Comparison of Immunoisolation Platforms for Pancreatic Islet Transplantation: Polyethylene Glycol Conformal Coating, Alginate Single and Double Capsules

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Statement of Purpose: Pancreatic islet transplantation improves metabolic control and prevents complications in patients with type-1 diabetes (T1D). However, chronic immunosuppression is required to prevent allograft rejection and recurrence of autoimmunity. The long-term side effects of immunosuppressive therapies limit the applicability of the procedure to patients with severe T1D. Islet encapsulation may eliminate the need for systemic and chronic immunosuppression, while providing glucose-stimulated insulin secretion (GSIS). So far, no encapsulation technology has demonstrated efficacy in humans. Here, we evaluated capsule permeability, *in silico* and *in vitro* dynamic GSIS, and biocompatibility of different encapsulation platforms that have been proven effective in preclinical models of T1D: polyethylene glycol (PEG) conformal coating (CC)[1,2] and alginate (ALG) single capsules (SC) without poly-L-lysine (PLL), and double capsules (DC) with PLL[3]. The overall goal of this work was to identify the strengths and weaknesses of these platforms, to develop a functional immunoisolating platform for islet transplantation in T1D.

Methods: Cell-free PEG and ALG capsules were used for permeability quantification. PEG capsules were obtained manually by suspension of 8arms-PEG-maleimide (10kDa, 75% functionalized, PEG-MAL, Jenkem) with HS-PEG-SH (2kDa, PEG-SH, Jenkem) or dithiothreitol (DTT, Sigma) (ratio 1:9) in polypropylene glycol (4k Mn, PPG, Sigma) + 10% Span80 (Sigma) + 0.02% triethanolamine (TEA, Sigma) to obtain 1 mm-diameter capsules. Cell-free ALG SC were obtained by extruding 2% low viscosity high mannuronic (LVM) or 1.2% medium viscosity high guluronate (MVG) ALG from a 0.5mm nozzle into a gelling $SrCl_2$ solution, using an electrostatic droplet generator. For DC, SC were coated with PLL, cultured overnight, resuspended in 1.26% LVM or 0.95% MVG ALG, and extruded through a 1.1mm nozzle, into the gelling solution. PEG and ALG capsules were dried and resuspended overnight in 1mg/mL FITC-insulin or 2-NBD-glucose solutions. The following day, FRAP analyses were conducted on the capsules to determine the diffusion coefficients (D). Encapsulated human islets (HI) were used for *in vitro* dynamic GSIS. For PEG CC encapsulation, 6.05% (w/v) PEG-MAL was partially crosslinked with 36.2% (w/v) PEG-SH. HI were resuspended in this viscous solution and extruded through a proprietary CC microfluidic device (Biorep) using a PPG + 10% Span80 external oil solution and a 1.65mg/ml DTT/PPG gelling emulsion that was flowed coaxially. For SC and DC encapsulation, HI were resuspended in ALG and extruded into the gelling bath as for cell-free capsules. Size of HI, and thickness of capsules were measured using ImageJ. D and size values were used for dynamic GSIS simulation on COMSOL Multiphysics as previously described[4]. For *in vitro* dynamic GSIS, encapsulated HI were stimulated sequentially with 2.2mM glucose (8min), 16.6mM glucose (20min), 2.2mM glucose (15min), 30mM KCl (10min) and 2.2mM glucose (10min) solutions flowing at 100µL/min. The insulin release profile was determined through ELISA (Mercodia). For biocompatibility studies, cell-free ALG capsules and CC polystyrene beads (PS) were transplanted in the omentum of Lewis Rats. After 21 days, the rats were euthanized, the tissues were harvested and processed for histological evaluation.

Results: *In silico* GSIS from encapsulated islets depended on the size of the islets and on the capsule thickness. The thicker the capsules, the larger the delay in insulin release. PLL coating resulted in further delay in insulin secretion. I*n vitro* dynamic GSIS validated the *in silico* results. While CC HI showed similar first- and second-phase GSIS to naked HI, for SC and DC, increasing ALG capsule thickness resulted in larger insulin delay, which was further increased by PLL coating (**Figure 1**). In biocompatibility studies, we observed that small CC capsules (200µm diameter) caused macrophage activation and accumulation of foreign body giant cells (FBGCs) on the surface of capsules. Instead, larger capsules (>500µm) led to pericapsular fibrosis independently of the coating material.

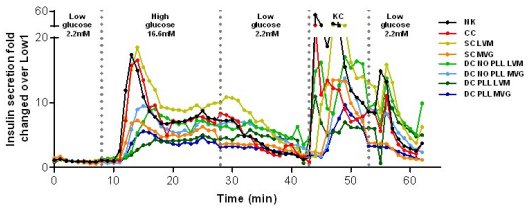

Figure 1. DGSIS of naked (NK) HI compared to HI in CC, SC, DC.

Conclusions: We demonstrated that small CC capsules are necessary for physiological GSIS because the larger the capsules, the higher the insulin delay. However, we found that minimizing the capsules size shifts the host responses from fibrotic encapsulation to frustrated phagocytosis as accumulation of FBGCs. These host responses to CC capsules may promote graft loss in the long term. Future studies will determine whether localized immunomodulation can improve CC biocompatibility.

References: [1]Tomei AA et al. *PNAS* 2014; [2]Manzoli V et al. *AJT* 2018; [3]Safley SA et al. *Xenotransplantation* 2018. [4]Buchwald, P et al. *Biotechnol. Bioeng*. 2018.