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Juvenile Porcine Islets Can Restore Euglycemia in Diabetic Athymic Nude Mice After Xenotransplantation

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Background. Porcine islet xenotransplantation has been demonstrated in many animal studies to cure experimentally induced diabetes. However, several issues currently impede the translation of porcine islet xenotransplantation to sustained insulin independence clinically. Although adult pigs have mature islets that secrete insulin in response to a glucose challenge, and are physiologically similar to humans, there are logistical considerations with adult porcine tissue that are not present with juvenile porcine tissue. To circumvent these issues, we have identified 18- to 21-day-old preweaned juvenile pigs as islet donors as we have previously demonstrated superior islet yields and function from juvenile pigs using our islet isolation protocols. **Methods.** We evaluated the efficacy of islets isolated from 18- to 24-day-old Yorkshire swine in vitro using a standard glucose-stimulated insulin response assay, and in vivo after xenotransplantation under the kidney capsule of streptozotocin-induced 8- to 10-week-old male athymic nude mice. The mice were monitored for a period of 60 days after transplantation, after which the grafts were explanted and analyzed. **Results.** Diabetic athymic nude mice transplanted with 1500 to 3000 islet equivalents (IEq) of islets achieved sustained normoglycemia for up to 60 days after islet transplantation. When the grafts were explanted with the kidney, a rapid return to hyperglycemia was observed. **Conclusions.** Efficacy and dose-titration studies evaluating these islets in immunocompetent and nonobese diabetic mouse models are underway. The results of these studies will permit application for nonhuman primate and pivotal clinical trials in human diabetic patients in the near future.

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Human islet allotransplantation as a cure for type 1 diabetes (T1D) has been demonstrated to be a viable therapeutic option, albeit for a select minority of patients with multiple comorbidities.[1] Despite some transplant recipients reporting insulin independence for longer than 15 months, the need for chronic immunosuppression, its attending adverse effects, eventual graft failure, and a rapid return to exogenous insulin therapy dissuade widespread application of this procedure to all T1D patients.[2,3] Furthermore, the availability of transplant-worthy islets isolated from healthy, deceased pancreas donors is scarce, severely limiting the number of recipients that may be able to benefit from this procedure. These limitations have stimulated investigators to look for alternative islet sources for transplantation. A clear choice as a prospective donor of xenogeneic tissue is the pig; porcine insulin was used to treat diabetes in the past and is structurally and physiologically similar to human insulin, seeing as it differs in only 1 amino acid from human insulin.[4]

However, translation of porcine islet xenotransplantation to clinical trials faces several stringent requirements that have to be met before securing approval from the Food and Drug Administration for clinical trials in humans.[5] The identification of the ideal donor source and strain, standardization of isolation and culture techniques, augmentation of islet yields, the need for specific pathogen-free (SPF) herds, and designated pathogen-free secure facilities where these pigs can be bred and housed are some of the standards that need to be met before this treatment modality can be offered to human patients.

For eventual translation to clinical practice, issues such as islet dosage, cost-effectiveness, and reproducibility are of paramount importance. Studies have compared adult, juvenile, neonatal, and fetal islets and reported that adult islets, being fully differentiated, are more responsive to a glucose challenge compared to islets isolated from younger porcine donors.[6] However, the prohibitive cost of housing and feeding donor pigs till they mature into adult swine negates any advantages

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observed in islet function. In a study comparing human, nonhuman primate, adult porcine and juvenile porcine islets, none of the recipient mice remained normoglycemic with 50% becoming diabetic in 100 days, and all returning to the diabetic state after 175 days.[7] Most studies that reported successful reversal of hyperglycemia used islet dosages between 2000 and 5000 IEq per recipient.[7–10] Dosages of less than 2000 IEq did not result in sustained normoglycemia or hyperglycemia reversal in even 50% of mice.[7]

In our study, we isolated islets from pancreases harvested from juvenile porcine donors using a novel gentle enzymatic digestion process that avoids the use of toxic purification gradient solutions and achieves islet purification over a 7- to 10-day in vitro culture period[11] and transplanted these islets into streptozocin-induced diabetic athymic nude mice. We demonstrate the ability of islets isolated and matured using our methods to be sufficient to achieve euglycemia, at dosages comparable to what has been previously reported with isolated adult-derived porcine pancreatic islets.[7]

MATERIALS AND METHODS

Islet Isolation

Islets were isolated from 18- to 21-day-old male Yorkshire swine (n = 5; S&S Farms, Ramona, CA) as previously described.[11] Briefly, the pancreas was harvested using rapid surgical procurement (<5 minutes) and placed in Organ Preservation Solution (Mediatech, Manassas, VA). Cold ischemia time was limited to less than 30 minutes. All animal procedures (monitoring, surgery, and euthanasia) were performed with approval from the University of California Institutional Animal Care and Use Committee at the University of Irvine. The pancreas was then washed in cold (4°C) Hanks balanced salt solution supplemented with HEPES (Corning Cellgro, Manassas, VA) and trimmed of surrounding adipose and lymphatic tissue in a sterile biosafety hood. The pancreatic tissue was then minced into 2 to 3 mm^2 pieces and digested at 37°C using Sigma Type V Collagenase (2.5 mg/mL in Hanks balanced salt solution; Sigma Aldrich, St. Louis, MO). The mean digestion time was 16.1 ± 0.6 minutes. The islet tissue clusters (50-500 μm) isolated using this method were allowed to mature into complete islets during in vitro culture at 37°C, 5% CO_2 first in Recovery Maturation Media (Optatio LLC, Chino, CA) supplemented with 215 mM aprotinin (Sigma-Aldrich), 0.5 mM Pefabloc (Sigma-Aldrich), 417 mM dornase alfa (Genentech, San Francisco, CA), and 10% porcine serum (Lampire Biological Laboratories Inc., Ottsville, PA), and 48 hours later, in a novel maturation media (Optatio LLC) supplemented with 10% porcine serum (Lampire Biological Laboratories Inc.) as outlined previously.[11] At the end of the 7-day (or 14-day) culture period, islet quality control assessment was performed (Table 1).

Islet Quality Control

Islet count and IEQ were determined by staining a 100-μL aliquot with 1 mL dithizone (MP Biomedicals), and observing islets at 25× on a standard stereomicroscope (Max Erb, Santa Ynez, CA) containing a 10× eye piece graticule. Dithizone staining was also used to determine percentage of purity over the maturation period. Islet viability was analyzed using Newport Green (Invitrogen, Carlsbad, CA) and propidium iodide, imaged using fluorescence microscopy (Nikon LSM510; Thornwood, NY), and quantified with a Microplate reader (Tecan Infinite F200, Magellan V7; Tecan, Männedorf, Switzerland).

Islet function during was determined using glucose-stimulated insulin release. One hundred to 150 porcine islets were incubated for 1 hour, in corresponding order, in low glucose (2.8 mM), high glucose (28 mM), high glucose plus 3-isobutyl-1-methylxanthine (28 mM, 50 μM), and then low glucose (D-(+)-glucose; Sigma Aldrich); stimulation index was calculated as the ratio of insulin secreted in high glucose over the amount of insulin secreted in low glucose. Insulin levels were measured using a standard porcine insulin enzyme-linked immunosorbent assay (Porcine Insulin ELISA; Mercodia, Winston Salem, NC), and absorbance was measured using a Microplate reader (InfiniteF200, Tecan, and Magellan V7).

Islets with a purity greater than 80% (assessed using Dithizone staining), viability greater than 90% (assessed using Newport Green/ Propidium Iodide staining) and stimulation index of 2 or higher were deemed suitable for transplantation.

Animals

Test Animals

Athymic nude mice (Charles River, Strain Code 490) with an initial target body weight of 25 g and between 8 to 10 old were used as transplant recipients.

Exclusion Criteria

All animals that failed to become hyperglycemic after Streptozotocin (STZ) administration or had lost more than 20% of their body weight during the course of the study were excluded.

Transplant Groups

Control Group

Nondiabetic and diabetic athymic nude mice were transplanted with 1.5 mL saline injected under the kidney capsule and monitored for 30 days after transplantation.

Treatment Groups—Islet Transplantation Under the Kidney Capsule

Athymic nude mice were rendered diabetic and transplanted with either 1500 or 3000 IEq under the kidney

TABLE 1.

Porcine pancreatic donor parameters—Animal weight, pancreas weight, islet yield (/g Pancreas), islet viability, Islet purity and function

Donor strain	No. animals	Age, days	Weaning status	Animal weight, kg	Islet yield, IE/g	Islet purity, %	Islet viability, %	SI (Hi/Low)
Yorkshire	5	20 ± 2.4	Pre-weaned	7.2 ± 1.1	5,120 ± 317	81 ± 1.7	92 ± 1.4	2.7 ± 0.2

SI, stimulation index.

capsule. Mice were monitored daily for 30 and 60 days, after which the kidneys were removed with the islet grafts, and blood glucose was measured until levels returned to hyperglycemia (>350 mg/dL).

Experimental Design Groups (Diabetic Models): Juvenile Porcine islets were isolated and matured for 7 (or 14) days and then transplanted under the kidney capsule of diabetic athymic nude mice (Table 3). Blood glucose levels were monitored three times a week, and animal weights were monitored weekly.

Monitoring and Diabetes Induction

On arrival, all animals were allowed to acclimate for 48 hours before induction of diabetes. Mice were rendered diabetic by a single intravenous injection of Streptozotocin (150 mg/kg Streptozocin; Sigma Aldrich), and diabetes was confirmed after 3 consecutive days of hyperglycemia (Blood Glucose >350 mg/dL). Blood glucose was measured using tail capillary blood using a standard glucose monitor (Bayer Health Care, Whippany, NJ). After diabetes confirmation, mice were adminstered subcutaneous insulin glargine (Lantus) to stabilize blood glucose levels.

After blood glucose levels were stabilized with insulin, mice were transplanted with either 1500 or 3000 IEq of juvenile porcine islets under the kidney capsule.

Islet Preparation for Kidney Capsule Transplant

Isolated islets were first washed in washed 0.9% normal saline (Baxter, Deerfield, IL) and then slowly placed into PE50 Tubing (Becton Dickinson, Franklin Lakes, NJ) by using a 1-mL TB slip-tip syringe with a 23G BD precision Needle (BD) to withdraw them into the tubing. Islets within PE50 tubing were placed into a 15-mL conical tube (Fisher Scientific, Pittsburgh, PA) and then centrifuged for 2 minutes at 200 g (Sorvall RT7, Thermo Scientific, Waltham, MA) to create 1 continuous pellet.

Islet Transplant Under the Kidney Capsule

Surgical field for transplant was cleaned before transplantation using sterile equipment. Mice were sedated using a 2% isoflurane (Piramal Healthcare, Bethlehem, PA)/O_2 mixture (Isotech by SurgiVet, Dublin, OH). Once full sedation was reached, the transplantation site was prepared using povidine-iodine prep pads (Applicare) and 70% ethanol. A small incision was made into the skin above the kidney to expose the peritoneum, followed by an incision through the peritoneum to expose the kidney. A small incision was made into the kidney capsule using a 25G precision Needle (BD). Using a glass rod, the space was widened carefully to create a subcapsular pocket where the islets were placed using a 23G Hamilton Syringe (SGE Analytical Science, Austin, TX). The capsule was closed using cautery (Gyrus Acmi, Southborough, MA) and spread evenly over the surface of the kidney using a sterile cotton tip applicator moistened with sterile saline. The surgical site was closed using a 3-0 silk suture on a 30" SH tapered needle (Ethicon, Somerville, NJ) and polymyxin B sulfate/bacitracin/neomycin sulfate antibiotic ointment (Target Corp, Minneapolis, MN) was applied to the surgical site. All mice were given a single injection of Buprenorphine dose 0.2 mg/kg (Reckitt Benckiser Pharmaceuticals, Parsippany, NJ; Schedule III/DEA 9064) and allowed to recover. All mice were also given 0.02 mg/mL ibuprofen (Advil; Pfizer Pharmaceuticals, New York City, NY) for 1 week in drinking water, and sutures were removed once incision site was completely healed.

Posttransplant Animal Monitoring

During the immediate posttransplant period (up to 7 days after transplantation), blood glucose was measured once daily using a standard glucose monitor (Bayer Health Care) and mice were given insulin glargine (Lantus) to stabilize blood glucose levels if and when required (<250 mg/dL, no insulin; 250-550 mg/dL, 1 unit; > 550 mg/dL, 2 units).

After the first week after transplantation, mice were monitored for clinical observations, blood glucose levels 3 times a week, and body weights weekly. No insulin was administered after the 7th day posttransplantation to any of the recipient animals, and any animals that showed 3 consecutive days of hyperglycemia (blood glucose, >350 mg/dL) were removed from the study, euthanized, and their kidneys were explanted for histological analysis.

For the purpose of this study, normoglycemia was defined as a random blood glucose level less than 200 mg/dL and a fasting blood glucose level less than 126 mg/dL.

Oral Glucose Tolerance Test

The recipient mice were fasted overnight (no insulin is administered) before the test. The mice were anesthetized using 2% isoflurane (Piramal Healthcare)/O_2 mixture (Isotech by SurgiVet) and placed on a heating pad. Using a 20G blunt-tipped gavage needle, a precalculated volume of 50% dextrose solution was injected into the esophagus. Blood was drawn from the tail vein at 0 (before injection), 5, 10, 15, 20, 30, 45, 60, 120, and 180 minutes after oral administration of glucose to measure changes in blood glucose levels. Blood glucose levels were measured using a standard glucometer and blood glucose strips (Contour, Bayer Health Care, Whippany, NJ).

Explant of Islet-Containing Kidneys via Nephrectomy

The surgical field for transplant was cleaned before transplantation using sterile equipment. Mice were sedated using a 2% isoflurane (Piramal Healthcare)/O_2 mixture (Isotech by SurgiVet). Once full sedation was reached, the transplantation site was prepared using povidine-iodine prep pads (Aplicare, Meriden, CT) and 70% ethanol. A small incision was made on the skin overlying the kidney containing the

TABLE 2.

Quality control performed on islet preparations during in vitro culture before transplant

S. No.	Culture period	Islet purity, %	Islet viability, %	SI (Hi/Low)	Islet size distribution, %				
					50–100 μm	100–150 μm	150–200 μm	200–250 μm	250–300 μm
1	7 days	79 ± 2.2	93 ± 2.1	2.6 ± 0.5	49.3 ± 9	34.3 ± 4	11.0 ± 5	2.7 ± 2	2.7 ± 3
2	14 days	82 ± 1.7	91 ± 1.7	2.9 ± 0.4	44.4 ± 3	33.3 ± 2	11.1 ± 4	8.9 ± 6	2.2 ± 1

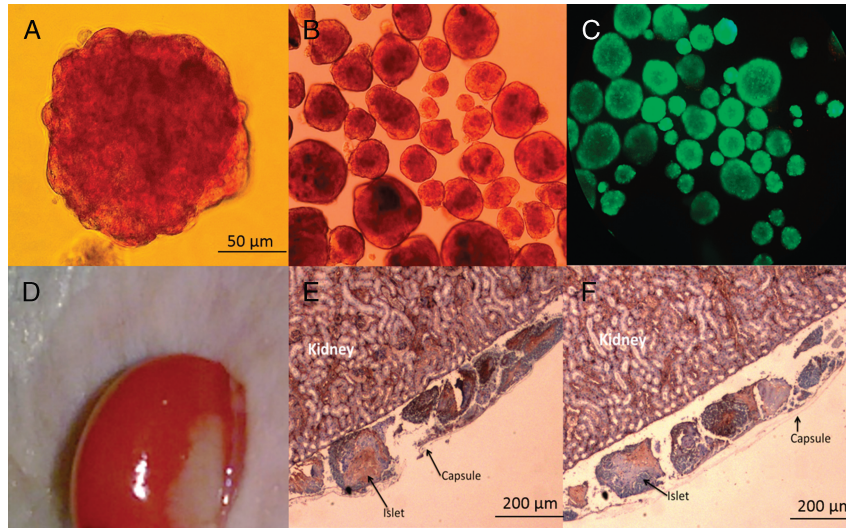

Figure 1. A, Young porcine islet stained with dithizone. B, Dithizone and (C) Newport Green/PI–stained islets. D, Islets under the kidney capsule. E, glucagon and (F) insulin immunostaining in diabetic athymic nude mice implanted with 1500 piglet islets under the kidney capsule at 60 days after transplantation. Arrowheads are islets, the thick arrow is the kidney, and the arrow indicates the kidney capsule.

islet transplant to expose the peritoneum, followed by an incision through the peritoneum to expose the kidney. A short length of 5-0 prolene suture (Ethicon, Somerville, NJ) was then looped under the kidney to securely tie off the renal vein, artery, and ureter. The kidney was then removed leaving a small piece behind to ensure the suture knot did not slip. The kidney was then placed in 10% neutral buffered formalin. The surgical site was closed using a 3-0 Silk suture on a 30" SH tapered needle (Ethicon) and polymyxin B sulfate/bacitracin/neomycin sulfate antibiotic ointment (Target Corp) was applied to the surgical site. A single injection of 0.2 mg/kg Buprenorphine (Reckitt Benckiser Pharmaceuticals, Schedule III/DEA 9064) was administered subcutaneously, and the mice were allowed to recover. Postoperative pain management also included 0.02 mg/mL ibuprofen (Advil; Pfizer) supplemented in the drinking water for the first week post-transplant. Blood glucose levels were monitored daily until mice showed 3 days of hyperglycemia (>350 mg/dL), after which they were euthanized. The pancreas was recovered and stored in 10% neutral buffered formalin.

All animal surgical procedures, care and euthanasia were in performed in accordance with the approval of the Institutional Animal Care and Use Committee (2008-2850) with efforts to minimize the number of animals needed for this study. All controlled substances were used and handled in accordance with the approved CSUA 2010-136.

Histology

Pancreas Histology

At euthanasia, pancreases were removed and fixed for histology with 10% Phosphate Buffered Formalin (Fisher Scientific). Samples were then embedded in paraffin, sectioned, and stained using hematoxylin-eosin and horse radish peroxidase (HRP) immunohistochemistry for insulin and glucagon. Both insulin and glucagon HRP stains were counterstained with hematoxylin.

Histological Examination of Islets Transplanted Under the Kidney Capsule

Explanted kidneys containing transplanted porcine islets were fixed in 10% phosphate-buffered formalin (Fisher Scientific). Kidneys were then sliced to remove excess kidney tissue, and samples were paraffin embedded, sliced, and stained using hematoxylin-eosin and HRP immunohistochemistry for insulin and glucagon. Both insulin and glucagon were counterstained with hematoxylin. Paraffin blocking and staining were performed by the University of California Irvine Medical Center Histology Core Facility.

Statistical Analysis

Data are presented as means ± SEM. Differences in results were assessed by a single-factor analysis of variance. A probability (P) value less than 0.05 was considered statistically

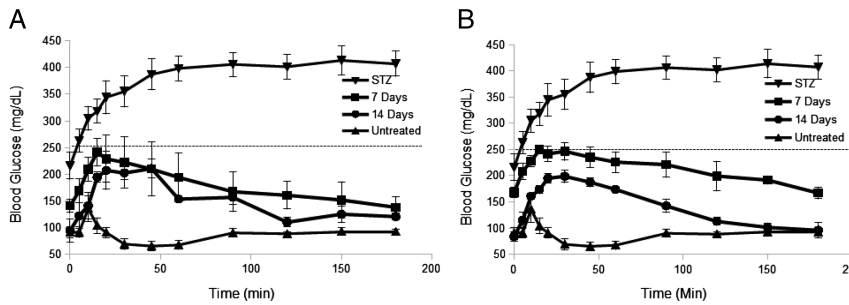

Figure 2. Oral glucose tolerance tests performed on mice. A, At 2 weeks and (B) 4 weeks after implantation in islets incubated at 7 (n = 10) and 14 (n = 7) days before being transplanted under the kidney capsule into STZ-induced diabetic athymic nude mice, compared to mice who received STZ (n = 6), and the control group who received neither islets or STZ (n = 5).

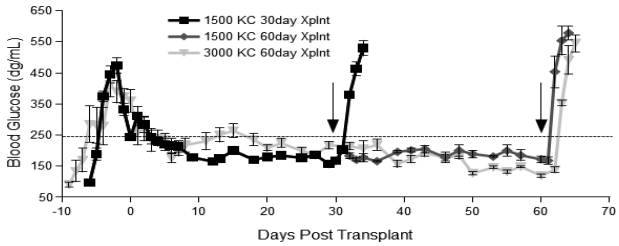

Figure 3. Daily blood glucose in STZ-induced diabetic athymic nude mice. The athymic nude mice either received no islets (ATN CTRL, n = 10), 1500 IEq (n = 8), or 3000 IEq (n = 10) under the kidney capsule. The arrows indicated when nephrectomy of the kidney containing islets was performed, with resulting hyperglycemia.

significant, and a *P* less than 0.001 was considered highly statistically significant.

RESULTS

Determination of Optimal Incubation Length—In Vitro Culture Duration

After the islets were incubated at 37°C, 5% CO_2 for either 7 or 14 days, appropriate quality control assays were performed (Table 2) as previously stated, and then islets were selected for transplant under the kidney capsule in STZ-induced diabetic athymic nude mice (Figure 1D). A dose of 3000 IEq was transplanted into each recipient mouse. At 2 weeks and 4 weeks (Figure 2A and B) after transplantation, oral glucose tolerance tests (OGTT) were performed on control mice, diabetic mice, and mice that had received islets incubated for 7 or 14 days. As expected, diabetic mice were unable to tolerate the glucose challenge and remained hyperglycemic for the 3-hour observation period, whereas control nondiabetic mice maintained normal blood glucose easily. In comparison, recipients of both the 7 and 14 days incubated islets never became significantly hyperglycemic, and demonstrated superior glycemic control to diabetic mice. There was no significant difference between either group in terms of blood glucose during the OGTT ($P = 0.5$). At 4 weeks, the results were similar, with again both transplanted groups maintaining normoglycemia; however, the 14-day group had significantly lower blood glucose than the 7-day group ($P < 0.05$). Despite this finding, since both groups still demonstrated normoglycemia, for the remaining experiments, we preferred a 7-day in vitro incubation period before transplantation, as it conferred logistical advantages over a 14-day period of in vitro culture.

Implantation of Islets into Immune Deficient Mice

After determining that 7 days incubation of our young pig islets was sufficient to produce normoglycemia in an immunosuppressed model, we decided to identify the islets marginal mass of juvenile porcine required to achieve reversal of STZ-induced hyperglycemia in athymic nude mice after transplantation of either 1500 IEq or 3000 IEq under the kidney capsule, and compared these groups to controls (Figure 3). All transplant groups were able to maintain normoglycemia effectively for up to 60 days, at which point, the experiment was terminated. There was no difference between the transplant groups based on analysis of variance after normoglycemia was established after transplantation (Table 3).

To show that the islet transplants were responsible for return to normoglycemia, the mice that had received transplants under the kidney capsule underwent unilateral nephrectomies on the transplanted side (Figure 3). In each group, when the kidney containing the islet grafts was removed, there was a rapid return to hyperglycemia, at both 30 and 60 days, regardless of the islet dose. Histology performed on these organs revealed positive staining for both glucagon (Figure 1E) and insulin (Figure 1F) in islet cells located under the kidney capsule, with no evidence of necrosis.

Survival Benefit of Transplantation and Return to Hyperglycemia

Excluding the mice that had died due to complications after transplant surgery or STZ injection, when compared to control diabetic mice, there was significantly improved survival in all transplant recipients (Figure 3, $P < 0.05$). Additionally, no mouse in our study reverted to hyperglycemia after establishing normoglycemia for up to 60 days until nephrectomy after which all experiments were terminated (Figure 3).

Histological Confirmation of Pancreas Specimens

At the conclusion of the study, the mice were euthanized and their pancreases examined for evidence of STZ-induced islet death. Compared to control nondiabetic mice, none of the mice from the diabetic control (untreated) and diabetic transplant groups (treated with either 1500 IEq or 3000 IEq) showed evidence of β cell regeneration (Figure 4) thereby demonstrating that the return to normoglycemia was a direct result of the islets transplanted under the kidney capsule and not due to β cell regeneration in the recipient's islets.

DISCUSSION

Previous studies have reported successful reversal of hyperglycemia after xenotransplantation of adult pig islets into nude mice.[9] Here, we have shown similar success with xenotransplantation of islets isolated from 18- to 21-day-old pigs for up to 60 days under the kidney capsule, which has repeatedly been demonstrated to be the preferred transplant site for

TABLE 3.

Number of athymic mice used in the study by experimental study group

S. No.	Experimental group	Islet dose	Recipient mice	Deaths/Exclusions	Successful reversal of hyperglycemia
1	7-day in vitro culture	1500 IEq	8	2[a]	8/8
2	7-day in vitro culture	3000 IEq	10	0	10/10
3	14-day in vitro culture	3000 IEq	7	3[a]	7/7
4	Diabetic control	—	6	4[a]	0/6
5	Nondiabetic control	—	4	1[b]	NA

[a] The mice were found very sick after STZ administration and were euthanized according to standard IACUC protocols. They did not receive islet transplants.
[b] The mouse was found dead.
IACUC, Institutional Animal Care and Use Committee; NA, not applicable.

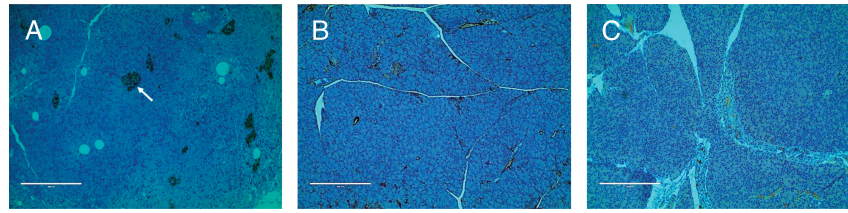

Figure 4. Insulin immunohistochemistry on pancreas specimens. Immunohistochemical staining was performed on mouse pancreas specimens at euthanasia using the HRP method. Positive staining for insulin (brown) is noted in the islets (arrows) in the nondiabetic control animals (A), whereas no staining is noted in the diabetic control (B) or diabetic animals treated with islet transplants (C). Magnification, 10×; scale, 400 μm. HRP, horse radish peroxidase.

evaluating islet efficacy and dosage owing to its high vascularity, ease of access, and the ability to demonstrate a return to hyperglycemia after nephrectomy.[10] There are numerous advantages to using juvenile porcine donors as opposed to older donors for the purposes of scalability. Type 1 diabetes has been increasing in incidence worldwide, and 1 in 300 children in the United States alone has T1D.[11,12] Porcine insulin's physiological similarity to human insulin has led to a concerted effort in the diabetes research community to develop an alternative islet source to combat the severe scarcity of healthy pancreas donors seen with human islet allotransplantation.[13] In order to develop an effective therapy for this disease, a consistent, reliable and scalable source of high-quality islets isolated from designated pathogen-free pigs bred and housed in an SPF facility is required. Pigs require over 2 years to reach adulthood, and reach weights in the hundreds of kilograms. The costs of housing and managing large herds of SPF pigs for prolonged periods of time would be prohibitive, severely limiting the applicability of this transplant model. The pigs used in this study were younger than 1 month, and weighed around 5 kg. Due to their smaller size and easier handling, pancreata harvested from these pigs suffered much less cold ischemia time (27 ± 2 min). One study noted that a lowering of cold ischemia time significantly improved islet function and yield and that the average cold ischemia time for young pig islets was around 10 minutes, whereas adult islets underwent up to 90 minutes of cold ischemia.[14]

The duration of in vitro culture and incubation time has been demonstrated to impact graft success.[12] A previous study reported significant detriment to incubating islets for an additional week over 7 days, that is, 14 days.[15] Our results demonstrate that even if the time in culture exceeds 7 days, the islets that were able to respond adequately to a glucose challenge during an OGTT. In our porcine islet xenotransplant model, we aim to demonstrate that it is feasible to generate consistent, high-quality islets within a month from farrowing. This has significant implications for translation to clinical trials as the improvements in cost-efficiency are appreciable. Also, in contrast to previous reports which had suggested that a dose of 2000 IEq would not be sufficient to maintain normoglycemia in recipient mice, here normoglycemia was achieved and maintained with a dose of only 1500 IEq per mouse. A lower islet requirement per recipient and a high islet yield per pancreas would mean fewer animal requirements per recipient, thereby scaling down costs and enabling wider application of this promising therapeutic tool.

CONCLUSIONS

From our results, we report that islets can be isolated from juvenile pigs using a simple, cost-effective isolation procedure without the need for purification with potentially toxic chemical gradients, which enables easy scalability. We have demonstrated consistently high islet yields (~30,000 IEq/pancreas) for a period of over 2 years, where over 88 isolation procedures were performed.[16] We have also demonstrated that the islets thus isolated are able to secrete adequate insulin to reverse hyperglycemia and maintain euglycemia in a diabetic mouse model for a prolonged time period, We believe that the next step in our search for a cure for T1D would be to work toward translating this method of isolation to larger animal models, with the eventual goal of evaluating the efficacy and safety of juvenile porcine islets in human clinical trials.[17]

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