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The impact of cell surface PEGylation and short-course immunotherapy on islet graft survival in an allogeneic murine model

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ABSTRACT

Islet transplantation is a promising therapy for Type 1 diabetes mellitus; however, host inflammatory and immune responses lead to islet dysfunction and destruction, despite potent systemic immunosuppression. Grafting of poly(ethylene glycol) (PEG) to the periphery of cells or tissues can mitigate inflammation and immune recognition via generation of a steric barrier. Herein, we sought to evaluate the complementary impact of islet PEGylation with a short-course immunotherapy on the survival of fully-MHC mismatched islet allografts (DBA/2 islets into diabetic C57BL/6J recipients). Anti-Lymphocyte Function-associated Antigen 1 (LFA-1) antibody was selected as a complementary, transient, systemic immune monotherapy. Islets were PEGylated via an optimized protocol, with resulting islets exhibiting robust cell viability and function. Following transplantation, a significant subset of diabetic animals receiving PEGylated islets (60%) or anti-LFA-1 antibody (50%) exhibited long-term (>100 d) normoglycemia. The combinatorial approach proved synergistic, with 78% of the grafts exhibiting euglycemia long-term. Additional studies examining graft cellular infiltrates at early time points characterized the local impact of the transplant protocol on graft survival. Results illustrate the capacity of a simple polymer grafting approach to impart significant immunoprotective effects via modulation of the local transplant environment, while short-term immunotherapy serves to complement this effect.

Statement of Significance

We believe this study is important and of interest to the biomaterials and transplant community for several reasons: 1) it provides an optimized protocol for the PEGylation of islets, with minimal impact on the coated islets, which can be easily translated for clinical applications; 2) this optimized protocol demonstrates the benefits of islet PEGylation in providing modest immunosuppression in a murine model; 3) this work demonstrates the combinatory impact of PEGylation with short-course immunotherapy (via LFA-1 blockage), illustrating the capacity of PEGylation to complement existing immunotherapy; and 4) it suggests macrophage phenotype shifting as the potential mechanism for this observed benefit.

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1. Introduction

Type 1 diabetes mellitus (T1DM) is an autoimmune disorder characterized by the destruction of the insulin producing beta cells within a patient's pancreatic islets of Langerhans [1]. Replacement of beta cells via intraportal infusion of allogeneic islets has the promise of providing a long-term cure for T1DM [2,3]. Recent clinical islet transplantation (CIT) trials have reported sustained improvement in metabolic control, with 57% of patients achieving insulin

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independence and ~70% with measureable c-peptide levels after 5 yrs [3–6]. It has become evident, however, that significant inflammatory and immunological host responses to the islets lead to islet dysfunction and destruction. It has been estimated that as much as 60% of the transplanted islets are lost during the first week due to a potent instant blood-mediated inflammatory reaction (IBMIR), activated when islets are in direct contact with blood [7–9]. Further, despite the use of immunosuppression regimens, a smoldering allogeneic and autoimmune response to the transplanted islets persists, ultimately resulting in rejection [10–13]. Consequently, the development of effective strategies for alleviating early inflammatory events and minimizing the burden of systemic immunosuppression would significantly improve long term efficacy of islet grafts and broaden clinical translation.

Islet encapsulation within biomaterials is an appealing approach to mitigate immunological responses to foreign grafts. Encapsulation consists of creating a biocompatible semipermeable barrier that serves to separate the tissue graft from the host's immune effectors, both cellular and humoral, while permitting the proper diffusion of nutrients such as oxygen and glucose, as well as metabolic waste and therapeutic cell products, such as insulin [14]. Traditional approaches of cell microencapsulation result in a significant void space between the islet and its surrounding environment, leading to hypoxia-induced necrosis and lags in glucose responsiveness, as well as substantial increases in graft volumes that limit implantation site options [15,16]. Thinner coatings (<100 μm) would minimize these problems, as described in recent publications [17–21], although further optimization and *in vivo* validations are needed.

An alternative approach to full barrier polymeric encapsulation is cell surface modification via poly(ethylene glycol) (PEG) conjugation. PEGylation, the conjugation of PEG to proteins or cell surfaces, is typically achieved using the heterofunctional PEG: NHS-PEG-CH_3 (NHS-mPEG). The N-hydroxysuccinimide (NHS) group permits spontaneous reactivity to free amines, while the methyl group (CH_3) provides an inert terminal end. It has long been established that the PEGylation of exogenous proteins increases their half-life and reduces immunogenicity without affecting function [22,23], while PEGylation of cell surfaces, specifically red blood cells, reduces antigenicity *in vitro* and *in vivo* [24]. Overall, PEGylation of the islet cell cluster is a highly attractive approach to mask graft recognition, as this simple and efficient conjugation strategy can be easily performed prior to transplantation without altering the transplant procedure (i.e. islets can still be infused into the liver). Given this appeal, islet surface PEGylation has been explored using varying approaches, with minimal adverse effects on islet function or viability observed [25–27]. *In vivo*, however, PEGylation alone has not been shown to significantly extend allograft survival in rodent models, with the exception of a single study exhibiting modest protection using a triple PEGylation procedure [28]. Further, the local impact of PEGylation on host cell responses to allografts has not been studied.

While islet PEGylation as a singular approach has not shown substantial promise, selected studies have demonstrated a synergistic impact when combined with low-dose or local immunosuppression [29–31]. In this study, we sought to explore the impact of a targeted and short-course immune intervention used in combination with islet PEGylation. Lymphocyte Function-associated Antigen-1 (LFA-1) is a surface integrin found on immune cells and is involved in cell trafficking to sites of injury and/or infection, immune synapse stabilization, and co-stimulation [32]. LFA-1 blockade as a monotherapy has demonstrated success in delaying, but not completely preventing, murine allograft rejection [33]. Further, it has shown to be synergistic with other immune interventions [34–37]. Herein, the effects of PEGylation, alone or in combination with a short-course LFA-1 blockade, on graft survival and the local impact on host cell phenotypes were explored.

2. Materials and methods

2.1. Reagents and polymer fabrication

All chemical reagents were purchased from Sigma-Aldrich, unless otherwise noted. All culture media was sourced from Mediatech.

2.2. Fabrication of NHS-PEG-CH_3

NHS-PEG-CH_3 was fabricated by dissolving NH_2-PEG-CH_3 (2 g, JenKem Technology USA, MW 5000 Da) in 4 mL of anhydrous N, N'-dimethylformamide (DMF) at 37 °C under Argon. A solution consisting of glutaric anhydride (50 mg) dissolved in 0.4 mL DMF was then injected drop-wise into the PEG solution, followed by drop-wise injection of a solution consisting of 112 μL triethylamine in 0.4 mL DMF. After stirring for 25 min, the product (COOH-PEG-CH_3) was precipitated with 60 mL cold diethyl ether, collected by centrifugation, and dissolved in 60 mL absolute ethanol at 37 °C. The solution was then filtered through a 5 mm silica gel plug inside a Pasteur pipet and the polymer was precipitated and collected by cooling in an ice-water bath and centrifugation. This product was rinsed by vortex-shaking with 60 mL cold diethyl ether, collected by centrifugation, and dried under reduced pressure.

The above product (925 mg) and N-hydroxysuccinimide (65 mg) were dissolved in 1.5 mL DMF at 37 °C under Argon. A solution of 176 μL diisopropylcarbodiimide (AnaSpec) was dissolved in 0.4 mL DMF and injected to the PEG solution. After stirring for 2 h under Argon, the product was precipitated with 40 mL cold diethyl ether and collected by centrifugation. This was subsequently dissolved in 32 mL absolute ethanol at 37 °C, precipitated by cooling in an ice-water bath, and collected by centrifugation. The product was rinsed with 40 mL cold diethyl ether, collected by centrifugation, and dried under reduced pressure. The final yield was 900 mg of NHS-mPEG powder. Chemical modifications throughout this process were monitored by FT-IR.

2.3. Islet isolation and culture

Studies involving animals were performed under protocols reviewed and approved by the University of Miami and the University of Florida IACUC. Male DBA/2 J (H-2^d) mice, between 10 and 12 weeks of age (Jackson Laboratory; Bar Harbor, Maine), were used as islet donors. Islets were isolated as previously described, counted, and scored for size using the islet equivalent (IEQ) method [38]. Islets were cultured at 37 °C, 5% CO_2 in CMRL 1066-based medium supplemented with 10% FBS, 20 mM Hepes Buffer, 1% penicillin-streptomycin, and 1% L-glutamine.

2.4. Pegylation

Isolated islets were cultured for 48 h prior to PEGylation. On the day of the procedure, islets were counted, washed thrice in DPBS, and incubated for 45 min at 37 °C in a freshly prepared 4 mM NHS-mPEG solution in DPBS (titrated to pH 7.8 before addition of mPEG) supplemented with Ca^{2+}/Mg^{2+} and 11 mM D-glucose at a cell density of 1500 IEQ/mL. Following PEGylation, the islets were washed thrice in full media and placed in the incubator for overnight culture prior to assessment or transplantation. For imaging of islet coating, NHS-PEG-FITC (5000 MW, NANOCS Co) was used in lieu of NHS-mPEG.

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