivation from embryos. iPS cells can also yield insulin-producing cells following similar approaches. Although two papers showed the generation of insulin-secreting islet-like clusters from human iPS cells[66,67], the efficiency of the method for generating these insulin-producing cells seems low. However, the method used for ES cells may represent a critical step in the development of insulin-producing cells from iPS cells.

CONCLUSION

Islet transplantation is an alternative method to whole pancreas transplantation in patients with type 1 diabetes because of its low invasiveness and safety for the recipient[68,69]. Significant progress in clinical islet transplantation has occurred during recent years, with a progressive improvement of short and long term outcomes. However, the overall long term function of transplanted islets is not satisfactory enough to merit widespread clinical application; at 5 years after transplantation, only 15% of islet recipients remain insulin independent[70]. Moreover, the risk for sensitization after islet transplantation has been reported recently[71]. The hope remains that 1 d islet transplantation will replace insulin therapy, but there is still much work that needs to be done before this goal can be reached. Improving the efficacy of islet transplantation seems to be the most realistic and prudent method to cure diabetes and further investigations to induce the differentiation of stem/progenitor cells into insulin-producing cells will help to establish cell-based therapies for diabetes.

REFERENCES

1 The effect of intensive treatment of diabetes on the development and progression of long-term complications in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial Research Group. *N Engl J Med* 1993; **329**: 977-986
2 The effect of intensive diabetes treatment on the progression of diabetic retinopathy in insulin-dependent diabetes mellitus. The Diabetes Control and Complications Trial. *Arch Ophthalmol* 1995; **113**: 36-51
3 **Dufrane D**, Gianello P. Pig islet xenotransplantation into non-human primate model. *Transplantation* 2008; **86**: 753-760
4 **Najarian JS**, Sutherland DE, Matas AJ, Steffes MW, Simmons RL, Goetz FC. Human islet transplantation: a preliminary report. *Transplant Proc* 1977; **9**: 233-236
5 **Ricordi C**, Strom TB. Clinical islet transplantation: advances and immunological challenges. *Nat Rev Immunol* 2004; **4**: 259-268
6 **Shapiro AM**, Lakey JR, Ryan EA, Korbutt GS, Toth E, Warnock GL, Kneteman NM, Rajotte RV. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* 2000; **343**: 230-238
7 **Langer RM**. Islet transplantation: lessons learned since the Edmonton breakthrough. *Transplant Proc* 2010; **42**: 1421-1424
8 **Robertson RP**. Islet transplantation as a treatment for diabetes - a work in progress. *N Engl J Med* 2004; **350**: 694-705
9 **Ikemoto T**, Noguchi H, Shimoda M, Naziruddin B, Jackson A, Tamura Y, Fujita Y, Onaca N, Levy MF, Matsumoto S. Islet cell transplantation for the treatment of type 1 diabetes in the USA. *J Hepatobiliary Pancreat Surg* 2009; **16**: 118-123
10 **D'Alessandro AM**, Stratta RJ, Sollinger HW, Kalayoglu M, Pirsch JD, Belzer FO. Use of UW solution in pancreas transplantation. *Diabetes* 1989; **38** Suppl 1: 7-9
11 **Munn SR**, Kaufman DB, Field MJ, Viste AB, Sutherland DE. Cold-storage preservation of the canine and rat pancreas

prior to islet isolation. *Transplantation* 1989; **47**: 28-31
12 **Noguchi H**, Ueda M, Hayashi S, Kobayashi N, Okitsu T, Iwanaga Y, Nagata H, Nakai Y, Matsumoto S. Ductal injection of preservation solution increases islet yields in islet isolation and improves islet graft function. *Cell Transplant* 2008; **17**: 69-81
13 **Noguchi H**, Matsumoto S, Onaca N, Naziruddin B, Jackson A, Ikemoto T, Shimoda M, Fujita Y, Chujo D, Iwanaga Y, Nagata H, Okitsu T, Kobayashi N, Ueno H, Chaussabel D, Grayburn P, Banchereau J, Levy MF. Ductal injection of JNK inhibitors before pancreas preservation prevents islet apoptosis and improves islet graft function. *Hum Gene Ther* 2009; **20**: 73-85
14 **Noguchi H**, Ueda M, Nakai Y, Iwanaga Y, Okitsu T, Nagata H, Yonekawa Y, Kobayashi N, Nakamura T, Wada H, Matsumoto S. Modified two-layer preservation method (M-Kyoto/PFC) improves islet yields in islet isolation. *Am J Transplant* 2006; **6**: 496-504
15 **Noguchi H**, Naziruddin B, Onaca N, Jackson A, Shimoda M, Ikemoto T, Fujita Y, Kobayashi N, Levy MF, Matsumoto S. Comparison of modified Celsior solution and M-kyoto solution for pancreas preservation in human islet isolation. *Cell Transplant* 2010; **19**: 751-758
16 **Noguchi H**, Ueda M, Hayashi S, Kobayashi N, Okitsu T, Iwanaga Y, Nagata H, Liu X, Kamiya H, Levy MF, Matsumoto S. Comparison of trypsin inhibitors in preservation solution for islet isolation. *Cell Transplant* 2009; **18**: 541-547
17 **Noguchi H**, Levy MF, Kobayashi N, Matsumoto S. Pancreas preservation by the two-layer method: does it have a beneficial effect compared with simple preservation in University of Wisconsin solution? *Cell Transplant* 2009; **18**: 497-503
18 **Tsujimura T**, Kuroda Y, Kin T, Avila JG, Rajotte RV, Korbutt GS, Ryan EA, Shapiro AM, Lakey JR. Human islet transplantation from pancreases with prolonged cold ischemia using additional preservation by the two-layer (UW solution/perfluorochemical) cold-storage method. *Transplantation* 2002; **74**: 1687-1691
19 **Ricordi C**, Fraker C, Szust J, Al-Abdullah I, Poggioli R, Kirlew T, Khan A, Alejandro R. Improved human islet isolation outcome from marginal donors following addition of oxygenated perfluorocarbon to the cold-storage solution. *Transplantation* 2003; **75**: 1524-1527
20 **Hering BJ**, Kandaswamy R, Harmon JV, Ansite JD, Clemmings SM, Sakai T, Paraskevas S, Eckman PM, Sageshima J, Nakano M, Sawada T, Matsumoto I, Zhang HJ, Sutherland DE, Bluestone JA. Transplantation of cultured islets from two-layer preserved pancreases in type 1 diabetes with anti-CD3 antibody. *Am J Transplant* 2004; **4**: 390-401
21 **Caballero-Corbalán J**, Eich T, Lundgren T, Foss A, Felldin M, Källen R, Salmela K, Tibell A, Tufveson G, Korsgren O, Brandhorst D. No beneficial effect of two-layer storage compared with UW-storage on human islet isolation and transplantation. *Transplantation* 2007; **84**: 864-869
22 **Kin T**, Mirbolooki M, Salehi P, Tsukada M, O'Gorman D, Imes S, Ryan EA, Shapiro AM, Lakey JR. Islet isolation and transplantation outcomes of pancreas preserved with University of Wisconsin solution versus two-layer method using preoxygenated perfluorocarbon. *Transplantation* 2006; **82**: 1286-1290
23 **Brandhorst D**, Iken M, Brendel MD, Bretzel RG, Brandhorst H. Long-term preservation of the pig pancreas by a one-layer method for successful islet isolation. *Transplant Proc* 2005; **37**: 229-230
24 **Brandhorst D**, Iken M, Brendel MD, Bretzel RG, Brandhorst H. Successful pancreas preservation by a perfluorocarbon-based one-layer method for subsequent pig islet isolation. *Transplantation* 2005; **79**: 433-437
25 **Ricordi C**, Lacy PE, Finke EH, Olack BJ, Scharp DW. Automated method for isolation of human pancreatic islets. *Diabetes* 1988; **37**: 413-420
26 **Noguchi H**, Matsushita M, Okitsu T, Moriwaki A, Tomizawa K, Kang S, Li ST, Kobayashi N, Matsumoto S, Tanaka K, Tanaka N, Matsui H. A new cell-permeable peptide allows successful allogeneic islet transplantation in mice. *Nat Med* 2004; **10**: 305-309
27 **Shapiro AM**, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, Cagliero E, Alejandro R, Ryan EA, DiMercurio B, Morel P, Polonsky KS, Reems JA, Bretzel RG, Bertuzzi F, Froud T, Kandaswamy R, Sutherland DE, Eisenbarth G, Segal M, Preiksaitis J, Korbutt GS, Barton FB, Viviano L, Seyfert-Margolis V, Bluestone J, Lakey JR. International trial of the Edmonton protocol for islet transplantation. *N Engl J Med* 2006; **355**: 1318-1330
28 **Froud T**, Ricordi C, Baidal DA, Hafiz MM, Ponte G, Cure P, Pileggi A, Poggioli R, Ichii H, Khan A, Ferreira JV, Pugliese A, Esquenazi VV, Kenyon NS, Alejandro R. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. *Am J Transplant* 2005; **5**: 2037-2046
29 **Hering BJ**, Kandaswamy R, Ansite JD, Eckman PM, Nakano M, Sawada T, Matsumoto I, Ihm SH, Zhang HJ, Parkey J, Hunter DW, Sutherland DE. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA* 2005; **293**: 830-835
30 **O'Gorman D**, Kin T, Imes S, Pawlick R, Senior P, Shapiro AM. Comparison of human islet isolation outcomes using a new mammalian tissue-free enzyme versus collagenase NB-1. *Transplantation* 2010; **90**: 255-259
31 **Goto M**, Eich TM, Felldin M, Foss A, Källen R, Salmela K, Tibell A, Tufveson G, Fujimori K, Engkvist M, Korsgren O. Refinement of the automated method for human islet isolation and presentation of a closed system for in vitro islet culture. *Transplantation* 2004; **78**: 1367-1375
32 **Markmann JF**, Deng S, Huang X, Desai NM, Velidedeoglu EH, Lui C, Frank A, Markmann E, Palanjian M, Brayman K, Wolf B, Bell E, Vitamaniuk M, Doliba N, Matschinsky F, Barker CF, Naji A. Insulin independence following isolated islet transplantation and single islet infusions. *Ann Surg* 2003; **237**: 741-749; discussion 749-750
33 **Ichii H**, Wang X, Messinger S, Alvarez A, Fraker C, Khan A, Kuroda Y, Inverardi L, Goss JA, Alejandro R, Ricordi C. Improved human islet isolation using nicotinamide. *Am J Transplant* 2006; **6**: 2060-2068
34 **Noguchi H**, Matsumoto S. Islet transplantation at the Diabetes Research Institute Japan. *J Hepatobiliary Pancreat Surg* 2008; **15**: 278-283
35 **Noguchi H**, Ikemoto T, Naziruddin B, Jackson A, Shimoda M, Fujita Y, Chujo D, Takita M, Kobayashi N, Onaca N, Levy MF, Matsumoto S. Iodixanol-controlled density gradient during islet purification improves recovery rate in human islet isolation. *Transplantation* 2009; **87**: 1629-1635
36 **Anazawa T**, Matsumoto S, Yonekawa Y, Loganathan G, Wilhelm JJ, Soltani SM, Papas KK, Sutherland DE, Hering BJ, Balamurugan AN. Prediction of pancreatic tissue densities by an analytical test gradient system before purification maximizes human islet recovery for islet autotransplantation/allotransplantation. *Transplantation* 2011; **91**: 508-514
37 **Ichii H**, Pileggi A, Molano RD, Baidal DA, Khan A, Kuroda Y, Inverardi L, Goss JA, Alejandro R, Ricordi C. Rescue purification maximizes the use of human islet preparations for transplantation. *Am J Transplant* 2005; **5**: 21-30
38 **Noguchi H**, Naziruddin B, Shimoda M, Chujo D, Takita M, Sugimoto K, Itoh T, Onaca N, Levy MF, Matsumoto S. A combined continuous density/osmolality gradient for supplemental purification of human islets. *Cell Medicine* 2011; In press
39 **Friberg AS**, Brandhorst H, Buchwald P, Goto M, Ricordi C, Brandhorst D, Korsgren O. Quantification of the islet product: presentation of a standardized current good manufac-

turing practices compliant system with minimal variability. *Transplantation* 2011; **91**: 677-683
40 **Loganathan G**, Dawra RK, Pugazhenthi S, Guo Z, Soltani SM, Wiseman A, Sanders MA, Papas KK, Velayutham K, Saluja AK, Sutherland DE, Hering BJ, Balamurugan AN. Insulin Degradation by Acinar Cell Proteases Creates a Dysfunctional Environment for Human Islets Before/After Transplantation: Benefits of α-1 Antitrypsin Treatment. *Transplantation* 2011; **92**: 1222-1230
41 **Keymeulen B**, Gillard P, Mathieu C, Movahedi B, Maleux G, Delvaux G, Ysebaert D, Roep B, Vandemeulebroucke E, Marichal M, In 't Veld P, Bogdani M, Hendrieckx C, Gorus F, Ling Z, van Rood J, Pipeleers D. Correlation between beta cell mass and glycemic control in type 1 diabetic recipients of islet cell graft. *Proc Natl Acad Sci USA* 2006; **103**: 17444-17449
42 **Kin T**, Senior P, O'Gorman D, Richer B, Salam A, Shapiro AM. Risk factors for islet loss during culture prior to transplantation. *Transpl Int* 2008; **21**: 1029-1035
43 **Ryan EA**, Paty BW, Senior PA, Bigam D, Alfadhli E, Kneteman NM, Lakey JR, Shapiro AM. Five-year follow-up after clinical islet transplantation. *Diabetes* 2005; **54**: 2060-2069
44 **Kedinger M**, Haffen K, Grenier J, Eloy R. In vitro culture reduces immunogenicity of pancreatic endocrine islets. *Nature* 1977; **270**: 736-738
45 **Ammendrup A**, Maillard A, Nielsen K, Aabenhus Andersen N, Serup P, Dragsbaek Madsen O, Mandrup-Poulsen T, Bonny C. The c-Jun amino-terminal kinase pathway is preferentially activated by interleukin-1 and controls apoptosis in differentiating pancreatic beta-cells. *Diabetes* 2000; **49**: 1468-1476
46 **King A**, Lock J, Xu G, Bonner-Weir S, Weir GC. Islet transplantation outcomes in mice are better with fresh islets and exendin-4 treatment. *Diabetologia* 2005; **48**: 2074-2079
47 **Olsson R**, Carlsson PO. Better vascular engraftment and function in pancreatic islets transplanted without prior culture. *Diabetologia* 2005; **48**: 469-476
48 **Noguchi H**, Naziruddin B, Shimoda M, Fujita Y, Chujo D, Takita M, Peng H, Sugimoto K, Itoh T, Tamura Y, Olsen GS, Kobayashi N, Onaca N, Levy MF, Matsumoto S. Comparison of fresh and cultured islets from human and porcine pancreata. *Transplant Proc* 2010; **42**: 2084-2086
49 **Noguchi H**, Naziruddin B, Jackson A, Shimoda M, Ikemoto T, Fujita Y, Chujo D, Takita M, Peng H, Sugimoto K, Itoh T, Kobayashi N, Onaca N, Levy MF, Matsumoto S. Fresh islets are more effective for islet transplantation than cultured islets. *Cell Transplantation* 2011; In press
50 **Noguchi H**, Yamada Y, Okitsu T, Iwanaga Y, Nagata H, Kobayashi N, Hayashi S, Matsumoto S. Secretory unit of islet in transplantation (SUIT) and engrafted islet rate (EIR) indexes are useful for evaluating single islet transplantation. *Cell Transplant* 2008; **17**: 121-128
51 **Ichii H**, Sakuma Y, Pileggi A, Fraker C, Alvarez A, Montelongo J, Szust J, Khan A, Inverardi L, Naziruddin B, Levy MF, Klintmalm GB, Goss JA, Alejandro R, Ricordi C. Shipment of human islets for transplantation. *Am J Transplant* 2007; **7**: 1010-1020
52 **Noguchi H**, Naziruddin B, Jackson A, Shimoda M, Ikemoto T, Fujita Y, Chujo D, Takita M, Kobayashi N, Onaca N, Levy MF, Matsumoto S. Low-temperature preservation of isolated islets is superior to conventional islet culture before islet transplantation. *Transplantation* 2010; **89**: 47-54
53 **Johansson H**, Lukinius A, Moberg L, Lundgren T, Berne C, Foss A, Felldin M, Källen R, Salmela K, Tibell A, Tufveson G, Ekdahl KN, Elgue G, Korsgren O, Nilsson B. Tissue factor produced by the endocrine cells of the islets of Langerhans is associated with a negative outcome of clinical islet transplantation. *Diabetes* 2005; **54**: 1755-1762
54 **Moberg L**, Johansson H, Lukinius A, Berne C, Foss A, Källen R, Østraat Ø, Salmela K, Tibell A, Tufveson G, Elgue G, Nilsson Ekdahl K, Korsgren O, Nilsson B. Production of tissue factor by pancreatic islet cells as a trigger of detrimental thrombotic reactions in clinical islet transplantation. *Lancet* 2002; **360**: 2039-2045
55 **Nanji SA**, Shapiro AM. Islet transplantation in patients with diabetes mellitus: choice of immunosuppression. *BioDrugs* 2004; **18**: 315-328
56 **Close N**, Alejandro R, Hering B, Appel M. Second annual analysis of the collaborative islet transplant registry. *Transplant Proc* 2007; **39**: 179-182
57 **Bellin MD**, Kandaswamy R, Parkey J, Zhang HJ, Liu B, Ihm SH, Ansite JD, Witson J, Bansal-Pakala P, Balamurugan AN, Papas KK, Sutherland DE, Moran A, Hering BJ. Prolonged insulin independence after islet allotransplants in recipients with type 1 diabetes. *Am J Transplant* 2008; **8**: 2463-2470
58 **Noguchi H**. Pancreatic stem/progenitor cells for the treatment of diabetes. *Rev Diabet Stud* 2010; **7**: 105-111
59 **Noguchi H**, Naziruddin B, Shimoda M, Fujita Y, Chujo D, Takita M, Peng H, Sugimoto K, Itoh T, Tamura Y, Olsen GS, Kobayashi N, Onaca N, Hayashi S, Levy MF, Matsumoto S. Induction of insulin-producing cells from human pancreatic progenitor cells. *Transplant Proc* 2010; **42**: 2081-2083
60 **Noguchi H**, Naziruddin B, Jackson A, Shimoda M, Ikemoto T, Fujita Y, Chujo D, Takita M, Kobayashi N, Onaca N, Hayashi S, Levy MF, Matsumoto S. Characterization of human pancreatic progenitor cells. *Cell Transplant* 2010; **19**: 879-886
61 **Noguchi H**. Production of pancreatic beta-cells from stem cells. *Curr Diabetes Rev* 2010; **6**: 184-190
62 **Noguchi H**, Oishi K, Ueda M, Yukawa H, Hayashi S, Kobayashi N, Levy MF, Matusmoto S. Establishment of mouse pancreatic stem cell line. *Cell Transplant* 2009; **18**: 563-571
63 **Hoffman LM**, Carpenter MK. Characterization and culture of human embryonic stem cells. *Nat Biotechnol* 2005; **23**: 699-708
64 **Soto-Gutiérrez A**, Kobayashi N, Rivas-Carrillo JD, Navarro-Alvarez N, Zhao D, Okitsu T, Noguchi H, Basma H, Tabata Y, Chen Y, Tanaka K, Narushima M, Miki A, Ueda T, Jun HS, Yoon JW, Lebkowski J, Tanaka N, Fox IJ. Reversal of mouse hepatic failure using an implanted liver-assist device containing ES cell-derived hepatocytes. *Nat Biotechnol* 2006; **24**: 1412-1419
65 **D'Amour KA**, Bang AG, Eliazer S, Kelly OG, Agulnick AD, Smart NG, Moorman MA, Kroon E, Carpenter MK, Baetge EE. Production of pancreatic hormone-expressing endocrine cells from human embryonic stem cells. *Nat Biotechnol* 2006; **24**: 1392-1401
66 **Tateishi K**, He J, Taranova O, Liang G, D'Alessio AC, Zhang Y. Generation of insulin-secreting islet-like clusters from human skin fibroblasts. *J Biol Chem* 2008; **283**: 31601-31607
67 **Zhang D**, Jiang W, Liu M, Sui X, Yin X, Chen S, Shi Y, Deng H. Highly efficient differentiation of human ES cells and iPS cells into mature pancreatic insulin-producing cells. *Cell Res* 2009; **19**: 429-438
68 **Noguchi H**. Pancreatic islet transplantation. *World J Gastrointest Surg* 2009; **1**: 16-20
69 **Noguchi H**, Iwanaga Y, Okitsu T, Nagata H, Yonekawa Y, Matsumoto S. Evaluation of islet transplantation from non-heart beating donors. *Am J Transplant* 2006; **6**: 2476-2482
70 **Kin T**, Shapiro AM. Surgical aspects of human islet isolation. *Islets* 2010; **2**: 265-273
71 **Campbell PM**, Senior PA, Salam A, Labranche K, Bigam DL, Kneteman NM, Imes S, Halpin A, Ryan EA, Shapiro AM. High risk of sensitization after failed islet transplantation. *Am J Transplant* 2007; **7**: 2311-2317

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