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Factor Affecting Transplant Outcomes in Diabetic Nude Mice Receiving Human, Porcine, and Non-Human Primate Islets: Analysis of 335 Transplantations

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Abstract

Background—In the absence of a reliable islet potency assay, nude mice transplant is the criterion standard to assess islet quality for clinical transplantation. There are factors other than islet quality that affect the transplant outcome.

Methods—Here, we analyzed the transplant outcomes in 335 nude mice (NM) receiving islets from human (n=103), porcine (n=205), and non-human primate (NHP) donors (n=27). The islets (750, 1000, and 2000 islet equivalents) were transplanted under the kidney capsule of streptozotocin (STZ) induced diabetic NM.

Results—The proportion of mice that achieved normoglycemia was significantly higher in the group implanted with 2000 IEQ of human, porcine, or NHP islets (75% normoglycemic) versus groups that were implanted with 750 IEQ (7% normoglycemic) and 1000 IEQ (30% normoglycemic). In this study, we observed that the purity of porcine islet preparations ($P = .001$), islet pellet size in porcine preparations ($P = .01$) and mice recipient body weight for human islets preparations ($P = .013$), was independently associated with successful transplant outcome. NHP islets of 1000 IEQ were sufficient to achieve normoglycemic condition (83%). An islet mass of 2000 IEQ, high islet purity, increased recipient body weight, and high islet pellet volume increased the likelihood of successful reversal of diabetes in transplanted mice. Also, higher insulin secretory status of islets at basal stimulus was associated with a reduced mouse cure rate. The cumulative incidence of graft failure was significantly greater in human islets (56.12%) compared with porcine islets 35.57% ($P = .001$).

Conclusion—Factors affecting NM bioassay were identified (islet mass, islet purity, pellet size, *in vitro* insulin secretory capability and mouse recipient body weight) and should be considered when evaluating islet function.

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Keywords

Islet cell transplantation; nude mice transplant; nude mice bioassay; human islets; porcine islets and non-human primate islets

Introduction

Islet transplant has become a promising treatment option for patients with type 1 diabetes mellitus, as it is a less-invasive procedure than whole organ transplant to restore pancreatic endocrine function (1, 2). Currently, the source of cells for patients in clinical trials is from only pre-screened, brain dead, human donors, creating a significant shortage of donor organs. Xenotransplantation is a promising alternative approach to alleviate this islet shortage and expand islet transplantation to more diabetic patients. Consequently, islets from porcine-to-non human primate (NHP) have become the model for xenotransplantation and there is an immediate need to assess the quality of isolated islet preparations from humans, porcine, and NHP to better determine their potential clinical function in humans (3).

Isolated islet qualities are assessed through inclusion/exclusion of fluorescent-dye staining for viability, glucose-stimulated insulin secretory response (using a static incubation technique or dynamic perfusion), and the nude mouse bioassay involving the transplantation of islets to streptozotocin (STZ) induced mice to observe the reversal of hyperglycemia (4). Among these methods, the nude mouse (NM) bioassay is considered to be the criterion standard for assessing isolated islet quality. However, there are many factors that affect the NM transplant outcome which should be carefully considered before attempting to correlate outcome with clinical expectations: transplanted islet characteristics, recipient mouse characteristics, and transplantation (surgical) techniques.

While the effects of islet characteristics and recipient mouse characteristics are being extensively studied, technical factors known to negatively influence transplant outcomes include damage to the islet cells in the capillary tube during packing, tearing of the kidney capsule during the implantation, loss of the pellet while infusing it under the kidney capsule, or excessive bleeding over the kidney capsule (5). The recipient mice were chosen based on age, weight, blood glucose level, and the duration of diabetes. Owing to the dynamic nature of the animal husbandry process and timing of the availability of islets, it is very difficult to standardize the recipient selection process, so minor variations are impossible to avoid among recipients. During the islet transplant preparation, several factors are measured and monitored to determine “islet quality,” because they have been shown to influence the success of the islet graft. Factors such as the purity of the islet preparation, quantity of islet equivalents transplanted, potency and relative size of the islets (including *in vitro* insulin secretory capability of islet cells), culture duration, and morphologic factors such as solidity, border integrity, and degree of fragmentation, all may play a role.

In this study, we performed 335 nude mouse transplants to assess the function of isolated islets of humans, porcine, and NHP. We studied numerous factors that might affect the transplant outcome in NM, and analyzed the role of these factors play in reversal of hyperglycemia. By using multivariate logistic regression, we evaluated donor, recipient, and islet processing factors that were independently associated with islet functioning in NM.

Results

Table 1A-G shows donor, islet and recipient characteristics. In human studies (n=103), the average donor age was 38 ± 12 years, body mass index was 32 ± 6 , cold ischemic time was 7 ± 4 hours, and the trimmed pancreas weight 109 ± 28 grams. In porcine studies (n=194),

the average donor age was 3.7 ± 0.8 , body weight was 590 ± 66 pounds, and the trimmed pancreas weight was 237 ± 45 grams. In NHP studies ($n=27$), the average donor body weight was 9.4 ± 3.3 kg, and the trimmed pancreas weight was 15.8 ± 6.2 grams.

To assess the isolated islet function, aliquots of 750, 1000, and 2000 IEQ human, porcine and NHP islets were transplanted under the kidney capsule of diabetic NM.

After transplant of human islets into NM, 87% of mice receiving 2000 IEQ ($n=67$), 17% with 1000 IEQ ($n=20$), and 7% with 750 IEQ ($n=16$) became normoglycemic. For porcine islets, 79% of mice receiving 2000 IEQ ($n=144$), 27% with 1000 IEQ ($n=33$), and 11% with 750 IEQ ($n=28$) became normoglycemic. For NHP islets, 80% of mice receiving 2000 IEQ ($n=10$) and 83% with 1000 IEQ ($n=7$) became normoglycemic, and no mice were cured when receiving 750 IEQ ($n=3$). In an independent analysis, when islets from the same preparation were transplanted at different doses (750, 1000, 2000 IEQ), 98% of the time the 750 IEQ islet dose failed to reverse diabetes for all 3 donor species. However, when 1000 IEQ was transplanted, the success rate for human and pig islets were only 7% and 13% respectively, however, NHP islets success rate was 83%.

Overall, 75% of mice receiving 2000 IEQ became normoglycemic, compared to only 30% and 7% of those receiving 1000 and 750 IEQ. The cumulative incidence of graft failure was significantly greater for human (56.12%) compared with porcine islets (35.57%) ($P < .001$).

For human islet transplants, 2 factors were independently associated with graft failure: total IEQ transplanted and recipient mouse body weight (Table 1B). Pellet volume, which was an independent risk factor for porcine islet transplants, was not statistically associated with graft failure. The multivariate logistic model (Table 1C) for the human islet transplant provided adequate discrimination across categories or risk but not as pronounced as the adjusted model. Between the upper and lower quartiles of risk (calculations not shown), the relative risk of graft failure only doubled. That means that compared to an islet dose of 2000 IEQ, along with a heavier mouse, the risk of graft failure was about 2 times higher among lighter-weight NM receiving less than 2000 IEQ.

For porcine islet transplants, only 2 factors were independently associated with graft failure: total IEQ transplanted and the pellet volume (Table 1D and E). Having an islet equivalent of 2000 IEQ decreased the likelihood of graft failure by 77%, whereas a pellet volume increase of one-standard deviation (4.07 cc) reduced the risk of graft failure by 74%. Summary statistics show that the reduced model discriminates (c statistic=0.867) between NM in different risk categories. For example, between the upper and lower quartiles of risk (calculations not shown), the relative risk of graft failure was 12.85%. This means that based on an islet dose of less than 2000 IEQ and a low pellet volume, the risk of graft failure was about 13 times higher than the risk among mice receiving 2000 IEQ and maximum pellet volume (Table 1F).

Islet isolated from human, porcine, and NHP showed varying responses to glucose challenges, with average stimulation indexes of 5.13, 4.17, and 9.14, respectively (Table 2A). NHP islets exhibited the highest insulin secretory response to glucose when compared to human and porcine islets. Total insulin content of NHP islets were four-fold higher than human and five-fold higher than porcine islets. Transplantation of 2000 IEQ from human, porcine, and NHP preparations reversed the diabetic condition in 70%, 64%, and 100% NM.

In Table 2B, further analysis of the insulin secretory response of human, pig, and NHP islets at basal (1.67 mM) and stimulated (16.7 mM) glucose were separated into low and high insulin secretory status groups in order to compare the mouse transplantation cure rate. Interestingly, human and porcine islets with higher levels of insulin secretion at both the

basal level and stimulated level of glucose stimulation were less likely to reverse diabetes in nude mouse transplantations, whereas a 100% cure rate was achieved by NHP islets. If islets release high amount of insulin at basal stimulus indicates unhealthy status of islets (6-8).

Human clinical islet preparations (n=28) were transplanted in NM for islet quality assessment (Table 3). Transplantation of 2000 IEQ human islets into diabetic NM resulted in a cure rate of 54% and failure rate of 46%. Upon further characterization of the islet product, statistical analysis of factors potentially affecting nude mouse transplantation outcomes were performed with islet purity, % beta cells, % alpha cells, % delta cells, %PP cells, % acinar cells, % other cells, 24 hours viability (%), 48 hours viability (%), stimulation index and endotoxin from final product; no significant differences were found when comparing groups.

Figure 1 graphically displays blood glucose measurements of cured and hyperglycemic mice after transplantation of 2000 IEQ human (A) and porcine (B) islets. In histological examination of porcine islet grafts (Figure 2) from normoglycemic (A, B) and hyperglycemic (C, D) mice, although both mice received 2000 IEQ's from the same islet preparation, both mice display positive insulin staining despite differing transplant outcomes.

Discussion

Positive islet graft function in diabetic NM is considered the gold standard for predicting isolated islet quality. However, as mentioned above, variable factors (surgical procedure, recipient mice characteristics, and quality of the islet preparation) affecting NM transplant outcomes can confound the interpretation of results. We have examined many of these factors in this retrospective analysis to suggest further standardization of the process. This is necessary to eliminate technical factors that confound assay results and eliminate variability between different centers when assaying islet products for transplant quality.

Among the technical factors leading to poor outcomes, surgical issues can be critical. Kidney capsule damage is associated with hemorrhage, inflammation, adhesion, and loss of islets, and can negatively affect the assay outcome. Other technical factors (eg, compaction of islets) artificially leads to poor graft function. The Edmonton group reported the effect of maintaining islets in a compact pellet for 30 minutes before transplant significantly reduces the proportion of transplant recipients that achieve normoglycemia from 100% to 38% ($P=0.026$) (9).

When islets are transplanted clinically into humans, there can be great variability in the transplant success based solely on recipient characteristics. For mouse recipients, this is certainly no different. The results from our analysis found that mice body weights correlated with the transplant success rates in the case of human islets ($P=0.013$) (Table 1B). Clinically healthy mice with a body weight > 25 grams could better tolerate the surgical procedure and experienced improved transplant outcomes in contrast to mice weighing less than 25g which had a lower survival rate.

The purity of islets is also a decisive factor for the success of transplant outcome. We found the purity of islets (especially porcine islets) to be strongly associated with transplant success (Table 1E). This agrees with another study by Migliavacca et al., which also found the purity of islet preparations and the insulin content correlated with mouse transplant success (10). Recent research suggests that, unlike portal venous infusion, impure islets transplanted under the renal capsule of mice are more closely associated with harmful acinar cell proteases which are known to degrade insulin which may explain why transplantation of impure islets never reversed diabetes in mice (11). In porcine islet transplants, with nearly

equivalent product purities, the pellet size also correlated with graft function (Table 1F). Although 2000 IEQ were present in all pellets, the pellet size varied in different preparations most likely due to the islet morphology, fragmentation, and fragile nature of porcine islets.

Similar histopathological findings from normoglycemic and hyperglycemic mice (Figure 2) which were identically transplanted with 2000 IEQ from a single islet preparation suggest the presence of secondary recipient characteristics which significantly affect the transplant outcome. Transplantation with 2000 IEQ in NM increased the reversal of diabetes from levels observed with 750 and 1000 IEQ transplantations. Comparatively, in syngenic islet transplantation, the minimum islet number that consistently achieves normoglycemia in diabetes C57 B1/6 mice is 300 IEQ (12, 13). In the case of xenotransplantation, it is not clear why higher doses of islets are required to reverse diabetes. Also, despite the presence of islet function and positive results from additional islet viability assays, many mice did not experience a reversal of the diabetic condition, implying the absence of a sole correlation between islet viability and mouse transplant outcome. Furthermore, islets from human clinical isolations used in mouse transplantation studies resulted in a 54% cure rate despite exhibiting no significant differences in islet viability, endotoxin, GSIR, and cellular composition (Table 3).

When comparing human, porcine and NHP islets, the highest insulin secretory response is exhibited by NHP islets (Table 2A). It is possible that the 100% cure rate achieved by NHP islets is due to the intrinsically higher insulin secretory capacity of NHP islets (14-16) and is supported by their observed high total insulin content. So our recommendation from this analysis was, whenever NHP islets are tested in nude mice, 1000 IEQ would be the optimal number to test islet function.

In Table 4b, interestingly higher levels of insulin secretion by human and porcine islets seems to contribute to lower cure rates when compared to islets secreting lower levels of insulin. This fact may be explained by damaged islets releasing higher levels of insulin as a result of islet fragmentation and cell membrane disruption (6-8). If islets secrete normal quantity of insulin at basal stimulus indicate proper sensing and releasing of insulin.

When NM is used for assessing islet quality, it is important to consider mouse characteristics along with islet characteristics when interpreting success and failed outcomes. Transplants of less than 2000 IEQ are not ideal when transplanting human or porcine islets as the mouse recipient requires more islets to achieve normoglycemia regardless of islet potency. However, 1000 NHP islets (IEQ) are sufficient to achieve normoglycemic condition. It is critical to transplant pure islets as acinar cell proteases may be harmful for islet engraftment and function (17). Selecting mice with body weights > 25g is important because these mice tolerate the surgical procedure appropriately. Smaller pellet volume, even with 2000 IEQ, is indicative of fragile and fragmented islets, especially with porcine islets. Failure of the nude mouse assay does not necessarily correlate with the islet quality of the transplant preparation and careful observation of other factors is required to more accurately deem the quality of clinical islet preparations. In this study, many important factors were identified such as islet mass, islet purity, islet pellet size, recipient body weight, and insulin secretory pattern at basal stimulus *in vitro* should be considered when evaluating islet function.

Materials and Methods

All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) and conducted according to National Institutes of Health guidelines. Historical mouse data was analyzed. Aliquots of 750, 1000, and 2000 IEQ of human, porcine, and NHP islets were transplanted under the kidney capsule of diabetic NM (Table 1). Then, the

animals were followed for 30 days, with repeated measurements of blood glucose and body weight (Figure 1 A and B).

Nude Mice

Athymic male NM, from weaning through adulthood, was obtained from the National Cancer Institute (Hartford, CT). Mice were separated and caged in a solid bottom caging, with sterile bedding, food, and water in a specific pathogen free environment, with a constant temperature range of 20°C to 23°C, in an approved animal care facility under the management of the University of Minnesota Research Animal Resources according to IACUC requirements.

Diabetes Induction in Nude Mice

Diabetes was induced in NM by administration of STZ (18) (Sigma, St. Louis, MO, USA) or clinical grade STZ (Zanosar, Sicor Pharmaceuticals, Irvine, CA), both herein referred to as STZ. Zanosar is a clinical-grade compound, made available in 2007, at which time STZ was replaced (19). Heavier mice (weighing over 25 g) were selected preferentially, as they are most suitable for STZ induction due to potential weight loss after induction. STZ was prepared in cold and delivered intravenous or intraperitoneal at a dose of 240 mg/kg to induce diabetes.

The day after induction, 1 mL of saline was injected subcutaneously on a daily basis. Blood glucose levels were measured by bleeding the tail vein on day 3 and 4 after induction. Mice with a blood glucose level > 300 mg/dL for 2 consecutive days were considered diabetic, at which time insulin injections were initiated. In diabetic mice, 1 mL of insulin (2 U isophane insulin (N) + 2 U regular insulin (R) in 12 cc saline) was injected subcutaneously, daily, until the islet transplant.

Islet Isolation

Human islet isolations were done at the University of Minnesota, Molecular and Cellular Therapeutics, GMP facility in compliance with FDA regulations. The islet isolation method has been reported previously (20). Isolated islets were cultured in CMRL 1066 medium containing 0.5% human serum albumin.

Porcine pancreases were recovered and isolated at the University of Minnesota isolation laboratory. Islets were isolated using Liberase PI (Roche Molecular Biochemical, Indianapolis, IN) enzyme. The islet isolation method has been described previously (21). Isolated islets were cultured at 37°C in complete medium E199 (Mediatech Inc., Manassas, VA) with 10% heat-inactivated porcine serum.

NHP islet isolation was done using modifications of the automated method for human islet isolation (22). The final islet suspension was cultured free-floating in CMRL-1066 with 10% fetal calf serum, in a humidified atmosphere of 95% air, 5% CO₂, at 37°C.

In vitro Glucose Stimulated Insulin Response: stimulation index and total insulin content of islets

The functional capacity of isolated human, porcine, and NHP islets was tested after overnight culture using the glucose stimulated insulin release (GSIR) test (previously described) (23). At the end of the incubation period, supernatants were collected for determination of insulin secretion and stimulation index by insulin ELISA (23). Total insulin content of islets and DNA measurements were also determined after GSIR test following the previously described method (23).

Beta cell composition and islet viability

Human clinical islet preparations (n=28) were further characterized for islet cell composition (23). Islet viability was measured after 24 and 48 hours culture by fluorescein diacetate/propidium iodide staining (24). Endotoxin level of the final islet product was characterized by the Charles River® Endosafe® method (25).

Nude Mouse Bioassay: Transplantation of Islets into Diabetic Mice

Diabetic mice were selected for transplant based on their blood glucose levels (at least 2 consecutive readings of > 300 mg/dL maintained throughout the diabetic period). Mice were used within 30 days after diabetes induction. On the day of transplant, mice were selected for by body weight (< 25 g) and were anesthetized with Avertin (0.01 mL/g, National Institutes of Health guidelines for Avertin dosage is 125-250 mg/kg [0.02 mL/g] and retinoic acid receptor guidelines is 225-240 mg/kg), and artificial tear ointment was applied. The left kidney was located by palpation, and the surgical area was scrubbed with a Betadine swab. After incision, the membrane was lifted using an angle-tipped glass rod; a separate blunt-tipped glass rod was used to detach the kidney capsule. The fresh or cultured (day 0 - day 7) human, porcine, or NHP islets (750-2000 IEQ) were placed under the kidney capsule, using PE 50 tubing attached to a Hamilton syringe. The islet pellet size in the PE 50 tubing was measured in millimeters (mm). The skin incision was sutured and stapled, and the mouse subsequently given a subcutaneous injection of 0.1 cc buprenorphine for analgesia before being placed back in a cage on a heating pad (18, 26).

After transplant, the blood glucose and body weight of the mice were measured up to 30 days. If the blood glucose level was persistently < 200 mg/dL post-transplant, the mice were considered normoglycemic. If the mice continued having high blood glucose values (> 300 mg/d), it was considered hyperglycemic. Kidneys from both study groups were preserved in 10% formalin for histology studies.

Histological Analysis of Islet Graft

Preserved kidney samples were processed for histological study. Five micron sections were cut for hematoxylin and eosin (H&E) and insulin staining. Islet cell grafts were examined for histopathological analysis (Figure 2).

Statistical Analyses

Continuous variables are summarized as means $\pm$ the standard error of the means, while categorical variables are summarized as a count and a percentage. Differences in the bivariate model are tested using either an independent sample *t* test or a chi-square statistic. Historical nude mouse transplantation data were used for analysis. There were 194 porcine islet transplants, 103 human islet transplants and 27 NHP islet transplants used for the analyses.

For the multivariate logistic regression, the following risk factors were tested for any graft failure in the first 28 days after initial reversal of hyperglycemia: [1] pellet size; [2] islet equivalents (IEQ); [3] processing era (year); [4] weight of donor (or body mass index, human only); [5] mouse body weight; [6] duration after diabetes induction; [7] blood glucose; [8] pancreas weight; [9] preprocessing, total, unpurified cell count; [10] post processing, total, unpurified cell count; [11] cold ischemia time; [12] donor age (human only); and [13] source of pancreas (human only).

An independent analysis was done when three different doses from the same islet preparation (human, pig and NHP islets) were transplanted. We carefully monitored how islets were reversing diabetes condition at 2000 IEQ to 750 IEQ. We also asked, when islets

were reversing diabetes, was it correlating with age of the donor, type of enzyme used to isolate islets, islet morphology score, or recipient mouse diabetic status before transplantation.

Multivariate logistic regression was used to test for an independent association of each factor and the cumulative incidence of graft failure in the NM (27). The final multivariate models were tested for discrimination, using the c statistics, and the Hosmer-Lemeshow chi-square statistic for calibration (28). All analyses were done using statistical procedures in SAS System (SAS Institute, Inc., version 9.2, Cary, NC).

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Abbreviations

NHP	Non-human primate
NM	Nude mice

STZ	Streptozotocin
FDA/PI	fluorescent diacetate/propidium iodide
GSIR	glucose-stimulated insulin release
IEQ	islet equivalent number
IC	islet count

Figure 1.

(1A) Changes in blood glucose after transplant of human islets (2000 IEQ) under the kidney capsule of diabetic NM (n=63). Blood glucose levels were monitored for up to 30 days (mg/dL; mean $\pm$ standard error of the mean). After 30 days, 48 mice were normoglycemic, and 15 mice remained hyperglycemic.

(1B): Blood glucose trend after transplantation of porcine islets (2000 IEQ) under the kidney capsule of diabetic NM (n=153). Blood glucose levels were monitored up to 30 days (mg/dL; mean $\pm$ standard error of the mean). After 30 days, 118 mice were normoglycemic, and 35 mice remained hyperglycemic.

(1C): Blood glucose trend after transplantation of NHP islets (2000 IEQ) under the kidney capsule of diabetic NM (n=10). Blood glucose levels were monitored up to 30 days (mg/dL; mean $\pm$ standard error of the mean). After 30 days, 8 mice were normoglycemic, and 2 mice remained hyperglycemic.

Figure 2.

Histology sections showing porcine islet graft under the kidney capsule of NM. Islets from a single preparation were transplanted into two mice (2000 IEQ each). One mouse was maintaining normoglycemia (A&B) and the other one was hyperglycemia (C&D) pictures A and B show H&E and insulin staining of islets. Picture B islets stain for insulin and shows abundant, normally granulated beta cells scattered throughout the islet graft. Pictures C and D were from hyperglycemic mouse. The normal cytologic appearance of the islet cells (H&E). (C) Insulin staining was light as compared to picture B showing beta cells health in failed graft.

Table 1A
Human islet preparations: Donor, isolation, and recipient mouse characteristics

Donor & Isolation Characteristics	Human (n=103)
Age (years)	38 ± 12 (20 - 61)
Body Mass Index (BMI)	32 ± 6 (21 - 46)
Cold Ischemia Time (CIT)	7 ± 4 (2.30 - 15)
Trimmed Pancreas Weight (grams)	109 ± 28 (67 - 239)
Enzyme used to Digest Pancreas	Liberase-HI (n=34); Serva (n=29); ViaCyte (n=23)
Digest Islet Equivalent (IEQ)	438,239 ± 179,409
Digest Islet Equivalent/gram Pancreas	4,064 ± 1830
Post Purification Islet Equivalent (IEQ)	344,663 ± 171,542
Post Purification Islet Equivalent/gram Pancreas	3,233 ± 1702
Percent Islet Purity (%)	85 ± 0.5
Islet Characteristics	
Islet Equivalent for Transplantation (IEQ)	750, 1000 and 2000
Pellet Volume in PE-50 tubing (mm) of 750 IEQ	3 ± 2
Pellet Volume in PE-50 tubing (mm) of 1000 IEQ	5 ± 2
Pellet Volume in PE-50 tubing (mm) of 2000 IEQ	10 ± 3
Recipient Mouse Characteristics	
Body Weight (gram)	28 ± 4
Duration of Diabetes Status (days)	23 ± 23
Average Blood Glucose before Tx (mg/dL)	510 ± 64

Data presented as Mean ± SD

Table 1B
Bivariate Association between Donor Characteristics, Nude Mouse Characteristics, Islet Processing Characteristics and Human Islet Graft Functioning

	Analysis of 750, 1000, and 2000 IEQ human islet transplantation in diabetic nude mice			Analysis of 2000 transplantation in human islet IEQ diabetic nude mice		
	Graft Cured (n = 43) Mean $\pm$ SEM	Graft Failure (n = 55) Mean $\pm$ SEM	P value	Graft Cured (n = 39) Mean $\pm$ SEM	Graft Failure (n = 27) Mean $\pm$ SEM	P value
Human Donor Characteristics						
Donor Age	37.1 $\pm$ 1.9	38.0 $\pm$ 1.7	0.739	37.3 $\pm$ 1.9	38.8 $\pm$ 2.1	0.610
Donor BMI (Kg/meter ²)	31.9 $\pm$ 0.9	32.8 $\pm$ 0.8	0.482	32.2 $\pm$ 1.0	33.2 $\pm$ 1.0	0.463
Nude Mouse Characteristics						
Body Weight (g)	29.4 $\pm$ 0.5	27.5 $\pm$ 0.5	0.013	29.6 $\pm$ 0.5	27.3 $\pm$ 0.8	0.015
Blood Glucose (mg/dL)	510.3 $\pm$ 9.6	509.7 $\pm$ 4.7	0.969	513.1 $\pm$ 10.0	535 $\pm$ 12.0	0.172
Processing Characteristics						
Average Transplanted Islet Equivalents (750, 1000, 2000 IEQ)	1918.6 $\pm$ 43.0	1445.5 $\pm$ 76.7	< 0.001	2000	2000	NA
Pellet Volume (cc)	14.1 $\pm$ 1.2	11.2 $\pm$ 1.6	0.148	14.8 $\pm$ 1.3	16.8 $\pm$ 2.7	0.456
Percent Islet Purity	85.8 $\pm$ 1.0	84.0 $\pm$ 1.5	0.351	83.3 $\pm$ 1.8	80.9 $\pm$ 1.5	0.331
Cold Ischemia Time (hours)	7.5 $\pm$ 0.5	7.1 $\pm$ 0.5	0.603	7.7 $\pm$ 0.6	8.2 $\pm$ 0.7	0.599
Culture Period (days)	2.4 $\pm$ 0.1	2.2 $\pm$ 0.1	0.269	2.3 $\pm$ 0.1	2.2 $\pm$ 0.2	0.512
Duration of Diabetes Status (days)	23.7 $\pm$ 4.0	22.0 $\pm$ 3.3	0.737	24.3 $\pm$ 4.2	20.6 $\pm$ 4.5	0.556
Isolated IE/Pancreas Weight (g)	2095.8 $\pm$ 241.8	2415.8 $\pm$ 217.8	0.328	2160 $\pm$ 260	2667 $\pm$ 317	0.219
IE/IC Ratio	1.3 $\pm$ 0.1	1.3 $\pm$ 0.1	0.678	1.3 $\pm$ 0.1	1.3 $\pm$ 0.1	0.410

Table 1C
Multivariate Logistic Regression Model Predicting Nude Mouse Graft Failure with Human Islets.

Human Islets	Odds Ratio	(95% CI)	P value
Islet Equivalent 2000	0.09	(0.03, 0.33)	0.004
Mouse Body Weight (per 3.7 g)	0.59	(0.37, 0.96)	0.034
Pellet Volume (per 10.0 cc)	1.23	(0.76, 2.00)	0.397
C statistic			0.784
Hosmer-Lemeshow Chi-Square			8.88
DF			8
p value			0.353

Table 1D
Pig islet preparations: Donor, isolation, islet and recipient mouse characteristics

Donor & Isolation Characteristics		Pig (n=194)
Age (years)		3.7 ± 0.8 (1.8-5.3)
Gender		Gilt
Body Weight (Pounds)		590 ± 66 (232-704)
Trimmed Pancreas Weight (grams)		237 ± 45 (102 - 340)
Enzyme used to Digest Pancreas		Liberase-HI (n=178); VitaCyte (n=16)
Digest Islet Equivalent (IEQ)		401,284 ± 169,946
Digest Islet Equivalent/gram Pancreas		1791 ± 809
Post Purification Islet Equivalent (IEQ)		374,876 ± 164,159
Post Purification Islet Equivalent/gram Pancreas		1639 ± 761
Percent Islet Purity (%)		90 ± 8
<u>Islet Characteristics</u>		
Islet Equivalent for Transplantation (IEQ)		750, 1000 and 2000
Pellet Volume in PE-50 tubing (mm) of 750 IEQ		5 ± 1
Pellet Volume in PE-50 tubing (mm) of 1000 IEQ		6 ± 2
Pellet Volume in PE-50 tubing (mm) of 2000 IEQ		16 ± 11
<u>Recipient Mouse Characteristics</u>		
Body Weight (gram)		29 ± 4
Duration of Diabetes Status (days)		23 ± 8
Average Blood Glucose before Tx (mg/dL)		517 ± 67

Data presented as mean ± SD

Table 1E
Bivariate Association between Donor Characteristics, Nude Mouse Characteristics, Islet Processing Characteristics and Pig Islet Graft Functioning

	Analysis of 750, 1000, and 2000 IEQ porcine islet transplantation in diabetic nude mice			Analysis of 2000 IEQ porcine islet transplantation in diabetic nude mice		
	Graft Cured (n = 43) Mean $\pm$ SEM	Graft Failure (n = 55) Mean $\pm$ SEM	P value	Graft Cured(n=39) Mean $\pm$ SEM	Graft Failure (n = 27) Mean $\pm$ SEM	P value
Porcine Donor Characteristics						
Porcine Body Weight (pounds)	540.5 $\pm$ 9.0	596.7 $\pm$ 12.8	< 0.001	540.0 $\pm$ 9.5	568.7 $\pm$ 23.8	0.234
Nude Mouse Characteristics						
Body Weight (g)	28.8 $\pm$ 0.3	29.5 $\pm$.6	0.337	28.7 $\pm$ 0.3	30.5 $\pm$ 0.7	0.029
Blood Glucose (mg/dL)	514.3 $\pm$ 5.7	521.5 $\pm$ 8.8	0.474	520.0 $\pm$ 5.8	529.5 $\pm$ 12.2	0.491
Processing Characteristics						
Average Transplanted Islet Equivalents (750, 1000, 2000 IEQ)	1916.0 $\pm$ 25.1	1274.6 $\pm$ 68.1	< 0.001	2000	2000	NA
Pellet Volume (cc)	10.1 $\pm$ 0.3	5.3 $\pm$ 0.4	< 0.001	10.5 $\pm$ 0.3	8.0 $\pm$ 0.7	< 0.001
Percent Islet Purity	92.5 $\pm$ 0.4	90.0 $\pm$ 0.5	< 0.001	92.5 $\pm$ 0.6	90.0 $\pm$ 0.5	< 0.001
Culture Period (days)	4.7 $\pm$ 0.4	4.4 $\pm$ 0.8	0.736	4.6 $\pm$ 0.4	3.8 $\pm$ 1.0	0.493
Duration of Diabetes Status (days)	22.7 $\pm$ 1.5	23.9 $\pm$ 2.4	0.666	21.9 $\pm$ 1.6	26.0 $\pm$ 5.2	0.455
Isolated IE/Pancreas Weight (g)	1612.1 $\pm$ 74.1	1697.6 $\pm$ 102.2	0.509	1578.1 $\pm$ 73.4	1388.9 $\pm$ 114.5	0.286

Table 1F
Multivariate Logistic Regression Model Predicting Nude Mouse Graft Failure with Porcine Islets.

Porcine Islets	Odds Ratio	(95% CI)	P value
Islet Equivalent 2000	0.23	(0.09, 0.63)	0.004
Mouse Body Weight (per 4.1 g)	1.34	(0.93, 1.93)	0.113
Pellet Volume (per 4.07 cc)	0.26	(0.14, 0.49)	<0.001
C statistic			
0.867			
Hosmer-Lemeshow Chi-Square			
5.89			
DF			
8			
p value			
0.660			

Table 1G
Non-Human Primate islet preparations: Donor, isolation, islet and recipient mouse characteristics

Donor & Isolation Characteristics		NHP (n=27)
Species	Cynomolgous (n=14); Rhesus (n=16); Baboons (n=13)	
Body Weight (kg)		9.4 ± 3.3
Trimmed Pancreas Weight (grams)		15.8 ± 6.2 (5-37)
Enzyme used to Digest Pancreas	Liberase-HI (n=27)	
Digest Islet Equivalent (IEQ)		55,935 ± 27,032
Digest Islet Equivalent/gram Pancreas		3771 ± 2031
Post Purification Islet Equivalent (IEQ)		53,444 ± 25,569
Post Purification Islet Equivalent/gram Pancreas		3667 ± 2094
Percent Islet Purity (%)		93 ± 6
Islet Characteristics		
Islet Equivalent for Transplantation (IEQ)		750, 1000 and 2000
Pellet Volume in PE-50 tubing (mm) for 750 IEQ		5 ± 2 (n=3)
Pellet Volume in PE-50 tubing (mm) for 1000 IEQ		7 ± 2 (n=7)
Pellet Volume in PE-50 tubing (mm) for 2000 IEQ		11 ± 3 (n=10)
Recipient Mouse Characteristics		
Body Weight (gram)		27 ± 4
Average Blood Glucose before Tx (mg/dL)		489 ± 67

Data presented as Mean ± SD

After transplantation of 750, 1000, and 2000 IEQ non-human primate islets under the kidney capsule of diabetic nude mice, 80% of mice receiving 2000 IEQ (n=10) and 83% with 1000 IEQ (n=7) became normoglycemic, and no mice were cured when receiving 750 IEQ (n=3). Due to less number of transplant cases the data was not showing any significant outcome when compared to donor and islet isolation characteristics.

Basal, stimulated insulin release and total insulin content of human, porcine and NHP islets and their mice transplantation cure rate.

Table 2A

Insulin secretory response and mouse transplantation outcome	Human (n=70)	Pig (n=67)	NHP (n=15)
Basal (1.67 mM) Insulin Release (ng/μg DNA/60 min)	6.28 ± 7.28	1.13 ± 1.72	3.06 ± 3.76
Stimulated (16.7 mM) Insulin Release (ng/μg DNA/60 min)	18.08 ± 13.62	3.20 ± 3.96	24.19 ± 31.27
Stimulation Index (Stimulated release/Basal release)	5.13 ± 5.50	4.71 ± 4.29	9.14 ± 8.41
Total Insulin Content (ng/μg DNA)	550 ± 410	672 ± 394	2,783 ± 3,784
% Mice cured (2000 IEQ)	70%	64%	100%

Data presented as Mean ± SD

Table 2B

During glucose stimulation of islets we observed variations in insulin response. So the insulin secretory response (Human, pig, NHP) was separated into low and high secretion and the mouse transplantation cure rate was compared

Insulin secretion after exposure to basal glucose (1.67 mM)				
	Low Insulin Secretion (ng/ μ g DNA/60 min)	% Mice Cured (2000 IEQ)	High Insulin Secretion (ng/ μ g DNA/60 min)	% Mice Cured (2000 IEQ)
Human	1.678 $\pm$ 0.784 (n=24)	80%	12.5 $\pm$ 5.37 (n=21)	57%
Porcine	0.214 $\pm$ 0.104 (n=12)	75%	3.116 $\pm$ 2.219 (n=3)	67%
NHP	1.097 $\pm$ 0.453 (n=6)	100%	5.304 $\pm$ 4.662 (n=7)	100%
Insulin secretion after exposure to stimulated glucose (16.7 mM)				
	Low Insulin Secretion (ng/ μ g DNA/60 min)	% Mice Cured (2000 IEQ)	High Insulin Secretion (ng/ μ g DNA/60 min)	% Mice Cured (2000 IEQ)
Human	4.85 $\pm$ 3.41 (n=19)	68%	29.1 $\pm$ 8.31 (n=19)	58%
Porcine	0.531 $\pm$ 0.231 (n=8)	63%	6.976 $\pm$ 5.037 (n=4)	50%
NHP	7.378 $\pm$ 5.470 (n=5)	100%	43.399 $\pm$ 37.939 (n=7)	100%

Data presented as Mean $\pm$ SD

Table 3

Nude mouse transplantation outcomes (n=28) were separated into cured and failed groups after transplantation of 2000 IEQ from human clinical islet preparations. There were no differences in % beta cells and islet cell viability.

Nude mouse transplantation(2000 IEQ)	Cured (54%)	Failed (46%)	P-Value
Characteristics of islet transplantation preparation			
Islet Purity (%)	63 ± 13	64 ± 13	0.7809
% Beta Cells	33 ± 7	30 ± 7	0.255
% Alpha Cells	16 ± 7	16 ± 8	0.9311
% Delta Cells	11 ± 4	12 ± 5	0.5355
% PP Cells	4 ± 2	6 ± 3	0.1628
% Acinar Cells	23 ± 6	20 ± 10	0.5099
% Ductal Cells	11 ± 5	7 ± 6	0.1363
% Other Cells	7 ± 7	5 ± 6	0.744
24 hours Viability (%) by FDA/PI	93 ± 4	94 ± 4	0.6668
48 hours Viability (%) by FDA/PI	96 ± 2	97 ± 4	0.3272
Endotoxin level in final product	0.7 ± 0.5	1.0 ± 1.1	0.3038
Stimulation index calculated from GSIR	4.5 ± 3.0	2.8 ± 2.5	0.1423

Data presented as Mean ± SD