Polyethylene Glycol Maleimide Hydrogels for Vascularization of Transplanted Pancreatic Islets

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Statement of Purpose: Type 1 diabetes affects one in every 400-600 children in the US. Standard therapy with exogenous insulin is burdensome, associated with significant hypoglycemia risk, and only partially efficacious in preventing long term complications. Pancreatic islet transplantation has emerged as a promising therapy for type 1 diabetes. However, this cell-based therapy is significantly limited by inadequate revascularization of transplanted islets resulting in reduced islet viability, function, and engraftment. We engineered polyethylene glycol-maleimide hydrogel matrices to incorporate pro-angiogenic signals as supportive matrices to improve pancreatic islet engraftment and vascularization. This hydrogel chemistry is advantagous for cell delivery due to mild crosslinking that occurs rapidly enough for *in situ* delivery, while easily lending itself to "plug-and-play" design variations. Combined with a novel transplantation model, we present a robust strategy incorporating advanced biomaterials for rapid vascularization of cell and tissue grafts.

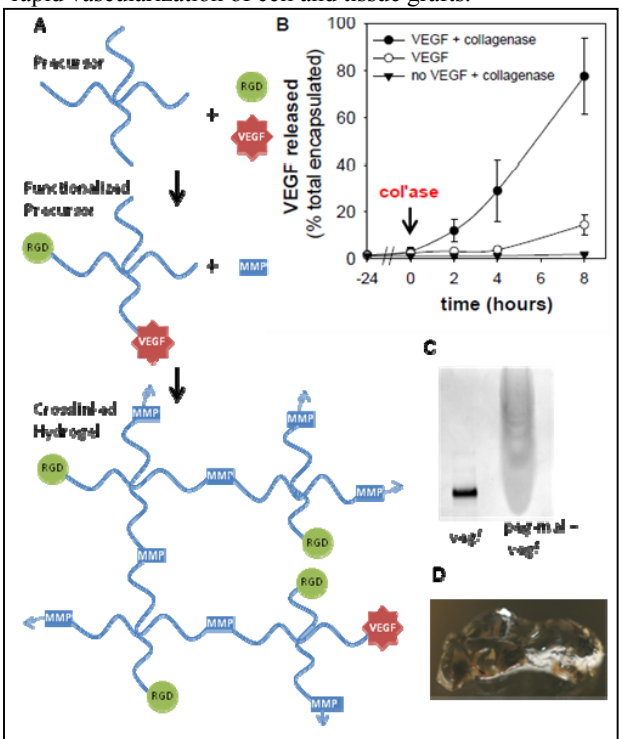

Figure 1. PEG-maleimide hydrogel: (A) Reaction scheme. (B) VEGF release from hydrogels is MMP-dependent. Hydrogels were incubated in collagenase for different time points and VEGF in supernatant was quantified by ELISA. No VEGF was released prior to treatment with collagenase, indicating minimal passive (diffusion) release. (C) Western blot confirming pegylation of growth factor. (D) Sample of PEG-maleimide hydrogel

Methods: Precursors solutions of 4-arm PEG-maleimide were pre-functionalized with RGDS adhesion peptide and growth factor. The precursor molecules were crosslinked into a hydrogel by addition of cyteine-flanked MMP-degradable peptide sequences[1] (Fig 1A). A collagenase gel degradation study measured release of growth factor from the matrix (Fig 1B). In a pilot *in vivo* study, approximately 500 islets isolated from inbred Lewis rats were transplanted into 3 sites in the small bowel mesentery of recipient Lewis rats by delivery in 5% (wt/vol) PEG-maleimide matrix with 2 μM RGDS adhesive peptide and 80 ng/mL VEGF or FGF. The matrix was crosslinked with MMP-degradable peptide *in situ* to adhere the graft to the mesentery tissue. After 7 days, the rats were perfused with fluorescein-lectin to label vasculature and the grafts were examined histologically for neovascularization, insulin expression, and inflammatory response.

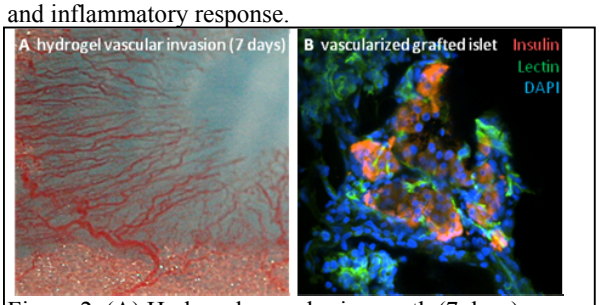

Figure 2. (A) Hydrogel vascular ingrowth (7 days) (B) Engrafted islet showing vascularization and insulin.

Results: Kinetic release studies indicate that PEG-maleimide bound growth factors incorporated into the matrix were released primarily in a proteolytic manner (Fig. 1B). Islets / matrix delivered to the small bowel mesentery exhibited vascular reperfusion (as shown by lectin staining) by 7 days *in vivo*. Furthermore the hydrogel itself exhibited a high degree of vascular invasion (Fig 2A) with markedly stronger vascular response in grafts with FGF or VEGF. Immuno-staining revealed a concentration of grafted islets expressing insulin at the transplant site (Fig 2B). Histological staining indicated vessel penetration into the bulk of the hydrogel with no fibrous encapsulation and low presence of primary inflammatory cells (negative for CD11b).

Conclusions: We present the use of an engineered matrix that supports islet activities and promotes vascularization *in vivo*. This project establishes novel biomaterial strategies for islet delivery that support islet viability and function via the induction of local vascularization. To our knowledge these PEG-maleimide hydrogels have never been used for cell culture or delivery.

References: [1] Patterson J. Biomaterials. 2010;31:7836-45.

Funding: CHoA/GT Center for Pediatric Healthcare Technology Innovation and GTEC/ACTSI