Layer-by-layer Assembly of PEG-rich, Nano-thin, Conformal Coatings for Intraportal Islet Transplantation

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Statement of Purpose: Islet encapsulation offers a rational approach for protecting transplanted islets from deleterious host responses that occur during clinical islet transplantation [1]. However, encapsulation strategies have traditionally utilized capsules which are too large for infusion into the portal vein of the liver, the clinically preferred site for islet transplantation [2]. Therefore, a need exists to develop islet encapsulation strategies that minimize transplant volume. The objective of this work is to develop nano-thin, conformal coatings on the surface of individual pancreatic islets through a process of layer-by-layer (LbL) polymer self-assembly.

Methods: Poly(L-lysine)-*g*-poly(ethylene glycol)biotin (PPB) was synthesized as previously described [3] with PEG(3.4kD)-biotin grafted to ~30% of lysine repeat units. To determine if multilayer thin films could be fabricated on negatively charged planar substrates through layer-by-layer (LbL) deposition of PPB and streptavidin (SA) (Figure 1), solid-state spectroscopy was used to monitor absorbance of Cy3-labeled SA as a function of layer number.

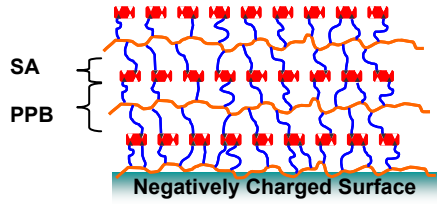

Figure 1. Multilayer thin film fabricated from PPB and SA

Pancreatic islets were isolated from B10 mice or human cadaveric donors and purified using established techniques. Thin films were fabricated on islets through serial incubation with PPB (1 mg/ml) and SA (0.1 mg/ml), washing with culture media between layers. Film formation, localization, and gross uniformity were assessed using fluorescently-labeled SA and confocal microscopy. Islet viability was assessed semi-quantitatively using ethidium homodimer and calcein AM, and islet function was characterized by measuring insulin secretion in response to a step-change in glucose. To confirm islet function *in vivo* and assess the capacity of PPB/SA multilayer films to improve islet engraftment, a sub-optimal islet mass was transplanted intraportally using a B10 → diabetic C57BL/6 mouse allograft model.

Results/Discussion: Multilayer films could be assembled on planar substrates through layer-by-layer deposition of PPB and SA (Figure 2). Incubation of islets with PPB facilitated specific binding of SA to both human and murine islets. Additionally, multilayer films terminated with PPB were capable of binding free SA, whereas those terminated with an outer layer of SA were not, indicating the formation of a multilayered structure on islets.

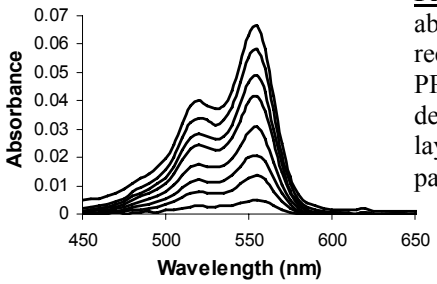

Figure 2. Solid-state absorbance spectra recorded after each PPB/Cy3-SA bilayer demonstrates regular layer-by-layer growth pattern.

Confocal microscopy also demonstrated that the film was localized extracellularly both on the islet surface (Figure 3A) as well as in the interstitial space between individual cells within the islet (Figure 3B). 3D reconstruction of optical sections of islets coated with PPB/SA films demonstrated gross film uniformity (Figure 3C).

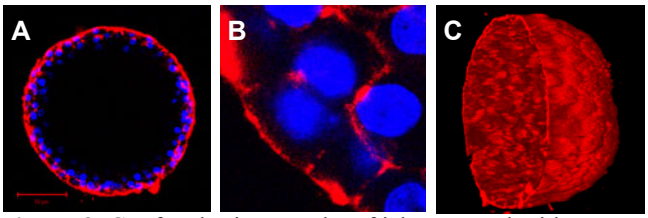

Figure 3. Confocal micrographs of islets coated with PPB/Cy3-SA films (blue: islet cell nuclei stained with Hoechst)

The viability of islets coated with a $(PPB/SA)_8$ multilayer film (93.8±2.5%) was statistically similar ($p > 0.05$) to non-coated islets (92.2±4.4%). Similarly, the glucose stimulation index of coated islets (4.5±1.2) was statistically similar ($p > 0.05$) to non-coated islets (3.9±1.1), indicating that film formation did not adversely influence islet function. Upon intraportal transplantation, the presence of a $(PPB/SA)_8$/PPB multilayer thin film tended to increase the fraction of mice that converted from diabetic to normoglycemic (44%) to a slightly greater extent than non-coated islets (38%). Though this difference is not statistically significant, these data suggest that *in vivo* islet function is maintained after film formation.

Conclusions: PPB/SA multilayer thin films can be assembled on the surface of individual islets while preserving viability and function, thereby providing a facile strategy for PEGylating and biotinylating pancreatic islets. Future efforts will focus on further characterizing and improving film properties, and incorporating biotinylated macromolecules with anti-inflammatory or immunoregulatory properties into the film. Overall, this work provides a novel strategy for resurfacing the biochemical landscape of cell and tissue interfaces.

References: [1] de Vos, P. Diabetologia. 2002; 45: 159-73. [2] Brendel, MDH. Intl Islet Transplant Registry. 1999; 8:5-18. [3] Huang, NP. Langmuir. 2002; 18:220-230.