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A bilaminated decellularized scaffold for islet transplantation: Structure, properties and functions in diabetic mice

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ABSTRACT

Ectopic transplantation of islets provides a beta cell-replacement approach that may allow the recovery of physiological regulation of the blood sugar level in patients with Type I diabetes (T1D). In development of new extrahepatic islet transplantation protocols in support of the islet engraftment, it is pivotal to develop scaffold materials with multifaceted functions to provide beneficial microenvironment, mediate host response in favor of vascularization/islet integration and maintain long-term islet function at the transplantation site. In this study, a new composite bilaminar decellularized scaffold (CDS) was fabricated with differential structural, degradation and mechanical properties by the combination of a fast-degrading porous collagen matrix and a mechanically supportive porcine pericardium. When investigated in the epididymal fat pad in syngeneic mouse models, it was shown that CDS could serve as superior scaffolds to promote islet adhesion and viability, and islet-CDS constructs also allowed rapid reversal of the hyperglycemic condition in the host. The engraftment and effects of islets were achieved at low islet numbers, accompanied by minimal adverse tissue reactions and optimal islet integration with the surrounding fat tissue. The bioactive surface, mechanical/chemical durability and biocompatibility of the CDS may all have played important roles in facilitating the engraftment of islets. Our study provided new insights into scaffold's function in the interplay of cells, materials and host tissue and the extracellular matrix-based scaffolds have potential for clinical translation in the beta cell-replacement therapy to treat T1D.

1. Introduction

Type I diabetes (T1D) is a chronic autoimmune condition that cause permanent destruction of β cells in pancreas and disordered elevation of blood and urine glucose levels [1]. The disease is one of the most common severe chronic illnesses diagnosed in children and young adults and currently affects over 30 million people worldwide [2]. Currently, treating T1D heavily relies on the injection of insulin matched to meals; the treatment, however, may only be passive, given that the ensuing control of glycemia is often inadequate to prevent debilitating complications, especially in the situation of hypoglycemic unawareness [3]. Replacement of insulin-producing cells has been proposed as a regenerative therapy that holds promise to restore the physiological regulation of the blood sugar level. In particular, the establishment of Edmonton Protocol in the beginning of the millennium—-transplantation of donor islets via intra-portal infusion into the hepatic vasculature—has been the hallmark of treating T1D through exogenous islets [4,5].

Despite the progress, the implementation of Edmonton Protocol on large clinical scales is associated with a number of difficulties. First, the islet source is limited and unsustainable, as transplantation has so far has been depending on tissues collected from cadavers and often 2 to 4 cadavers are needed to treat one patient. Second, inefficient engraftment or malfunction of the transplanted islets is implicated—only a minority (around 10%) of patients remained insulin-independent five-year post-transplantation [6]. In fact, the transplanted islets could be threatened or eliminated due to host environment/reaction including immediate blood-mediated inflammatory reaction [5–9]. In addition to the search for reliable islet source for transplantation, it is therefore a critical

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task to address questions pertinent to how to generate environment that can effectively promote the islet engraftment.

Among the efforts, alternative, extrahepatic transplantation sites were studied which include omentum, intra-muscle, subcutaneous space and spleen etc. [10,11] On the other hand, one important strategy to help the islets cope with the transplantation conditions is to develop biomaterial-based scaffolds to improve the microenvironment and promote islet viability/function post-transplantation [12–15]. Indeed, in previous studies, scaffolds were found to have abilities to 1) help confine the islets at the transplantation site and 2) provide mechanical support and protection during implantation [16–18]. Despite the understanding, the role and the function of the scaffolds during transplantation still need to be investigated for developing transplantation materials/protocols for clinical applications. It is noted that most of the scaffold materials investigated so far were based on synthetic polymers or hydrogels, which include poly(lactide-*co*-glycolide) (PLG) [16,19–22], polydimethylsiloxane (PDMS) [23–25], polyurethane (PU) [26] and polyethylene glycol (PEG) [27] and fibrin gels [28,29] et al. In many of these paradigms, exogenous growth factors, such as vascular endothelial growth factor (VEGF) and platelet-derived growth factor-β (PDGF-β), were incorporated into the scaffolds for optimal vascularization, islet function or transplantation results [22,24,28,30], which may be difficult for clinical translation.

Here, we are interested in investigating a composite scaffold fabricated from decellularized matrices in the aim to create materials with temporal and spatial characteristics and multiple functions necessitated by islet transplantation. In particular, a decellularized scaffold (CDS) with a bilaminar structure was created with a fast-degrading macroporous top layer and a relatively stable bottom layer. The scaffolds loaded with islets were investigated for its ability to support islet transplantation in the epididymal fad pad (EFP) in a syngeneic mouse model. Our studies show that the use of CDS in transplantation allowed rapid reversal of hyperglycemia with optimal vascularization and integration of islets within the fat tissue. The property-function relationships and the role of the scaffold were analyzed and CDS can be further developed for islet transplantation and cell-based therapy.

2. Materials and methods

Water was distilled and deionized at 18 MΩ resistance (Gelante Pure Water, Shijiazhuang, China). All chemicals were obtained from Sigma Aldrich (St. Louis, MO, USA) unless otherwise noted. The reagents for cell culture and biological assays, including Dulbecco's modified Eagle's medium (DMEM), Dulbecco's phosphate buffered saline (DPBS), fetal bovine serum (FBS), penicillin/streptomycin (P/S), dimethyl sulfoxide (DMSO), 4′, 6-diamidino-2-phenylindole (DAPI) and Quant-iT™ PicoGreen® dsDNA were all purchased from Invitrogen (Carlsbad, CA, USA) unless otherwise specified.

2.1. Fabrication of CDS

Pericardium and tendon from porcine were washed up, immersed in benzalkonium bromide for 15 h and then extracted using the 3% acetone solution for 24 h to remove the adipose tissues and impurities. The tissues were decellularized using 0.1% Triton-100 solutions with 0.1% pepsin and ultrasonically digested under 30 kHz for 48 h to remove cells and debris. In order to remove antigen, the tissues were reacted with amide under pH 7 for 48 h and guanidine hydrochloride solutions under pH 8 for 24 h to achieve antigen-free matrix. Then the matrixes were stirred in 0.5% polypropylene oxide solutions for 2 days to crosslink the collagen molecules in matrix. The decellularized tendon tissue was dissolved in 0.2% acetic acid and then grinded into powders. The collagen was obtained by dissolving these powders in 0.4% pepsin for more than 2 days and then combined onto the matrix obtained from pericardium. Finally, the resulting decellularized scaffolds were freeze-dried and sterilized with gamma ray. The final products were cut into 7-mm disks for use.

2.2. Structural and biochemical characterization of CDS

To observe the morphology of CDS, samples were gold sputter-coated and examined by the scanning electron microscope (SEM) (S-4800, Hitachi, Japan). ImageJ (NIH, Bethesda, USA) was applied to quantitatively characterize the pore size of the upper layer of the membrane. For each sample, all the pores were measured in each image, and a total of 6 images were counted. The thickness of CDS was measured using a vernier caliper (n = 4).

To determine the porosity of CDS (ϕ), the volume of scaffold (V_s) and the total volume of pores inside the scaffold (V_p) were measured through the fluid saturation and gravity differential experiments. Specifically, a clean and dried scaffold sample was first weighted (M_1). The sample was then immersed in 50 ml water contained in a volumetric flask and the total weight recorded as M_2. The sample was impregnated fully with water at reduced pressure and kept for 24 h at room temperature to reach equilibrium. Water was then carefully aspirated out so that the total volume of the liquid and scaffold in the flask remained at 50 ml. The flask containing water with the sample was reweighted (M_3). The weight differential was calculated (M_3-M_2) to derive V_s. Finally, the sample was removed from the flask, blotted dry on the surface and reweighted (M_4). The V_p was calculated based on the weights of the scaffold before and after immersion (M_4-M_1). ϕ was derived based on the equation below. (n = 3)

$$\phi = \frac{V_p}{V_P + V_s} = \frac{(M_4 - M_1)/\rho_w}{(M_4 - M_1)/\rho_w + (M_3 - M_2)/\rho_w} = \frac{M_4 - M_1}{(M_4 - M_1) + (M_3 - M_2)}$$

where ρ_w represents the density of water.

To measure the residual α-gal content of CDS, scaffolds were grinded and lysed with radio immunoprecipitation assay (RIPA) Lysis Buffer for 1 h. The samples were centrifuged at 4000 rpm and the supernatants were collected. The positive control is porcine liver and the negative control is human placenta. The samples were first reacted with α-gal epitope (Galα1-3Galβ1-4GlcNAc-R) monoclonal antibody (M86) (Enzo Life Sciences, Switzerland), washed and then reacted with goat anti-mouse IgM-HRP (sc-2064, Santa Cruz) according to manufacturers' protocol. Then the samples were added with 3, 3′, 5, 5′-tetramethylbenzidine (TMB) solutions and the absorbance was read at 470 nm with microplate reader (M2e, Molecular Devices). The α-gal contents of the samples were then determined based on the standard curve, which was obtained through the gradient dilutions of α-gal-BSA standard solutions (n = 3).

To determine the residual DNA contents of CDS, scaffolds were cut into pieces and digested with proteinase K. After digestion, the samples were centrifuged and the supernatants were collected. DNA in the supernatants was extracted using Omega Bio-tek's Mag-Bind® Blood and Tissue DNA HDQ 96 Kit (M6399) according to the manufacturer's protocol. 100 μl of the DNA samples were then mixed with an equal volume of PicoGreen® reagent according to the manufacturer's protocol and reacted for 5 min. The fluorescence