Methacrylic acid-based hydrogel and tuned inflammatory response enable subcutaneous allogeneic islet survival

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Introduction: Subcutaneous delivery of insulin producing cells (primary islets or stem cell derived cells) is a promising regenerative therapy for type 1 diabetes. The subcutaneous space is a uniquely advantageous site because it is easily accessible and large enough to accommodate the cell mass necessary to reverse diabetes. However, subcutaneous transplant survival is precluded by the lack of vasculature and innate immune response. The Sefton lab has previously shown that pancreatic islets subcutaneously injected in an inherently vascularizing[1], immunomodulating[2], degradable, and synthetic methacrylic acid (MAA)-based hydrogel ("MAA-PEG") survived, revascularized and reversed diabetes in immunocompromised mice[3]. No pre-vascularization was required. Here we further the use of this platform in immune competent C57BL/6 mice.

Methods: MAA-PEG hydrogel precursor solutions were prepared as previously reported [3]. Primary islets (600 equivalent units, IEQ) were isolated from BALB/c mice and injected into the subcutaneous space of streptozotocin (STZ)-induced diabetic C57BL/6J mice (allograft) in a MAA-PEG hydrogel precursor solution; MAA-PEG gels within minutes after injection via dithiothreitol (DTT). Non-fasting blood glucose levels are used to track implant function and diabetes reversal. Flow cytometry and protein measurements (LEGENDplex) of explants were used to characterize the host response.

Results: Allo-islets subcutaneously injected in MAA-PEG reversed diabetes in STZ-C57BL/6J mice (similar to our previous work [1,3]), but only with added peri-operative neutrophil "depletion" therapy (Figure 1). Neutrophil "depletion" was Ly6G antibody (clone 1A8) treatment from day -3 to 0, or -3 to 3. Other forms of immunosuppression ("other IS," including CTLA4-Ig and rapamycin) or using syngeneic islets without anti-Ly6G had little effect (not shown). We presume that islets were destroyed by the innate neutrophil response to the material, not by a specific rejection. Islets injected in PEG alone (no MAA) but with anti-Ly6G did not survive (as expected [3]). These results highlight the need for the vascularizing, regenerative effects of MAA and a dampened early neutrophil response.

Strikingly, some implants survived for weeks (and more than 50 days in some cases, not shown) without any other immunosuppression. However, results were variable and only ~50% of implants survived (reduced glucose levels) after day 3 (Figure 1).

Towards a more reproducible method, we measured graft-associated proteins on day 1 and found high levels of CXCL1 (Figure 2A), a neutrophil-recruiting chemokine. We expect that more specific, targeted inhibition of the early inflammatory response (neutrophils) will reduce the variation we see in Figure 1. Moreover, targeting specific signaling molecules or cytokines would be more translatable; the Ly6G antibody will not work in humans since Ly6G is a mouse protein.

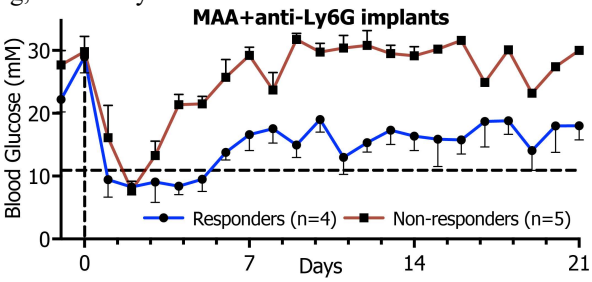

Figure 1 – Allogeneic islets subcutaneously injected in degradable MAA-PEG with neutrophil depletion (anti-Ly6G) survive and reverse diabetes in C57BL/6J mice, but results are variable. Data shows non-fasting blood glucose readings from treated mice segregated into responding and non-responding animals. 600 BALB/c IEQ subcutaneously injected with MAA-PEG. Hydrogels degrade within ~7 days. Error bars are S.E.M.

Targeting CXCL1 signaling via its receptor (CXCR2 inhibition) led to a significant (~10 fold) reduction in neutrophil recruitment to a subcutaneous MAA-PEG implant (Figure 2B) on day 1. The extent of reduction was not as great as that seen with anti-Ly6G (although this may be an artifact from epitope "masking" [4]); further optimization of this protocol is necessary.

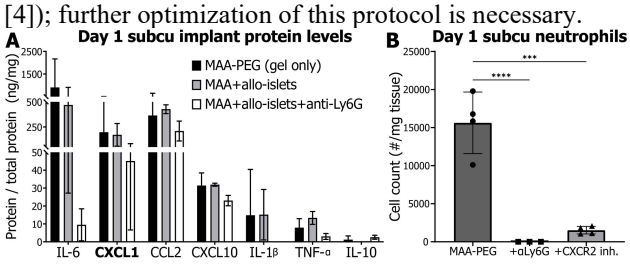

Figure 2 – Disrupting CXCL1 signaling reduces neutrophils at the subcutaneous MAA hydrogel implant on day 1. (A) Protein levels from allo-islet MAA-PEG grafts explanted on day 1, normalized to total protein content. N=2-3. (B) The number of neutrophils recovered from subcutaneous MAA-PEG hydrogels (no islets) explanted on day 1. CXCR2 inhibitor was dosed on day -1 and 0 at 10 mg/kg. n=4. Anti-Ly6G was dosed at 250µg per day from day -3 to 0. ***p<0.001, ****p<0.0001. Error bars are SD.

Conclusions: The unique, vascularizing host response to MAA materials allows islets injected subcutaneously in MAA hydrogels to survive and reverse diabetes. We found that the neutrophil response had to be dampened in C57BL/6J mice for islet survival. Targeted inhibition of neutrophil signaling achieves similar results to a broad, mouse-specific antibody. Islet transplant experiments with the inhibitor are ongoing. Our work is a step towards a clinically-relevant, biomaterial-based approach for islet transplantation as a treatment for diabetes.

References:[1] Mahou, R. *Biomaterials*. 2017;131:27-35
[2] Kinney, SM. *Biomaterials.* 2023; 301:122265.
[3] Kinney, SM. *Biomaterials.* 2021; 281:121342.
[4] Boivin, G. *Nat Commun*. 2020; 11: 2762.