Cell Transplantation, Vol. 25, pp. 1763–1775, 2016
Printed in the USA.

0963-6897/16 $90.00 + .00
DOI: http://dx.doi.org/10.3727/096368916X691475
E-ISSN 1555-3892
www.cognizantcommunication.com

Plasticity and Aggregation of Juvenile Porcine Islets in Modified Culture: Preliminary Observations

Bradley P. Weegman,*†[1] Michael J. Taylor,†‡§[1] Simona C. Baicu,‡¶ Kate Mueller,#
Timothy D. O'Brien,** John Wilson,†† and Klearchos K. Papas‡‡

*Department of Radiology, Center for Magnetic Resonance Research, University of Minnesota, Minneapolis, MN, USA
†Sylvatica Biotech, LLC, N. Charleston, SC, USA
‡Tissue Testing Technologies, LLC, N. Charleston, SC, USA
§Department of Mechanical Engineering, Carnegie Mellon University, Pittsburgh, PA, USA
¶LifePoint, Inc., Charleston, SC, USA
#Department of Surgery, University of Minnesota, Minneapolis, MN, USA
**Veterinary Population Medicine Department, University of Minnesota, St. Paul, MN, USA
††Wilson Wolf Manufacturing, New Brighton, MN, USA
‡‡Department of Surgery, University of Arizona, Tucson, AZ, USA

Diabetes is a major health problem worldwide, and there is substantial interest in developing xenogeneic islet transplantation as a potential treatment. The potential to relieve the demand on an inadequate supply of human pancreata is dependent upon the efficiency of techniques for isolating and culturing islets from the source pancreata. Porcine islets are favored for xenotransplantation, but mature pigs (>2 years) present logistic and economic challenges, and young pigs (3–6 months) have not yet proven to be an adequate source. In this study, islets were isolated from 20 juvenile porcine pancreata (~3 months; 25 kg Yorkshire pigs) immediately following procurement or after 24 h of hypothermic machine perfusion (HMP) preservation. The resulting islet preparations were characterized using a battery of tests during culture in silicone rubber membrane flasks. Islet biology assessment included oxygen consumption, insulin secretion, histopathology, and in vivo function. Islet yields were highest from HMP-preserved pancreata (2,242 ± 449 IEQ/g). All preparations comprised a high proportion (>90%) of small islets (<100 μm), and purity was on average 63 ± 6%. Morphologically, islets appeared as clusters on day 0, loosely disaggregated structures at day 1, and transitioned to aggregated structures comprising both exocrine and endocrine cells by day 6. Histopathology confirmed both insulin and glucagon staining in cultures and grafts excised after transplantation in mice. Nuclear staining (Ki-67) confirmed mitotic activity consistent with the observed plasticity of these structures. Metabolic integrity was demonstrated by oxygen consumption rates = 175 ± 16 nmol/min/mg DNA, and physiological function was intact by glucose stimulation after 6–8 days in culture. In vivo function was confirmed with blood glucose control achieved in nearly 50% (8/17) of transplants. Preparation and culture of juvenile porcine islets as a source for islet transplantation require specialized conditions. These immature islets undergo plasticity in culture and form fully functional multicellular structures. Further development of this method for culturing immature porcine islets is expected to generate small pancreatic tissue-derived organoids termed "pancreatites," as a therapeutic product from juvenile pigs for xenotransplantation and diabetes research.

Key words: Islet transplantation; Porcine islets; Islet culture; Diabetes; Xenotransplantation

INTRODUCTION

A recent report on worldwide markets and emerging technologies for tissue engineering and regenerative medicine emphasizes the alarming statistics concerning the rapidly growing worldwide problem of diabetes as an estimated 366 million cases that will be diagnosed in the next 20 years (21). Type 1 diabetes accounts for 5%–10% of all diagnosed cases, but 27% of type 2 diabetes patients also require insulin. Therefore, about a third of all diabetics could potentially benefit from a cell-based therapy that restores insulin production, particularly one that is minimally invasive such as isolated islet infusion into a

Received June 3, 2015; final acceptance August 3, 2016. Online prepub date: April 22, 2016.
[1]These authors provided equal contribution to this work.
Address correspondence to Bradley P. Weegman, Sylvatica Biotech Inc., 2231 Technical Parkway, Charleston, SC 29406, USA. Tel: +1-612-567-2723; E-mail: weegman@umn.edu

heterotopic site (especially if this can be accomplished without the need for systemic immunosuppression). This situation clearly demonstrates a major clinical/commercial impact for new technologies that provide desperately needed improved methods of pancreas preservation to produce better yields of high-quality islets.

Ever since the first experimental attempts to ameliorate type 1 diabetes by transplantation of allograft donor islets, the field has been challenged by the need for improved methods of retrieving and/or obtaining islets from donor pancreata. There is a considerable worldwide effort to further develop the concept for treating type 1 diabetes by transplanting islets, but clinical application of the techniques developed in animal models is fraught with many challenges. The source of the islets remains a primary concern, and isolation from donor pancreata demands resolution of questions concerning the source, supply, and condition of the donor organs. Reliance upon an adequate supply of human organs for this purpose is considered limited, such that alternative sources are actively being sought (6,20).

Various mammals are considered potential candidates for xenogeneic islet transplantation. Of these, pigs are considered the donor species of choice for xenogeneic islet transplantation for a number of compelling reasons. Pigs share many physiological similarities to humans, and porcine insulin has demonstrated clinical efficacy for many years. Pigs are raised as a food source and provide an ethical source of donor islets by being housed in a controlled environment to ensure safety for porcine islet xenotransplantation (18,19). However, experiences in many laboratories over the past decades show that isolation of porcine islets appears to be more difficult (15,16,30,33,49) compared with the isolation of human (24), bovine (14), or rodent islets (44). For example, porcine islets are less compact and tend to fragment during the isolation procedure and during prolonged periods of in vitro culture (3,39). Moreover, the age, and even the strain, of the donor pig has been documented by several groups to markedly influence the islet isolation process, with young, so-called market-size pigs (≤6 months old) proving to be particularly difficult as a source of transplantable islets (3,7,13,22,49). Islets from adult pigs (>2 years old) offered higher yields and retained better morphology during the isolation process and culture, in association with higher functional properties after transplantation (7). Despite the challenge encountered by many groups attempting to isolate islets from young pigs, donor pigs of market weight (<50 kg, ≤6 months old) are preferred to retired breeders (>200 kg, ≥2 years old) due to their abundance and relative cost efficiency with lower animal and husbandry costs. They are much easier to handle than >200 kg adults and more suitable to meet regulatory guidelines for donor tissue for xenotransplantation (3,7,11,13,43,49). If the supply of islet cells could be augmented by culturing and improving the therapeutic capacity of donated islets from more readily available sources (such as young pigs), these new sources would provide sufficient material to become a new treatment for insulin-dependent diabetes, assuming immunological issues (rejection) are eventually addressed.

Preservation of the pancreas prior to islet isolation is also a critical step in the procedure, and we have recently described a technique for 24-h hypothermic machine perfusion (HMP) of pancreata that facilitates islet isolation from young pigs (46–48). The research described here combines this technology with a culture method involving silicone–rubber membrane (SRM), gas-permeable flasks (34) in an attempt to develop a system for culture and shipping of these fragile juvenile porcine islets for research and possible future xenotransplantation. In so doing, the immature porcine islets were observed to undergo plasticity during 7 days in culture resulting in larger aggregate structures comprising both endocrine and exocrine cells. For convenience, we refer to these aggregates as "pancreatites" to reflect their apparent structure as small pancreatic tissue-derived organoids, not previously described to our knowledge.

MATERIALS AND METHODS

Ethics Statement

For the respective work at each institution, the Department of Comparative Medicine at the Medical University of South Carolina (Charleston, SC, USA) Institutional Animal Care and Use Committee (IACUC) approved the research protocols with pigs, and the University of Minnesota (Twin Cities, MN, USA) IACUC approved the research protocols with mice, as described in this article. Every effort was made to minimize suffering, and all studies complied with the USDA Animal Welfare Act Regulations, the Public Health Service Policy on Humane Care and Use of Laboratory Animals, and the recommendations in the Guide for the Care and Use of Laboratory Animals (NIH).

Islet Isolation and Culture

Islets were isolated by conventional mammalian tissue-free collagenase (Roche Custom Biotech, Indianapolis, IN, USA) digestion of juvenile porcine pancreata (2–3 months old, 25–30 kg Yorkshire pigs) either between 2 and 3 h after procurement (fresh) or after 24 h of HMP using the methods we have previously reported (46–48). Briefly, pancreata preserved by HMP were perfused using the Lifeport Kidney Transporter and KPS1 perfusion solution, both from Organ Recovery Systems (Itasca, IL, USA). The liberated islet tissue was purified by Optiprep density-gradient separation (Sigma-Aldrich, St. Loius, MO, USA)

and cultured for up to 7 days at 37°C. The isolation and culture protocols were identical for both fresh and HMP pancreata. ME199 medium (Mediatech, Herndon, VA, USA) was used for the duration of the culture period and modified based on our experience with the addition of an antioxidant, 50 mM Trolox (Roche, Basel, Switzerland), a broad spectrum antiapoptotic agent, quinoline-Val-Asp-difluorophenoxymethylketone (Q-VD-OPH; 10 μM from MP Biomedical, Santa Ana, CA, USA), and 10% porcine serum (Gibco, Life Technologies, Carlsbad, CA, USA) (9). The isolation product was then characterized using a battery of tests at various time points during culture in SRM flasks (Wilson Wolf Manufacturing, New Brighton, MN, USA). Islet morphology was assessed daily by phase contrast microscopy (Olympus IX70 inverted microscope) in the presence of dithizone (diphenyl thiocarbazone (DTZ)) (Sigma-Aldrich) stain, and the dynamic cell morphology was recorded in digital micrographs. Viability, functional status, and islet biology were assessed using established islet quality assays at various time points during the culture period, including measurements of oxygen consumption rate normalized to DNA (OCR/DNA), static insulin secretion [glucose-stimulated insulin secretion (GSIS)], immunohistochemistry (IHC), and confocal microscopy. Extended quality assessment entailed shipment of pancreatite structures overnight by standard courier to the laboratories of our collaborators at the University of Minnesota (UMN; Minneapolis, MN, USA) and Yale University (New Haven, CT, USA). For these shipments, we used the shipping version of the Wilson Wolf silicone membrane flasks (34), using a temperature-controlled shipping system (42). Perifusion insulin release measurements were done at Yale as previously reported (31), and postshipment evaluation and diabetic nude mouse transplantation was done at the UMN. The extended assessments at Yale and UMN did not compare fresh versus HMP pancreatites due to limited data sets. All data are reported as the mean and the standard error of the mean (SEM), with statistical comparisons between two groups using a two-tailed unpaired *t*-test, and comparison between multiple groups using one-way repeated measures analysis of variance (ANOVA) and Tukey's multiple comparison post hoc test. Statistical tests were done using Prism 5.0 (GraphPad Software Inc, La Jolla, CA, USA).

Islet Assessments

Islet Quantification. Following islet isolation and purification, the total number of islets was determined using standard published techniques (3,40). A volume of 50 μl of the purified prep was added to 2 ml of DTZ stain inside a 35×10-mm tissue dish with grid (Sarstedt AG & Co., Nümbrecht, Germany). Thus, islet tissue clusters were stained in contrast to exocrine tissue and then counted and converted to islet equivalents (IEQs) according to standard convention (3,40). Counting the pancreatites using the conventional method was challenging given the colocalized nature of stained and unstained tissues in each sample. To ameliorate observer bias, all counts were performed in duplicate by two independent observers, and reported values are averages of both counts. The purity of the islet preparation was also assessed by comparing DTZ-stained tissue to unstained exocrine tissue.

OCR/DNA. The OCR/DNA assay is a measure of isolated islet functional viability and is used as a predictor of islet function in vivo (35–38). Oxygen consumption rate measurements of the islets were done on the same day as isolation, after 7 days in culture, and after shipment to the University of Minnesota (day 8). Designated islet samples (~5,000 IEQ/sample) were collected, washed, and suspended in Dulbecco's modified Eagle's medium (Mediatech) containing 4.5 g/L of L-glutamine and supplemented with 100 U/ml of penicillin, 100 μg/ml of streptomycin (Sigma-Aldrich), and 10 mM N-2-hydroxyethylpiperazine-N′-2-ethanesulfonic acid (HEPES) (Sigma-Aldrich) without serum. Each islet suspension was divided into three or more 200-μl titanium chambers. The chambers were sealed and maintained at 37°C. The time-dependent oxygen partial pressures (pO_2) within the chambers were recorded over time using a fluorescent-based fiber optic oxygen sensor (Micro Oxygen Uptake System; FO=SYSZ-P250; Instech Laboratories, Plymouth Meeting, PA, USA). The OCR was then calculated as previously described (35). The cell suspensions were then carefully removed from the chambers and processed for DNA quantification as described below.

DNA Quantification. Islet samples analyzed by the OCR assays were subsequently sampled for quantification of DNA content. To measure DNA content, islet samples were diluted in an aqueous cell lysis solution of 1 M of ammonium hydroxide (Thermo Fisher Scientific, Pittsburgh, PA, USA) and 3.4 mM of Triton X-100 (Sigma-Aldrich) and sonicated to ensure adequate cell lysis. DNA content was determined using the Quant-iT PicoGreen dsDNA kit (Molecular Probes, Eugene, OR, USA) as per the manufacturer's instructions. Fluorescence readings were taken on a SpectraMax M5 microplate reader (Molecular Devices, Sunnyvale, CA, USA)

Islet Insulin Content and Stimulated Secretion Assay. Islet insulin release upon exposure to low and high glucose concentrations was determined following an initial recovery of 1 h at 37°C in low, 2 mM of glucose (in RPMI-1640; Gibco, Life Technologies) solution. Consecutive 30-min islet incubation periods (37°C water bath shaker) in 2.5, 16.7, and then 2.5 mM glucose solution, respectively, were performed, each followed by careful supernatant removal and freezing (0.5 ml), and

islet resuspension in the next glucose concentration solution. A total of 12×1.5-ml conical tubes, each containing 25 IEQs in 1 ml of glucose solution, were employed. Gravity-driven sedimentation of islets was used prior to supernatant removal. The insulin content of frozen supernatants and samples was determined using porcine insulin enzyme-linked immunosorbent assay (ELISA) kits (Alpco Diagnostics, Windham, NH, USA) and normalized to islet equivalents.

Histology, Immunohistochemistry, and Confocal Microscopy

Islet samples containing 1,000–2,000 IEQs were collected from cultures 7–8 days following isolation. The samples were immediately fixed in 10% buffered formalin (Thermo Fisher Scientific) for at least 24 h and then transferred to a 70% ethanol solution. Fixed samples were embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E) (Thermo Fisher Scientific). For insulin, glucagon, and Ki-67 immunohistochemical staining, sections were cut at 4 μm, deparaffinized, and rehydrated, followed by incubation with 3% hydrogen peroxide to quench endogenous peroxidase activity. Following appropriate antigen retrieval, sections were incubated with the primary antibody (guinea pig anti-swine insulin; 1:300 dilution; cat. #A0564; Dako, Carpinteria, CA, USA; rabbit anti-human glucagon; cat. #NB120-1846; Novus Biologicals, Littleton, CO, USA; mouse anti-human Ki-67 monoclonal antibody clone MiB-1; 1:50 dilution; cat. #M7240; Dako). Antigen detection was done with anti-rabbit polymer (EnVision by Dako), and color visualization was done using 3,3′-diaminobenzidine (DAB; Dako). Amylase labeling was done with a goat polyclonal antibody (cat. #SC-12821 from Santa Cruz Biotechnology, Santa Cruz, CA, USA), and the detection kit was PoLink-2 Plus HRP (cat. #D43-110 from GBI Labs, Mukilteo, WA, USA). The same protocols were used for examining explanted graft tissues. Double immunofluorescence staining for insulin/cytokeratins was done on formalin-fixed, paraffin-embedded sections cut at 4 μm. For insulin labeling, sections were first deparaffinized, rehydrated, and incubated with guinea pig anti-swine insulin (Dako) for 60 min. Next, sections were incubated in the dark with fluorescein isothiocyanate (FITC)-conjugated, goat anti-guinea pig immunoglobulin (Novus; cat # NB7397) for 60 min. Sections were then incubated with antibodies to wide spectrum cytokeratin (rabbit anti-cytokeratin; cat. #Z0622; Dako) followed by incubation in the dark with streptavidin-Alexa Fluor-594 (ThermoFisher Scientific; cat #21207) for 60 min. Finally, nuclear staining was done using TOPRO-3 (Molecular Probes). Labeled sections were examined with a laser confocal microscope (Bio-Rad Laboratories Inc., Hercules, CA, USA).

Nude Mouse Bioassay

Islets from six different preparations were shipped to the University of Minnesota for implantation into diabetic nude mice ($n=17$). Mice (Crl:NU-Foxn1nu mice from Charles River Laboratories, Wilmington, MA, USA) were rendered diabetic by a 240-mg/kg intraperitoneal (IP) injection of streptozotocin (STZ; Zanosar, Pfizer, New York, NY, USA) (17). After diabetes establishment (hyperglycemia) was confirmed, and based upon our previous experience using this model, a dose of 2,000 IEQ of islets was transplanted into the renal subcapsular space of each mouse, and blood glucose and body weights were observed between 30 and 60 days following transplantation. The standard 30-day follow-up period was extended for a subgroup ($n=8$) of these implants because it was suspected that these underdeveloped pancreatite structures may continue to mature in vivo and may offer delayed graft function. In some animals, the observation period was extended if the glucose levels were observed to decrease following transplant but had not yet reached the critical conclusive levels. Mice were considered to be diabetic when three consecutive blood glucose measurements of >300 mg/dl were observed, and a graft was considered functional upon a return to stable glycemic control when three consecutive blood glucose measurements were <200 mg/dl during the observation period. Mice with functioning grafts were nephrectomized following the observation period to ensure that blood glucose measurements returned to diabetic levels, after which the mice were sacrificed (17,29,35).

RESULTS

Yields and purities for both fresh and HMP islets are reported in Table 1, along with culture recoveries and insulin data. The average purified islet yield per gram of digested tissue was notably higher for islets isolated from HMP organs when compared to islets isolated from fresh controls with a 57% improvement observed for these studies, but due to the limited number of isolations studied and large variability this difference was not statistically significant ($p=0.285$). Following culture for 1 week, pancreatites from both conditions were demonstrated to be fully functional in terms of insulin secretion in response to glucose challenge (Table 1). When compared using a two-tailed unpaired *t*-test, average yield, average purity, culture duration, culture recovery, insulin stimulation index, and insulin content were not different between groups ($p>0.05$). These observations were consistent with our recently published findings describing islet isolation from juvenile porcine pancreata after 24-h HMP preservation (46–48). Islets isolated from HMP and fresh pancreata exhibited similar behavior in culture and yielded comparable functional outcomes (OCR/DNA, IHC, nude mouse transplants);

Table 1. Isolation and Culture Results

	Fresh Control ($n=9$)	HMP (KPS1) ($n=11$)
Islet yield (IEQ/g, day 0)	1,425±610	2,242±449*
Purity (day 0)	69.2±7.6	63.8±6.1
Total culture time (days)	6.7±0.1	5.7±0.6
End culture recovery (% of day 0)	71.7±12.5	75.5±14.2
End culture stimulation index	6.54±1.19	5.26±0.77
End culture insulin content (ng/IEQ)	2.55±0.33	2.10±0.28*

HMP, hypothermic machine perfusion; IEQ, islet equivalent.
*Difference is significant ($p<0.05$).

therefore, the results reported on these aspects represent the observations of islets from both groups.

As illustrated in Figure 1A, islet preparations from both fresh and HMP pancreata comprised a high proportion (>90%) of small islets (<100 μm) immediately following isolation. The DTZ-stained islets appeared as "grape-like" clusters on day 0. Figure 1 also shows the plasticity observed during subsequent postisolation culture of the immature islets. The term "plasticity" is used according to the definition of the ability of a biological system to be altered into different shapes and/or behavior according to varying environmental conditions. Each panel (A–E) shows the typical appearance of the β-cells (stained red with DTZ) and exocrine (unstained, gray-brown) at various time points during 1 week of culture postisolation and after shipment (Fig. 1F). The gross morphology of the islets was observed to change during the first 24–48 h in culture (compare the looser more fragmented nature of the day 1 cultured islets in Fig. 1B, with the "grape-like" clusters of islets on day 0 immediately post-isolation in Fig. 1A).

During the initial 48 h of culture, the islets appeared to disaggregate to form strings of cells, and then they associated with residual non-DTZ-stained tissue (Fig. 1B and C). The most significant observation is the transition of the tissue from this loosely disaggregated appearance at 24 h (day 1) to more condensed, aggregated structures comprising both exocrine and endocrine cells by days 4–7 (Fig. 1D and E).

The pancreatite structures shipped to Yale University maintained their structural integrity and demonstrated dynamic insulin secretory responses to a standard panel of secretagogues as described in a previous publication (31). Moreover, pancreatite samples shipped to the University of Minnesota ($n=5$) retained their morphology and their metabolic capability as demonstrated by the oxygen consumption measurements, which were not significantly different to the preshipment values. The OCR/DNA was measured to be (mmol/min/mg DNA) = 134±19 at day 0; 159±25 on day 7 (preshipment), and 175±16 on day 8 (postshipment), and analysis by one-way ANOVA ($p=0.3391$) with Tukey's multiple comparisons posttest concluded that no significant differences were observed between timepoints.

Upon arrival in Minnesota, IHC of the tissue showed cells with insulin, glucagon, or amylase immunoreactivity among cells in the cultured aggregates (Fig. 2A–C, respectively). Confocal microscopy of double immunofluorescently stained pancreatites showed strong, nonoverlapping staining for insulin and wide spectrum cytokeratins indicating the presence of both β-cells and ductal epithelial cells within the cultured tissue (Fig. 2D). Furthermore, nuclear staining for Ki-67-MiB confirmed mitotic activity within the cell aggregates during culture (Fig. 3A) and within the graft posttransplant (Fig. 3B). These observations suggest that the pancreatites are composed of both endocrine and exocrine cells prior to transplantation and that their formation was not simply due to aggregation but also due to growth by cell division and possibly maturation in culture and in vivo.

The pancreatites maintained metabolic integrity, demonstrated by oxygen consumption rates (175±16 nmol/min/mg DNA), and physiological function, confirmed by both static and previously published dynamic glucose stimulation (31). Furthermore, in vivo function was examined by transplantation under the kidney capsule in nude mice. Figure 4A shows the average blood glucose measurements for the eight animals that achieved glycemic control (blood glucose levels ≤200 mg/dl for 3 consecutive days), as well as the average blood glucose measurements of the nine animals that did not (shown separately for clarity). Glycemic control was achieved in 8/17 mice (47%) on average 25 days posttransplantation (for these mice) with seven animals achieving control 10 or more days following transplant (Fig. 4B). Postexplant glucose measurements were excluded from this figure due to variable explant timing, but all animals returned to hyperglycemic states (≥3 consecutive BG measurements ≥300 mg/dl) following graft explant. The average mass of all animals was observed to decrease by 0.3 g from the time of transplant until nephrectomy or sacrifice, which was not statistically significant ($p=0.3722$ for paired Student t-test comparing starting mass and final mass), and no animal was observed to lose more than 10% of starting body mass. Histopathology of explanted grafts confirmed persistent islet

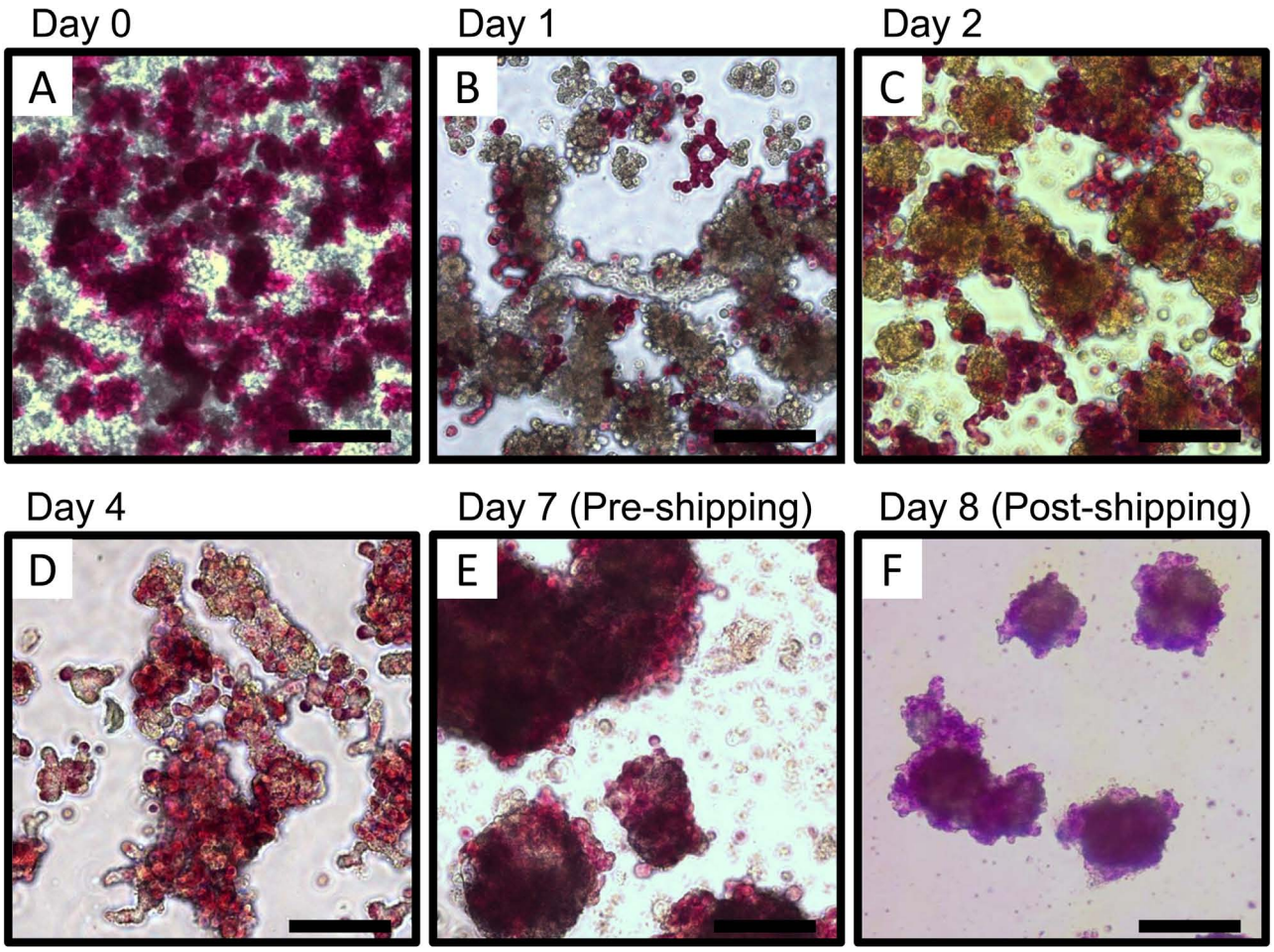

Figure 1. Micrographs demonstrating the plasticity of pancreatite structures during 7 days of culture and after shipment. Representative phase contrast micrographs of dithizone (DTZ)-stained (red) islet cultures at critical times in culture (A–E) and after shipment (F). Exocrine tissue remains unstained (light gray/yellow). Day 0 purified islets (A) show the familiar irregular shape of "grape-like" islet clusters. During the first 24 h in culture (B), the islets appear to disaggregate into strings of loosely clustered islets associated with the residual exocrine tissue. Between 2 and 4 days of culture (C and D), this process continues, and the clusters become larger and more condensed aggregate structures comprising both exocrine and endocrine cells. By 7 days, the larger reaggregated structures, which we termed "pancreatites," were clearly more dense and compact. The final panel (F) shows that the pancreatites remained morphologically intact following ambient shipment to Minnesota in Wilson Wolf flasks. Scale bars: 100 μm.

tissue within the graft, and sections showed strong insulin and glucagon staining (Fig. 5A and B, respectively); however, minimal staining was observed for amylase and cytokeratin (data not shown).

DISCUSSION

Clinical trials are under way at several centers around the world to assess the efficacy of implantation of functional pancreatic islet cells as a potentially curative treatment for type 1 diabetes (1,45). Nevertheless, the limited availability of donor human pancreata means that clinical islet transplantation may not reach its full potential without reliance upon alternative sources of islets. Porcine islets are favored for xenotransplantation for a variety of reasons (33), but mature pigs (>2 years old) present logistic and economic challenges, and young pigs (3–6 months) have not yet proved to be an adequate source. As discussed by several investigators, experiences in many laboratories over several decades show that isolation of porcine islets appears more difficult than other mammals including humans, and the economically favored juvenile pig has proved even more problematic due to poor yields and extreme fragility of the immature islets (4,7,27,41). Despite some reports of successful isolation of islets from young market pigs (4–6 months old) the studies did not involve culturing the islets (27), which remains a significant challenge. For example, Rijkelijkhuizen et al. report 80% attrition of adult porcine islets after 1 day of culture (41). More recently, Lamb et al. has reported successful postisolation culturing of partially digested pancreatic

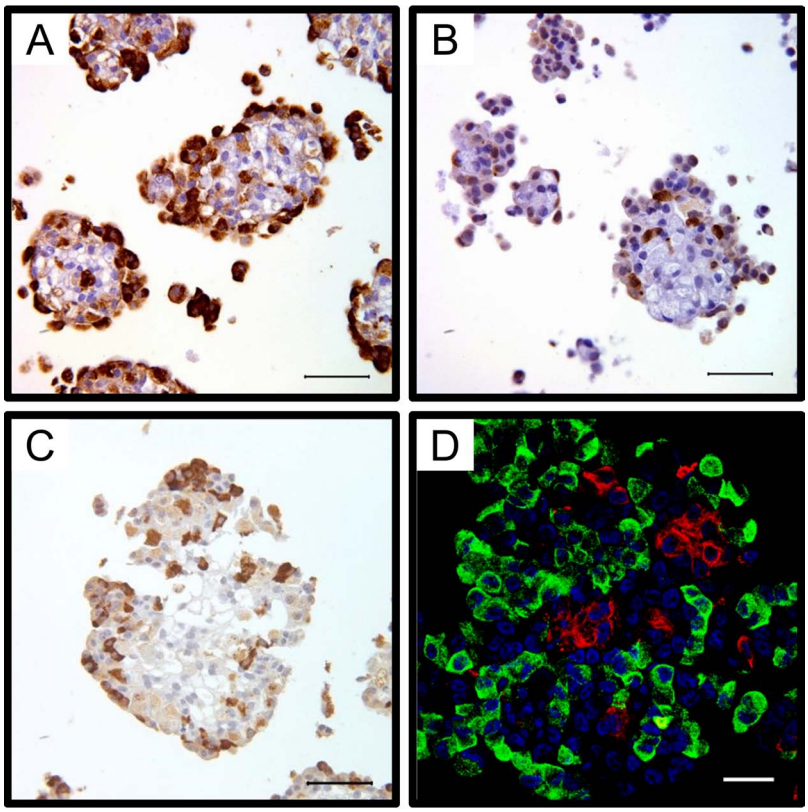

Figure 2. Immunohistochemistry staining of 7-day cultured pancreatite tissue shows staining for endocrine and exocrine markers. Upon arrival at Minnesota, pancreatites showed strong staining of insulin (A), glucagon (B), and amylase (C). Confocal microscopy of double immunofluorescently stained pancreatites (D) showed staining for insulin and wide spectrum cytokeratins indicating the presence of both β-cells and ductal epithelial cells within the tissue. Scale bars: 50 μm (A–C) and 25 μm (D).

fragments from infant pigs (<4 weeks old) (28). This model produces small numbers of islets compared with conventional models using older pigs, but the study demonstrates a robust capacity for continued in vitro development in culture thus supporting the basic premise of our study.

Preservation of the pancreas prior to islet isolation is also a critical step in the procedure, and we have recently described a technique for a 24-h HMP of the pancreas that facilitates islet isolation from young pigs (46–48). The research described here combines this technology with an

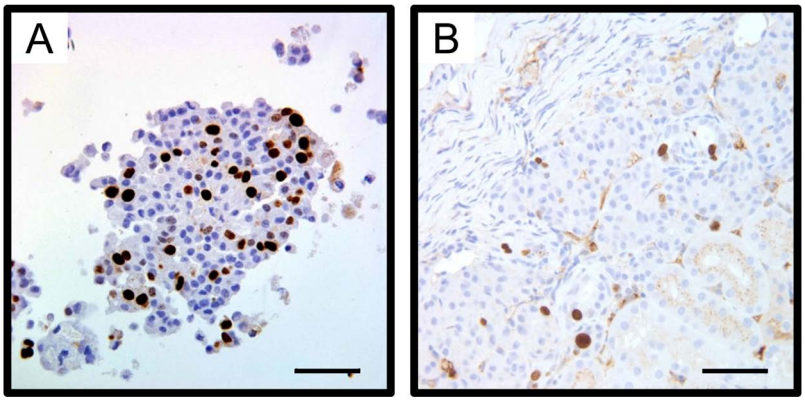

Figure 3. Immunohistochemistry staining of 7-day cultured pancreatite tissue shows evidence of proliferation markers. Ki-67-MiB nuclear staining shows mitotic activity in these structures after 7 days in culture (A) and in explanted grafts (B) providing evidence of cell proliferation in culture and in vivo. Scale bars: 50 μm.

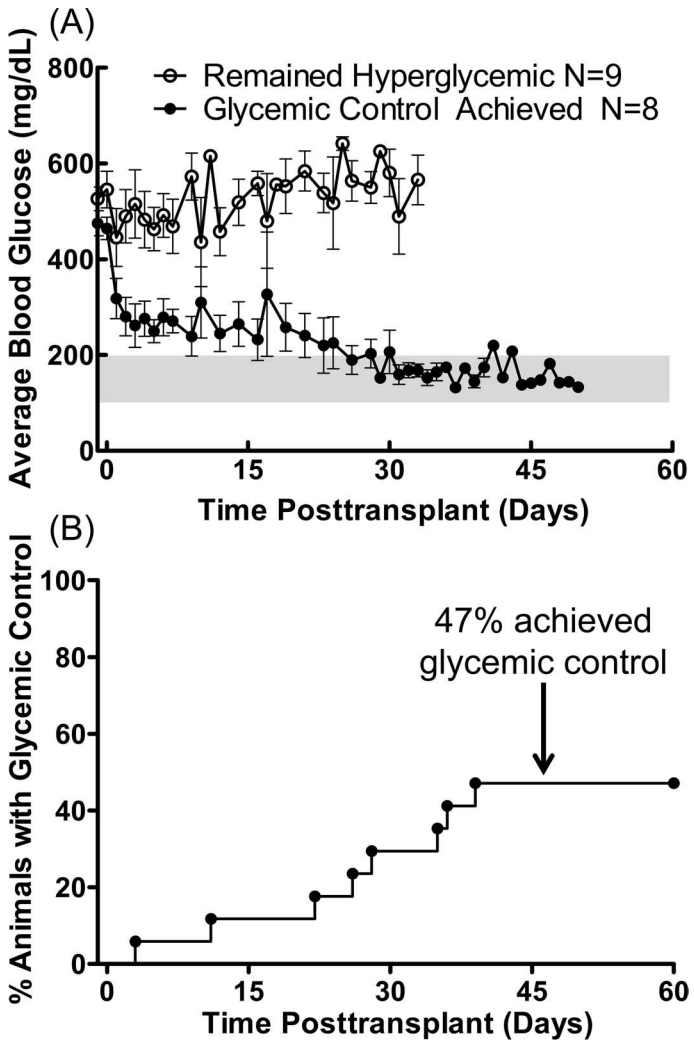

Figure 4. Mean blood glucose measurements (A) and diabetes reversal rate plot (B) from diabetic nude mice. Data from mice that received a renal subcapsular transplant of pancreatites shipped to Minnesota for assessment. Blood glucose data shown are grouped by outcome; 8 of 17 animals achieved glycemic control after an average of 25 days. Blood glucose measurements following explant are not shown for clarity due to differing points of euthanasia, although all animals reverted to hyperglycemia following explantation of the graft. Achievement of glycemic control was defined as three consecutive blood glucose measurements ≤300 mg/dl following transplant. The delayed control of blood glucose levels is suspected to reflect possible generation of a critical mass of insulin-secreting cells by neogenesis as evidenced by the positive Ki-67-MiB immunostaining in the explanted grafts.

effective culture method involving silicone membrane, gas-permeable flasks, in an attempt to develop a system for culture and shipping of juvenile porcine islets for research and possible future xenotransplantation. This contrasts sharply with conventional culture of porcine islets from pigs of any age, especially juvenile, which have proved to be too fragile and labile in culture to be of practical utility (7,13,39,41).

Special Conditions for Culture of Juvenile Porcine Islets

An important achievement of the studies reported here is the successful isolation and culture of juvenile porcine islets, which are notoriously difficult to establish and maintain in culture (7,13,41). Until the introduction of the use of gas-permeable culture flasks (34) (Wilson Wolf Manufacturing) our experience was the same as many in the field in that islets from young market-size pigs are very difficult to maintain in culture. Conventional culture in regular flasks led to the demise of the islets within 12–24 h of culture. Even the purest preparations resulted in disappearance of islets within the first 24 h as reported previously by other investigators (13,39,41).

Although considerable attention has been given to the formulation of media for islet culture as reviewed by Clayton and London (10) and Murdoch et al. (32), very little attention was given to the role of oxygen transport

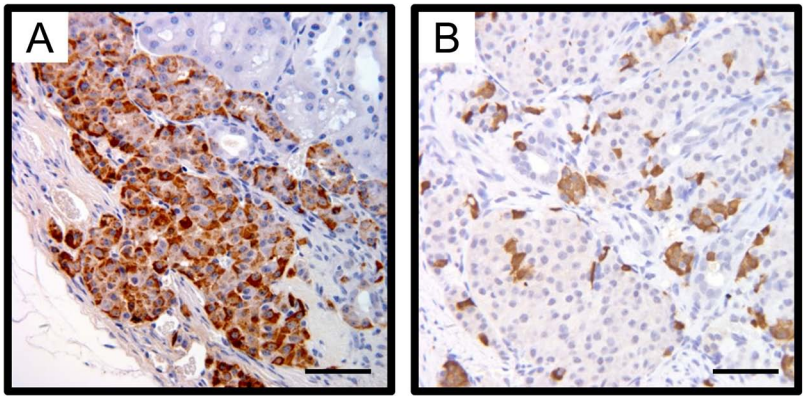

Figure 5. Insulin (A) and glucagon (B) staining in explanted pancreatite grafts. Sections from explanted grafts show strong staining for both insulin (A) and glucagon (B), which confirms the preservation of endocrine cell phenotypes within these pancreatites in vivo. Future studies with colocalized staining to investigate the mitotic activity observed in these grafts will further illuminate the behavior and plasticity of these pancreatites in vivo. Scale bars: 50 μm.

until the introduction of gas-permeable substrates in the form of silicone–rubber membrane flasks (34). Islet density and aggregation have been shown to negatively impact islet survival in culture such that a marked improvement was demonstrated by increasing external pO_2 and culturing on an SRM (34). This new technology was therefore introduced in this study to complement changes to the culture medium and islet isolation after HMP (46,47,51).

The ME199 medium was specially modified for porcine islet culture through the addition of various supplements and porcine serum (9). Using this scheme, it was observed that the juvenile islets did not behave the same as conventional cultures of adult pig islets or even human islets. As described above, the immature porcine islets underwent plasticity during 7 days in culture resulting in larger, reaggregated structures. This contrasts markedly with the typical behavior of islets from adult species, which maintain their islet individuality and postisolation structure throughout conventional tissue culture. In cultures of adult islets, the contaminating exocrine tissue tends to disintegrate and disappear by autolysis, leaving an even purer preparation of individual islets (4). In contrast, the tissue from juvenile pigs appeared to reaggregate into larger, new structures comprising endocrine and exocrine cells in coexistence, hence the term "pancreatite." The aggregates demonstrated strong immunohistochemical staining for insulin, glucagon, and amylase (Fig. 2A–C, respectively) and furthermore, confocal double staining (Fig. 2D) clearly demonstrated the heterogeneous aggregated tissue character and confirmed the presence of both β-cells and ductal epithelial cells in the structures after 7 days in culture. For convenience, we refer to these aggregates as "pancreatites" to reflect their apparent structure as small pancreatic tissue-derived organoids, not previously described to our knowledge. These preliminary observations have been achieved by combining previously reported developments for modified preservation solutions and juvenile porcine islet isolation after HMP, with the use of SRM flasks. This phenomenological discovery of pancreatite formation using the combined technologies has been generated without specific knowledge of the relative contributions of each of the component systems. Hence, this new phenomenon provides the basis for further research to employ this combination of technologies for the synergistic development of "pancreatites" as a potentially new solution for generating a therapeutic product from juvenile pigs for xenotransplantation and diabetes research.

In Vitro Functional Assessment

The pancreatite cultures were monitored daily for 1 week to observe these changes in morphology and were assessed at critical points for quality by OCR/DNA, insulin secretion, histopathology, and IHC. The islet tissue function was also tested in vivo by nude mouse bioassay. The OCR/DNA results showed high tissue viability following culture (159 ± 25 nmol/min/mg DNA), and this viability was maintained following shipment to the University of Minnesota (175 ± 16 nmol/min/mg DNA). These observations are consistent with reports in the literature using similar shipping methods (25). The OCR/DNA assay in conjunction with the total transplanted OCR dose has been shown to be an accurate predictor of diabetes reversal in nude mice transplanted with human islets. The viability level of the pancreatite structures was comparable with high-viability human islets reported in the literature (35) and sufficiently higher than the reported minimum OCR/DNA boundary (150 nm/min/mg DNA) required to reverse diabetes in mice with rat islets (36). This comparison can only be considered as a reference

because, as discussed above, these preparations exhibit notable morphological and functional differences between adult pig islets and are also different from observations with human or rat islets. These differences, along with the limited reports in literature, warranted a brief examination of these structures in vivo and suggest a need for further studies examining the viability and function of these pancreatites.

In Vivo Functional Assessment

Further examination of the pancreatite functionality was assessed with a limited number of nude mouse transplants ($n=17$). The average blood glucose of all the animals is shown in Figure 4A (separated into animals that remained hyperglycemic and those that achieved glycemic control) and demonstrates a gradual decrease in average fasting blood glucose levels with reversal of glycemic control achieved on average 25 days posttransplantation. Despite strong staining for insulin and glucagon in culture (Fig. 2A and B, respectively) delayed function was observed in seven of the eight animals that showed glycemic control. These animals achieved glycemic control 10 or more days following transplant, as shown in Figure 4B. This is likely due to an inadequate functional β-cell mass immediately following transplant with possible islet neogenesis contributing to the primary graft function after 10 or more days in vivo. These results are consistent with the findings of Korbutt et al. describing the delayed graft function of neonatal porcine islet cell (NIC) aggregate transplants in nude mice (26). Neonatal pigs represent a much earlier stage of development (1–3 days old) compared to juvenile pigs used in our studies (1–3 months old). The study found a dose-dependent return to normoglycemia in nude mice transplanted with NIC aggregates and reported 100% achieved normoglycemia after 8 weeks for grafts with 2,000 aggregates (26). However, only 30% of these animals achieved normoglycemia after 4 weeks, suggesting that pancreatite grafts may offer expedited graft function compared to NIC grafts. It should also be noted that these in vivo results are highly dependent on the initial amount of grafted tissue (e.g., islet dose), and more rigorous methods for quantification of pancreatite tissue (e.g., DNA) or total β-cell mass would allow for a better understanding of the dose-dependent nature of glycemic control. Future studies to parallel these findings, which extend the transplant follow-up period of mice with pancreatite grafts, would provide a valuable comparison of these two similar cell sources. Furthermore, pancreatites exhibited notable staining for Ki-67-MiB in culture and in explanted grafts (Fig. 3A and B, respectively), indicating an elevated level of mitotic activity. Separate sections from explanted grafts were also stained for insulin and glucagon (Fig. 5) to confirm the presence of endocrine islet tissue in the explanted graft. Strong staining for both hormones indicated that the endocrine phenotypes of these pancreatite cells were maintained in vivo. This finding supports the hypothesis of in vivo islet neogenesis, but quantitative morphometric analysis and colocalization with insulin staining would be required to confirm this mechanism.

Observed Pancreatite Potential for Regeneration

Over the past decade, significant advances have been made in developmental biology of the pancreas and other endoderm-derived organs. Studies on islet morphogenesis show that the majority of islet cells are formed in late gestation by the process of neogenesis from precursor cells. The changes observed in the early periods of our juvenile porcine islet culture system closely resemble the budding of islet cells from the ducts and reorganization to form clusters during the process of islet morphogenesis seen both in vitro and in vivo (8,12,23). Bonner-Weir et al. among others have reviewed the plasticity of adult pancreatic cells and the ultimate feasibility of in vivo neogenesis (6,20,23). In vitro differentiation of stem/progenitor cells into β-cells is being pursued as an important approach to generate a reliable and replenishable source of β-cells. Pancreatic ductal cell lines and primary ductal cells have been successfully differentiated into insulin-expressing cells by in vitro approaches, including treatment with growth factors (e.g., EGF, gastrin, exendin), expression of pancreatic transcription factors, and aggregation (23). The aggregation and plasticity of the pancreatic cell cultures derived in our preliminary studies using juvenile porcine pancreas is consistent with this approach and is remarkably complementary to the parallel studies of Bonner-Weir et al. For example, her group has shown that discarded digested pancreatic tissue from human islet isolations can be successfully processed to expand and differentiate the ductal cells over a period of 3–4 weeks in vitro to cultivate human islet buds consisting of cytokeratin-19-positive duct cells and hormone-positive islet cells (5). On the basis of these findings it may be important to extend the culture period of the juvenile porcine islets to 28 days to parallel Bonner-Weir's studies using human tissue. Since it is now believed that in vitro neogenesis may recapitulate development of the embryonic pancreas, the system we propose to develop using immature porcine pancreatic tissue will not only be an important, readily available, and economically viable source of therapeutic material but also will generate a new model to identify potential markers of neogenesis and to determine the molecular mechanisms underlying the process.

Pancreatite Size

The notable growth or size change of these structures is demonstrated by our observations in Figure 1. In our 2010 study of HMP of juvenile pig pancreata, we

included an analysis of the size distribution of the islets in the purified preps showing that >90% of the young pig islets fall within the range 50–100 μm and very rarely were any islets found with diameters greater than 200 μm (47). In contrast, the micrographs here (Fig. 1) show that most of the pancreatite structures are much bigger (>100 μm) and often substantially larger, as illustrated by the large (387 μm) structure in Figure 1E. It is likely that the changing size, morphology, and cellular composition of the pancreatite structures gave rise to some error in the estimate of IEQs for the purpose of tissue quantification. The reported data are based on the usual convention of counting individual islets that are primarily composed of DTZ-stained endocrine cells only, while these pancreatites were composed of both exocrine and endocrine cells. This complication undoubtedly led to an overestimate of the islet mass transplanted in the nude mouse assay and may in part be responsible for the delayed graft function observed in some recipients as noted above. This is also consistent with the observations in several other studies involving the transplantation of adult pig islets in which the numbers of IEQ transplanted show considerable variation and range 10-fold from 1,000 IEQ/mouse to 10,000 IEQ/mouse. Moreover, the time-to-normoglycemia showed considerable variance, often taking >50 days (41). Hence achieving glycemic control in 47% (8 of 17 transplanted mice) in this study is comparable with reports in the literature for adult pig islets as discussed by Rijkelijkhuizen et al. (41). Further extended studies will necessitate a titration of the pancreatite dose in the transplanted mice to resolve this issue of the critical β-cell mass within the pancreatites necessary for achieving glycemic control.

Advantages of the Juvenile Pancreas Model Over Existing Models of Neogenesis and Transdifferentiation

A variety of strategies are currently being investigated to deliver regulatory factors to pancreatic cells, in vitro, aimed at increasing β-cell mass for transplantation. Transdifferentiation of α-cells into β-cells is another approach that has merit for in vivo regeneration and neogenesis (23). The processes of neogenesis of β-cells in vitro are currently inefficient for adult human cells and do not generate sufficient β-cell mass to normalize hyperglycemia (23). In contrast, we demonstrate here preliminary data showing that the pancreatite cell clusters derived from juvenile porcine pancreata are able to reverse diabetes in a diabetic nude mouse model (Fig. 4). The association of multiple cell types in the formation of the pancreatite cell clusters is another important characteristic with potential therapeutic benefits since there is mounting evidence for improved transplantation outcome using impure islet preparations containing non-endocrine cells, notably ductal epithelial cells (50). In the broad context of processing tissue to generate a readily available cost-effective product for therapeutic treatment of diabetes, the novel "pancreatite" structures derived from juvenile pigs are potentially an innovative solution to the inevitable deficiency of supply of human tissue.

The delayed return to glycemic control, which took, on average, 25 days, may be an indication of the generation of a critical mass of insulin-secreting cells by cell division as evidenced by the positive Ki-67-MiB staining in the explanted grafts (Fig. 3B). There is insufficient evidence in this preliminary study however, which did not include a rigorous correlation of insulin immunostaining colocalized with the evident Ki-67 staining, to fully support this hypothesis. Nevertheless, the phenomenological appearance of strong insulin, glucagon, and Ki-67 staining in the explants supports the notion of ongoing islet development and maturation in the graft and provides encouragement for further, more in-depth studies to test this hypothesis.

CONCLUSIONS

The pancreatites (pancreatic cell aggregates) derived from the preservation, isolation, and culture of juvenile porcine pancreas tissue using the described techniques could provide an unlimited source of β-cells to integrate into a reproducible, economic, and safe system for therapeutic and research applications. Moreover, the model we propose here meets the stringent economic demands that have emerged in the healthcare marketplace and could be a cost-efficient alternative to standard management of diabetes (2). We conclude from these preliminary observations that immature islets isolated from juvenile porcine pancreata form cellular aggregates comprising both exocrine and endocrine cells in modified tissue culture. This phenomenon has not previously been reported to our knowledge, presumably because of the notorious difficulty of maintaining juvenile pig islets in conventional tissue culture.

ACKNOWLEDGMENTS: The research described here was supported in part by grants from US National Institute of Health (R44DK065508-02 – M.J.T., 1 R44 DK076326-0 and 5 R44DK076326-03 – M.J.T., and R44DK069865 – J.W.) and The Richard M. Schulze Family Foundation. The authors gratefully acknowledge the assistance of Elizabeth Greene with the preparation of the figures and Dr. Melanie Graham with the analysis of the nude mouse bioassay results. Dr. Michael J. Taylor was a paid employee of Cell and Tissue Systems (CTS) during the course of this study. John Wilson is a stakeholder and paid employee of Wilson Wolf Manufacturing. The remaining authors declare no conflicts of interests.

REFERENCES

1. Alejandro, R.; Barton, F. B.; Hering, B. J.; Wease, S. 2008 Update from the collaborative islet transplant registry. Transplantation 86:1783–1788; 2008.

2. Beckwith, J.; Nyman, J. A.; Flanagan, B.; Schrover, R.; Schuurman, H. J. A health-economic analysis of porcine islet xenotransplantation. Xenotransplantation 17:233–242; 2010.
3. Bertera, S.; Marigliano, M.; Bottino, R.; Trucco, M. Pancreatic islet isolation from swine. In: Soto-Gutierrez, A.; Navarro-Alvarez, N.; Fox, I. J., eds. Methods in bioengineering: Cell transplantation. Boston, MA: Artech House; 2011:77–99.
4. Bonner-Weir, S.; Davalli, A. M.; Scaglia, L.; Hollister, J.; Weir, G. C. Myths about the structure and function of porcine islets. Xenotransplantation 2:207–212; 1995.
5. Bonner-Weir, S.; Taneja, M.; Weir, G. C.; Tatarkiewicz, K.; Song, K. H.; Sharma, A.; O'Neil, J. J. In vitro cultivation of human islets from expanded ductal tissue. Proc. Natl. Acad. Sci. USA 97:7999–8004; 2000.
6. Bonner-Weir, S.; Weir, G. C. New sources of pancreatic beta-cells. Nat. Biotechnol. 23:857–861; 2005.
7. Bottino, R.; Balamurugan, A. N.; Smetanka, C.; Bertera, S.; He, J.; Rood, P. P. M.; Cooper, D. K. C.; Trucco, M. Isolation outcome and functional characteristics of young and adult pig pancreatic islets for transplantation studies. Xenotransplantation 14:74–82; 2007.
8. Bouwens, L. Islet morphogenesis and stem cell markers. Cell Biochem. Biophys. 40:81–88; 2004.
9. Campbell, L. H.; Taylor, M. J.; Brockbank, K. G. Development of pancreas storage solutions: Initial screening of cytoprotective supplements for β-cell survival and metabolic status after hypothermic storage. Biopreserv. Biobank. 11:12–18; 2013.
10. Clayton, H. A.; London, N. J. Survival and function of islets during culture. Cell Transplant. 5:1–12; discussion 13–17, 19; 1996.
11. Cozzi, E.; Tallacchini, M.; Flanagan, E. B.; Pierson, R. N.; Sykes, M.; Vanderpool, H. Y. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 1: Key ethical requirements and progress toward the definition of an international reg. Xenotransplantation 16:196–202; 2009.
12. Dodge, R.; Loomans, C.; Sharma, A.; Bonner-Weir, S. Developmental pathways during in vitro progression of human islet neogenesis. Differentiation 77:135–147; 2009.
13. Dufrane, D.; Goebbels, R.; Fdilat, I.; Guiot, Y.; Gianello, P. Impact of porcine islet size on cellular structure and engraftment after transplantation: Adult versus young pigs. Pancreas 30:138–147; 2005.
14. Figliuzzi, M.; Zappella, S.; Morigi, M.; Rossi, P.; Marchetti, P.; Remuzzi, A. Influence of donor age on bovine pancreatic islet isolation. Transplantation 70:1032–1037; 2000.
15. Finke, E.; Marchetti, P.; Falqui, L.; Swanson, C.; McLear, M.; Olack, B.; Scharp, D.; Lacy, P. Large scale isolation, function, and transplantation of islets of Langerhans from the adult pig pancreas. Transplant. Proc. 23:772–773; 1991.
16. Giannarelli, R.; Marchetti, P.; Villani, G.; di Carlo, A.; Cosimi, S.; Andreozzi, M.; Cruschelli, L.; Masiello, P.; Coppelli, A.; Navalesi, R. Preparation of pure, viable porcine and bovine islets by a simple method. Transplant. Proc. 26:630–631; 1994.
17. Graham, M. L.; Janecek, J. L.; Kittredge, J. A.; Hering, B. J.; Schuurman, H. The streptozotocin-induced diabetic nude mouse model: Differences between animals from different sources. Comp. Med. 61:356–360; 2011.
18. Hering, B. J.; Cooper, D. K. C.; Cozzi, E.; Schuurman, H. J.; Korbutt, G. S.; Denner, J.; O'Connell, P. J.; Vanderpool, H. Y.; Pierson, R. N., 3rd. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Executive summary. Xenotransplantation 16:196–202; 2009.
19. Hering, B. J.; Wijkstrom, M.; Graham, M. L.; Hårdstedt, M.; Aasheim, T. C.; Jie, T.; Ansite, J. D.; Nakano, M.; Cheng, J.; Li, W.; Moran, K.; Christians, U.; Finnegan, C.; Mills, C. D.; Sutherland, D. E.; Bansal-Pakala, P.; Murtaugh, M. P.; Kirchhof, N.; Schuurman, H. J. Prolonged diabetes reversal after intraportal xenotransplantation of wild-type porcine islets in immunosuppressed nonhuman primates. Nat. Med. 12:301–303; 2006.
20. Inada, A.; Bonner-Weir, S.; Toschi, E. How can we get more beta cells? Curr. Diab. Rep. 6:96–101; 2006.
21. InteLab Corporation. Report I601, Worldwide Markets and Emerging Technologies for Tissue Engineering and Regenerative Medicine; 2013. http://www.intelabcorp.com/markettechnologyreports/reportil600.html
22. Jay, T. R.; Heald, K. A.; Carless, N. J.; Topham, D. E.; Downing, R. The distribution of porcine pancreatic beta-cells at ages 5, 12 and 24 weeks. Xenotransplantation 6:131–140; 1999.
23. Juhl, K.; Bonner-Weir, S.; Sharma, A. Regenerating pancreatic beta-cells: Plasticity of adult pancreatic cells and the feasibility of in-vivo neogenesis. Curr. Opin. Organ Transplant. 15:79–85; 2010.
24. Kenmochi, T.; Miyamoto, M.; Une, S.; Nakagawa, Y.; Moldovan, S.; Navarro, R. A.; Benhamou, P. Y.; Brunicardi, F. C.; Mullen, Y. Improved quality and yield of islets isolated from human pancreata using a two-step digestion method. Pancreas 20:184–190; 2000.
25. Kitzmann, J. P.; Pepper, A. R.; Gala-Lopez, B.; Pawlick, R.; Kin, T.; O'Gorman, D.; Mueller, K. R.; Gruessner, A. C.; Avgoustiniatos, E. S.; Karatzas, T.; Szot, G. L.; Posselt, A. M.; Stock, P. G.; Wilson, J. R.; Shapiro, A. M.; Papas, K. K. Human islet viability and function is maintained during high-density shipment in silicone rubber membrane vessels. Transplant. Proc. 46:1989–1991; 2014.
26. Korbutt, G. S.; Elliott, J. F.; Ao, Z.; Smith, D. K.; Warnock, G. L.; Rajotte, R. V. Large scale isolation, growth, and function of porcine neonatal islet cells. J. Clin. Invest. 97:2119–2129; 1996.
27. Krickhahn, M.; Bühler, C.; Meyer, T.; Thiede, A.; Ulrichs, K. The morphology of islets within the porcine donor pancreas determines the isolation result: Successful isolation of pancreatic islets can now be achieved from young market pigs. Cell Transplant. 11:827–838; 2002.
28. Lamb, M.; Laugenour, K.; Liang, O.; Alexander, M.; Foster, C. E.; Lakey, J. R. T. In vitro maturation of viable islets from partially digested young pig pancreas. Cell Transplant. 23:263–272; 2014.
29. Loganathan, G.; Graham, M. L.; Radosevich, D. M.; Soltani, S. M.; Tiwari, M.; Anazawa, T.; Papas, K. K.; Sutherland, D. E. R.; Hering, B. J.; Balamurugan, A. N. Factors affecting transplant outcomes in diabetic nude mice receiving human, porcine, and nonhuman primate islets: Analysis of 335 transplantations. Transplantation 95:1439–1447; 2013.
30. Marchetti, P.; Finke, E. H.; Gerasimidi-Vazeou, A.; Falqui, L.; Scharp, D. W.; Lacy, P. E. Automated large-scale isolation, in vitro function and xenotransplantation of porcine islets of Langerhans. Transplantation 52:209–213; 1991.

31. Mueller, K. R.; Balamurugan, A. N.; Cline, G. W.; Pongratz, R. L.; Hooper, R. L.; Weegman, B. P.; Kitzmann, J. P.; Taylor, M. J.; Graham, M. L.; Schuurman, H.-J.; Papas, K. K. Differences in glucose-stimulated insulin secretion in vitro of islets from human, nonhuman primate, and porcine origin. Xenotransplantation 20:75–81; 2013.
32. Murdoch, T. B.; McGhee-Wilson, D.; Shapiro, A. M. J.; Lakey, J. R. T. Methods of human islet culture for transplantation. Cell Transplant. 13:605–617; 2004.
33. O'Neil, J. J.; Stegemann, J. P.; Nicholson, D. T.; Gagnon, K. A.; Solomon, B. A.; Mullon, C. J. The isolation and function of porcine islets from market weight pigs. Cell Transplant. 10:235–246; 2001.
34. Papas, K. K.; Avgoustiniatos, E. S.; Tempelman, L. A.; Weir, G. C.; Colton, C. K.; Pisania, A.; Rappel, M. J.; Friberg, A. S.; Bauer, A. C.; Hering, B. J. High-density culture of human islets on top of silicone rubber membranes. Transplant. Proc. 37:3412–3414; 2005.
35. Papas, K. K.; Colton, C. K.; Nelson, R. A.; Rozak, P. R.; Avgoustiniatos, E. S.; Scott, W. E.; Wildey, G. M.; Pisania, A.; Weir, G. C.; Hering, B. J. Human islet oxygen consumption rate and DNA measurements predict diabetes reversal in nude mice. Am. J. Transplant. 7:707–713; 2007.
36. Papas, K. K.; Colton, C. K.; Qipo, A.; Wu, H.; Nelson, R. A.; Hering, B. J.; Weir, G. C.; Koulmanda, M. Prediction of marginal mass required for successful islet transplantation. J. Invest. Surg. 23:28–34; 2010.
37. Papas, K. K.; Pisania, A.; Wu, H.; Weir, G. C.; Colton, C. K. A stirred microchamber for oxygen consumption rate measurements with pancreatic islets. Biotechnol. Bioeng. 98:1071–1082; 2007.
38. Papas, K. K.; Suszynski, T. M.; Colton, C. K. Islet assessment for transplantation. Curr. Opin. Organ Transplant. 14:674–682; 2009.
39. Ricordi, C.; Finke, E. H.; Lacy, P. E. A method for the mass isolation of islets from the adult pig pancreas. Diabetes 35:649–653; 1986.
40. Ricordi, C.; Hering, B. J.; Londo, N. J. Islet isolation assessment. In: Ricordi, C., ed. Pancreatic islet cell transplantation, 1892-1992: One century of transplantation for diabetes. Austin, TX: R.G. Landes; 1992:132–142.
41. Rijkelijkhuizen, J. K. R. A.; van der Burg, M. P. M.; Töns, A.; Terpstra, O. T.; Bouwman, E. Pretransplant culture selects for high-quality porcine islets. Pancreas 32:403–407; 2006.
42. Rozak, P. R.; Weegman, B. P.; Avgoustiniatos, E. S.; Wilson, J. R.; Welch, D. P.; Hering, B. J.; Papas, K. K. Devices and methods for maintenance of temperature and pressure during islet shipment. Transplant. Proc. 40:407–410; 2008.
43. Schuurman, H. J. The International Xenotransplantation Association consensus statement on conditions for undertaking clinical trials of porcine islet products in type 1 diabetes—Chapter 2: Source pigs. Xenotransplantation 16: 215–222; 2009.
44. Shapiro, A. M.; Hao, E.; Rajotte, R. V; Kneteman, N. M. High yield of rodent islets with intraductal collagenase and stationary digestion—A comparison with standard technique. Cell Transplant. 5:631–638; 1996.
45. Shapiro, A. M.; Ricordi, C.; Hering, B. J.; Auchincloss, H.; Lindblad, R.; Robertson, R. P.; Secchi, A.; Brendel, M. D.; Berney, T.; Brennan, D. C.; Cagliero, E.; Alejandro, R.; Ryan, E. A.; DiMercurio, B.; Morel, P.; Polonsky, K. S.; Reems, J. A.; Bretzel, R. G.; Bertuzzi, F.; Froud, T.; Kandaswamy, R.; Sutherland, D. E.; Eisenbarth, G.; Segal, M.; Preiksaitis, J.; Korbutt, G. S.; Barton, F. B.; Viviano, L.; Seyfert-Margolis, V.; Bluestone, J.; Lakey, J. R. International trial of the Edmonton protocol for islet transplantation. N. Engl. J. Med. 355:1318–1330; 2006.
46. Taylor, M.; Baicu, S. Hypothermic perfusion of pancreas: Emphasis on preservation prior to islet isolation. In: Uygun, K.; Lee, C. Y., eds. Methods in bioengineering: Organ preservation and reengineering. Norwood, MA: Artech House; 2011; 85–103.
47. Taylor, M. J.; Baicu, S.; Greene, E.; Vazquez, A.; Brassil, J. Islet isolation from juvenile porcine pancreas after 24-h hypothermic machine perfusion preservation. Cell Transplant. 19:613–628; 2010.
48. Taylor, M. J.; Baicu, S.; Leman, B.; Greene, E.; Vazquez, A.; Brassil, J. Twenty-four hour hypothermic machine perfusion preservation of porcine pancreas facilitates processing for islet isolation. Transplant. Proc. 40:480–482; 2008.
49. Toso, C.; Brandhorst, D.; Oberholzer, J.; Triponez, F.; Buhler, L.; Morel, P. Isolation of adult porcine islets of Langerhans. Cell Transplant. 9:297–305; 2000.
50. Webb, M. A.; Dennison, A. R.; James, R. F. The potential benefit of non-purified islets preparations for islet transplantation. Biotechnol. Genet. Eng. Rev. 28:101–114; 2012.
51. Weegman, B. P.; Taylor, M. J.; Baicu, S. C.; Scott, W. E.; Mueller, K. R.; Kitzmann, J. D.; Rizzari, M. D.; Papas, K. K. Hypothermic perfusion preservation of pancreas for islet grafts: Validation using a split lobe porcine model. Cell Med. 2:105–110; 2012.