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Local release of dexamethasone from macroporous scaffolds accelerates islet transplant engraftment by promotion of anti-inflammatory M2 macrophages

Kaiyuan Jiang [a, b], Jessica D. Weaver [b], Yangjunyi Li [a], Xiongjian Chen [a], Jiapu Liang [a], Cherie L. Stabler [a, b, *]

[a] J. Crayton Pruitt Family Department of Biomedical Engineering, College of Engineering, University of Florida, Gainesville, FL, USA
[b] Diabetes Research Institute, University of Miami, Miami, FL, USA

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ABSTRACT

Transplant-associated inflammatory responses generate an unfavorable microenvironment for tissue engraftment, particularly for cells susceptible to inflammatory stress, such as pancreatic islets. The localized delivery of anti-inflammatory agents, such as glucocorticoids, offers a promising approach to minimize the detrimental side effects associated with systemic delivery; however, the dosage must be carefully tailored to avoid deleterious responses, such as poor engraftment. Herein, we employed a polydimethylsiloxane (PDMS)-based three-dimensional scaffold platform for the local and controlled delivery of dexamethasone (Dex). Incorporation of 0.1% or 0.25% Dex within the scaffold was found to significantly accelerate islet engraftment in a diabetic mouse model, resulting in improved control of blood glucose levels during the early transplant period. Investigation into the mechanism of this impact found that local Dex delivery promotes macrophage polarization towards an anti-inflammatory (M2) phenotype and suppresses inflammatory pathways during the first week post-implantation. Alternatively, higher Dex loadings (0.5% and 1%) significantly delayed islet engraftment and function by impairing host cell migration into the implanted graft. Our results demonstrate the dose-dependent impact of local glucocorticoid delivery on the modulation of inflammatory responses at the implant site *in vivo*. Outcomes highlight the potential of this platform for generating favorable host responses that improve overall cellular transplant outcomes.

1. Introduction

Type 1 diabetes (T1D) is an autoimmune disease resulting in the targeted destruction of insulin-secreting beta cells. Clinical islet transplantation, where allogeneic pancreatic islets are infused into the portal vein of the liver, has achieved insulin independence for individuals suffering from labile diabetes with severe hypoglycemic unawareness [1]. Graft efficacy is unstable, however, as multiple transplant-related factors, such as poor engraftment and host inflammatory and immunological responses, result in the significant loss of islets [2]. In an effort to improve graft duration, our laboratory developed a three-dimensional macroporous polydimethylsiloxane (PDMS) scaffold for housing transplanted islets [3]. Placement of islet-loaded scaffolds within an extrahepatic site (i.e. the omentum) results in stable efficacy in syngeneic and allogeneic diabetic rodent and nonhuman primate models [4]. The highly interconnected and porous structure of the PDMS scaffold facilitates positive interactions between graft and host tissue, resulting in a supportive microenvironment [3]. Further, the localization of islets within a defined implant permits ease of monitoring and retrieval, as well as the integration of beneficial factors, such as proangiogenic hydrogels [5].

With the potential of this 3-D platform to locally instruct host responses to the islet graft, efforts were focused on decreasing inflammatory events instigated post-transplant. In clinical studies, inflammation has been implicated as a prominent player in decreasing islet viability and graft efficacy [6]. Activation of the inflammatory cascade starts early, whereby pro-inflammatory mediators, such as tissue factor and high-mobility group box-1

* Corresponding author. J. Crayton Pruitt Family Department of Biomedical Engineering, College of Engineering, University of Florida, Gainesville, FL, USA.
E-mail address: cstabler@bme.ufl.edu (C.L. Stabler).

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protein, are upregulated by stressed islets following pancreatic isolation. The transplantation of islets subsequently triggers thrombosis, complement cascade, and an instant blood-mediated inflammatory reaction (IBMIR), which results in early islet loss (~60% of the islet mass) and the promotion of adaptive immune responses [7,8]. When biomaterials are added to the transplant microenvironment, activation of inflammatory processes can be further amplified, due to foreign body responses (FBR) [9]. To mitigate inflammation in clinical islet transplantation, supplemental administration of TNFα and IL-1β blockers (etanercept and anakinra) has been shown to dampen pro-inflammatory effectors and lead to improved allograft survival [10]. The intravenous delivery of these agents, however, results in detrimental systemic impacts such as an increased risk of serious infections.

The engineering of a localized drug delivery platform could avoid the side effects of systemic anti-inflammatory regimens, while also precisely modulating inflammatory responses at the transplant site. An optimal drug and dosage would suppress active inflammatory pathways and instruct local host responses towards desirable phenotypes. Directing macrophage phenotype is of particular interest, as macrophages are key contributors in determining the overall outcome of the host response to an implant [11]. During normal activation and resolution of inflammatory processes, the macrophage population covers a balanced spectrum of phenotypes. On one end of this spectrum are classically activated macrophages (termed M1), which generate a microenvironment rich in reactive oxygen species, degradative enzymes, and pro-inflammatory cytokines (e.g. IL-1β, IL-6, and TNFα) [12]. On the other end are the alternatively activated subtype (termed M2) that is associated with a different portfolio of cytokines (e.g. IL-10 and TGFβ) that facilitate healthy tissue remodeling [13]. Local pro-inflammatory instigators and undesirable implants tip this balance towards M1, which can result in detrimental downstream events such as the generation of a fibrotic capsule, the promotion of detrimental Th1 responses, and the destruction of the graft [14]. Alternatively, modest promotion of the M2 phenotype has been shown to direct positive tissue remodeling, resulting in functional engraftment of the implant [15]. These M2 macrophages impart additional benefits to islet transplants, as they have been shown to promote islet function and beta cell proliferation [16].

A strong drug candidate for this approach is dexamethasone (Dex). Dex, an immunosuppressive glucocorticoid capable of potent suppression of inflammatory pathways, has been shown to polarize human blood-derived monocytes toward the anti-inflammatory (M2) phenotype, while retaining their migratory function [17,18]. The Dex dosage must be carefully tailored, however, to prevent overloading at the implant site, as elevated glucocorticoids can severely impair cell mobility, resulting in compromised engraftment and vascularization [19]. Further, Dex has also been shown to impair the glucose responsiveness of beta cells at high concentrations [20]. In this study, we utilized our macroporous PMDS scaffold platform to locally deliver dexamethasone in a controlled manner within the desired therapeutic range (Fig. 1). This streamlined approach eliminated the need for disparate drug depots, as previously explored [21]. Evaluation of kinetic release of Dex from the 3-D scaffold permitted tailoring of drug loading to desired doses. Following optimization, the impact of this drug-release platform on the efficiency of islet engraftment and subsequent host cell responses were investigated.

2. Materials and methods

2.1. Fabrication of Dex-PDMS scaffolds and characterization of Dex-releasing kinetics

Macroporous PDMS scaffolds of 85% total porosity were fabricated by particle leaching and solvent casting, as described previously [3]. In brief, the PDMS matrix was prepared by mixing PDMS part A with cross-linker part B (RTV 615 A/B kit, Momentive) at a ratio of 4:1 (A:B). Dexamethasone (Enzo life sciences) was subsequently added to the PDMS mixture at the desired percentage (1, 0.5, 0.25, and 0.1% weight-to-weight, which results in a total mass of 182, 90.5, 45.1, and 18 μg Dex per scaffold implant). Sodium chloride crystals (Fisher Chemicals), which served as porogens, were then added into the Dex-PDMS mixture and the resulting slurry was mixed and degassed using automated mixer (Thinky). Control (drug-free) and Dex-PDMS mixtures were then loaded within a 25 mm diameter mold and cured at 40 °C overnight under 500 psi compression using a compression molder (Across International). The cured disks (25 mm) were soaked in 1.5 L of deionized water and agitated for 3 days to leach out the salt particles, with complete water exchange twice a day. Scaffolds (4.5 × 4.5 mm square by 1.5 mm height) were cut from the 25 mm disk, as previously described [5].

To evaluate Dex-releasing kinetics, samples (n = 3 per group) were incubated in 5 mL 1% benzalkonium chloride (BKC; Sigma-Aldrich) solution on a rotary shaker (Thermo Fisher) for 30 days, with total fluid exchanges and samples collected daily. 1, 0.5, 0.25, 0.1% w/w Dex-PDMS scaffolds, as well as Dex-free PDMS control scaffolds, were included in the experiment. BKC was used as a solubilizing agent to promote a high sink condition and mimic the clearance of the steroid *in vivo* [22]. Released Dex was quantitated by dexamethasone ELISA kit (Neogen; sensitivity 0.23 ng/mL). Dex release was represented as ng of Dex released per day per scaffold (transplant volume size).

2.2. Islet isolation and transplantation within scaffolds at extrahepatic site

All animal procedures were performed under protocols approved by the University of Miami or University of Florida IACUC and in accordance with National Institutes of Health guidelines. Drug-free and 1, 0.5, 0.25, and 0.1% w/w Dex PDMS scaffolds were fabricated, as described above. Scaffold batches tested negative for endotoxins (<0.25 EU/mL; Lonza). Male C57BL/6 mice (The Jackson Laboratory) were used as transplant recipients. Diabetes was induced by intravenous injection of streptozotocin (200 mg/kg; Sigma-Aldrich), as previously described [5], and confirmed by three consecutive days of non-fasting blood glucose levels > 350 mg/dL. Diabetic recipients were transplanted within 10 days post-induction. The body weight of recipients averaged 22.64 ± 2.66 g, with no significant deviation in average body weight between groups. Donor pancreatic islets were isolated from heathy male C57BL/6 mice (The Jackson Laboratory, USA), as described elsewhere [5]. Prior to islet loading, scaffolds were pre-incubated with human plasma fibronectin (0.25 mg/mL; Life Technologies), to permit ease in islet loading within the highly hydrophobic PDMS scaffold, as previously described [5]. Diabetic recipient mice were prepared for surgery, the right epididymal fat pad (EFP) in the lower intraperitoneal cavity was gently spread out, and a PDMS scaffold piece (size equivalent to 4.4 mm × 4.4 mm square) was placed on the unfolded EFP. The syngeneic islets (500 IEQ) were collected in a Hamilton syringe and pipetted on the top of the scaffold, where they distributed into the pores via gravity-driven fluid flow, as outlined previously [5]. The implant was subsequently wrapped in the EFP and sealed by fibrin gel (7.5 μL fibrinogen solution; 8 mg/mL; added to 7.5 μL of thrombin solution; thrombin (2 U/mL), aprotinin (85 mg/mL), 5 mM $CaCl_2$, 150 mM NaCl, and 20 mM HEPES), as described elsewhere [21]. The groups and number of animals used in efficacy studies were as follows: drug-free control PDMS scaffolds (n = 14), and 1% Dex (n = 4), 0.5% Dex (n = 4), 0.25%