Methacrylic Acid-based Synthetic Hydrogel Enhances Immunomodulation by Tolerogenic Dendritic Cells

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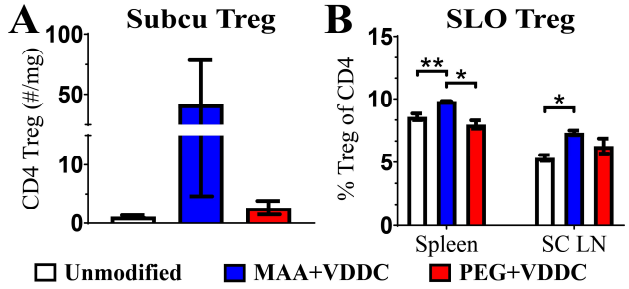
Statement of purpose: Type 1 Diabetes (T1D) is an autoimmune disease that results from the destruction of the insulin-secreting pancreatic islet cells. Islet transplantation is an alternate, regenerative therapy compared to insulin injections due to the innate ability of islets to monitor and regulate blood glucose, although the persistent islet-targeting autoimmune and allograft rejection immune responses are still detrimental to long-term islet graft survival (even with immunosuppression)[1]. Immune suppression can attenuate acute rejection and prolong graft survival but is often non-specific, increasing the risk of infection and other complications[1,2]. In this work we study the efficacy of tolerogenic dendritic cells (tolDCs) delivered in an inherently regenerative degradable poly(sodium methacrylate)-poly(ethylene glycol) (MAA-PEG) hydrogel as a therapeutic immunomodulating platform, in autoimmune and allo-islet graft T1D mouse models. The Sefton lab has previously shown that MAA based biomaterials are inherently vascularizing[3] and regenerative[4]; these effects are dependent on polarization of the foreign body response by MAA towards an "alternatively activated," regenerative state[4]. Of particular relevance, we have found that these regenerative properties of MAA biomaterials, including MAA-PEG, improve the outcomes of islet transplantation in the subcutaneous space[5,6]. Here we focus on the ability of tolDC delivered in MAA-PEG to expand antigen-specific $CD4^+CD25^+FoxP3^+$ Treg populations at the implant site and related lymph tissues; these Tregs will attenuate autoimmune responses[7] and allo-islet rejection[8]. **We hypothesize that expansion of Tregs by VD-DCs will be enhanced by delivery in MAA-PEG, with benefits in both autoimmune and allograft transplant contexts.**

Methods: Tolerogenic dendritic cells were obtained by culturing bone marrow in the presence of the active form of vitamin D_3 (10^8 M) ("VDDC")[9]; VDDC were purified with $CD11c^+$ beads and primed for 4h with islet lysate. Flow cytometry was used to determine the impact of VDDC delivery on T cell populations (focusing on Tregs). To track migration, DC were stained with a far-red membrane dye (Xenolite DiR) that could be visualized by daily imaging on an IVIS spectrum *in vivo* imager.

Results and Discussion: Subcutaneous delivery in a degradable MAA-PEG hydrogel enhanced the expansion of Tregs by VDDCs at the subcutaneous implant site (Fig 1A) and secondary lymph tissues (Fig 1B) by day 7. Pilot experiments tracking migration of VDDC delivered subcutaneously in MAA-PEG suggest that by day 7, a portion of VDDCs remain at the subcutaneous site, while others migrate to secondary lymph organs (Fig 1C); consistent with the expansion of Tregs seen in Fig 1A,B. These results show that VDDCs expand relevant Treg populations in an autoimmune context, and that this action appears to be enhanced by delivery in MAA-PEG. Follow-up experiments will further explore immunomodulation by VDDCs in MAA-PEG in NOD mice (autoimmune model), and allo-islet grafts in C57BL/6 mice (allograft model). We will also validate migration of VDDC in both contexts using imaging techniques and DCs derived from animals with unique markers that can be verified by flow cytometry and qPCR. **Together, these results will show that synthetic MAA-based hydrogels are effective platforms for tolDC-based immunomodulation strategies that could help to make regenerative, insulin injection-free therapies a feasible treatment for T1D.**



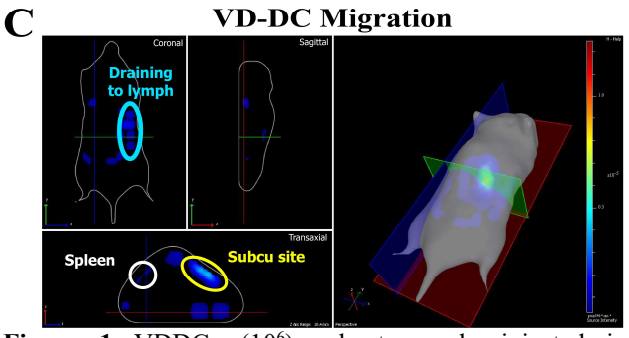


Figure 1 VDDCs (10^6) subcutaneously injected in degradable MAA-PEG hydrogels migrate and expand Treg populations by day 7. $CD4^+CD25^+FoxP3^+$ Treg populations in mice treated with VDDCs in MAA-PEG were greater on day 7 at the subcutaneous implant site (A), and (B) the spleens and subcutaneous lymph nodes than those treated with VDDC in PEG, or untreated controls. (C) Migration of VDDC delivered in MAA-PEG from the subcutaneous implant site (yellow circles), highlighting VDDC signal appearing in the spleen (white circle) and migrating towards the subcutaneous draining lymph nodes (blue circles). 8 w/o, female NOD mice were used as VDDC donors and recipients. n=3. * $p<0.05$, ** $p<0.01$.

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