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A macroporous heparin-releasing silk fibroin scaffold improves islet transplantation outcome by promoting islet revascularisation and survival

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ABSTRACT

Islet transplantation is considered the most promising therapeutic option with the potential to cure diabetes. However, efficacy of current clinical islet transplantation is limited by long-term graft dysfunction and attrition. We have investigated the therapeutic potential of a silk fibroin macroporous (SF) scaffold for syngeneic islet transplantation in diabetic mice. The SF scaffold was prepared via lyophilisation, which enables incorporation of active compounds including cytokines, peptide and growth factors without compromising their biological activity. For the present study, a heparin-releasing SF scaffold (H-SF) in order to evaluate the versatility of the SF scaffold for biological functionalisation. Islets were then co-transplanted with H-SF or SF scaffolds in the epididymal fat pad of diabetic mice. Mice from both H-SF and SF groups achieved 100% euglycaemia, which was maintained for 1 year. More importantly, the H-SF-islets co-transplantation led to more rapid reversal of hyperglycaemia, complete normalisation of glucose responsiveness and lower long-term blood glucose levels. This superior transplantation outcome is attributable to H-SF-facilitated islet revascularisation and cell proliferation since significant increase of islet endocrine and endothelial cells proliferation was shown in grafts retrieved from H-SF-islets co-transplanted mice. Better intra-islet vascular reformation was also evident, accompanied by VEGF upregulation. In addition, when H-SF was co-transplanted with islets extracted from *vegfr2-luc* transgenic mice *in vivo*, sustained elevation of bioluminescent signal that corresponds to *vegfr2* expression was collected, implicating a role of heparin-dependent activation of endogenous VEGF/VEGFR2 pathway in promoting islet revascularisation and proliferation. In summary, the SF scaffolds provide an open platform as scaffold development for islet transplantation. Furthermore, given the pro-angiogenic, pro-survival and minimal post-transplantation inflammatory reactions of H-SF, our data also support the feasibility of clinical implementation of H-SF to improve islet transplantation outcome.

Statement of Significance

1) The silk fibroin scaffold presented in the present study provides an open platform for scaffold development in islet transplantation, with heparinisation as an example.
2) Both heparin and silk fibroin have been used clinically. The excellent *in vivo* therapeutic outcome reported here may therefore be clinically relevant and provide valuable insights for bench to bed translation.
3) Compared to conventional clinical islet transplantation, during which islets are injected via the hepatic portal vein, the physical/mechanical properties of silk fibroin scaffolds create a more accessible transplantation site (i.e., within fat pad), which significantly reduces discomfort.

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4) Islet implantation into the fat pad also avoids an instant blood mediated inflammatory response, which occurs upon contact of islet with recipient's blood during intraportal injection, and prolongs survival and function of implanted islets.

1. Introduction

Type 1 diabetes is an autoimmune disorder caused by destruction of insulin-producing β-cells within the pancreatic islets [1]. Allogeneic islet transplantation is considered a viable therapeutic approach for diabetic patients with the potential to cure the disease. Despite remarkable improvements that have been made with the development of the "Edmonton protocol", long-term insulin independence remains to be desired as majority of recipients eventually reversed to insulin replacement [2,3]. The lack of sustained therapeutic efficacy of islet transplantation is mainly due to early depletion of functional islets post-transplantation followed by a gradual decline of islet function and viability.

Islets are highly vascularised requiring 10–20% of the pancreatic blood supply while accounting for merely 2–3% of the total pancreatic mass [4–8]. The highly enriched vascularisation within each islet allows rapid exchange of oxygen, nutrients, islet hormones and other cellular effectors [5,7]. During the isolation procedure prior to clinical islets transplantation, the extracellular matrix (ECM) that is essential to maintain islet vascularisation and innervations is often damaged due to enzymatic digestion, resulting in disrupted islet microvasculature and impaired islet function and survival [4,5]. Given that accessibility to nutrition, adequate physical support and vascular regeneration are essential factors for successful engraftment, the possibility of incorporating biomaterials to co-deliver islets during transplantation have been explored.

Macroporous scaffolds have been extensively used as temporary artificial ECM for cell therapy by providing an optimal *in situ* site for cell accommodation, subsequent proliferation and differentiation. Due to their three-dimensional (3D) and highly porous structures, they also facilitate nutrients exchange, cell infiltration and revascularisation *in vivo* [4–6,8–19], which are important factors for improving post-transplantation islet survival and function. Multiple scaffolds have been engineered with surface-coated ECM proteins, growth factors and anti-inflammatory cytokines to enhance cell survival and neovascularisation during islet transplantation [14,15,18,20,21]. Previous studies reported reversal of diabetes after extrahepatic islets transplantation using a poly (lactic-co-glycolide) (PLGA)-based scaffold in syngeneic and tolerance-inducing allogeneic models [9,12,15]. However, despite the promising potential shown by the PLGA-based scaffolds, degradation of the polymer releases acidic by-products causing substantial pro-inflammatory responses *in vivo* [16]. The hydrophobic nature of PLGA also interferes with cell infiltration, and would be detrimental to post-transplantation vascular regeneration when lacking porosity [13]. Recent reports also demonstrated excellent outcome post islet transplantation using poly(dimethylsiloxane) (PDMS)-based and polyurethane (PU) scaffolds [17,19], although PU lacks suppleness and flexibility while PDMS tends to become hydrophobic following polymerisation and require extra processes for surface adaptation. In addition, porous hydrogel-based scaffolds fabricated using fibrin and PEG have all been employed to assist islet transplantation. However, the challenge of reproducibility and graft retrieval compared to conventional 3D scaffolds impedes their potential for clinical implementation.

Silk fibroin (SF) is a natural structural protein derived from *Bombyx mori*. Known for its excellent biodegradability and low immunogenicity, SF-based scaffolds have demonstrated exceptional advantages over conventional synthetic and other natural biomaterials in various aspects of tissue engineering [22–26]. Indeed, co-encapsulation of islets with the mesenchymal stem cells (MSCs) and two components of ECM (laminin and collagen IV) by an SF-based hydrogel was reported in which prolonged islet survival and enhanced function were observed *in vitro* [10]. Moreover, an SF matrix incorporated with an endothelial cell binding motif was also proven to be facilitative in "pseudoislets" formation using either MIN6 β-cell or human endocrine cells [27], although *in vivo* potential of using silk fibroin scaffold for islet transplantation has never been reported. We have previously reported fabrication of an interconnected macroporous SF scaffold via lyophilisation, which enables incorporation of cellular mediators such as cytokines, growth factors and proteins without compromising their biological activity [28]. Since heparin has been shown to enhance islet angiogenesis by stabilising VEGF and other growth factors [29] while dampen local inflammatory reactions [30], we have also prepared a heparin-releasing SF scaffold to evaluate the versatility of the SF scaffold for biological functionalisation in islet transplantation. Thus, a "proof-of-concept" study was conducted by investigating the *in vivo* therapeutic efficacy of the SF scaffolds (heparinised and non-heparinised) in syngeneic islet transplantation. Moreover, islets were also obtained from *vegfr2-luc* and *β-actin-luc* transgenic mice for bioluminescence and imaging analysis to further illustrate the mechanistic role of heparin in islet revascularisation and survival after co-transplantation with the heparin-SF scaffold.

2. Materials and methods

2.1. Scaffold preparation and characterisation

Fabrication of the macroporous SF scaffold was carried out as previously detailed [28]. Briefly, SF solution was concentrated to 20 wt% by dialysis against 15 wt% poly (ethylene oxide) (Mw = 20,000) aqueous solution. Ethanol was added to adjust SF concentration. The solution was then poured into a cylinder-shaped polytetrafluoroethylene mold (height 1 cm, diameter 1.5 cm) and frozen at 20, −70 or −196 °C, followed by lypophilisation to form a porous SF scaffold. The scaffolds were immersed in 90% methanol for 30 min to induce SF crystallization, and dried at room temperature in vacuum for 2 days. All scaffolds were cut into cylinder-shaped slices (height 0.5 mm, diameter 5 mm) for islet transplantation. The scaffolds were examined by scanning electronic microscope (SEM). For Heparin-SF scaffolds, 2 wt% heparin sodium salt extracted from porcine intestinal (Mw 14,000 Da, Pierce) was added to the SF solution. Heparin-releasing kinetics was determined as previously described by toluidine blue assay [28].

2.2. Animals and induction of T1

Male C57BL/6 mice and GFP transgenic C57BL/6 mice (8 weeks, 20 g) were purchased from the Laboratory Animal Centre of the Academy of Military Medical Sciences (Beijing, China). The