Development of Controlled Drug-Releasing Constructs to Mediate Inflammation in an Islet Transplant Site

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Statement of Purpose: Clinical islet transplantation has demonstrated promise for the reversal of Type 1 Diabetes Mellitus; however, inflammation at the transplant site contributes to significant graft loss post transplantation. Administration of systemic anti-inflammatory drugs has improved this outcome, but typically contributes to adverse effects on patient health. Alternatively, the localized, sustained delivery of anti-inflammatory/immune-modulatory agents at the transplant site has the potential to reduce detrimental inflammation while also avoiding potential systemic complications. Previous studies on the incorporation of hydrophobic drugs within hydrophobic materials have demonstrated the potential of this strategy for controlled, long-term release. Critical design parameters in the development of sustained drug delivery platforms include material selection, surface area, volume, geometry and drug loading. In our model system, we evaluate the local delivery of the anti-inflammatory corticosteroid dexamethasone from a polydimethylsiloxane (PDMS) implantation device in an islet transplant site, and the capacity of this platform to reduce inflammatory cell infiltration within islet grafts.

Methods: *PDMS-Dexamethasone (Dexa) construct fabrication:* PDMS-Dexa was fabricated by mixing PDMS and dexamethasone (Dexa) to homogeneity and curing at 40°C for > 4hrs. Constructs were formed by punching 3.8mm diameter disks from a 2mm thick slabs or extruding rods from 2.8mm diameter tygon tubing. Dexa release was measured over 30 days. *Il-6 Suppression in Macrophages (MØ) by PDMS-Dexa disks*: THP-1 Monocytes (ATCC) were activated by PMA (Sigma) and cultured to *MØ* differentiation for 8 days. Two PDMS-Dexa or PDMS-only disks were added and cells stimulated for 6 hrs with LPS (Sigma). *Inflammatory cell suppression by PDMS-Dexa rods in a syngeneic mouse transplant model:* Blank PDMS or Dexa-loaded (1 or 2, 10%) PDMS rods were transplanted with syngeneic islets housed in a PDMS scaffold in the epididymal fat pad (EFP) of diabetic mice. Blood glucose levels were tracked to assess diabetes reversal. Explants were removed at day 90 and histologically assessed (Masson's Trichrome, CD45, and Insulin). For short-term studies, whereby constructs were harvested on days 3 and 6 for phenotypical analysis of inflammatory cell populations (CD45, CD11b, F4/80, CD86) by flow cytometry.

Results: PDMS-Dexa construct design parameters (surface area, volume, drug load) were optimized to achieve sustained drug release within a targeted therapeutic range (0.05-0.5µg/day) for both disk and rod geometries, demonstrating release within this range for a minimum of 30 days. PDMS-Dexa constructs (5, 10, 20% load) co-incubated with activated THP-1 *MØ* suppressed overall levels of IL-6 release upon stimulation with LPS. In vivo drug release from PDMS-Dexa rods demonstrated no detrimental impact on time-to-diabetes-reversal in mice. Phenotypical assessment of early (acute) inflammation at days 3 and 6 revealed a strong effect of the presence of PDMS-Dexa rods on monocyte and *MØ* migration and activation. Specifically, overall CD45 expression and CD86 activation was lower ($p < 0.05$) in both rod groups on day 3 compared to blank controls, indicating infiltration of fewer leukocytes due to the presence of released Dexa. Further, a significant ($p < 0.05$) decrease in the percentage of monocytes (CD11b+F4/80-) and overall CD11b cells in both PDMS-Dexa rod groups was found at days 3 and 6. Also, significant ($p < 0.05$) decrease in *MØ* (CD11b+F4/80+) populations and overall F4/80 cells was observed on day 3 for the 2 PDMS-Dex rod group only. Masson's Trichrome staining of explants at day 90 demonstrated reduced overall host cell infiltration in PDMS-Dexa rod groups, while immunofluorescence staining of sections exhibit reduced presence of infiltrating CD45+ leukocytes in PDMS-Dexa rod groups compared with controls. Transplanted islets stabilized blood glucose levels, and displayed positive insulin immunostaining.

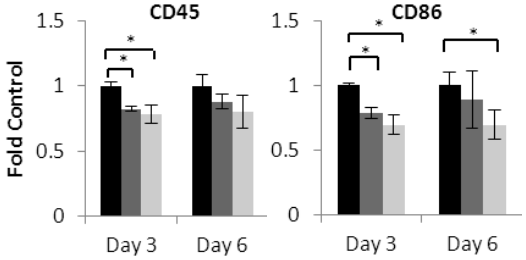

Figure 1. FACS assessment of cells in an islet transplant site with blank PDMS rods (black) vs. 1 (dark gray) or 2 (light gray) PDMS-Dexa rods demonstrate reduced levels of leukocytes (CD45) and cell activation (CD86) (*$p<0.05$).

Conclusions: Herein, we demonstrate the safety and efficacy of PDMS-Dexa constructs custom-designed for controlled and sustained drug release through optimization of key design parameters. These drug-releasing constructs exhibit the capacity to suppress inflammatory cell activation in vitro and in vivo, without demonstrable adverse effects on islet engraftment or function. This model platform provides an effective tool for localized delivery of anti-inflammatory drugs to an islet transplant site, whereby reduced inflammatory cell infiltration may improve islet engraftment in more complex models. Future studies will investigate the effectiveness of this strategy in allogeneic rodent models.

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