Endocrine Pancreas Engineered Using Porcine Islets and Partial Pancreatic Scaffolds

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•Running title

Engineered Pancreas with Islets & Native Scaffolds

•Acknowledgement

The authors thank Mr. Toshio Otake, Mr. Koji Matsumoto and Ms. Keiko Kumagai from the Laboratory Animal Center, Dr. Kana Sugiyama, and Ms. Makiko Hieda from Department of Surgery, School of Medicine, Keio University. This study was supported by Japan Agency for Medical Research and Development (AMED), Research Center Network for Realization of Regenerative Medicine, Projects for Technological Development to Y.K., and was partly supported by the Takeuchi Biohybrid Innovation Project, Exploratory Research for Advanced Technology (ERATO), Japan Science and Technology (JST), Japan.

Abstract:

Objectives: Because therapeutic options for severe diabetes are currently limited, there is a continuing need for new therapeutic strategies, especially in the field of regenerative medicine. Collaborative efforts across the fields of tissue engineering technology and islet biology may be able to create functionally engineered islets capable of restoring endocrine function in patients with insulin-dependent diabetes.

Methods: This engineered scaffold was seeded with isolated primary porcine islets via the pancreatic duct using a multi-step infusion technique. Endocrine function of perfusion-cultured islets in the native scaffold was analyzed by immunohistochemical staining of insulin and glucagon as well as by the insulin stimulation test.

Results: The pancreas in this large animal could be uniformly decellularized by perfusion with trypsin and Triton X-100 via the pancreatic duct, as shown by positive staining of extracellular matrix (ECM) components. These scaffolds derived from porcine pancreas were able to maintain the cellular integrity of islets that had repopulated the parenchymal space, which is fundamental for the restoration of endocrine function. Insulin release up to four days after islet infusion was maintained.

Conclusions: This scaffold from a large animal maintained islet survival and function in the short-term, retaining the cells as a solid organ in the parenchymal space after infusion through the pancreatic duct. These results suggest that this scaffold is suitable for further fabrication of fully functional bioengineered endocrine pancreases when implanted *in vivo*. Therefore, it may represent a key improvement in the field of beta-cell replacement therapy. Nonetheless, the facilitation of longer-term islet survival and studies of implantation *in vivo* is required for successful clinical translation.

Key words: recellularization, cell transplantation, tissue engineering, large animal, extracellular matrix

Introduction

In spite of numerous investigations seeking appropriate treatment options, currently diabetes mellitus is one of the most serious worldwide health problems¹⁻². Although exogenous insulin therapy, islet transplantation or whole pancreas transplantation are effective means for preventing acute metabolic decompensation in patients with severe diabetes³⁻⁷, only limited numbers of patients achieve their therapeutic targets. Hyperglycemia-related organ injury including kidney or heart damage remains a significant cause of morbidity and mortality among these severe diabetics^{8,9}.

Currently, the most common treatment options for diabetes, such as pharmaceutical interventions and insulin supplementation, have not resulted in a curative effect and can potentially lead to long-term complications^{10,11}. Progress in tissue engineering technology has facilitated the development of replacement tissues for the treatment of organ dysfunction¹²⁻¹⁴. Among these, the development of a bioengineered pancreas using an appropriate combination of cells and ECM scaffolds will provide an alternative approach to diabetes therapy. Indeed, cell-ECM interactions have been reported to be fundamental for the regulation of islet survival and insulin production. Native tissues are characterized by their unique ECM composition making an appropriate micro-environment¹⁵⁻¹⁷. Hence, three-dimensional (3D) scaffolds will play a critical role in pancreatic regenerative medicine by providing a similar micro-environmental niche for the seeded islet cells. At present, investigations of pancreatic scaffolds have been focused on artificially constructing those similar to the native ECM environment¹⁸. Using different synthetic biomaterials, many different types of scaffold have been developed, but none was found to accurately mimic the complexity of the actual pancreatic ECM composition and ultrastructure^{19,20}. Very recently, tissue decellularization technology has been explored as an alternative; it was demonstrated that eliminating all the viable cells but preserving the native ECM composition from tissues or organs including vessels²¹, valves²², skin²³, heart²⁴, liver^{25,26}, kidney²⁷ and pancreas²⁸ is feasible. This unique technology can meet the requirement for functional pancreas tissue engineering by providing

a physiologically relevant ECM scaffold that represents the complex in vivo micro-environment²⁹. Indeed, we have reported the efficacy of this native organ scaffold in the liver context, showing that the decellularized scaffold could support cell function and viability. Importantly, it could be applied on a large scale, such as in porcine-derived organ scaffolds³⁰. Investigations by others have also reported improved islet functionality when cultured on decellularized matrices derived from small intestinal submucosa or pancreatic slices¹⁵. Therefore, we hypothesized that three-dimensional native pancreas ECM from pigs would support the viability, phenotype, function, and tissue assembly of islets freshly isolated from porcine pancreas. Very recently, Mirmale-Sani et al. demonstrated the efficacy of pancreas scaffolds along these lines³¹. Optimized protocols for production of the scaffold as well as cell infusion remain to be determined, because the pancreas is basically a fragile tissue and the islet itself is too delicate to be efficiently infused into the parenchymal space.

Based on these considerations, the objectives of this study were to determine whether porcine pancreas-derived scaffolds could serve as suitable platforms for the reconstruction of an endocrine pancreas, with emphasis on its insulin- producing capacity. Although islet transplantation is already an established therapeutic option, viability of infused islets engrafted mostly in the portal vein decreased to less than 50% soon after their infusion^{32,33}. This is due to the methodological limitation inherent in islet infusion via the portal vein, which triggers rapid destruction of the cells not only by mechanical shear stress caused by blood flow but also by an immediate blood-mediated inflammatory reaction (IBMIR) which is defined as an innate inflammatory response activating complement and infiltration of polymorphonuclear cells into “foreign” material^{34,35}.

To overcome these limitations to clinical translation, we developed an effective method for the decellularization and recellularization of intact porcine pancreases which can maintain freshly isolated porcine islets in the parenchymal space, resulting in the construction of three-dimensional ECM scaffold-based bioengineered tissues with efficient insulin production, at least in the short-term.

The methods and techniques established in this study demonstrate an effective approach for pancreas decellularization and recellularization by bio-engineering for human-scale transplantation.

Material and Methods

Animals

Female LWD pigs weighing 20-23 kg were used for pancreas harvesting to prepare decellularized matrices as a scaffold for islet maintenance. Female pigs were also the source of pancreases harvested for the isolation of islet cells. All protocols were reviewed and approved by the local Ethics Committee of Keio University.

Whole pancreas harvest and decellularization

The pigs were anesthetized by midazolam 0.2 mg/kg (Astellas, Tokyo, Japan) and medetomidine 0.08 mg/kg (Zenoaq, Fukushima, Japan), followed by isoflurane inhalation to maintain anesthesia during the procedure. They were connected to a standard respiratory system, consisting of endotracheal tube for continuing inhalation. Using a vertical midline incision, the pancreas was excised en-bloc together with the upper duodenal segment. The latter was then removed from the pancreas, which was cannulated via the main pancreatic duct and superior mesenteric vein using 18 Fr Safeed Tubes (Terumo, Tokyo, Japan). The pancreas was flushed with PBS at 20 ml/min for 3-4 hours and then frozen at -80°C for a minimum of 24 hours prior to decellularization. The frozen/thawed pancreas was washed with PBS at 20 ml/min for 6 hours to remove blood via the main pancreatic duct and then perfused with 0.05% trypsin at 20 ml/min for 6 hours, and rinsed with PBS at the same flow rate for a further 3 hours. It was then decellularized with 0.1% TritonX-100 and 0.05% EGTA for 24-36 hours by perfusion at 20 ml/min via the main pancreatic duct. The resulting pancreas bioscaffold was finally rinsed with PBS for 12 hours.

Vascular Corrosion Casting

Corrosion casts were prepared using Baton's #17 anatomical corrosion kit following the manufacturer's recommendations (Polysciences, INC., Warrington, PA, USA). Polymer mixture

(20-40 ml depending on pancreas size) was injected via tubes inserted into the main pancreatic duct, superior mesenteric vein, and superior mesenteric artery. Polymerization took at least 2 hours at 4°C and was followed by maceration in 1N KOH solution for 3-4 hours at 50°C.

Histological Analyses

Normal pancreas, decellularized pancreas matrix and recellularized pancreas samples at day 4 were fixed with 10% formalin, embedded in paraffin, and processed for hematoxylin and eosin staining. Additional samples were permeabilized and incubated with rabbit polyclonal anti-collagen IV, rabbit polyclonal anti-fibronectin, and rabbit polyclonal anti-laminin (Abcam, Cambridge, MA, USA). Samples were then incubated overnight at 4°C with monoclonal anti-insulin (1:50, Sigma) or anti-glucagon (1:3,000, Dako, Glostrup, Denmark) antibodies. The secondary antibody was goat anti-rabbit IgG (Life Technologies, Grand Island, NY, USA). After all procedures, specimens were counterstained with DAPI (DAPI Fluoromount-G, Southern Biotech).

Porcine islet cell isolation

Immediately after receiving the pancreas, we inserted a cannula into the main pancreatic duct for infusion of modified ET-Kyoto solution consisting of 50,000U of ulinastatin (Mochida Pharmaceutical Co Ltd, Tokyo, Japan) added to 1L of Kyoto solution (Otsuka Pharmaceutical Co Ltd, Tokyo, Japan) for ductal protection, and placed the pancreas into a two-layer (modified ET-Kyoto solution/PFC) preservation container. Islets were isolated using a simple method optimized for adult pigs, as described elsewhere ³⁶.

Histological evaluation of islets

The number of islets in each field of view was determined by counting them after dithizone

staining (3 mg/ml final concentration, Sigma Chemical Co., St. Louis, MO) using an optical graticule. The number was then converted to a standard number of islet-equivalents (IE; diameter standardizing to 150 μ m). The decellularized pancreas was transferred to a customized-built organ culture chamber, specially constructed for large-scale organ perfusion; the perfusion system was designed based on a previously developed system for porcine liver that consisted of a peristaltic pump, bubble trap, and oxygenator³⁰. The apparatus was placed in an incubator to control temperature and the oxygenator was connected to an atmospheric gas mixture source. The organ was continuously perfused through the pancreatic duct at 2-4 ml/min with continuous oxygenation delivering an inflow partial oxygen tension of ~300 mmHg. The medium was changed daily. The perfusate consisted of basal medium (DMEM), 100 U/ml penicillin, and 100 mg/L streptomycin (Invitrogen, Carlsbad, CA, USA). The cells were seeded by an intra-portal multistep infusion method, as previously reported by H.Yagi³⁰; a total of 4.0×10^5 IEQ (islet cells) was infused into the circuit in multisteps at 10 min intervals through a port located in the perfusion system just before the location of the perfused decellularized pancreas matrix (n = 4 for histological evaluations and n = 2 for functional analysis). To calculate engraftment efficiency, the perfusate was collected, and the number and viability of cells not retained in the pancreas was determined using a hemacytometer and trypan blue exclusion. The total number of cells retained in the decellularized pancreas represented the difference between the initial number of cells seeded and the number of cells present in the perfusate after seeding.

Functional analysis of the recellularized pancreas

Islet function was assessed by monitoring the insulin secretion response of purified islets during glucose stimulation according to a procedure described by Shapiro and colleagues⁶. Briefly, 1200 IE were incubated with either 2.8 mM or 25 mM glucose in RPMI-1640 for 2 h at 37°C and 5% CO₂. The supernatants were collected and insulin levels were determined using a commercially-available enzyme-linked immunosorbent assay (ELISA) kit (ALPCO Insulin ELISA).

Results

Whole-Organ Porcine Pancreas Homogeneous Decellularization

Whole pancreas decellularization was achieved by pancreatic duct perfusion with 0.1% TritonX-100, an anionic detergent that simultaneously lyses cells and solubilizes cytoplasmic components. The protocol and system was optimized for porcine pancreas based on experience with porcine liver originally reported in the work published by Yagi et al. and Kadota et al. (Fig. 1a)³⁷. Figure 1b shows macroscopic images of the pancreas prior to decellularization and sequentially at different times during the decellularization process. This optimized protocol resulted in the production of an acellular scaffold which retained the gross shape of the whole organ. Corrosion casting of the decellularized pancreas from the pancreatic duct revealed an intact network of pancreatic ducts from the main duct to peripheral capillaries after decellularization (Fig. 1c). H&E staining demonstrated that compared to normal pancreas (Fig. 1d), the decellularized pancreas showed no visible nuclear material but had an intact extracellular matrix (Fig. 1e).

Immunohistochemical Evaluation of ECM Components of the Decellularized Pancreas

An advantage of using tissue-specific native ECM is the preservation of its natural composition, providing a unique micro-environment. Specifically, the pancreatic parenchyma is composed of ECM molecules that have a significant role in islet maintenance and secretory function. To first characterize the decellularized pancreas, immunohistochemical staining was performed for the evaluation of preserved ECM components, compared to native pancreas. Immuno-labeling characterization of the pancreas ECM showed specific distribution of collagen VI in the basement membrane of the pancreatic vessels and the acini, and fibronectin or laminin around the vascular structures and in discrete structures in the acellular pancreas. This appeared similar (Fig. 2b) to the structural composition of the matrix in the intact porcine pancreas (Fig. 2a). The images are high power fields (first row) and low power fields (second row). Importantly, these findings indicate

complete removal of cellular components while maintaining ECM integrity.

Porcine Islet Isolation and Recellularization

Isolated porcine islet cells were infused through the main pancreatic duct using the perfusion chamber (Fig. 3a). First, in a preliminary investigation, fibroblasts (NIH 3T3; Sigma-Aldrich, MO, USA) were infused in the same way to test whether this system worked satisfactorily. After 4 days of culture-perfusion, fibroblasts were found to have migrated towards the parenchymal space of the decellularized pancreas (Fig. 3b). Isolated porcine islet cells with $88.2 \pm 5.6\%$ viability ($n=10$) were infused through the pancreatic duct using the perfusion chamber (Fig. 3c). The medium after the first perfusion was collected and the number of single islet cells in the pellet after centrifugation was counted. These very few cells not captured by the scaffold numbered only ca. 6% of infused cells, indicating an approximate 94% efficiency of deposition of the islet cells within the parenchymal space and occasionally in the pancreatic duct, either clustering together or repopulating as isolated single cells. Four days after infusion, most of the cells retained in the pancreatic duct had also migrated into the parenchymal space of the decellularized pancreas scaffold. To demonstrate the potential of the decellularized pancreas ECM to support pancreatic function, isolated porcine islets were seeded into the decellularized pancreas ECM and insulin secretion was measured over time from day 0 to day 4. H&E staining showed that individual islets settled inside the decellularized pancreas (Fig. 3e). Islets seeded into the decellularized pancreas produced slightly less insulin on day 1 through day 4 relative to freshly isolated islets. Insulin release was measured at the end of 2 h incubation in medium containing 22 mmol/l glucose ($714 \pm 62.4 \mu\text{U/IEQ}$) (Fig. 3d). Islets cultured in tissue culture-treated dishes also showed a progressive reduction of insulin release ($395 \pm 33.3 \mu\text{U/IEQ}$) (Fig. 3d).

Maintaining Islet Functionality on Pancreas ECM

We applied freshly isolated porcine islets to porcine-derived scaffolds to optimize the whole system on a large scale for evaluating endocrine function of the islets after perfusion recellularization. Immunohistochemical analyses showed that islets in the porcine pancreas stained for insulin and glucagon (Fig. 4a, 4c), as did individual islets settled inside the acellular pancreas (Fig. 4b, 4d). To demonstrate the potential of the decellularized pancreas ECM to support pancreatic secretory function, isolated porcine islet cells were recellularized into decellularized pancreas scaffolds and insulin secretion was measured at day 0 and day 4 (Fig. 4e). The numbers of islet cells found in the decellularized pancreas scaffold decreased from $4.11 \pm 0.63 \times 10^5$ to $3.16 \pm 0.92 \times 10^5$ IEQ between days 0 and 4, whereas islet cells seeded in liquid medium decreased from $3.92 \pm 0.65 \times 10^5$ to $2.24 \pm 0.89 \times 10^5$ IEQ over the same time. Thus, both the islet cells recellularized into the decellularized pancreas scaffold and those seeded in liquid medium showed a decrease in numbers over time, but the former decreased more slowly than the latter (Fig. 4e). Insulin secretion by islets at day 1 in the recellularized pancreas ECM scaffold was measured at 420 mM/IEQ under basal glucose conditions and 700 mM/IEQ under high glucose conditions. This compared with insulin secretion by islets after day 1 in liquid medium of 700 mM/IEQ under basal conditions and 1200 mM/IEQ at day 1 under high glucose conditions. After 4 days, insulin secretion by islets in the recellularized pancreas ECM scaffold was 200 mM/IEQ and 420 mM/IEQ under basal and high glucose conditions, respectively. After 4 days in liquid medium these values were 360 and 790 mM/IEQ, respectively. Insulin secretion by islets after 4 days fluctuated more in liquid medium under both basal and high glucose conditions than in the recellularized pancreas (Fig. 4f).

Discussion

Current best-practice guidelines for the management of diabetes are focused on strict metabolic control throughout the day and pharmaceutical intervention. While these therapeutic options reduce the incidence of progressive diabetic complications, such as neural, vascular, retinal, and renal dysfunction, they do not offer the possibility of cure. β -cell replacement, through pancreas or islet transplantation, is the sole treatment capable of establishing long-term, stable euglycemia in severely diabetic patients⁶. Whole pancreas transplantation was first reported by Kelly et al.^{38,39} providing sufficient rates of insulin independence proven to control secondary complications of diabetes relative to insulin treatment. Despite these advantages, donor shortage, surgical morbidity and the requirement for life-long immunosuppression seriously limits routine clinical application. Meanwhile, clinical trials of islet transplantation have been performed in the last three decades which showed that restoration of β -cell function allows reproducible achievement of a more physiological release of endocrine hormone than exogenous insulin substitution in subjects with diabetes³. Isolated islets are transplanted using minimally-invasive techniques with lower morbidity than vascularized pancreas transplantation, which requires major surgery⁴⁰. In addition, islet transplantation represents an excellent platform for the development of cellular therapies aimed at the restoration of β -cell function using ECM in the near future. However, no studies have yet reported improved outcomes relative to pharmaceutical interventions and insulin supplementation.

Native ECM scaffolds are composed primarily of networks of collagens and other structural proteins filled with a hydrogel of proteoglycans. They have the potential to provide an appropriate micro-environment for islets. In addition, the 3D structure of native ECM has been shown to determine the structural arrangement of pancreatic endocrine cells, which influences islet secretory activity⁴¹. Although successful recellularization of ECM scaffolds has been reported in several organ systems, pancreas bioengineering lags behind other organs in the field, as only a few studies to date report successful recellularization of native pancreatic ECM²⁸. A suitable scaffold for functional

tissue engineering should be easily explanted and decellularized, retain native structural relationships and vascular networks, and be accessible to repopulation via perfusion. The excellent work of Goh et al. has demonstrated successful recellularization of mouse-derived pancreas scaffolds using murine pancreatic cell lines²⁸. Similar work was reported by Mirmalek-Sani using porcine scaffolds³¹, but the function of the recellularized organ was not evaluated. Moreover, it is important to determine whether islets can repopulate the scaffold and retain their spheroidal form not in the vessel but in the parenchymal space, in order to prevent shear stress and IBMIR both of which are reported to be critical issues of naked islet transplantation^{34,35}. This is of fundamental importance for maintaining islet function. We believe that the construction of a human-scale system which can sustain large numbers of islets is essential for further clinical application development. In the current, study we demonstrated that acellular porcine pancreas fulfills these criteria by preserving all three major ECM properties, and thus serves as an ideal platform for further bioengineering investigations to address the clinical need for insulin supplementation. To obtain an acellular porcine pancreas as a three-dimensional scaffold, we optimized a protocol that builds on the technique developed for porcine liver perfusion decellularization by Yagi et al.³⁰ This decellularization protocol showed (i) preservation of the three-dimensional architecture, as well as the functional capillary network of the original organ including the pancreatic duct; and (ii) the multi-step cell seeding method allowed high-efficiency engraftment of functional islet cells in the parenchymal space using a customized organ culture system. After decellularization, the pancreas was translucent while preserving the 3D architecture shown in corrosion casting of the pancreatic ducts. Although these methodologies for whole-organ decellularization have been reported for different organs, they have been scaled up to a clinically relevant size for humans only in a limited number of studies^{42,43}. Moreover, most in vivo implantation experiments in rodents have been limited to a few hours due to problems such as hemorrhage, thrombosis, and surgical technical difficulties^{24,26}. The method for whole-pancreas decellularization reported herein using porcine pancreas facilitates approaches to some of these

issues (e.g., scalability, pancreatic duct reliability) that might overcome some of the clinical problems.

In the current study, the multistep islet perfusion technique resulted in approximately 94% cell retention efficiency, assessed by calculating the number of cells in the perfusate after cell infusion. Retained cells were deposited within the parenchymal space and occasionally in the pancreatic duct, either clustering together or repopulating as isolated single cells. After four days, most of the cells were no longer found in the pancreatic duct but had migrated into the parenchymal space. We demonstrated the efficacy of the decellularized pancreas as a biological scaffold for pancreatic islets to maintain cell function. The architecture of the scaffold was stable, including parenchymal, vessel and ductal structures supporting large numbers of functional primary islet throughout the perfusion culture period. Our data showed the preservation of glucose-related insulin secretion in islets cultured on pancreatic matrix over 4 days of *in vitro* incubation. Levels of secreted insulin remained stable at >1000 pmol/IEQ over the 4 days of culture, but decreased after 5 days. We hypothesize that further optimization of speed and pressure of medium perfusion, which might be a factor contributing to mechanical stress to the cells, is required to prevent rapid deterioration of beta cell function. That the culture system lacks intra-islet vascularization and innervation may also contribute. Thus, our results are limited to demonstrating that pancreatic acellular matrix is a valuable scaffold able to maintain short-term islet secretory function with retention of their spheroidal shape *ex vivo*. Several previous reports, including our own, have suggested that these native scaffolds have a high affinity for endothelial cells and could contribute positively to angiogenesis^{25,37}. We propose that these scaffolds will promote angiogenesis and revascularization when implanted *in vivo*. In the present study, vascular endothelial cells were not co-infused into the scaffold, because the purpose of the study was to evaluate whether ECM from the native scaffold itself could have positive effects on maintaining primary islets at least prior to implantation of this large bioengineered tissue. Our results did indeed confirm the feasibility of this approach.

Future studies will be needed to extend the duration of islet cell survival and function with or without co-infusion of endothelial cells, and by implantation *in vivo* to achieve insulin secretion levels higher than those seen in liquid culture medium or after naked islet transplantation.

The current study describes a minimally disruptive method for whole-organ decellularization in a large animal model that produces a three-dimensional pancreas matrix suitable for supporting functional islet cells. Characterization of the decellularized pancreas matrix showed maintenance of the pancreas matrix components such as fibronectin, collagen IV and laminin, and the preservation of the pancreatic duct tree. We showed that the possibility of reintroducing primary islets into the organ matrix via the pancreatic duct is possible, with retention of their spheroidal formation in a decellularized large animal pancreas. Although islet transplantation is already an established therapeutic option, viability of the infused islets that were engrafted mostly in the portal vein decreased to less than 50% soon after their infusion in earlier studies^{32,33}. To overcome the clinical problem of shear stress and IBMIR that contribute to this rapid destruction of naked islets, we developed an effective method for the decellularization and recellularization of intact porcine pancreases. This approach avoided loss of the vascular network which can maintain freshly isolated porcine islets in the parenchymal space, resulting in the construction of three-dimensional ECM scaffold-based bioengineered tissues with efficient insulin production in the short-term. This suggests that this scaffold is suitable for further fabrication of fully functional bioengineered endocrine pancreas when implanted *in vivo* because of its stability, vascular/ductal structure and capacity to maintain islets in a native parenchymal architecture. Therefore, it may represent a key improvement in the field of beta-cell replacement therapy, but studies of extended islet survival and implantation *in vivo* are still required prior to successful clinical translation.

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Figure legends

FIGURE 1. Decellularization of the porcine pancreas.

(a) Representative image of the pancreas decellularization system. (b) Representative images of porcine pancreas during the decellularization process at 0 h, 8 h, 24 h and 48 h. (c) Whole pancreas bioscaffold as a corrosion cast of the pancreatic duct. (d) H&E staining of the native pancreas at low (top row) and high magnification (bottom row). (e) H&E staining of the decellularized pancreas at 48 h at low (top row) and high magnification (bottom row). Scale bar: 100 μ m.

FIGURE 2. Histological comparison of native pancreas and decellularized pancreas matrix

(a) Immunohistochemical staining of native porcine pancreas (top row) and decellularized porcine pancreas scaffolds (bottom row) for collagen IV, fibronectin, and laminin at low and high magnification. (b) Immunohistochemical staining of native porcine pancreas (top row) and decellularized porcine pancreas scaffolds (bottom row) of collagen IV, fibronectin, and laminin at low and high magnification. Scale bar: 100 μ m.

FIGURE 3. Islet isolation from porcine pancreas and infusion protocol via pancreatic duct

(a) Representative image of the pancreas perfusion system (1: closed chamber, 2: peristaltic pump, 3: bubble trap). (b) H&E staining of fibroblasts in the decellularized pancreas at low and high magnification. (c) Gross morphology of recellularized porcine partial pancreas scaffold with isolated porcine islets. (d) Sequential infusion protocol of isolated islets via pancreatic duct. (e) Dithizone staining of isolated islet cells. Scale bar: 100 μ m.

FIGURE 4. Islet viability and functionality in the scaffold

(a) H&E staining of infused cells in the native pancreas at low (left) and high magnification (right). (b) Comparison of insulin secretion by islet cells in culture media and in the decellularized porcine pancreas matrix through day 4 (n=3). (c-f) Representative image of the pancreas perfusion system. (c) Insulin staining of normal porcine pancreas. (d) Insulin staining of recellularized porcine pancreas. (e) Glucagon staining of normal porcine pancreas. (f) Glucagon staining of recellularized porcine

pancreas. (g) Comparison of number of islet cells under floating culture condition and in the decellularized porcine pancreas matrix at day0 and day 4. (n=3) (h) Comparison of the glucose challenge test of islet cells in culture media and in the decellularized porcine pancreas matrix through day 4 (n=3). Scale bar: 100 μ m.

Figure 1

a

b

c

d

e

Figure 2

a

b

Figure 3

Figure 4

a

c

d

e

f

b

g

h