Collagen IV-Modified Scaffolds Improve Islet Survival and Function and Reduce Time to Euglycemia

Woon Teck Yap, MEng,^{1,2,*} David M. Salvay, MD, PhD,^{2,3,*} Michael A. Silliman, BS,^{2,3}
Xiaomin Zhang, MD,⁴ Zachary G. Bannon,^{2,3} Dixon B. Kaufman, MD, PhD,⁵
William L. Lowe, Jr., MD,⁶ and Lonnie D. Shea, PhD^{2,3,7,8}

Islet transplantation on extracellular matrix (ECM) protein-modified biodegradable microporous poly(lactide-co-glycolide) scaffolds is a potential curative treatment for type 1 diabetes mellitus (T1DM). Collagen IV-modified scaffolds, relative to control scaffolds, significantly decreased the time required to restore euglycemia from 17 to 3 days. We investigated the processes by which collagen IV-modified scaffolds enhanced islet function and mediated early restoration of euglycemia post-transplantation. We characterized the effect of collagen IV-modified scaffolds on islet survival, metabolism, and insulin secretion *in vitro* and early- and intermediate-term islet mass and vascular density post-transplantation and correlated these with early restoration of euglycemia in a syngeneic mouse model. Control scaffolds maintained native islet morphologies and architectures as well as collagen IV-modified scaffolds *in vivo*. The islet size and vascular density increased, while β -cell proliferation decreased from day 16 to 113 post-transplantation. Collagen IV-modified scaffolds promoted islet cell viability and decreased early-stage apoptosis in islet cells *in vitro*—phenomena that coincided with enhanced islet metabolic function and glucose-stimulated insulin secretion. These findings suggest that collagen IV-modified scaffolds promote the early restoration of euglycemia post-transplantation by enhancing islet metabolism and glucose-stimulated insulin secretion. These studies of ECM proteins, in particular collagen IV, and islet function provide key insights for the engineering of a microenvironment that would serve as a platform for enhancing islet transplantation as a viable clinical therapy for T1DM.

Introduction

ISLET TRANSPLANTATION OFFERS the potential to improve glycemic control and reduce insulin dependence in patients with type 1 diabetes mellitus (T1DM), an autoimmune disease that affects over 1.5 million Americans.^{1,2} Although initial transplant results utilizing the Edmonton Protocol were encouraging, with over 70% of transplant recipients requiring no exogenous insulin for more than 1 year,³ less than 10% of recipients remained insulin independent after 5 years.⁴ In particular, the shortage of donors and the necessity for multiple rounds of islet infusion limit the widespread use of islet transplantation and compels the development of strategies to improve the survival and function of transplanted islets.^{5–10}

Tissue-engineering scaffolds have been employed as a means to support the function of transplanted islets; however, to date, the focus of using biomaterials for islet transplantation has been largely on immunoisolation of islets to prevent rejection,¹¹ which has faced challenges related to the fibrotic response to the implant. Abrogating the allo- and autoimmune responses of islet transplantation remains an important problem, but the need for multiple islet infusions and islet failure over time also relates, in part, to the current approach for islet transplantation, intrahepatic infusion. Islet exposure to blood leads to cell death secondary to the immediate blood-mediated inflammatory reaction. Long-term exposure to toxins and high concentrations of orally administered immunosuppressive drugs in the portal circulation likely contributes to islet cell dysfunction and death over

¹Department of Biomedical Engineering, ²Institute for BioNanotechnology in Medicine, and ³Department of Chemical and Biological Engineering, Northwestern University, Evanston, Illinois.

⁴Division of Transplantation, Feinberg School of Medicine, Northwestern University, Chicago, Illinois.

⁵Division of Transplantation, Department of Surgery, School of Medicine and Public Health, Clinical Science Center, University of Wisconsin, Madison, Wisconsin.

⁶Division of Endocrinology, Metabolism and Molecular Medicine, Department of Medicine, Feinberg School of Medicine, Northwestern University, Chicago, Illinois.

⁷Robert H. Lurie Comprehensive Cancer Center, Northwestern University, Chicago, Illinois.

⁸Chemistry of Life Processes Institute, Northwestern University, Evanston, Illinois.

*Both authors contributed equally to this work.

the longer term. Thus, developing a new extrahepatic platform for islet transplantation will be important for enhancing this therapeutic modality for diabetes. To that end, the concept of using tissue engineering to integrate islets with host tissue emerged. Transplantation of islets on microporous scaffolds composed of biodegradable poly(lactide-co-glycolide) (PLG) or polydimethylsiloxane or within biodegradable hydrogels, all of which allow for direct contact between host cells and the transplanted islets, has been reported.^{12–15} Avid revascularization and the lack of a robust fibrotic response, as were observed for islets transplanted on biodegradable microporous PLG scaffolds, supported long-term islet survival and function and likely facilitated glucose sensing and the distribution of secreted insulin.¹⁶ Furthermore, the degradation of microporous PLG scaffolds did not adversely affect the function of transplanted islets.¹⁷ Research is currently underway to elaborate on and refine the design of the microenvironment to which the transplanted islets are exposed so as to provide signals that promote islet engraftment, e.g., replacement and presentation of extracellular matrix (ECM) proteins that are removed during islet isolation.¹⁶ Microporous scaffolds thus offer a clinically translatable platform upon which to investigate processes affecting islet engraftment and function postisolation and -transplantation.¹⁷

In this report, we investigated the processes by which collagen IV-modified scaffolds enhanced islet function and mediated early restoration of euglycemia post-transplantation. We previously reported that collagen IV enhanced the engraftment and function of islets transplanted on microporous PLG scaffolds.¹⁶ The engraftment and function of transplanted islets can be impaired by the isolation process that severs critical vascular connections and disrupts interactions between islet cells and their ECM.^{18–27} These interactions regulate many aspects of islet physiology, including survival, proliferation, and insulin secretion.^{15,23,28} Reconstruction of islet cell-ECM relationships following isolation mitigates the loss of differentiated islet structure and function, reduces apoptosis and necrosis of β -cells,²⁹ and increases insulin secretion.^{15,21,29–32} Herein the effect of collagen IV-modified scaffolds on islet survival, metabolism, and insulin secretion *in vitro* and early- and intermediate-term islet mass and vascular density post-transplantation were characterized and these processes were correlated with the early restoration of euglycemia in a syngeneic mouse model. The syngeneic mouse model allows focus on effects specific to the islet microenvironment on engraftment, while avoiding immune-mediated phenomena, including an allogenic response or the autoimmune response found in the nonobese diabetic mouse model. These studies of collagen IV-modified scaffolds on islet engraftment and function contribute to the design of a microenvironment that can serve as a platform for enhancing islet transplantation as a therapy for T1DM.

Materials and Methods

Fabrication of microporous scaffolds

PLG scaffolds were prepared and treated as previously described on the day before islet isolation and seeding.¹⁶ All scaffolds used were 5 mm in diameter and 2 mm in height. Briefly, dry scaffolds were immersed in 0.5 N NaOH for 30 s followed by immersion in an excess of phosphate-buffered

saline (PBS).³³ Scaffolds were dried at room temperature before being placed in 70% ethanol and dried again before being placed into individual wells of a 24-well tissue culture dish. Collagen IV (25 μ L at 1 mg/mL; Sigma), fibronectin (25 μ L at 1 mg/mL; Sigma), laminin-332 (formerly termed laminin-5 and hereafter referred to as “laminin”; 25 μ L of conditioned media from 804G cells containing $\sim$ 1 mg/mL of laminin-332³⁴), or 25 μ L of islet culture media (ICM; RPMI-1640 media [Life Technologies] supplemented with 10% heat-inactivated fetal calf serum [Hyclone], 100 U/mL penicillin-G, 100 mg/mL streptomycin sulfate, 1 mM L-glutamine, and containing 11 mM glucose; henceforth known as islet ICM) were added to the scaffold and incubated at room temperature for 2 h, followed by the addition of 25 μ L of the same component to each scaffold. The dose of 50 μ g of each protein was selected as per the manufacturer’s recommendations for tissue culture plates and to be consistent with our previous report that demonstrated enhancement in islet function following transplantation on ECM protein-modified scaffolds.¹⁶ Scaffolds modified with serum proteins were used as controls, as serum proteins have been widely used for *in vitro* culture of islets.^{32,35} Scaffolds were then incubated with 95% humidity at 37°C overnight to facilitate protein adsorption to the scaffold surface. Control and ECM protein-modified scaffolds were fabricated using the same process and a common batch of PLG microparticles and salt crystals.¹² Thus, the pore diameters and the diameters of the interconnections between the pores for the control and ECM protein-modified scaffolds were expected to be similar.

Animals and induction of diabetes

Male C57BL/6 mice (Jackson Laboratories) between 8 and 12 weeks of age were used as islet donors and transplant recipients. Diabetes was induced in graft recipient mice by streptozotocin (Sigma) treatment and verified as previously described.¹⁶ Mice were used in these studies only if they had blood glucose measurements greater than 300 mg/dL on consecutive days before transplantation. The blood glucose levels of donor mice were also checked before islet isolation to verify that they were metabolically normal. All studies were approved by the Northwestern University Animal Care and Use Committee.

Islet isolation and scaffold seeding

Islet isolation and scaffold seeding were performed as previously described.^{12,16,36,37} Briefly, before seeding on scaffolds, 100 μ L of fresh ICM was applied to the top of all scaffolds for rewetting. Islets were seeded onto each scaffold in a minimal volume of isolation media by applying them to the top surface and allowing them to filter into the microporous structure.

In vitro islet culture on microporous scaffolds

For all *in vitro* studies, 50 islets were seeded onto each scaffold. Islet-seeded scaffolds were incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 30 min. Every 30 min thereafter, for a total of 2 h, a drop of ICM was added to the top of each scaffold and returned to the incubator. After 2 h, 2 mL of ICM was added to each tissue culture well in which a scaffold was placed (12 wells/dish) and returned

to the incubator for culture. ICM were replaced every 24 h with 2 mL of fresh media.

Islet localization on scaffold

Islet localization was assessed by incubating islet-seeded scaffolds with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT; Fisher). MTT is reduced to an insoluble purple formazan product by the mitochondria of living cells.³⁸ Scaffolds incubated overnight in ICM were seeded with islets, as described above. After the initial 2-h incubation in a minimal volume of media, the media were aspirated and 2 mL of ICM containing a final concentration of 0.5 mg/mL MTT was added to each well. The scaffolds were then incubated at 37°C in a humidified atmosphere containing 5% CO₂ for 4 h, gently washed in PBS, and fixed in 4% w/v paraformaldehyde (PFA) overnight at 4°C. After washing thoroughly in PBS, scaffolds were cut along their long axis with a razor blade. Digital images were acquired using a Spot camera via the accompanying image acquisition software (Diagnostic Instruments, Inc.) attached to a standard dissecting microscope.

Islet cell metabolic activity, viability/cytotoxicity, and apoptosis assays

The islet cell metabolic activity was measured using the CellTiter-Blue Assay Kit (Promega). The ratio of live/dead cells was determined using the MultiTox-Fluor Multiplex Cytotoxicity Assay Kit (Promega). Islet cell apoptosis was quantified using the Caspase-Glo 3/7 Assay (Promega). Islet-seeded scaffolds modified with collagen IV, fibronectin, or laminin were incubated in ICM for 24, 48, or 72 h before transferring into 96-well, flat-bottom plates (Greiner Bio-One).

Glucose-stimulated insulin secretion

The insulin secretory capacity of islet cells on scaffolds, in response to the glucose challenge, was investigated. Islet-seeded scaffolds were kept in culture plate inserts with mesh sizes of 12 μ m and diameters of 12 mm (Millipore, Inc.), which were placed in individual wells of a 24-well polystyrene plate (1 mL of ICM per well) and subsequently incubated in ICM containing multiple concentrations of glucose. At each time point, samples were transferred from the standard ICM (11 mM glucose) to ICM with 2.8 mM glucose and incubated for 1 h, then transferred to ICM with 28 mM glucose and incubated for 1 h, then transferred back to 1 mL of fresh ICM (11 mM glucose) and allowed to incubate for 24 h until the next stimulation was performed. The ICM from the 2.8 and 28 mM glucose conditions were collected at the end of each incubation period and stored at -80°C until needed. Insulin levels in the collected samples were determined using a radioimmunoassay with a guinea pig anti-rat insulin 1° antibody and a goat anti-guinea pig immunoglobulin 2° antibody (Linco Research). Stimulation indices were calculated by dividing the insulin concentration in the ICM containing 28 mM glucose by that in the ICM containing 2.8 mM glucose.

In vivo islet transplantation

For all *in vivo* studies, only scaffolds modified with collagen IV or control scaffolds were used. Each scaffold was seeded with 100 islets and incubated at 37°C in a humidified atmo-

sphere containing 5% CO₂ for 30 min. Every 30 min thereafter, for a total of 2 h, a drop of ICM was added to the top of each scaffold and returned to the incubator. After 2 h, each recipient was transplanted with one islet-seeded scaffold as previously described.¹⁶ Briefly, recipients were anesthetized with an intraperitoneal injection of ketamine (Ketaset®; Fort Dodge) and xylazine (Anased®; Lloyd Labs). The abdomen was shaved and sterile prepped. A midline incision was made and the epididymal fat pad (EFP) was identified and spread on the abdominal surface. Islet-seeded scaffolds were wrapped in the EFP and returned to the abdominal cavity. The wound was closed in two layers. Mice were allowed free access to food and water postoperatively and were routinely checked throughout the duration of the study for any signs of infection around the surgical site. After transplantation, nonfasting blood glucose levels and weights were measured as previously described, using the following schedule: daily during the first 3 postoperative weeks, once at the end of each week from weeks 4 to 6, and once per fortnight starting at the end of week 7 until the conclusion of the study.¹⁶ Animals were considered to be euglycemic if nonfasting blood glucose levels did not revert to hyperglycemic levels and were maintained at less than 200 mg/dL for the duration of the study.

Explant of islet grafts and preparation for histological analysis

To assess the effects of collagen IV on the time course of islet survival, growth, and revascularization following transplantation, islet-seeded scaffolds modified with collagen IV or islet-seeded control scaffolds were explanted on day 16 or 113 post-transplantation as described below. Each mouse was anesthetized with an intraperitoneal injection of tribromoethanol (250 mg/kg body weight; Sigma) and 100 μ L of biotin-conjugated tomato lectin (*Lycopersicon esculentum*, 1 mg/mL; Vector Laboratories) was slowly infused into the left femoral vein over 2 min and allowed to circulate for 5 min.³⁹ Perfusion with and subsequent histological analysis for biotin-conjugated tomato lectin allowed for the specific identification of recanalized vasculature within the transplanted islets. To prevent hemorrhage, a cotton-tipped swab was used to tamponade the wound. Subsequently, the thoracic cavity was rapidly opened, the right atrium was cut, and a small incision was made in the left ventricle to allow for the insertion of a catheter through which 30 mL of 4% w/v PFA, which had been chilled to 4°C, was perfused over 3 min. The abdominal cavity was then opened to explant the islet graft, along with the entire fat pad. For comparison, pancreata were isolated from three healthy mice that were not previously manipulated. Pancreata or fat pads containing islet grafts were fixed in 4% w/v PFA overnight at 4°C and embedded in paraffin. Five micrometer-thick sections were then prepared from the paraffin-embedded specimens.

Histological analysis of islet architecture, morphology, vascular basement membrane, and proliferating β -cells

Histological analyses were performed to characterize the morphologies and architectures of the transplanted islets, relative to those of islets within pancreata from healthy mice. Each graft or pancreas was completely sectioned and three sections from regions containing insulin-positive cells were

randomly chosen and immunofluorescence (IF) staining was performed to qualitatively determine the relative distributions and quantities of α -, β -, and δ -cells, proliferating β -cells, and laminin α 4-containing vascular basement membrane (VBM) within the individual islets. All DyLight[®] antibodies were purchased from Jackson Immunoresearch Laboratories. The following combinations of 1° and 2° antibodies were used to stain for the following features: α -cells—mouse anti-mouse glucagon antibody (1:500; R&D Systems)/DyLight 405 donkey anti-mouse immunoglobulin G antibody (1:500); β -cells—guinea pig anti-swine insulin antibody (1:250; Dako)/DyLight 488 donkey anti-guinea pig immunoglobulin G antibody (1:500); δ -cells—rabbit anti-mouse somatostatin antibody (1:500; Millipore)/DyLight 549 donkey anti-rabbit immunoglobulin G antibody (1:500); proliferating cell nuclei—rabbit anti-mouse Ki-67 antibody (1:100; Abcam)/DyLight 549 donkey anti-rabbit immunoglobulin G antibody (1:500); and laminin α 4—goat anti-mouse laminin α 4 antibody (1:100; R&D Systems)/DyLight 549 donkey anti-goat immunoglobulin G antibody (1:500). Cell nuclei were counter-stained with Hoechst 33258 (1:2000; Invitrogen). Digital images were acquired using a 20 $\times$ objective and a Photometrics CoolSnap cf high-resolution interline CCD camera (Photometrics) via MetaVue[®] Imaging System, Version 5.0 (Molecular Devices) attached to a Leica DM IRB inverted contrasting microscope (Leica Microsystems). Composite images of islets were assembled using PTgui software (New House Internet Services B.V.).

Quantification of islet area and functional vascular density

Assessments of the total islet area, total intra/peri-islet functional vascular areas, and intra/peri-islet functional vascular density were performed in grafts removed after 16 or 113 days of implantation. For each condition, that is, collagen IV or control, 5 postoperative day 16 and 4 postoperative day 113 paraffin-embedded grafts were serially sectioned to completion, as described above. Starting with the first section containing insulin-positive cells, and then with sections at 100 μ m-intervals thereafter, until the last section containing insulin-positive cells, IF staining for insulin and lectin was performed to identify functional islet and intra/peri-islet vascular areas, respectively. The following combinations of 1°

and 2° antibodies were used to stain for the functional islet area—guinea pig anti-swine insulin antibody (1:25; Dako)/Alexa[®] Fluor 546 goat anti-rabbit immunoglobulin G antibody (1:500; Invitrogen) and the functional vascular area—fluorescein-conjugated goat anti-biotin antibody (1:200; Vector Laboratories). Cell nuclei were counterstained with Hoechst 33258 (1:2000; Invitrogen). Digital images were acquired and assembled into composite images as described above. Using ImageJ software (<http://rsb.info.nih.gov/ij/>), the total functional islet and intra/peri-islet vascular areas were measured by gating on areas with fluorescence intensities above an observer-determined threshold. The thresholds for all sections were determined by three independent observers who were blind to the conditions being evaluated.

Statistical analysis

All values are reported as the mean $\pm$ the standard error of the mean (SEM). Statistical analyses for comparison of groups were performed by one-way analysis of variance with a *post hoc* Tukey's honestly significant difference (HSD) test using Prism 5.0 software (GraphPad Software). A *p*-value of less than 0.05 was considered statistically significant.

Results

Islet localization on scaffolds

Following seeding, the majority of islets remained localized to the top surface of the scaffold, with minimal infiltration into the interior (Fig. 1A). Additionally, islets clustered around the center of the top surface of the scaffold where they were initially deposited (Fig. 1B). Following seeding, the culture medium was examined for detached, free-floating islets both before and after addition of the MTT reagent. An average of 3 ± 2 islets was observed, indicating a minimal islet loss (<10%) during the seeding and hydration steps. No islets were observed on the curved sides or on the bottom of the scaffold.

Metabolic activity and glucose-stimulated insulin secretion

At all time points tested, islets cultured on all ECM protein-modified scaffolds exhibited enhanced metabolic

FIG. 1. Localization of islets seeded on microporous poly(lactide-co-glycolide) (PLG) scaffolds. **(A)** View of the interior of an islet-seeded scaffold (top-to-bottom cut made through scaffold mid-line) showing the extent of islet infiltration into the scaffold through the interconnected pore structure of the scaffold. **(B)** View of the top surface of an islet-seeded scaffold. Scale bar = 1 mm, *n* = 3. Color images available online at www.liebertpub.com/tea

activity relative to islets cultured on control scaffolds (Fig. 2A). At 24 h, islets cultured on collagen IV-modified scaffolds exhibited significantly higher metabolic activity compared to islets cultured on fibronectin- and laminin-modified scaffolds and control scaffolds ($p < 0.05$). At 48 and 72 h, islets cultured on all three of the ECM protein-modified scaffolds exhibited a significantly greater metabolic activity compared to islets cultured on control scaffolds ($p < 0.05$). At 48 and 72 h, no significant differences in metabolic activities were detected among islets cultured on the various ECM protein-modified scaffolds.

At 24 and 48 h, the stimulation indices for glucose-stimulated insulin secretion for islets cultured on collagen IV-modified scaffolds were significantly higher than those for islets cultured on control scaffolds ($p < 0.05$) (Fig. 2B). After 72 h in culture, the stimulation indices for islets cultured on collagen IV- and fibronectin-modified scaffolds were significantly higher than those for islets cultured on control scaffolds ($p < 0.05$).

Quantification of live/dead and apoptotic islet cells

At all time points tested using the live/dead assay, islets cultured on collagen IV-modified scaffolds had a significantly higher ratio of live to dead cells compared to islets cultured on fibronectin- and laminin-modified scaffolds as well as control scaffolds ($p < 0.05$) (Fig. 3A). Although the live-cell measure trended higher for islets cultured on all ECM protein-modified scaffolds and was highest for islets cultured on collagen IV-modified scaffolds relative to islets cultured on control scaffolds, the dead-cell measure was significantly lower for islets cultured on collagen IV-modified scaffolds compared to islets cultured on fibronectin-

and laminin-modified scaffolds and control scaffolds (data not shown). The reason for the increase in the ratio of live to dead cells for all ECM protein-modified scaffolds at 48 h was due to a significant decrease in the dead-cell measure and not an increase in the live-cell measure (data not shown).

At 24 h, islets cultured on collagen IV-modified scaffolds exhibited significantly reduced levels of caspase-3/7 activities relative to islets cultured on fibronectin- and laminin-modified scaffolds and control scaffolds ($p < 0.05$) (Fig. 3B). At 48 h, islets cultured on all ECM protein-modified scaffolds exhibited significantly reduced levels of caspase-3/7 activities relative to islets cultured on control scaffolds ($p < 0.05$). At 72 h, islets cultured on all ECM protein-modified scaffolds exhibited nonsignificant reductions in caspase-3/7 activities compared to islets cultured on control scaffolds ($p > 0.05$).

Islet evaluation post-transplantation

We subsequently investigated several markers associated with islet engraftment and function using collagen IV-modified scaffolds *in vivo*. *In vivo* studies focused on comparing collagen IV-modified scaffolds to control scaffolds at multiple time points because our previous report demonstrated that 125 islets transplanted on collagen IV-modified scaffolds restored euglycemia more rapidly than islets transplanted on laminin- and fibronectin-modified scaffolds.¹⁶ Furthermore, collagen IV-modified scaffolds were used for these studies since islets cultured on these scaffolds *in vitro* exhibited the highest metabolic activity, stimulation index for insulin secretion, and live to dead cell ratio and the lowest caspase-3/7 activities compared to islets

FIG. 2. (A) Metabolic activity of islets cultured on extracellular matrix (ECM)-modified microporous PLG scaffolds. Fifty islets were cultured for 24, 48, and 72 h on collagen IV-, fibronectin-, or laminin-modified scaffolds. A fluorescence assay, based on the metabolic reduction of resazurin to resorufin, was performed to quantify islet cell metabolic activity relative to that of islets seeded onto control scaffolds. Fluorescence was measured using excitation and emission wavelengths of 560 nm and 590 nm, respectively. * $p < 0.05$, $n = 5$ for each condition. (B) Insulin secretion, in response to a glucose challenge, by islets cultured on ECM protein-modified microporous PLG scaffolds. Fifty islets were cultured for 24, 48, and 72 h on collagen IV-, fibronectin-, or laminin-modified scaffolds. A radioimmunoassay for insulin was performed to quantify islet insulin secretion in response to a glucose challenge relative to that of islets seeded onto control scaffolds. The glucose challenge was performed by incubating islets that had been cultured on scaffolds, in low glucose concentration media (2.8 mM) for 1 h, followed by incubation in high glucose concentration media (28 mM) for 1 h. The stimulation index is defined as the amount of insulin secreted under the high glucose condition divided by the amount of insulin secreted under the low glucose condition. * $p < 0.05$, $n = 3$ for each condition.

FIG. 3. (A) Viability/cytotoxicity of islets cultured on ECM protein-modified microporous PLG scaffolds. Fifty islets were cultured for 24, 48, and 72 h on collagen IV-, fibronectin-, or laminin-modified scaffolds. A live/dead fluorescence assay, based on the presence of specific proteases in intact (viable) or lysed (dead) cells in culture, was performed to quantify islet cell viability and cytotoxicity relative to that of islets seeded onto control scaffolds. For the live cell marker, fluorescence was measured using excitation and emission wavelengths of 400 and 505 nm, respectively. For the dead cell marker, fluorescence was measured using excitation and emission wavelengths of 485 and 520 nm, respectively. * $p < 0.05$, $n = 5$ for each condition. (B) Apoptosis of islets cultured on ECM protein-modified microporous PLG scaffolds. Fifty islets were cultured for 24, 48, and 72 h on collagen IV-, fibronectin-, or laminin-modified scaffolds. A luminescence assay, based on caspase-3/7 activities, was performed to quantify islet cell apoptosis relative to that of islets seeded onto control scaffolds. Luminescence signal was integrated over 1 s. * $p < 0.05$, $n = 6$ for each condition.

cultured on fibronectin- and laminin-modified scaffolds (Figs. 2 and 3). Male C57BL/6 mice transplanted with 100 islets on collagen IV-modified scaffolds achieved euglycemia significantly more rapidly than mice transplanted with 100 islets on control scaffolds ($p < 0.05$) (Fig. 4). The mean time required for restoring euglycemia in the collagen IV-modified scaffold group was 3.0 ± 1.8 days (100% converted, $n = 6$), while that in the control scaffold group was 17.7 ± 2.7 days (100% converted, $n = 6$).

Analysis of the explants' histology obtained using IF indicated that islets transplanted on both collagen IV-modified

scaffolds (Fig. 5B, D) and control scaffolds (Fig. 5C, E) maintained morphologies and architectures similar to islets found within pancreata from healthy mice ($n = 3$) (Fig. 5A), on both days 16 (Fig. 5B, C) and 113 (Fig. 5D, E) post-transplantation ($n = 4$ per group). Specifically, insulin-positive cells formed the bulk of the islets with glucagon- and somatostatin-positive cells present on the periphery. In addition, there was no evidence of dissociation or central necrosis of islets transplanted on the scaffolds (Fig. 5B–E).

On both days 16 and 113 post-transplantation, the collagen IV-modified and control scaffold groups had similar

FIG. 4. (A) Nonfasting blood glucose regulation after islet transplantation. Nonfasting blood glucose levels from day 0 (day of transplantation) through day 113 post-transplantation. ■, islets transplanted on collagen IV-modified scaffolds ($n = 6$); □, islets transplants on control scaffolds ($n = 6$). Data are presented as mean glucose level $\pm$ standard error of the mean (SEM). Euglycemia is defined as a sustained nonfasting blood glucose value of ≤ 200 mg/dL as indicated by the dashed line. (B) Mean time required for restoring euglycemia after islet transplantation. ■, islets transplanted on collagen IV-modified scaffolds ($n = 6$); □, islets transplants on control scaffolds ($n = 6$). Data are presented as mean time to euglycemia $\pm$ SEM.

FIG. 5. Islets transplanted on PLG scaffolds are morphologically and architecturally similar to islets in native pancreata. (A–E) Representative images of islets stained using immunofluorescence (IF) demonstrate the maintenance of islet morphologies and architectures 16 and 113 days post-transplantation, $n=4$ per group. Green = insulin, β -cell marker, red = somatostatin, δ -cell marker, blue = glucagon, α -cell marker. (A) Islets in native stroma of pancreas, scale bar = 133 μ m. (B, C) Islets transplanted on collagen IV-modified scaffolds or control scaffolds, respectively, and explanted on day 16 post-transplantation, scale bar = 100 μ m. (D, E) Islets transplanted on collagen IV-modified scaffolds or control scaffolds, respectively, and explanted on day 113 post-transplantation, scale bar = 200 μ m. (F) Total islet area per section was quantified and presented as mean $\pm$ SEM for islets transplanted on collagen IV-modified scaffolds or control scaffolds and explanted on days 16 and 113 post-transplantation, $n=5$ for each condition on day 16, $n=4$ for each condition on day 113. Color images available online at www.liebertpub.com/tea

mean total area of insulin-positive cells per section ($p>0.05$) (Fig. 5F). For each section, the area occupied by insulin-positive cells was quantified. The total area occupied by insulin-positive cells, which we term the total islet area per section, was averaged over the total number of sections containing insulin-positive cells. Both the collagen IV and the control scaffold groups on day 113 exhibited significantly greater mean total islet areas per section relative to both the collagen IV and the control scaffold groups on day 16 ($p<0.05$) (Fig. 5F). Insulin-positive cells with Ki-67-positive nuclei were identified as proliferating β -cells (Fig. 6A–E). For islets transplanted on collagen IV-modified and control scaffolds, more proliferating β -cells were observed on day 16 than day 113 ($p<0.05$) (Fig. 6E). At each time point, that is, day 16 or 113, similar numbers of proliferating β -cells were observed for islets transplanted on collagen IV-modified and control scaffolds ($p>0.05$) (Fig. 6E).

Evaluation of functional vasculature and vascular basement membrane

Islets transplanted on both collagen IV-modified scaffolds and control scaffolds exhibited a dense, functional, (i.e., re-canalized) vasculature, which was visualized by IF staining for perfused biotin-conjugated tomato lectin (Fig. 7A). Functional vasculature was re-established in both the collagen IV-modified scaffold and the control scaffold groups as early as day 16 post-transplantation. The basement membrane architecture was visualized by staining for laminin $\alpha 4$,

a marker for VBM derived from endothelial cells. The VBM promotes β -cell proliferation and insulin gene expression.^{40,41} Specifically, laminin $\alpha 4$ was detected around the vasculature of islets transplanted on both collagen IV-modified scaffolds and control scaffolds (Fig. 7C–F), on both days 16 and 113 post-transplantation ($n=4$ per group), in a similar fashion to that in islets found within the native stroma of pancreata ($n=3$) (Fig. 7B). On both days 16 and 113 post-transplantation, the mean total vascular area per section for islets transplanted on the control scaffold group was similar to that for islets transplanted on the collagen IV-modified scaffold group ($p>0.05$) (data not shown). Both the collagen IV and the control scaffold groups on day 113 exhibited a significantly greater mean total vascular area per section relative to both the collagen IV and the control scaffold groups on day 16 ($p<0.05$) (data not shown). On both days 16 and 113 post-transplantation, the control scaffold group and the collagen IV-modified scaffold group had a similar mean functional vascular density per section ($p>0.05$) (Fig. 7G).

Discussion

Islet transplantation is a potential curative treatment for T1DM. However, the current percutaneous transhepatic approach is not optimal, owing to complications specific to the intravascular hepatic site and the inability to remove the islet graft should serious complications develop.^{42–45} We applied biodegradable, microporous PLG scaffolds for extrahepatic

FIG. 6. Proliferating β -cells in islets transplanted on PLG scaffolds. (A–D) Representative images of islets stained using IF demonstrate the presence of proliferating β -cells in islets 16 and 113 days post-transplantation, $n=4$ per group. Green=insulin, β -cell marker, red=Ki-67 antigen, proliferating cell nuclear marker. Nuclei counterstaining with Hoechst 33258 was omitted from the overlay to enhance clarity of insulin and Ki-67 staining. (A, C) Islets transplanted on collagen IV-modified scaffolds and explanted on days 16 or 113 post-transplantation, respectively, scale bar=100 μ m. (B, D) Islets transplanted on control scaffolds and explanted on days 16 or 113 post-transplantation, respectively, scale bar=133 μ m. (E) Percentage of β -cells that were proliferating was quantified and presented as mean $\pm$ SEM for islets transplanted on collagen IV-modified scaffolds or control scaffolds and explanted on days 16 and 113 post-transplantation, $n=5$ for each condition on day 16, $n=4$ for each condition on day 113. Color images available online at www.liebertpub.com/tea

islet transplantation.¹² The scaffold had a thickness of 2 mm, a diameter of 5 mm, and a pore size in the range of 250 to 425 μ m. Islets were seeded onto the scaffold, and their distribution within the scaffolds was determined by the pore structure. Islets settled into individual pores of the scaffold on the outer 500 μ m of the scaffold (Fig. 1) as the pores were larger than the islets.¹⁷ The islets remained near the outer edge of the scaffold as the diameter of the interconnections between the pores were smaller than the islets. The distri-

bution of islets on the ECM-coated and control scaffolds was similar, as protein deposition on the PLG surface is on the order of nanometers and is therefore unlikely to significantly influence the diameters of the pores or interconnections between the pores.⁴⁶ Although host cells and blood vessels ultimately penetrated throughout the scaffold (data not shown), the islets on the outer 500 μ m of the scaffold directly contacted host tissues and blood vessels post-transplantation. This proximity to host tissues is essential because, following isolation, but before revascularization, islets rely solely upon diffusion to obtain nutrients from and remove wastes to their environment.

Our current studies investigated the processes underlying enhanced islet function and engraftment post-transplantation, with particular emphasis on collagen IV-modified scaffolds *in vivo*. Collagen IV is the principal component of the basement membrane⁴⁷ and we previously reported that islets transplanted on collagen IV-modified scaffolds exhibited the most dramatic reduction in time to euglycemia and enhancement in islet function *in vivo* relative to islets transplanted on scaffolds coated with other ECM proteins or control scaffolds.¹⁶ Matrigel or combinations of ECM proteins have enhanced glucose-induced insulin secretion *in vitro*,¹⁵ and represent a compelling direction for future studies; however, using this approach will require significant characterization of the scaffold surface as the composition of the adsorbed proteins will likely not match the composition of the proteins in a solution due to different adsorption kinetics and thermodynamics for each protein.

Control and collagen IV-modified scaffolds preserved islet morphology and architecture at the intermediate stages post-transplantation. The relative proportions and spatial distributions of α -, β -, and δ -cells have been intimately linked to islet function and insulin demand and were similar pre- and post-transplantation on both days 16 and 113 (Fig. 5A–E).⁴⁸ At both time points, control scaffolds maintained the mean total islet area per section as well as collagen IV-modified scaffolds. Between day 16 and 113, significant increases in the islet area were observed (Fig. 5F). The greater total area of insulin staining suggests β -cell proliferation between days 16 and 113, consistent with the higher percentage of proliferating β -cells on day 16 relative to day 113 (Fig. 6). Our results are consistent with a previous report that syngeneic islets double in mass $\sim$ 30 days after transplantation.⁴⁹

Control scaffolds were comparable to collagen IV-modified scaffolds with respect to the re-establishment of functional vasculature, vascular density, and VBM structure at the intermediate stages post-transplantation (Fig. 7). The VBM is essential for the survival and proliferation of β -cells and for insulin gene expression as β -cells are unable to synthesize their own BM.^{40,41} The VBM contains collagen IV, laminins, and fibronectin that signal through integrin-mediated pathways and are essential for promoting β -cell proliferation and insulin gene expression.^{40,41} Islets on both collagen IV-modified scaffolds and control scaffolds, explanted on either day 16 or 113 post-transplantation (Fig. 7C–F), displayed functional vasculatures (Fig. 7A) that expressed laminin α 4, a marker for VBM derived from endothelial cells, similar to islets within the native stroma of the pancreas (Fig. 7B). On days 16 and 113 post-transplantation, the vascular densities were similar for islets transplanted on collagen IV-modified scaffolds and control scaffolds (Fig.

FIG. 7. Functional revascularization of islets and maintenance of VBMs in islets transplanted on PLG scaffolds. **(A)** Representative image of islets stained using IF demonstrate functional vasculature in islets post-transplantation, scale bar=200 μ m. Green=perfused tomato lectin, functional vasculature marker, red=insulin. Nuclei counterstaining with Hoechst 33258 was omitted from the overlay to enhance clarity of insulin and perfused tomato lectin staining. **(B–F)** Representative images of islets stained using IF demonstrate the maintenance of VBMs in islets 16 and 113 days post-transplantation, $n=4$ per group, scale bar=133 μ m. Green=insulin, β -cell marker, red=laminin $\alpha 4$, VBM marker. Nuclei counterstaining with Hoechst 33258 was omitted from the overlay to enhance clarity of insulin and laminin $\alpha 4$ staining. **(B)** Islets in native stroma of pancreas. **(C, D)** Islets transplanted on collagen IV-modified scaffolds or control scaffolds, respectively, and explanted on day 16 post-transplantation. **(E, F)** Islets transplanted on collagen IV-modified scaffolds or control scaffolds, respectively, and explanted on day 113 post-transplantation. **(G)** Functional vascular density (vascular area per unit islet area) per section was quantified and presented as mean $\pm$ SEM for islets transplanted on collagen IV-modified scaffolds or control scaffolds and explanted on days 16 and 113 post-transplantation, $n=5$ for each condition on day 16, $n=4$ for each condition on day 113. Color images available online at www.liebertpub.com/tea

7G). By day 297, the vascular densities were significantly greater for islets transplanted on collagen IV-modified scaffolds than those on control scaffolds.¹⁶ The vascular density appears to increase between days 113 and 297. This observation is consistent with previous reports in which dynamic changes (e.g., reorganization of blood vessels) within islet grafts continued for at least 6 months post-transplantation, significantly longer than the duration of the current study.⁵⁰

Islets transplanted on collagen IV-modified scaffolds restored euglycemia significantly more rapidly, than islets transplanted on control scaffolds (Fig. 4A). Most of the mice transplanted with islets on collagen IV-modified scaffolds achieved euglycemia within 2 days post-transplantation, while most of the mice transplanted with islets on control scaffolds achieved euglycemia only after 18 days post-transplantation (Fig. 4B). We assessed the effects that ECM

proteins exerted on islets at early stages *in vitro*, that is, 24 to 72 h postisolation because *in vivo* islet morphology, architecture, total β -cell mass, functional vascular density, and VBM structure were similar for islets transplanted on collagen IV-modified and control scaffolds. Time points beyond 72 h were not investigated due to the decline of islets cultured *in vitro*, consistent with previous reports in the literature.⁵¹ Compared to islets cultured on control scaffolds, islets cultured on ECM protein-modified scaffolds exhibited significantly enhanced metabolic activity (Fig. 2A), glucose-stimulated insulin secretion (Fig. 2B), and viability (Fig. 3A) and decreased early-stage apoptosis (Fig. 3B), as measured by caspase-3/7 activities. Integrin signaling has been implicated as important for the metabolism of and insulin secretion by islet β -cells.²⁴ Integrin signaling through collagen IV also enhanced islet cell resistance to apoptosis²⁸ and is consistent with decreased cell death in islets on collagen IV-modified scaffolds. The improved metabolism and glucose-stimulated insulin secretion on collagen-IV scaffolds relative to control scaffolds likely led to an earlier restoration of euglycemia. ECM proteins directly enhanced islet function immediately postisolation—a critical period for islet engraftment that likely determines the ultimate success or failure of a transplant.⁵²

In conclusion, the restoration of islet cell-ECM contacts led to enhanced islet cell viability, decreased islet cell apoptosis, and enhanced islet metabolism and glucose-stimulated insulin secretion. The increased insulin secretion on collagen IV-coated scaffolds relative to controls is consistent with a reduction in time to euglycemia that was observed *in vivo*.²⁴ Collagen IV likely provided key signals that bridged the time between islet transplantation and concomitant islet revascularization and re-establishment of VBMs. Islets transplanted on other biomaterial scaffolds or into other sites may exhibit different responses to ECM proteins.

Acknowledgments

The authors acknowledge Samantha Holland, Marina Zelivyanskaya, Ho Hyun Sun, Ashley G. Goodman, and Ali M. Falouji for their histology work. We acknowledge Romie F. Gibly and Aline M. Thomas for helpful discussions on image processing and analysis within ImageJ. This work was supported by the National Institutes of Health grants F31 EB007118 (D.M.S.), R01 EB003806, and R01 EB009910. W.T.Y. gratefully acknowledges support from the Ryan Fellowship and the Northwestern University International Institute for Nanotechnology and was partially funded by the Chicago Biomedical Consortium with support from the Searle Funds at the Chicago Community Trust.

Disclosure Statement

No competing financial interests exist.

References

- Eiselein, L., Schwartz, H.J., and Rutledge, J.C. The challenge of type 1 diabetes mellitus. *ILAR J* **45**, 231, 2004.
- Kay, T.W., Thomas, H.E., Harrison, L.C., and Allison, J. The beta cell in autoimmune diabetes: many mechanisms and pathways of loss. *Trends in endocrinology and metabolism: TEM* **11**, 11, 2000.
- Shapiro, A.M., Lakey, J.R., Ryan, E.A., Korbitt, G.S., Toth, E., Warnock, G.L., Kneteman, N.M., and Rajotte, R.V. Islet transplantation in seven patients with type 1 diabetes mellitus using a glucocorticoid-free immunosuppressive regimen. *N Engl J Med* **343**, 230, 2000.
- Ryan, E.A., Paty, B.W., Senior, P.A., Bigam, D., Alfadhli, E., Kneteman, N.M., Lakey, J.R., and Shapiro, A.M. Five-year follow-up after clinical islet transplantation. *Diabetes* **54**, 2060, 2005.
- Hering, B.J. Achieving and maintaining insulin independence in human islet transplant recipients. *Transplantation* **79**, 1296, 2005.
- Hering, B.J., Kandaswamy, R., Ansit, J.D., Eckman, P.M., Nakano, M., Sawada, T., Matsumoto, I., Ihm, S.H., Zhang, H.J., Parkey, J., et al. Single-donor, marginal-dose islet transplantation in patients with type 1 diabetes. *JAMA* **293**, 830, 2005.
- Balamurugan, A.N., Bottino, R., Giannoukakis, N., and Smetanka, C. Prospective and challenges of islet transplantation for the therapy of autoimmune diabetes. *Pancreas* **32**, 231, 2006.
- Hogan, A., Pileggi, A., and Ricordi, C. Transplantation: current developments and future directions; the future of clinical islet transplantation as a cure for diabetes. *Front Biosci* **13**, 1192, 2008.
- Robertson, R.P. Update on transplanting beta cells for reversing type 1 diabetes. *Endocrinol Metab Clin North Am* **39**, 655, 2010.
- Robertson, R.P. Islet transplantation a decade later and strategies for filling a half-full glass. *Diabetes* **59**, 1285, 2010.
- Dong, H., Fahmy, T.M., Metcalfe, S.M., Morton, S.L., Dong, X., Inverardi, L., Adams, D.B., Gao, W., and Wang, H. Immuno-isolation of pancreatic islet allografts using pegylated nanotherapy leads to long-term normoglycemia in full MHC mismatch recipient mice. *PLoS One* **7**, e50265, 2012.
- Blomeier, H., Zhang, X., Rives, C., Brissova, M., Hughes, E., Baker, M., Powers, A.C., Kaufman, D.B., Shea, L.D., and Lowe, W.L., Jr. Polymer scaffolds as synthetic microenvironments for extrahepatic islet transplantation. *Transplantation* **82**, 452, 2006.
- Pedraza, E., Coronel, M.M., Fraker, C.A., Ricordi, C., and Stabler, C.L. Preventing hypoxia-induced cell death in beta cells and islets via hydrolytically activated, oxygen-generating biomaterials. *Proc Natl Acad Sci U S A* **109**, 4245, 2012.
- Phelps, E.A., Enemchukwu, N.O., Fiore, V.F., Sy, J.C., Murthy, N., Sulchek, T.A., Barker, T.H., and Garcia, A.J. Maleimide cross-linked bioactive PEG hydrogel exhibits improved reaction kinetics and cross-linking for cell encapsulation and *in situ* delivery. *Adv Mater* **24**, 64, 2012.
- Nagata, N., Gu, Y., Hori, H., Balamurugan, A.N., Touma, M., Kawakami, Y., Wang, W., Baba, T.T., Satake, A., Nozawa, M., et al. Evaluation of insulin secretion of isolated rat islets cultured in extracellular matrix. *Cell Transplant* **10**, 447, 2001.
- Salvay, D.M., Rives, C.B., Zhang, X., Chen, F., Kaufman, D.B., Lowe, W.L., Jr., and Shea, L.D. Extracellular matrix protein-coated scaffolds promote the reversal of diabetes after extrahepatic islet transplantation. *Transplantation* **85**, 1456, 2008.
- Gibly, R.F., Zhang, X., Graham, M.L., Hering, B.J., Kaufman, D.B., Lowe, W.L., Jr., and Shea, L.D. Extrahepatic islet transplantation with microporous polymer scaffolds in syngeneic mouse and allogeneic porcine models. *Biomaterials* **32**, 9677, 2011.

18. Vaithilingam, V., Sundaram, G., and Tuch, B.E. Islet cell transplantation. *Curr Opin Organ Transplant* **13**, 633, 2008.
19. Vantyghem, M.C., Kerr-Conte, J., Arnalsteen, L., Sergent, G., Defrance, F., Gmyr, V., Declerck, N., Raverdy, V., Vandewalle, B., Pigny, P., *et al.* Primary graft function, metabolic control, and graft survival after islet transplantation. *Diabetes Care* **32**, 1473, 2009.
20. Thomas, F., Wu, J., Contreras, J.L., Smyth, C., Bilbao, G., He, J., and Thomas, J. A tripartite anoikis-like mechanism causes early isolated islet apoptosis. *Surgery* **130**, 333, 2001.
21. Thomas, F.T., Contreras, J.L., Bilbao, G., Ricordi, C., Curiel, D., and Thomas, J.M. Anoikis, extracellular matrix, and apoptosis factors in isolated cell transplantation. *Surgery* **126**, 299, 1999.
22. Paraskevas, S., Duguid, W.P., Maysinger, D., Feldman, L., Agapitos, D., and Rosenberg, L. Apoptosis occurs in freshly isolated human islets under standard culture conditions. *Transplant Proc* **29**, 750, 1997.
23. Paraskevas, S., Maysinger, D., Wang, R., Duguid, T.P., and Rosenberg, L. Cell loss in isolated human islets occurs by apoptosis. *Pancreas* **20**, 270, 2000.
24. Kaido, T., Yebra, M., Cirulli, V., Rhodes, C., Diaferia, G., and Montgomery, A.M. Impact of defined matrix interactions on insulin production by cultured human beta-cells: effect on insulin content, secretion, and gene transcription. *Diabetes* **55**, 2723, 2006.
25. van Deijnen, J.H., Hulstaert, C.E., Wolters, G.H., and van Schilfgaarde, R. Significance of the peri-insular extracellular matrix for islet isolation from the pancreas of rat, dog, pig, and man. *Cell Tissue Res* **267**, 139, 1992.
26. Davalli, A.M., Scaglia, L., Zangen, D.H., Hollister, J., Bonner-Weir, S., and Weir, G.C. Vulnerability of islets in the immediate posttransplantation period. Dynamic changes in structure and function. *Diabetes* **45**, 1161, 1996.
27. Morini, S., Brown, M.L., Cicalese, L., Elias, G., Carotti, S., Gaudio, E., and Rastellini, C. Revascularization and remodelling of pancreatic islets grafted under the kidney capsule. *J Anat* **210**, 565, 2007.
28. Pinkse, G.G., Bouwman, W.P., Jiawan-Lalai, R., Terpstra, O.T., Bruijn, J.A., and de Heer, E. Integrin signaling via RGD peptides and anti-beta1 antibodies confers resistance to apoptosis in islets of Langerhans. *Diabetes* **55**, 312, 2006.
29. Hammar, E., Parnaud, G., Bosco, D., Perriraz, N., Maedler, K., Donath, M., Rouiller, D.G., and Halban, P.A. Extracellular matrix protects pancreatic beta-cells against apoptosis: role of short- and long-term signaling pathways. *Diabetes* **53**, 2034, 2004.
30. Navarro-Alvarez, N., Rivas-Carrillo, J.D., Soto-Gutierrez, A., Yuasa, T., Okitsu, T., Noguchi, H., Matsumoto, S., Takei, J., Tanaka, N., and Kobayashi, N. Reestablishment of microenvironment is necessary to maintain *in vitro* and *in vivo* human islet function. *Cell Transplant* **17**, 111, 2008.
31. Ris, F., Hammar, E., Bosco, D., Pilloud, C., Maedler, K., Donath, M.Y., Oberholzer, J., Zeender, E., Morel, P., Rouiller, D., *et al.* Impact of integrin-matrix matching and inhibition of apoptosis on the survival of purified human beta-cells *in vitro*. *Diabetologia* **45**, 841, 2002.
32. Jalili, R.B., Moen Rezakhanlou, A., Hosseini-Tabatabaei, A., Ao, Z., Warnock, G.L., and Ghahary, A. Fibroblast populated collagen matrix promotes islet survival and reduces the number of islets required for diabetes reversal. *J Cell Physiol* **226**, 1813, 2011.
33. Park, G.E., Pattison, M.A., Park, K., and Webster, T.J. Accelerated chondrocyte functions on NaOH-treated PLGA scaffolds. *Biomaterials* **26**, 3075, 2005.
34. Baker, S.E., DiPasquale, A.P., Stock, E.L., Quaranta, V., Fitchmun, M., and Jones, J.C. Morphogenetic effects of soluble laminin-5 on cultured epithelial cells and tissue explants. *Exp Cell Res* **228**, 262, 1996.
35. Zhang, Y., Jalili, R.B., Warnock, G.L., Ao, Z., Marzban, L., and Ghahary, A. Three-dimensional scaffolds reduce islet amyloid formation and enhance survival and function of cultured human islets. *Am J Pathol* **181**, 1296, 2012.
36. Hyon, S.H., Tracey, K.J., and Kaufman, D.B. Specific inhibition of macrophage-derived proinflammatory cytokine synthesis with a tetravalent guanlylhydrazide CNI-1493 accelerates early islet graft function posttransplant. *Transplant Proc* **30**, 409, 1998.
37. Kaufman, D.B., Gores, P.F., Field, M.J., Farney, A.C., Gruber, S.A., Stephanian, E., and Sutherland, D.E. Effect of 15-deoxyspergualin on immediate function and long-term survival of transplanted islets in murine recipients of a marginal islet mass. *Diabetes* **43**, 778, 1994.
38. Mosmann, T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. *J Immunol Methods* **65**, 55, 1983.
39. Dickie, R., Bachoo, R.M., Rupnick, M.A., Dallabrida, S.M., Deloid, G.M., Lai, J., Depinho, R.A., and Rogers, R.A. Three-dimensional visualization of microvessel architecture of whole-mount tissue by confocal microscopy. *Microvasc Res* **72**, 20, 2006.
40. Nikolova, G., Strilic, B., and Lammert, E. The vascular niche and its basement membrane. *Trends Cell Biol* **17**, 19, 2007.
41. Nikolova, G., Jabs, N., Konstantinova, I., Domogatskaya, A., Tryggvason, K., Sorokin, L., Fassler, R., Gu, G., Gerber, H.P., Ferrara, N., *et al.* The vascular basement membrane: a niche for insulin gene expression and Beta cell proliferation. *Dev Cell* **10**, 397, 2006.
42. Robertson, R.P. Intrahepatically transplanted islets—strangers in a strange land. *J Clin Endocrinol Metab* **87**, 5416, 2002.
43. Bhargava, R., Senior, P.A., Ackerman, T.E., Ryan, E.A., Paty, B.W., Lakey, J.R., and Shapiro, A.M. Prevalence of hepatic steatosis after islet transplantation and its relation to graft function. *Diabetes* **53**, 1311, 2004.
44. Guan, J., Behme, M.T., Zucker, P., Atkison, P., Hramiak, I., Zhong, R., and Dupre, J. Glucose turnover and insulin sensitivity in rats with pancreatic islet transplants. *Diabetes* **47**, 1020, 1998.
45. Robertson, R.P. Islet transplantation as a treatment for diabetes—a work in progress. *N Engl J Med* **350**, 694, 2004.
46. Pai, S.S., Przybycien, T.M., and Tilton, R.D. Protein PEGylation attenuates adsorption and aggregation on a negatively charged and moderately hydrophobic polymer surface. *Langmuir* **26**, 18231, 2010.
47. LeBleu, V.S., Macdonald, B., and Kalluri, R. Structure and function of basement membranes. *Exp Biol Med* (Maywood) **232**, 1121, 2007.
48. Kim, A., Miller, K., Jo, J., Kilimnik, G., Wojcik, P., and Hara, M. Islet architecture: a comparative study. *Islets* **1**, 129, 2009.
49. Biarnes, M., Montolio, M., Nacher, V., Raurell, M., Soler, J., and Montanya, E. Beta-cell death and mass in syngeneically transplanted islets exposed to short- and long-term hyperglycemia. *Diabetes* **51**, 66, 2002.

50. Rooth, P., Dawidson, I., Lafferty, K., Diller, K., Armstrong, J., Pratt, P., Simonsen, R., and Taljedal, I.B. Prevention of detrimental effect of cyclosporin A on vascular ingrowth of transplanted pancreatic islets with verapamil. *Diabetes* **38 Suppl 1**, 202, 1989.
51. Noguchi, H., Naziruddin, B., Jackson, A., Shimoda, M., Ike-moto, T., Fujita, Y., Chujo, D., Takita, M., Peng, H., Sugimoto, K., *et al.* Fresh islets are more effective for islet transplantation than cultured islets. *Cell Transplant* **21**, 517, 2012.
52. Eckhardt, T., Jahr, H., Federlin, K., and Bretzel, R.G. Endotoxin impairs the engraftment of rat islets transplanted beneath the kidney capsule of C57BL/6-mice. *J Mol Med* **77**, 123, 1999.

Address correspondence to:

Lonnie D. Shea, PhD

Department of Chemical and Biological Engineering

Northwestern University

2170 Campus Drive

Silverman Hall, Room 3619

Evanston, IL 60208

E-mail: l-shea@northwestern.edu

Received: January 16, 2013

Accepted: May 13, 2013

Online Publication Date: June 27, 2013