Improving the use of donor organs in pancreas and islet of Langerhans transplantation

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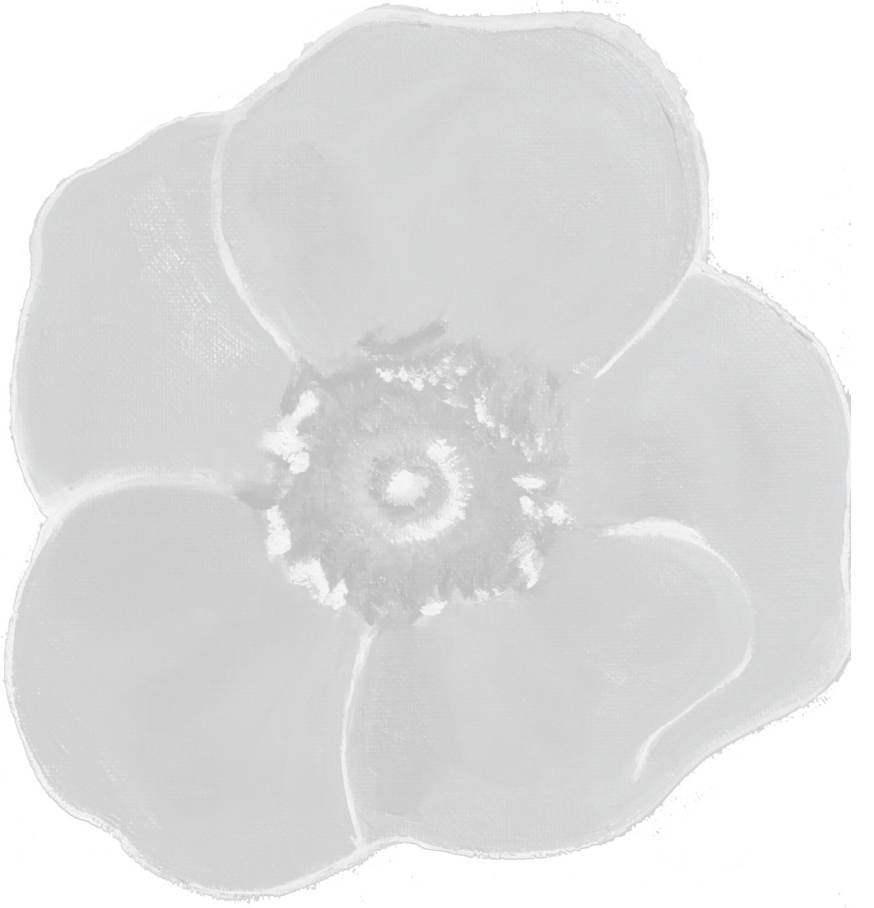
Chapter 1

Introduction



INTRODUCTION

Type 1 diabetes mellitus is caused by autoimmune destruction of insulin-producing beta cells in the islets of Langerhans of the pancreas. In patients with type 1 diabetes, insulin treatment is the only life-saving therapy. Long-term prognosis and quality of life of these patients is largely determined by the occurrence and severity of secondary diabetic complications. However, even when insulin treatment is well tolerated and carried out in a diligent way, metabolic derangements and long-term complications still occur, resulting in reduced patient survival (1, 2). Beta cell replacement, by transplantation of whole pancreas or isolated islets of Langerhans to restore endogenous insulin secretion, has emerged as a logical alternative to insulin injections. However, there is a shortage of donor pancreata relative to the needs of potential transplant recipients (3). Therefore, optimal use of the available donor organs is vital. In contribution to optimize the use of available organs, the focus of this thesis was on the improvement of pancreas graft survival in pancreas transplantation and to optimize islet isolation outcomes in islet of Langerhans transplantation. Furthermore, since porcine islet transplantation is an alternative to compensate for the shortage of human donor organs, we focused on optimizing porcine islet isolation outcome as well.

Pancreas transplantation

Transplantation of the whole pancreas is a complex procedure that can lead to good long term metabolic control and prolong survival of both nephropathic and neuropathic diabetic patients (4-7). The first clinical pancreas transplantation was performed in 1966, simultaneous with a kidney transplant in an uremic diabetic patient at the University of Minnesota (8). The success rate (long-term insulin independence) of pancreas transplantation was initially low, but increased dramatically in the 1980's. Pancreas graft and patient survival have further improved in recent years due to improved procurement and transplantation techniques, immunosuppression regimes and more emphasis on donor management and careful recipient selection (9-12).

The majority of pancreas transplantations are performed simultaneously with a kidney transplantation (simultaneous pancreas kidney transplantation: SPK), in patients with type 1 diabetes with end-stage or pre-emptive renal disease. Other possibilities are: pancreas after kidney transplantation (PAK), in which a pancreas from a deceased donor is transplanted in an insulin-dependent diabetic patient with a good functioning kidney transplant, and pancreas transplantation alone (PTA), in a type 1 diabetic patient with frequent and severe episodes of hypoglycemia, hyperglycemia or ketoacidosis but with preserved renal function. SPK transplants are performed more frequently than solitary pancreas transplants (211 SPK vs. 92 solitary pancreas transplants, in 2010 in the Eurotransplant region (3)). However, there is an increase in solitary pancreas transplantations, particularly PAK, reflecting an emphasis on living donor kidney

transplants in uremic diabetic patients to preempt the need for dialysis (13). This is also seen in the Netherlands where in 2005 a single solitary pancreas transplantation was performed, with an increase to 11 in 2010, as reported by Eurotransplant (3). The International Pancreas Transplant Registry (IPTR) maintains a database of all reported pancreas transplants worldwide. In their annual report of 2004, they reported 1 year pancreas graft survival rates of 80-85% and patient survival rates 95% for both SPK and PAK and 98% for PTA (13). In the Leiden University Medical Center, where 85% of all pancreas transplantations in the Netherlands are performed, even better graft survival rates are obtained, in particular with primary bladder-drainage followed by elective enteric conversion 6-12 months later, used in most of the patients. In these patients 1 year pancreas graft survival rate was 88% (14).

Pancreas graft and patient survival rates are influenced by several factors, e.g. procurement, transplantation technique, immunosuppression regimes and donor and recipient related factors. Many donor and recipient characteristics have been reported to influence pancreas graft survival (11, 12, 14-30). This raises the question on how the impact of donor characteristics relate to that of the recipient. In order to further improve pancreas graft and patient survival, do we have to focus on donor selection, optimize recipient condition or donor-recipient matching? No studies so far have examined the contribution of donor and recipient factors to graft survival. We therefore aimed in **chapter 2** to identify donor and recipient factors influencing pancreas graft survival, to evaluate the impact of donor and recipient factors on pancreas graft survival, and to compare their contribution in explaining graft survival differences between pancreas recipients.

The procurement technique of a pancreas graft has also been shown to influence pancreas graft and patient survival. Surgical injuries that occur during pancreas procurement may lead to complications after transplantation, impaired function of the allograft, graft loss or even death of the patient. These injuries may be so severe that the pancreas is not transplanted in order to protect the recipient. Liposis of the graft and critical vessel injuries have been reported as reasons for pancreas refusal after procurement (31). However, only few studies have addressed this issue. We therefore assessed how often pancreata were refused for transplantation during back-table inspection in our center and which type of problems were responsible for the decision not to transplant the pancreas (**chapter 3**). A better understanding of the type of problems that occur could lead to a higher awareness for injuries. In combination with training this could potentially lead to avoidance of these injuries. This would result in the use of more donor pancreata that would otherwise have been discarded because of the injuries. Furthermore the quality of the transplanted pancreata with minor injuries would improve when these are avoided. This would eventually result in better pancreas graft and patient survival.

Islet transplantation

Whole pancreas transplantation, however, is not devoid of complications, mainly secondary to surgery and immunosuppressive therapy (24). The alternative to transplantation of the pancreas is transplantation of isolated islets as a free graft. Islet transplantation is minimally invasive and has low morbidity because the islets are infused percutaneously into the hepatic portal vein. Furthermore, a pancreas graft can still be used for islet isolation and transplantation when rejected for pancreas transplantation. The first clinical islet allograft was performed in 1974 in a diabetic recipient who previous to the islet transplant received a kidney transplant (32). Since the late 1980s, the feasibility of isolating and purifying human islets from pancreatic organs of deceased donors raised hope that purified pancreatic islet cells, rather than an entire gland, could cure diabetes (33). However, a limiting factor in islet transplantation is the islet isolation yield that can be obtained from donor pancreata. In some cases, sufficient islet numbers can be obtained from a single donor, but even in the most successful studies, multiple transplantations are necessary to obtain (temporary) normalization of hyperglycemia in the recipients (34-39). Therefore, the supply of human donor pancreata as source of islets is insufficient.

In order to potentially enable the use of a single organ, several strategies were developed to maximize islet yield, e.g. by choosing better culture conditions, and improving donor and recipient selection. Many donor and recipient factors have been reported to have an influence on islet isolation yield (40-72). However, no uniformity is to be found in factors that are reported. Because of the scattered information, valuable information is potentially missed because there is insufficient power to determine the independent effect of the donor factors on islet isolation outcome in a single study. **Chapter 4** offers a review of the literature; identifying donor and recipient factors influencing islet isolation yield and provides recommendations for standardized reports of donor and recipient factors in order to provide better comparisons in the future and to improve the power by providing enough data to perform a meta-analysis.

Despite significant efforts to improve the yield of isolated islets by optimizing donor and recipient factors, isolation protocols and culture conditions, islet isolation yields in human pancreata remain unpredictable and variable. Histomorphological aspects (e.g. collagen and other matrix elements) of the pancreas are thought to play a role in these variations (73-79). When studying histological characteristics of human donor pancreata, a remarkably high number of hyperemic islets (HIs) was encountered. Similar islets have only been reported anecdotally in the literature but no mechanisms were described regarding their origin and no relevance has been determined from the perspective of islets isolation for transplantation (80-84). We therefore aimed to determine the relevance of the presence of HIs in human donor pancreata for isolation outcome and to identify donor and procurement factors associated with the occurrence of HIs (**chapter 5**).

Xenotransplantation

Xenotransplantation of porcine islets of Langerhans is another way to overcome the shortage of human donor pancreata. For various reasons, the pig is considered to be the preferred source of pancreatic xeno-islets. Pig insulin, which differs from the human type by only one amino acid, is active and well tolerated in humans. For years prior to the production of human recombinant insulin, patients were successfully treated with insulin injections extracted from swines. Transplantation of porcine islets has been proven to be successful in non-human primates as well as in humans (85-88). Moreover, pig islets can be successfully isolated and purified from adult pigs with a method that is similar to the one used for human islets (89). Advantages of using pigs as a source of islets for transplantation are, at least in theory, numerous. Besides the benefit of unlimited tissue supply, a higher quality of donor organs could be expected by planned elective organ harvesting, therefore minimizing cold ischemia and consequently improving islet yields. However, porcine islet isolation procedures have been shown to be notoriously difficult and provide unpredictable and variable islet isolation yields, even more so than in human pancreata (90-92). Because pancreata from adult pigs have resulted in large yields, a possible explanation could be related to donor age and to the relative fragility of the islets of juvenile pigs islet isolation procedures (90-94).

Furthermore, the amount of endocrine tissue present in a specific pancreas is undoubtedly an important factor in determining the islet isolation outcome. However, a high endocrine content does not ensure a high isolation yield. Despite improvement of isolation procedures, islet isolation is still associated with a considerable loss of endocrine tissue. This indicates that collagenase digestion of the pancreas is not limited to the exocrine pancreas but affects the islets as well. Because collagen is the major target in the enzymatic dissociation of the pancreas, the collagen substrate within the pancreas is one of the variables that could account for the unpredictable, highly variable islet yields. Also other matrix elements are thought to play a role (77-79, 92, 95). We have assessed the total amount and distribution of collagen within a large study population of adult and juvenile porcine pancreata and assessed the relation of these determinants to the outcome of islet isolation in adult pigs in **chapter 7**.

Another explanation for the unpredictable islet isolation outcomes could lie in morphological characteristics of porcine islets. Similar to human pancreata, we found a high number of hyperemic islets (HIs) when studying histological characteristics of porcine pancreata. We assessed the frequency of HIs in porcine pancreata compared to human pancreata. Furthermore, we studied the occurrence of HIs in relation to the outcome of islet isolation similar to the study in human pancreata (**chapter 6**). Besides the presence of HIs, we have observed morphological changes of islets after infusing the pancreas with collagenase during the isolation process. Previous studies have shown collagenase located within the islets after standard intraductal infusion

of collagenase in human and also at lower perfusion pressures in porcine pancreata (96, 97). The observed morphological changes could therefore be a result of either volume expansion of collagenase entering in the islet, leading to disruption of cell-cell contacts or be the result of the digestive effect of collagenase, subsequently leading to islet fragmentation. Both scenarios would eventually lead to lower islet isolation outcomes. In **chapter 8** we aimed to discriminate between these two hypotheses.

Finding answers to these questions will contribute to further optimization of pancreas graft survival in pancreas transplantation and improved islet isolation outcomes in islet of Langerhans transplantation, eventually leading to better use of available organs.

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; 329:977-86.
2. Epidemiology of Diabetes Interventions and Complications (EDIC). Design, implementation, and preliminary results of a long-term follow-up of the Diabetes Control and Complications Trial cohort. Diabetes Care 1999; 22:99-111.
3. Eurotransplant. http://www.eurotransplant.org/cms/index.php?page=annual_reports 2010.
4. Navarro X, Sutherland DE, Kennedy WR. Long-term effects of pancreatic transplantation on diabetic neuropathy. Ann Neurol 1997; 42:727-36.
5. Rayhill SC, D'Alessandro AM, Odorico JS, et al. Simultaneous pancreas-kidney transplantation and living related donor renal transplantation in patients with diabetes: is there a difference in survival? Ann Surg 2000; 231:417-23.
6. Smets YF, Westendorp RG, van der Pijl JW, et al. Effect of simultaneous pancreas-kidney transplantation on mortality of patients with type-1 diabetes mellitus and end-stage renal failure. Lancet 1999; 353:1915-9.
7. Tyden G, Bolinder J, Solders G, et al. Improved survival in patients with insulin-dependent diabetes mellitus and end-stage diabetic nephropathy 10 years after combined pancreas and kidney transplantation. Transplantation 1999; 67:645-8.
8. Kelly WD, Lillehei RC, Merkel FK, et al. Allotransplantation of the pancreas and duodenum along with the kidney in diabetic nephropathy. Surgery 1967; 61:827-37.
9. Andreoni KA, Brayman KL, Guidinger MK, et al. Kidney and pancreas transplantation in the United States, 1996-2005. Am J Transplant 2007; 7:1359-75.
10. Odorico JS, Becker YT, Groshek M, et al. Improved solitary pancreas transplant graft survival in the modern immunosuppressive era. Cell Transplant 2000; 9:919-27.
11. Gruessner RW, Dunn DL, Gruessner AC, et al. Recipient risk factors have an impact on technical failure and patient and graft survival rates in bladder-drained pancreas transplants. Transplantation 1994; 57:1598-606.
12. Axelrod DA, Sung RS, Meyer KH, et al. Systematic evaluation of pancreas allograft quality, outcomes and geographic variation in utilization. Am J Transplant 2010; 10:837-45.
13. Gruessner AC, Sutherland DE. Pancreas transplant outcomes for United States (US) and non-US cases as reported to the United Network for Organ Sharing (UNOS) and the International Pancreas Transplant Registry (IPTR) as of June 2004. Clin Transplant 2005; 19:433-55.
14. Marang-van de Mheen PJ, Nijhof HW, Khairoun M, et al. Pancreas-kidney transplantations with primary bladder drainage followed by enteric conversion: graft survival and outcomes. Transplantation 2008; 85:517-23.
15. Douzdjian V, Gugliuzza KG, Fish JC. Multivariate analysis of donor risk factors for pancreas allograft failure after simultaneous pancreas-kidney transplantation. Surgery 1995; 118:73-81.
16. Humar A, Ramcharan T, Kandaswamy R, et al. The impact of donor obesity on outcomes after cadaver pancreas transplants. Am J Transplant 2004; 4:605-10.
17. Gruessner RW, Sutherland DE, Kandaswamy R, et al. Over 500 solitary pancreas transplants in nonuremic patients with brittle diabetes mellitus. Transplantation 2008; 85:42-7.
18. Gruessner RW, Troppmann C, Barrou B, et al. Assessment of donor and recipient risk factors on pancreas transplant outcome. Transplant Proc 1994; 26:437-8.
19. Kapur S, Bonham CA, Dodson SF, et al. Strategies to expand the donor pool for pancreas transplantation. Transplantation 1999; 67:284-90.
20. Stegall MD, Dean PG, Sung R, et al. The rationale for the new deceased donor pancreas allocation schema. Transplantation 2007; 83:1156-61.
21. Vinkers MT, Rahmel AO, Slot MC, et al. Influence of a donor quality score on pancreas transplant survival in the Eurotransplant area. Transplant Proc 2008; 40:3606-8.
22. Hartgrink HH, van Bockel JH, Hansen B, et al. Effect of blood group and HLA matching on pancreas graft survival with the use of UW solution. Transpl Int 1995; 8:366-73.
23. Lo A, Stratta RJ, Alloway RR, et al. A multicenter analysis of the significance of HLA matching on outcomes after kidney-pancreas transplantation. Transplant Proc 2005; 37:1289-90.

24. Sutherland DE, Gruessner RW, Dunn DL, et al. Lessons learned from more than 1,000 pancreas transplants at a single institution. Ann Surg 2001; 233:463-501.
25. Colling C, Stevens RB, Lyden E, et al. Greater early pancreas graft loss in women compared with men after simultaneous pancreas-kidney transplantation. Clin Transplant 2005; 19:158-61.
26. Gaston RS, Alveranga DY, Becker BN, et al. Kidney and pancreas transplantation. Am J Transplant 2003; 3 Suppl 4:64-77.
27. Ris F, Toso C, Veith FU, et al. Are criteria for islet and pancreas donors sufficiently different to minimize competition? Am J Transplant 2004; 4:763-6.
28. Stewart ZA, Cameron AM, Singer AL, et al. Histidine-tryptophan-ketoglutarate (HTK) is associated with reduced graft survival in pancreas transplantation. Am J Transplant 2009; 9:217-21.
29. Salvalaggio PR, Schnitzler MA, Abbott KC, et al. Patient and graft survival implications of simultaneous pancreas kidney transplantation from old donors. Am J Transplant 2007; 7:1561-71.
30. Stratta RJ, Thacker LR, Sundberg AK. Multivariate analysis of the influence of donor and recipient cytomegalovirus sero-pairing on outcomes in simultaneous kidney-pancreas transplantation: the South-Eastern Organ Procurement Foundation Experience. Transplant Proc 2005; 37:1271-3.
31. Schulz T, Flecken M, Schenker P, et al. [Pancreas removal by external teams]. Chirurg 2005; 76:581-6; discussion 586-7.
32. Najarian JS, Sutherland DE, Matas AJ, et al. Human islet transplantation: a preliminary report. Transplant Proc 1977; 9:233-6.
33. Ricordi C, Lacy PE, Scharp DW. Automated islet isolation from human pancreas. Diabetes 1989; 38 Suppl 1:140-2.
34. Shapiro AM, Lakey JR, Ryan EA, et al. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. N Engl J Med 2000; 343:230-8.
35. Ryan EA, Paty BW, Senior PA, et al. Five-year follow-up after clinical islet transplantation. Diabetes 2005; 54:2060-9.
36. Froud T, Ricordi C, Baidal DA, et al. Islet transplantation in type 1 diabetes mellitus using cultured islets and steroid-free immunosuppression: Miami experience. Am J Transplant 2005; 5:2037-46.
37. Shapiro AM, Ricordi C. Unraveling the secrets of single donor success in islet transplantation. Am J Transplant 2004; 4:295-8.
38. Hering BJ, Kandaswamy R, Ansite JD, et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. JAMA 2005; 293:830-5.
39. Markmann JF, Deng S, Huang X, et al. Insulin independence following isolated islet transplantation and single islet infusions. Ann Surg 2003; 237:741-9; discussion 749-50.
40. Benhamou PY, Watt PC, Mullen Y, et al. Human islet isolation in 104 consecutive cases. Factors affecting isolation success. Transplantation 1994; 57:1804-10.
41. Brandhorst D, Brandhorst H, Hering BJ, et al. Islet isolation from the pancreas of large mammals and humans: 10 years of experience. Exp Clin Endocrinol Diabetes 1995; 103 Suppl 2:3-14.
42. Brandhorst H, Brandhorst D, Hering BJ, et al. Body mass index of pancreatic donors: a decisive factor for human islet isolation. Exp Clin Endocrinol Diabetes 1995; 103 Suppl 2:23-26.
43. Briones RM, Miranda JM, Mellado-Gil JM, et al. Differential analysis of donor characteristics for pancreas and islet transplantation. Transplant Proc 2006; 38:2579-81.
44. Bucher P, Mathe Z, Morel P, et al. Assessment of a novel two-component enzyme preparation for human islet isolation and transplantation. Transplantation 2005; 79:91-7.
45. Close NC, Hering BJ, Anand R, et al. Collaborative iIslet Transplant Registry. Clin Transpl 2003:109-18.
46. Close NC, Hering BJ, Eggerman TL. Results from the inaugural year of the Collaborative Islet Transplant Registry. Transplant Proc 2005; 37:1305-8.
47. Fiedor P, Goodman ER, Sung RS, et al. The effect of clinical and biochemical donor parameters on pancreatic islet isolation yield from cadaveric organ donors. Ann Transplant 1996; 1:59-62.
48. Hanley SC, Paraskevas S, Rosenberg L. Donor and isolation variables predicting human islet isolation success. Transplantation 2008; 85:950-5.

49. Hubert T, Gmyr V, Arnalsteen L, et al. Influence of preservation solution on human islet isolation outcome. Transplantation 2007; 83:270-6.
50. Kenmochi T, Miyamoto M, Une S, et al. Improved quality and yield of islets isolated from human pancreata using a two-step digestion method. Pancreas 2000; 20:184-90.
51. Kim SC, Han DJ, Kang CH, et al. Analysis on donor and isolation-related factors of successful isolation of human islet of Langerhans from human cadaveric donors. Transplant Proc 2005; 37:3402-3.
52. Kin T, Mirbolooki M, Salehi P, et al. Islet isolation and transplantation outcomes of pancreas preserved with University of Wisconsin solution versus two-layer method using preoxygenated perfluorocarbon. Transplantation 2006; 82:1286-90.
53. Kin T, Shapiro AM, Lakey JR. Pancreas divisum: a study of the cadaveric donor pancreas for islet isolation. Pancreas 2005; 30:325-7.
54. Kneteman NM, Lakey JR, Warnock GL, et al. Human islet isolation after prolonged cold storage. Diab Nutr Metab 1992; 5:33-37.
55. Lakey JR, Rajotte RV, Warnock GL, et al. Human pancreas preservation prior to islet isolation. Cold ischemic tolerance. Transplantation 1995; 59:689-94.
56. Lakey JR, Warnock GL, Shapiro AM, et al. Intraductal collagenase delivery into the human pancreas using syringe loading or controlled perfusion. Cell Transplant 1999; 8:285-92.
57. Liu X, Matsumoto S, Okitsu T, et al. Analysis of donor- and isolation-related variables from non-heart-beating donors (NHBDs) using the Kyoto islet isolation method. Cell Transplant 2008; 17:649-56.
58. Markmann JF, Deng S, Desai NM, et al. The use of non-heart-beating donors for isolated pancreatic islet transplantation. Transplantation 2003; 75:1423-9.
59. Matsumoto I, Sawada T, Nakano M, et al. Significant impact of two layer (Perfluorochemical/ University of Wisconsin solution (PFC/UW)) method on islet yield and function for short-term preservation of human donor pancreata prior to islet isolation and transplantation. Transplantation 2003; 76:S58.
60. Matsumoto I, Sawada T, Nakano M, et al. Improvement in islet yield from obese donors for human islet transplants. Transplantation 2004; 78:880-5.
61. Matsumoto S, Qualley SA, Goel S, et al. Effect of the two-layer (University of Wisconsin solution-perfluorochemical plus O2) method of pancreas preservation on human islet isolation, as assessed by the Edmonton Isolation Protocol. Transplantation 2002; 74:1414-9.
62. Matsumoto S, Rigley TH, Qualley SA, et al. Efficacy of the oxygen-charged static two-layer method for short-term pancreas preservation and islet isolation from nonhuman primate and human pancreata. Cell Transplant 2002; 11:769-77.
63. Matsumoto S, Rigley TH, Reems JA, et al. Improved islet yields from Macaca nemestrina and marginal human pancreata after two-layer method preservation and endogenous trypsin inhibition. Am J Transplant 2003; 3:53-63.
64. Nagata H, Matsumoto S, Okitsu T, et al. Procurement of the human pancreas for pancreatic islet transplantation from marginal cadaver donors. Transplantation 2006; 82:327-31.
65. Nano R, Clissi B, Melzi R, et al. Islet isolation for allotransplantation: variables associated with successful islet yield and graft function. Diabetologia 2005; 48:906-12.
66. Ponte GM, Pileggi A, Messinger S, et al. Toward maximizing the success rates of human islet isolation: influence of donor and isolation factors. Cell Transplant 2007; 16:595-607.
67. Sabek OM, Cowan P, Fraga DW, et al. The effect of isolation methods and the use of different enzymes on islet yield and in vivo function. Cell Transplant 2008; 17:785-92.
68. Sakuma Y, Ricordi C, Miki A, et al. Factors that affect human islet isolation. Transplant Proc 2008; 40:343-5.
69. Takei S, Teruya M, Grunewald A, et al. Isolation and function of human and pig islets. Pancreas 1994; 9:150-6.
70. Toso C, Oberholzer J, Ris F, et al. Factors affecting human islet of Langerhans isolation yields. Transplant Proc 2002; 34:826-7.
71. Tsujimura T, Kuroda Y, Avila JG, et al. Influence of pancreas preservation on human islet isolation outcomes: impact of the two-layer method. Transplantation 2004; 78:96-100.
72. Witkowski P, Liu Z, Guo Q, et al. Two-layer method in short-term pancreas preservation for successful islet isolation. Transplant Proc 2005; 37:3398-401.

73. Hughes SJ, McShane P, Contractor HH, et al. Comparison of the collagen VI content within the islet-exocrine interface of the head, body, and tail regions of the human pancreas. Transplant Proc 2005; 37:3444-5.
74. Hughes SJ, Clark A, McShane P, et al. Characterisation of collagen VI within the islet-exocrine interface of the human pancreas: implications for clinical islet isolation? Transplantation 2006; 81:423-6.
75. Uscanga L, Kennedy RH, Stocker S, et al. Immunolocalization of collagen types, laminin and fibronectin in the normal human pancreas. Digestion 1984; 30:158-64.
76. Tons HA, Terpstra OT, Bouwman E. Heterogeneity of human pancreata in perspective of the isolation of the islets of langerhans. Transplant Proc 2008; 40:367-9.
77. van Deijnen JH, Hulstaert CE, Wolters GH, et al. Significance of the peri-insular extracellular matrix for islet isolation from the pancreas of rat, dog, pig, and man. Cell Tissue Res 1992; 267:139-46.
78. Van Deijnen JH, Van Suylichem PT, Wolters GH, et al. Distribution of collagens type I, type III and type V in the pancreas of rat, dog, pig and man. Cell Tissue Res 1994; 277:115-21.
79. van Suylichem PT, van Deijnen JE, Wolters GH, et al. Amount and distribution of collagen in pancreatic tissue of different species in the perspective of islet isolation procedures. Cell Transplant 1995; 4:609-14.
80. Basta G, Falorni A, Osticioli L, et al. Method for mass retrieval, morphologic, and functional characterization of adult porcine islets of Langerhans: a potential nonhuman pancreatic tissue resource for xenotransplantation in insulin-dependent diabetes mellitus. J Investig Med 1995; 43:555-66.
81. Coleman R, Silbermann M. Erythrocytes within pancreatic B-cells of corticosteroid-treated mice. Experientia 1978; 34:1049-50.
82. Imaoka M, Satoh H, Furuhama K. Age- and sex-related differences in spontaneous hemorrhage and fibrosis of the pancreatic islets in Sprague-Dawley rats. Toxicol Pathol 2007; 35:388-94.
83. Kaduk B, Husslein EM, Siegfried A. Morphology of the chronic toxicity of busulfan on the islets of Langerhans in the rat. Hepatogastroenterology 1987; 34:108-12.
84. Lucocq JM, Findlay JA. Islet organ, blood glucose and glucose tolerance of lean and obese Mongolian gerbils. A quantitative study. Cell Tissue Res 1981; 220:623-36.
85. Groth CG, Korsgren O, Tibell A, et al. Transplantation of porcine fetal pancreas to diabetic patients. Lancet 1994; 344:1402-4.
86. Rijkelijkhuizen JK, Tons A, Terpstra OT, et al. Transplantation of long-term cultured porcine islets in the rat: prolonged graft survival and recipient growth on reduced immunosuppression. Cell Transplant 2010; 19:387-98.
87. Hering BJ, Walawalkar N. Pig-to-nonhuman primate islet xenotransplantation. Transpl Immunol 2009; 21:81-6.
88. Hering BJ, Wijkstrom M, Graham ML, et al. Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. Nat Med 2006; 12:301-3.
89. Ricordi C, Socci C, Davalli AM, et al. Isolation of the elusive pig islet. Surgery 1990; 107:688-94.
90. Heiser A, Ulrichs K, Muller-Ruchholtz W. Influence of porcine strain, age, and pH of the isolation medium on porcine pancreatic islet isolation success. Transplant Proc 1994; 26:618-20.
91. Rood PP, Buhler LH, Bottino R, et al. Pig-to-nonhuman primate islet xenotransplantation: a review of current problems. Cell Transplant 2006; 15:89-104.
92. Socci C, Ricordi C, Davalli AM, et al. Selection of donors significantly improves pig islet isolation yield. Horm Metab Res Suppl 1990; 25:32-4.
93. Marchetti P, Finke EH, Gerasimidi-Vazeou A, et al. Automated large-scale isolation, in vitro function and xenotransplantation of porcine islets of Langerhans. Transplantation 1991; 52:209-13.
94. Bottino R, Balamurugan AN, Smetanka C, et al. Isolation outcome and functional characteristics of young and adult pig pancreatic islets for transplantation studies. Xenotransplantation 2007; 14:74-82.
95. White SA, Hughes DP, Contractor HH, et al. An investigation into the distribution of different collagen types within adult and juvenile porcine pancreata. J Mol Med (Berl) 1999; 77:79-82.

96. Cross SE, Hughes SJ, Partridge CJ, et al. Collagenase penetrates human pancreatic islets following standard intraductal administration. Transplantation 2008; 86:907-11.
97. Toso C, Brandhorst D, Oberholzer J, et al. Isolation of adult porcine islets of Langerhans. Cell Transplant 2000; 9:297-305.