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## Layer-by-Layer Assembly of a Conformal Nanothin PEG Coating for Intraportal Islet Transplantation

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### Abstract

Encapsulation of cells and tissue offers a rational approach for attenuating deleterious host responses toward transplanted cells, but a need exists to develop cell encapsulation strategies that minimize transplant volume. In this report, we describe the formation of nanothin, PEG-rich conformal coatings on individual pancreatic islets via layer-by-layer self-assembly of poly(L-lysine)-*g*-poly(ethylene glycol)(biotin) (PPB) and streptavidin (SA). Through control of grafting ratio, PPB could be rendered nontoxic and facilitated growth of PPB/SA multilayer thin films that conformed to the heterogeneous islet surface. (PPB/SA)<sub>8</sub> multilayer films could be assembled without loss of islet viability or function, and coated islets performed comparably to untreated controls in vivo in a murine model of allogeneic intraportal islet transplantation.

Islet transplantation has emerged as a promising treatment for diabetes.<sup>1</sup> However, widespread clinical application of islet transplantation remains limited, in part, by the deleterious side effects of immunosuppressive therapy necessary to prevent host rejection of transplanted cells.<sup>2</sup> Decades of extensive research have led to the development of semipermeable microcapsules capable of protecting donor cells from the host immune system while allowing transport of glucose, insulin, and other essential nutrients.<sup>3–5</sup> To date, most microencapsulation approaches have employed 400–800 μm diameter microcapsules of diverse composition, formed via various drop generating processes, to randomly entrap 50–250 μm diameter islets.<sup>4,6,7</sup> Unfortunately, the relatively large size of conventional microcapsules imposes consequential mass transport limitations and produces transplant volumes not suitable for infusion into the portal vein of the liver,<sup>8–10</sup> the clinically preferred and currently most successful site for islet transplantation.<sup>1,11</sup> Consequently, most microencapsulated islets are transplanted into sites with a limited vascular supply, such as the omentum<sup>12</sup> or peritoneal cavity,<sup>13–15</sup> which ultimately contributes to cell hypoxia and subsequent graft failure.<sup>16</sup> Therefore, encapsulation strategies for intraportal islet transplantation must minimize capsule void volume.

To reduce capsule size, several investigators have developed approaches to deposit coatings of defined thickness that conform to the surface of individual islets.<sup>17–20</sup> Transplant volume

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**Supporting Information Available:** Experimental details. This material is available free of charge via the Internet at <http://pubs.acs.org>.

is, therefore, defined only by the size of the islet and the thickness of the coating, significantly reducing void volume while retaining the presence of a protective polymer barrier. Such conformal coatings have been fabricated using a number of processes including emulsification,<sup>17</sup> discontinuous gradient density centrifugation,<sup>18</sup> selective withdrawal,<sup>19</sup> and interfacial polymerization<sup>20</sup> to generate 5–50  $\mu\text{m}$  thick polymeric coatings. Attempts to further reduce coating thickness often lead to incomplete encapsulation or coating defects. Additionally, islet loss<sup>19</sup> and limited process scalability<sup>18</sup> are obstacles that must be addressed to coat a clinically relevant number of islets.

Layer-by-layer (LbL) polymer self-assembly has emerged as an attractive alternative to traditional thin film fabrication techniques due to its ability to generate films of nanometer thickness on chemically and geometrically diverse substrates.<sup>21–23</sup> Of particular relevance to cell encapsulation, film properties may be tailored to inhibit molecular recognition between complementary molecules on opposite sides of films. For example, Caruso et al. assembled multilayer films of poly(sodium styrenesulfonate) and poly(allylamine hydrochloride) on the surface of catalase crystals to protect the encapsulated enzyme from protease degradation.<sup>24</sup> Similarly, Hubbell and co-workers have assembled alginate/poly(L-lysine) films on gelatin to limit cell adhesion to the proteinaceous surface,<sup>25</sup> while Thierry et al. have coated deendothelialized blood vessels with chitosan/hyaluronic acid films to inhibit platelet deposition.<sup>26</sup> Moreover, through proper control of film constituents, multilayer films may also be used to elicit specific biochemical responses. Enzymes and other proteins,<sup>27,28</sup> DNA,<sup>29</sup> lipid vesicles,<sup>30</sup> drug-containing nanoparticles,<sup>31</sup> and polymers covalently functionalized with bioactive motifs<sup>32,33</sup> have been used as film components to control the local biochemical milieu. Such capabilities hold considerable promise for generating biologically active cell and tissue coatings with the potential to abrogate deleterious inflammatory and immune responses to encapsulated islet grafts.<sup>6</sup> All told, LbL polymer self-assembly represents a rational approach for coating cells and cell aggregates with nanothin films of tailored surface chemistry, permeability, and bioactivity.

Polyelectrolyte multilayer (PEM) films, in particular poly(L-lysine) (PLL)/alginate PEM films, have been widely employed to confer permselectivity to conventional microcapsules.<sup>13,34</sup> Therefore, it was hypothesized that such films could be assembled directly on the surface of pancreatic islets in an analogous manner, using the negatively charged cell surface as a substrate for film assembly. Islets were first incubated with PLL (MW 15–30 kD, 1 mg/mL in RPMI 1640) for 5 min, rinsed 3 times with RPMI 1640, and subsequently incubated with alginate (2 mg/mL in RPMI 1640) for 5 min to form a single PLL/alginate bilayer. Maintenance of cell viability is critical to effective islet transplantation, and accordingly, islet viability was assessed shortly after film formation using confocal microscopy to image a representative population of islets stained with calcein AM (live) and ethidium homodimer (dead). Formation of even a single PLL/alginate bilayer exerted significant toxicity (data not shown). The toxicity of PLL, as well as many other polycations, toward a variety of cell types has been well documented.<sup>36–39</sup> In accord with these reports, incubation of islets with 1 mg/mL PLL for 15 min resulted in a ~60% decrease in islet viability relative to untreated controls (Figure 1). Hence, direct contact between PLL and islets significantly decreases viability and precludes the use of PLL to initiate film growth on the islet surface. The toxicity of several other commonly employed polycations, including poly(allylamine hydrochloride) (PAH), poly(diallyldimethylammonium) (PDDA), and protamine was also assessed and all were found to exert significant toxicity after  $\leq 15$  min at both 1 and 0.1 mg/mL (data not shown); similar findings have been recently reported by Lee et al.<sup>39</sup> To the contrary, Krol et al. maintain that a PAH/poly(sodium 4-styrenesulfonate)/PAH (PAH/PSS/PAH) film may be assembled on the surface of human islets without significantly influencing islet viability or function.<sup>40</sup> To explore this apparent inconsistency, human islets were coated with a PAH/PSS/PAH multilayer using identical polyion properties (PAH, 15 kDa; PSS, 70 kDa), concentrations (2 mg/mL),

incubation times (5 min), and solvent (RPMI 1640, dissolved one day in advance) as previously reported. Islet viability was assessed after film formation via calcein AM and ethidium homodimer-1 (live/dead) staining and imaging with confocal microscopy (Figure 2A–E). The majority of cells within islets coated with a PAH/PSS/PAH film were found to be nonviable (Figure 2B), as indicated by a significant decrease in intercellular esterase activity (live, green) and an increase in ethidium homodimer (EthD-1) staining (red, dead). Consistent with the binding of EthD-1 to nucleic acids, punctate staining was distributed within cell nuclei (Figure 2D). Indeed, image analysis of confocal micrographs revealed a significant difference in viability between untreated and PAH/PSS/PAH coated islets (Figure 2E). Comparable results were obtained when film assembly was performed on murine islets (data not shown). The toxicity of PAH/PSS/PAH films was further confirmed through an MTS assay, which demonstrated that the viability of PAH/PSS/PAH coated islets was significantly less than that of untreated controls ( $30.7 \pm 0.8\%$  vs  $103.7 \pm 8\%$ ,  $p < 0.01$ , Figure 2E). These data were consistent with those obtained using an independent islet isolation (PAH/PSS/PAH,  $29.9 \pm 2.5\%$ ; control,  $100.7 \pm 11.0\%$ ). As an additional confirmation of toxicity, the cytosolic enzyme, lactose dehydrogenase (LDH), could be detected in coating and wash solutions. Specifically, islets were found to release significantly more LDH during the initial PAH coating step relative to those exposed solely to cell culture media but otherwise treated in a similar manner ( $296 \pm 21$  vs  $4.7 \pm 4.2$   $\mu\text{U}/\text{islet}$ ,  $p < 0.01$ , Figure 2F). Additionally, LDH continued to leak from islets during a 3 h period immediately after PAH/PSS/PAH coating, whereas significantly less was released from controls ( $76.8 \pm 5.9$  vs  $5.5 \pm 0.6$   $\mu\text{U}/\text{islet}$ ,  $p < 0.01$ , Figure 2F). Human islet isolations are highly variable by nature, and consequently, the susceptibility of islets to toxic agents, including polycations, may depend on the unique characteristics of an islet preparation or islet subpopulation, including size, viability, metabolic capacity, purity, and integrity of the peri-insular extracellular matrix. Nonetheless, results obtained using three viability assays (calcein AM/EthD-1, MTS, and LDH) and three independent islet isolations obtained from separate transplant centers with extensive islet isolation experience indicate that PAH/PSS/PAH multilayer films cannot be assembled on islets without significant adverse effects on islet viability.

Poly(L-lysine)-g-poly(ethylene glycol) copolymers have been used to modify the surface of synthetic and natural implantable materials<sup>41–45</sup> and, importantly, have been reported to exert minimal toxicity toward fibroblasts in culture.<sup>41</sup> Therefore, to reduce the toxicity of PLL, NHS-PEG<sub>3.4kDa</sub>(biotin) was grafted to primary amines on the PLL backbone to generate PLL-g-[x]-PEG<sub>3.4kDa</sub>(biotin) (PPB) graft copolymers<sup>46,47</sup> with grafting ratios,  $x$ , of 5 and 2.5 (PPB[5] and PPB[2.5], respectively), where  $x$  is the average number of modified and unmodified lysine residues per grafted side chain. Islets were incubated with PPB[5] and PPB[2.5] at 1 mg/mL for 15 min, 1 h, 4 h, and 12 h and islet viability was assessed until significant decreases in islet viability were observed relative to untreated controls. Incubation of islets with PPB[5] resulted in a statistically significant ( $p < 0.01$ ) 6.5% decrease in viability after 4 h which was largely due to death of cells on the islet periphery (Figure 1). While such small changes in viability may not have a significant impact on overall islet function or engraftment, death of peripheral cells and concomitant changes in islet morphology (Figure 1B) will likely compromise film assembly and properties. By contrast, islets could be incubated in PPB[2.5] for at least 12 h without adversely influencing islet viability or morphology (Figure 1). Therefore, cytotoxicity tends to decrease (PPB[2.5] < PPB[5] < PLL) with decreasing polycation charge density (PPB[2.5] < PPB[5] < PLL), a phenomenon in accord with previous findings.<sup>38</sup> The dramatic reduction in toxicity achieved with increased PEG grafting might be explained by differences in the three-dimensional arrangement of cationic monomers on the cell membrane. Ryser suggested that the membrane permeabilization potential of polyamines decreased as the space between amino groups increased.<sup>48</sup> Interestingly, it was speculated that a three-point attachment mechanism was necessary to invoke membrane pore formation, and, therefore, it is perhaps not coincidental that the toxicity of PLL is abrogated as the grafting

ratio decreases below 3 (i.e., charge neutralization of one in every three lysine residues). Alternatively, Hartmann et al. suggested that PLL transitions from a random coil in solution to an alpha helical conformation at the cell surface in order to maximize interfacial contact,<sup>49</sup> a phenomenon that may be sterically interrupted by grafted PEG chains.

PLL-g-PEG(biotin) copolymers adsorb to surfaces through Coulombic interactions between positively charged backbone lysine monomers and negatively charged surfaces, causing PEG chains terminated with biotin to extend into solution.<sup>46,47</sup> To demonstrate adsorption of PPB [2.5] on islets, Cy3-labeled SA (Cy3-SA, 0.1 mg/mL, 30 min) was used to identify accessible biotin groups. Incubation with PPB[2.5] (1 mg/mL, 15 min) facilitated the specific binding of Cy3-SA to the islet surface (Figure 3A), as islets incubated with only Cy3-SA demonstrated no fluorescent emission (Figure 3B). Islets incubated with nonmodified PLL (1 mg/mL, 15 min) prior to Cy3-SA demonstrated sporadic and concentrated domains of fluorescent emission (Figure 3C), likely a result of membrane permeabilization by PLL and subsequent diffusion of Cy3-SA into the cytoplasm.<sup>36</sup> Therefore, unlike PLL, PPB provides a foundation for initiating growth of multilayer thin films on the surface of viable pancreatic islets.

As an alternative to PEM film formation, receptor–ligand interactions have been used to fabricate multilayer architectures.<sup>50,51</sup> A ligand-derivatized polymer adsorbed to a surface creates a ligand-rich interface capable of binding soluble receptors. Provided each receptor has multiple binding sites for the ligand, a fraction of binding sites may remain unoccupied, facilitating binding of the ligand-derivatized polymer and regeneration of a ligand-rich interface. Such films have commonly been assembled through alternating deposition of biotin derivatized polycations and (strept)avidin.<sup>51–53</sup> Many of these films, however, have utilized polycations of high charge density<sup>52–54</sup> and, therefore, are likely unsuitable for assembly on living cells or tissues. Moreover, PEG-rich multilayer films have not been constructed in this manner. To determine if multilayer thin films could be fabricated through layer-by-layer deposition of PPB[2.5] and SA (Scheme 1), solid-state spectroscopy was used to monitor the absorbance of Cy3-SA as a function of layer number. Figure 4 shows a series of representative absorption spectra, with each successive curve corresponding to a different bilayer. Plotting absorbance at 554 nm (Figure 4, inset), which corresponds to the amount of surface-bound Cy3-SA, as a function of layer number demonstrates that film growth occurs in a linear manner. This behavior is in accord with previously published spectroscopic measurements of biotin-PEI/avidin multilayer films.<sup>52</sup> From the approximate molecular dimensions of streptavidin ( $5.4 \times 5.8 \times 4.8$  nm),<sup>51</sup> the molar extinction coefficient of Cy3 ( $1.3 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ ), and the fluorophore:protein ratio of the Cy3-SA conjugate (7.0), the absorbance of a monolayer of Cy3-SA is estimated to be  $5.7 \times 10^{-3}$ . The absorbance change per PPB/Cy3-SA layer was found to be  $5.4 \times 10^{-3}$ , indicating that just under a monolayer of streptavidin is bound after each deposition.

Confocal microscopy was next used to demonstrate multilayer film growth on the surface of individual pancreatic islets (Figure 5). Islets were incubated in PPB[2.5] for 15 min, rinsed three times with culture media, and incubated in Cy3-SA for 30 min. After formation of a single PPB[2.5]/Cy3-SA bilayer, islets were divided into two groups: one group was incubated in PPB[2.5] for an additional 15 min (Figure 5A) while the other was placed in RPMI 1640 (Figure 5B). Both groups were then incubated in FITC-labeled SA (FITC-SA) for 5 min and imaged with two-channel confocal microscopy. Receptor–ligand binding kinetics predicts that the initial rate of streptavidin binding increases with increased surface density of free biotin. Therefore, islets incubated with a second layer of PPB would be expected to bind more FITC-SA than islets that were not due to regeneration of accessible biotin groups in the former. Indeed, fluorescent emission from FITC-SA was observed around the periphery of islets that were incubated with a second layer of PPB, while the signal was essentially absent for islets

that were not. These observations indicate that multilayer architectures can be assembled on the surface of islets via alternating deposition of PPB[2.5] and streptavidin.

Live cell confocal microscopy was also used to assess the localization, distribution, and gross uniformity of PPB/SA multilayer films assembled on islets. Three-dimensional reconstructions of serial optical sections of islets coated with a (PPB[2.5]/Cy3-SA)<sub>4</sub> film (Figure 6) demonstrate that the film conforms to undulations on the islet surface and is grossly uniform at the resolution used here. Using Hoechst nuclear stain to identify individual cells within islets, FITC-labeled PPB (FITC-PPB), and Cy3-SA, confocal microscopy demonstrated that the film is localized both on the periphery of the islet (Figure 7A) and within the interstitial space between individual cells within the core of the islet (Figure 7B). Hence, all surfaces which are accessible to film constituents may be coated, reflecting the truly conformal nature of such nanoassembled films and demonstrating the potential to encapsulate and/or modify individual cells within a multicellular tissue such as islets. Importantly, film constituents were concentrated predominately on the surface of cells (i.e., in the extracellular space), as fluorescent emission from both Cy3-SA and FITC-PPB did not colocalize with cell nuclei, was not distributed throughout the cytoplasm of cells, and existed in discrete domains consistent with the extracellular architecture of isolated pancreatic islets. In contrast, FITC-labeled PLL (1 mg/mL, 15 min) was found colocalized with cell nuclei and distributed throughout the cytoplasm of individual cells (Figure 7C), which adopted an extended morphology, likely due to cell necrosis.<sup>38</sup> PLL and many other polycations have been shown to induce pore formation in the plasma membrane, a phenomenon which often mediates cell death and enables transport of molecules, including the polycation itself, across the cell membrane.<sup>36,38,48,55</sup> The extracellular localization of PPB/SA films, in particular the PPB component, suggests that conjugation of PEG<sub>3.4kDa</sub>(biotin) to the PLL backbone inhibits or reduces its capacity to form pores in the cell membrane and/or diffuse into the cytoplasm, consistent with the observed reduction in toxicity. Interestingly, Krol et al. also observed polycation (PAH) penetration into the cytoplasm of cells within islets,<sup>40</sup> consistent with the cytotoxic effects exerted by PAH reported herein.

As a consequence of cell encapsulation, diffusive transport of essential nutrients may be hampered, potentially resulting in decreased cell viability and/or improper temporal response to physiological stimuli.<sup>56</sup> Furthermore, fabrication of PPB[2.5]/SA multilayer films is anticipated to concentrate PPB on the cell surface, potentially generating locally toxic concentrations. Therefore, islet viability and function were assessed after fabrication of a (PPB/SA)<sub>8</sub> multilayer film. Coating islets did not affect islet viability (Figure 8A) indicating that neither the polymers employed nor the coating process caused damage to islets. Of clinical significance, human islets could also be coated with a (PPB[2.5]/SA)<sub>8</sub> multilayer film without compromising islet viability (Figure 8A); this was further confirmed using an MTS assay whereby the viability of coated and untreated islets was indistinguishable ( $p \gg 0.1$ ). Additionally, the coating process did not result in islet loss. Islet function was assessed in vitro by measuring insulin secretion in response to a step change in glucose concentration. As shown in Figure 8B, islets coated with a (PPB[2.5]/SA)<sub>8</sub> multilayer film function comparably to nontreated islets in response to glucose stimulation. Impaired in vitro insulin secretion has been observed for a variety of conventional microcapsule formulations<sup>3,57,58</sup> due to significant void space which glucose and insulin must cross prior to transport across the membrane. Due to the nanothin and conformal nature of PPB/SA coatings, this behavior was not observed. While no attempts were made to fabricate more than eight bilayers, it is anticipated that considerably more layers may be formed without compromising islet viability given the lack of toxicity exerted by PPB[2.5].

Finally, islets coated with a (PPB[2.5]/SA)<sub>8</sub>/PPB[2.5] multilayer film were transplanted into the portal vein of mice in a B10 to B6 allograft model; a final PPB layer was used to generate



a terminal PEG layer to help prevent nonspecific binding of serum proteins to the film.<sup>47,59</sup> In this model of islet transplantation, a suboptimal number of islets (250) are infused into the portal vein of the liver, resulting in transient reversal of diabetes (euglycemic for >2 consecutive days) in only a fraction of recipients during the initial 2 weeks post-transplant.<sup>60,61</sup> Therefore, differences in rates of conversion to euglycemia reflect changes in islet survival and function in the immediate post-transplant period. Of the 16 mice transplanted with untreated islets, 6 converted to euglycemia (37.5%), whereas 7 of 15 mice (46.7%) converted when receiving islets coated with a multilayer film. This difference was not statistically significant ( $\chi^2 = 0.11$ ), indicating that islets coated with a (PPB/SA)<sub>8</sub>/PPB multilayer thin film maintain islet viability and function in vivo, and suggesting that the film itself does not invoke a deleterious nonspecific inflammatory response. This is significant as intraportal transplantation of islets encapsulated in 350  $\mu$ m microcapsules has been found to *impair* islet engraftment relative to nonencapsulated controls due, in part, to inflammatory responses elicited against the implant.<sup>10</sup> Moreover, the observed trend toward increased conversion to euglycemia suggests a potential beneficial impact of the film, an effect which may be rendered more pronounced by increasing film thickness, optimizing barrier permeability, or by incorporating bioactive film constituents, efforts which are currently ongoing. Significantly, this is the first study to report in vivo survival and function of nanoencapsulated cells or cell aggregates.

Covalent conjugation of PEG to islet surface proteins and carbohydrates has recently been explored as a strategy for attenuating host responses to transplanted allo- and xenografts.<sup>62,63</sup> However, the efficacy of PEGylation may be limited, in part, by the lack of a defined pore structure, with primary dependence on barrier function through a steric exclusion effect. In principle, such limitations may be addressed through use of PPB/SA multilayer films, which are anticipated to generate PEG-rich networks rather than a monolayer of grafted PEG on the cell surface. Reports demonstrating in vivo efficacy of islet surface PEGylation have utilized different, in some cases less rigorous, animal models and/or adjunctive immunosuppressive therapy.<sup>39,63,64</sup> Therefore, PPB/SA multilayer films may demonstrate increased efficacy in other animal models of islet transplantation or may act in synergy with systemic administration of immunomodulatory agents.

PPB/SA films may also provide important advantages over covalent biotinylation strategies employed to immobilize bioactive molecules to the islet surface.<sup>65,66</sup> As a multilayered structure, PPB/SA films may allow biotinylated or streptavidin-linked molecules to be embedded within the film, thereby facilitating greater loading than might be accomplished using a single layer of immobilized biotin moieties. Moreover, multilayer films assembled via (strept)-avidin/biotin interactions may be disintegrated using excess biotin,<sup>53,54</sup> thereby allowing triggered release of embedded agents.

In conclusion, PPB/SA multilayer films provide a novel approach to generating nanothin, PEG-rich conformal islet coatings through a self-assembly process. While further characterization and optimization of properties is necessary to generate films capable of significantly improving in vivo islet engraftment, this work helps establish a new paradigm for encapsulating and/or modifying islets prior to portal vein transplantation. Additionally, this work provides mechanistic insight regarding the relationships between polycation charge density, cell surface localization, and cytotoxicity, with important implications for the design of cell and tissue surface-supported nanostructures. All told, PPB/SA multi-layer films offer a unique approach to resurfacing the biochemical landscape of living cell and tissue interfaces with broad applications in tissue-targeted chemistry, biosensing, in situ tissue engineering, and targeted cell delivery.

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## References

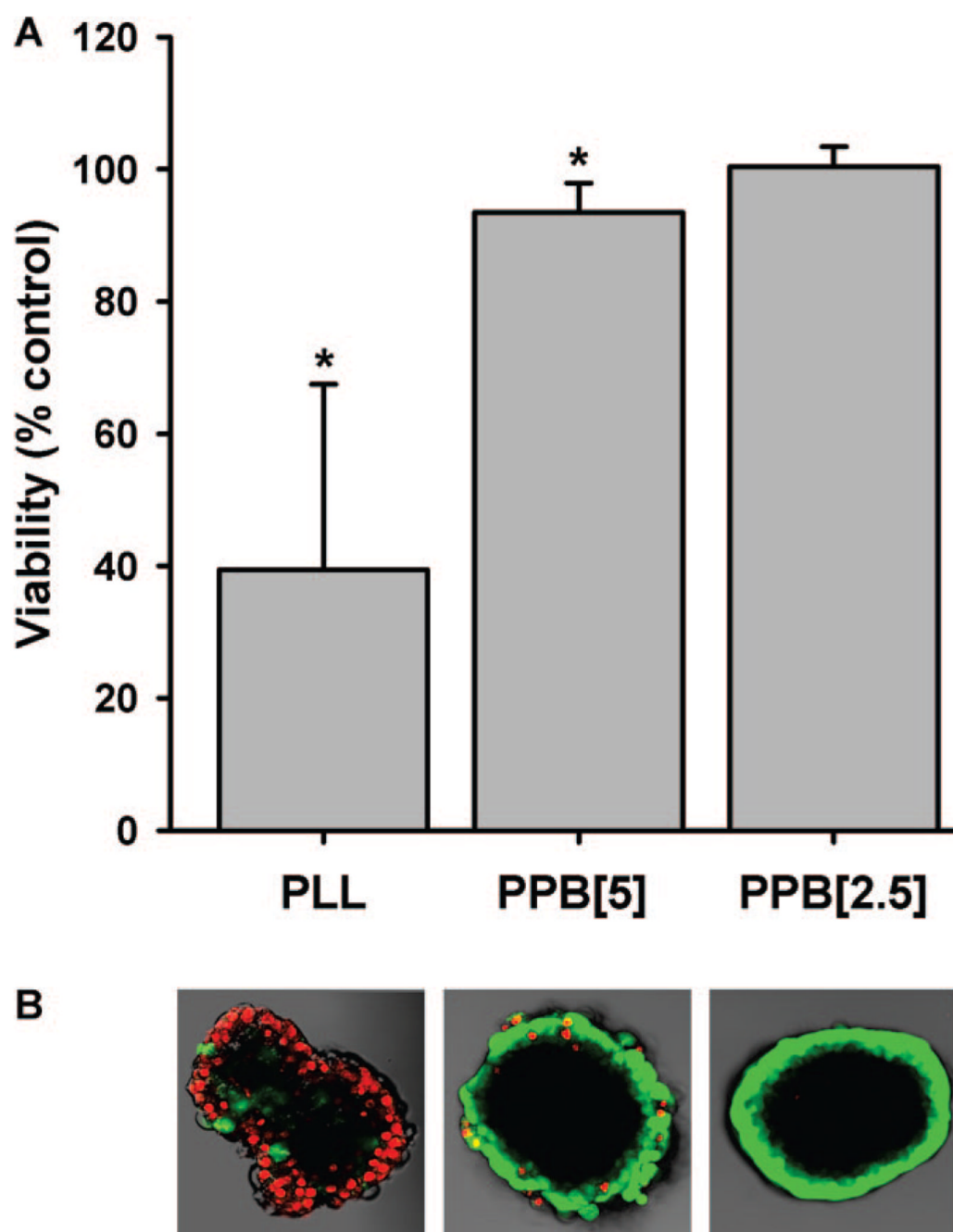
1. Shapiro AM, Ricordi C, Hering BJ, Auchincloss H, Lindblad R, Robertson RP, Secchi A, Brendel MD, Berney T, Brennan DC, Cagliero E, Alejandro R, Ryan EA, DiMercurio B, Morel P, Polonsky KS, Reems JA, Bretzel RG, Bertuzzi F, Froud T, Kandaswamy R, Sutherland DE, Eisenbarth G, Segal M, Preiksaitis J, Korbitt GS, Barton FB, Viviano L, Seyfert-Margolis V, Bluestone J, Lakey JR. International trial of the Edmonton protocol for islet transplantation. *N. Engl. J. Med* 2006;355(13):1318–1330. [PubMed: 17005949]
2. Ricordi C, Strom TB. Clinical islet transplantation: advances and immunological challenges. *Nat. Rev. Immunol* 2004;4(4):259–268. [PubMed: 15057784]
3. Lim F, Sun AM. Microencapsulated islets as bioartificial endocrine pancreas. *Science* 1980;210(4472):908–910. [PubMed: 6776628]
4. Lanza RP, Hayes JL, Chick WL. Encapsulated cell technology. *Nat. Biotechnol* 1996;14(9):1107–1111. [PubMed: 9631060]
5. Barnett BP, Arepally A, Karmarkar PV, Qian D, Gilson WD, Walczak P, Howland V, Lawler L, Lauzon C, Stuber M, Kraitichman DL, Bulte JWM. Magnetic resonance-guided, real-time targeted delivery and imaging of magnetocapsules immunoprotecting pancreatic islet cells. *Nat. Med* 2007;13(8):986–991. [PubMed: 17660829]
6. Wilson JT, Chaikof EL. Challenges and emerging technologies in the immunoisolation of cells and tissues. *Adv. Drug Delivery Rev* 2008;60(2):124–145.
7. de Vos P, Faas MM, Strand B, Calafiore R. Alginate-based microcapsules for immunoisolation of pancreatic islets. *Biomaterials* 2006;27(32):5603–5617. [PubMed: 16879864]
8. Calafiore R, Basta G, Luca G, Boselli C, Bufalari A, Giustozzi GM, Moggi L, Brunetti P. Alginate/polyaminoacidic coherent microcapsules for pancreatic islet graft immunoisolation in diabetic recipients. *Ann. N.Y. Acad. Sci* 1997;831:313–322. [PubMed: 9616723]
9. Leblond FA, Simard G, Henley N, Rocheleau B, Huet PM, Halle JP. Studies on smaller (approximately 315  $\mu\text{m}$ ) microcapsules: IV. Feasibility and safety of intrahepatic implantations of small alginate poly-L-lysine microcapsules. *Cell Transplant* 1999;8(3):327–337. [PubMed: 10442745]
10. Schneider S, von Mach MA, Kraus O, Kann P, Feilen PJ. Intraportal transplantation of allogenic pancreatic islets encapsulated in barium alginate beads in diabetic rats. *Artif. Organs* 2003;27(11):1053–1056. [PubMed: 14616525]
11. Brendel MDHBJ, Schultz AO, Bretzel RG. Insulin independence following islet transplantation: a comparison of different recipient categories. *Int. Islet Transplant Registry* 1999;8:5–18.
12. Kobayashi T, Aomatsu Y, Iwata H, Kin T, Kanehiro H, Hisanga M, Ko S, Nagao M, Harb G, Nakajima Y. Survival of microencapsulated islets at 400 days posttransplantation in the omental pouch of NOD mice. *Cell Transplant* 2006;15(4):359–365. [PubMed: 16898230]
13. Calafiore R, Basta G, Luca G, Lemmi A, Montanucci MP, Calabrese G, Racanicchi L, Mancuso F, Brunetti P. Microencapsulated pancreatic islet allografts into nonimmunosuppressed patients with type 1 diabetes: first two cases. *Diabetes Care* 2006;29(1):137–138. [PubMed: 16373911]
14. Soon-Shiong P, Heintz RE, Merideth N, Yao QX, Yao Z, Zheng T, Murphy M, Moloney MK, Schmehl M, Harris M. Insulin independence in a type 1 diabetic patient after encapsulated islet transplantation. *Lancet* 1994;343(8903):950–951. [PubMed: 7909011]
15. Sun Y, Ma X, Zhou D, Vacek I, Sun AM. Normalization of diabetes in spontaneously diabetic cynomolgus monkeys by xenografts of microencapsulated porcine islets without immunosuppression. *J. Clin. Invest* 1996;98(6):1417–1422. [PubMed: 8823307]
16. De Vos P, Van Straaten JF, Nieuwenhuizen AG, de Groot M, Ploeg RJ, De Haan BJ, Van Schilfgaarde R. Why do microencapsulated islet grafts fail in the absence of fibrotic overgrowth. *Diabetes* 1999;48(7):1381–1388. [PubMed: 10389842]



17. Calafiore R, Basta G, Luca G, Boselli C, Bufalari A, Bufalari A, Cassarani MP, Giustozzi GM, Brunetti P. Transplantation of pancreatic islets contained in minimal volume microcapsules in diabetic high mammals. *Ann. N.Y. Acad. Sci* 1999;875:219–232. [PubMed: 10415570]
18. Sefton MV, May MH, Lahooti S, Babensee JE. Making microencapsulation work: conformal coating, immobilization gels and in vivo performance. *J. Controlled Release* 2000;65(1–2):173–186.
19. Wyman JL, Kizilel S, Skarbek R, Zhao X, Connors M, Dillmore WS, Murphy WL, Mrksich M, Nagel SR, Garfinkel MR. Immunoisolating pancreatic islets by encapsulation with selective withdrawal. *Small* 2007;3(4):683–690. [PubMed: 17340661]
20. Cruise GM, Hegre OD, Scharp DS, Hubbell JA. A sensitivity study of the key parameters in the interfacial photopolymerization of poly(ethylene glycol) diacrylate upon porcine islets. *Biotechnol. Bioeng* 1998;57(6):655–665. [PubMed: 10099245]
21. Decher G. Fuzzy nanoassemblies: Toward layered polymeric multi-composites. *Science* 1997;277(5330):1232–1237.
22. Quinn JF, Johnston APR, Such GK, Zelikin AN, Caruso F. Next generation, sequentially assembled ultrathin films: beyond electrostatics. *Chem. Soc. Rev* 2007;36(5):707–718. [PubMed: 17471396]
23. Hammond PT. Form and function in multilayer assembly: New applications at the nanoscale. *Adv. Mater* 2004;16(15):1271–1293.
24. Caruso F, Trau D, Mohwald H, Renneberg R. Enzyme encapsulation in layer-by-layer engineered polymer multilayer capsules. *Langmuir* 2000;16(4):1485–1488.
25. Elbert DL, Herbert CB, Hubbell JA. Thin polymer layers formed by polyelectrolyte multilayer techniques on biological surfaces. *Langmuir* 1999;15(16):5355–5362.
26. Thierry B, Winnik FM, Merhi Y, Tabrizian M. Nanocoatings onto arteries via layer-by-layer deposition: Toward the in vivo repair of damaged blood vessels. *J. Am. Chem. Soc* 2003;125(25):7494–7495. [PubMed: 12812471]
27. Lvov Y, Ariga K, Ichinose I, Kunitake T. Assembly of Multi-component Protein Films by Means of Electrostatic Layer-by-Layer Adsorption. *J. Am. Chem. Soc* 1995;117(22):6117–6123.
28. Jessel N, Atalar F, Lavalle P, Mutterer J, Decher G, Schaaf P, Voegel JC, Ogier J. Bioactive coatings based on a polyelectrolyte multilayer architecture functionalized by embedded proteins. *Adv. Mater* 2003;15(9):692–695.
29. Jewell CM, Zhang JT, Fredin NJ, Lynn DM. Multilayered polyelectrolyte films promote the direct and localized delivery of DNA to cells. *J. Controlled Release* 2005;106(1–2):214–223.
30. Michel M, Vautier D, Voegel JC, Schaaf P, Ball V. Layer by layer self-assembled polyelectrolyte multilayers with embedded phospholipid vesicles. *Langmuir* 2004;20(12):4835–4839. [PubMed: 15984239]
31. Benkirane-Jessel N, Schwinte P, Falvey P, Darcy R, Haikel Y, Schaaf P, Voegel JC, Ogier J. Build-up of polypeptide multilayer coatings with anti-inflammatory properties based on the embedding of piroxicam-cyclodextrin complexes. *Adv. Funct. Mater* 2004;14(2):174–182.
32. Picart C, Elkaim R, Richert L, Audoin T, Arntz Y, Cardoso MD, Schaaf P, Voegel JC, Frisch B. Primary cell adhesion on RGD-functionalized and covalently crosslinked thin polyelectrolyte multilayer films. *Adv. Funct. Mater* 2005;15(1):83–94.
33. Chluba J, Voegel JC, Decher G, Erbacher P, Schaaf P, Ogier J. Peptide hormone covalently bound to polyelectrolytes and embedded into multilayer architectures conserving full biological activity. *Biomacromolecules* 2001;2(3):800–805. [PubMed: 11710034]
34. de Vos P, van Hoogmoed CG, van Zanten J, Netter S, Strubbe JH, Busscher HJ. Long-term biocompatibility, chemistry, and function of microencapsulated pancreatic islets. *Biomaterials* 2003;24(2):305–312. [PubMed: 12419632]
35. Orive G, Tam SK, Pedraz JL, Halle JP. Biocompatibility of alginate-poly-L-lysine microcapsules for cell therapy. *Biomaterials* 2006 27;20:3691–3700. [PubMed: 16574222]
36. Hong S, Leroueil PR, Janus EK, Peters JL, Kober MM, Islam MT, Orr BG, Baker JR Jr, Banaszak Holl MM. Interaction of polycationic polymers with supported lipid bilayers and cells: nanoscale hole formation and enhanced membrane permeability. *Bioconjugate Chem* 2006;17(3):728–734.
37. Chanana M, Gliozzi A, Diaspro A, Chodnevskaja I, Huewel S, Moskalenko V, Ulrichs K, Galla HJ, Krol S. Interaction of polyelectrolytes and their composites with living cells. *Nano. Lett* 2005;5(12):2605–2612. [PubMed: 16351223]

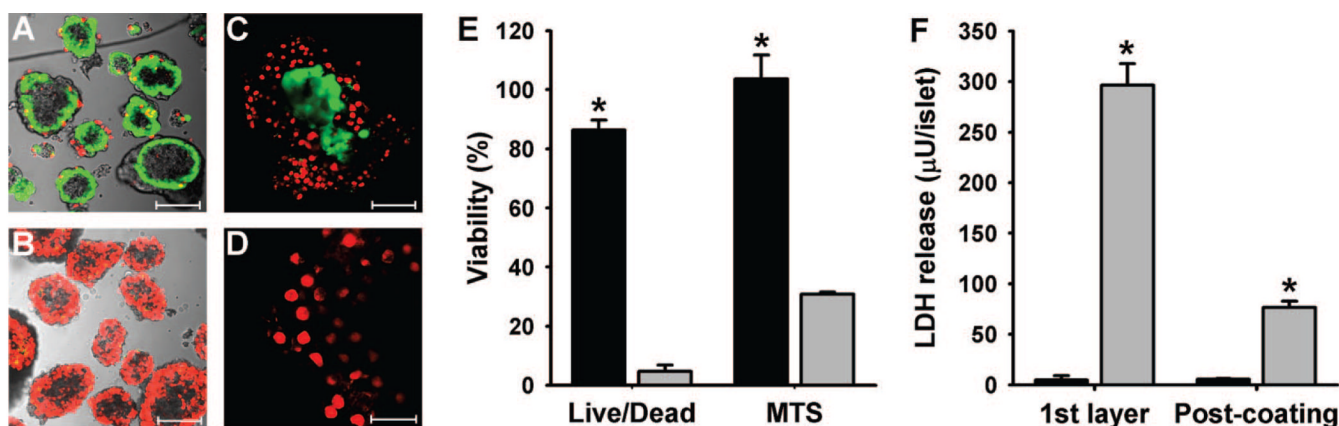
38. Fischer D, Li YX, Ahlemeyer B, Kriegelstein J, Kissel T. In vitro cytotoxicity testing of polycations: influence of polymer structure on cell viability and hemolysis. *Biomaterials* 2003;24(7):1121–1131. [PubMed: 12527253]
39. Lee DY, Park SJ, Lee S, Nam JH, Byun Y. Highly poly(ethylene) glycolylated islets improve long-term islet allograft survival without immunosuppressive medication. *Tissue Eng* 2007;13:2133–2141. [PubMed: 17516853]
40. Krol S, del Guerra S, Grupillo M, Diaspro A, Gliozzi A, Marchetti P. Multilayer nanoencapsulation. New approach for immune protection of human pancreatic islets. *Nano Lett* 2006;6(9):1933–1939. [PubMed: 16968004]
41. Elbert DL, Hubbell JA. Self-assembly and steric stabilization at heterogeneous, biological surfaces using adsorbing block copolymers. *Chem. Biol* 1998;5(3):177–183. [PubMed: 9545428]
42. Hansson KM, Tosatti S, Isaksson J, Wettero J, Textor M, Lindahl TL, Tengvall P. Whole blood coagulation on protein adsorption-resistant PEG and peptide functionalised PEG-coated titanium surfaces. *Biomaterials* 2005;26(8):861–872. [PubMed: 15353197]
43. Heuberger R, Sukhorukov G, Voros J, Textor M, Mohwald H. Biofunctional polyelectrolyte multilayers and microcapsules: Control of non-specific and bio-specific protein adsorption. *Adv. Funct. Mater* 2005;15(3):357–366.
44. Kenausis GL, Voros J, Elbert DL, Huang NP, Hofer R, Ruiz-Taylor L, Textor M, Hubbell JA, Spencer ND. Poly(l-lysine)-*g*-poly(ethylene glycol) layers on metal oxide surfaces: Attachment mechanism and effects of polymer architecture on resistance to protein adsorption. *J. Phys. Chem. B* 2000;104(14):3298–3309.
45. Billinger OM, Buddeberg F, Hubbell JA, Elbert DL, Schaffner T, Mettler D, Windecker S, Meier B, Hess OM. Polymer stent coating for prevention of neointimal hyperplasia. *J. Invasive Cardiol* 2006;18(9):423–426. [PubMed: 16954581]discussion 427.
46. Huang NP, Voros J, De Paul SM, Textor M, Spencer ND. Biotin-derivatized poly(l-lysine)-*g*-poly(ethylene glycol): A novel polymeric interface for bioaffinity sensing. *Langmuir* 2002;18(1):220–230.
47. Ruiz-Taylor LA, Martin TL, Zaugg FG, Witte K, Indermuhle P, Nock S, Wagner P. Monolayers of derivatized poly(L-lysine)-grafted poly(ethylene glycol) on metal oxides as a class of biomolecular interfaces. *Proc. Natl. Acad. Sci. U.S.A* 2001;98(3):852–857. [PubMed: 11158560]
48. Ryser HJ. A membrane effect of basic polymers dependent on molecular size. *Nature* 1967;215(5104):934–936. [PubMed: 6055419]
49. Hartmann W, Galla HJ. Binding of polylysine to charged bilayer membranes: molecular organization of a lipid-peptide complex. *Biochim. Biophys. Acta* 1978;509(3):474–490. [PubMed: 207323]
50. Anzai J, Akase S. Preparation of an organized film composed of polymers, avidin, and concanavalin A and its binding properties. *Macromol. Biosci* 2002;2(8):361–364.
51. Cassier T, Lowack K, Decher G. Layer-by-layer assembled protein/polymer hybrid films: nanoconstruction via specific recognition. *Supramol. Sci* 1998;5(3–4):309–315.
52. Anzai J, Kobayashi Y, Nakamura N, Nishimura M, Hoshi T. Layer-by-layer construction of multilayer thin films composed of avidin and biotin-labeled poly(amine)s. *Langmuir* 1999;15(1):221–226.
53. Inoue H, Anzai J. Stimuli-sensitive thin films prepared by a layer-by-layer deposition of 2-iminobiotin-labeled poly(ethyleneimine) and avidin. *Langmuir* 2005;21(18):8354–8359. [PubMed: 16114942]
54. Inoue H, Sato K, Anzai J. Disintegration of layer-by-layer assemblies composed of 2-iminobiotin-labeled poly(ethyleneimine) and avidin. *Biomacromolecules* 2005;6(1):27–29. [PubMed: 15638499]
55. Leroueil PR, Hong S, Mecke A, Baker JR Jr, Orr BG, Banaszak Holl MM. Nanoparticle interaction with biological membranes: does nanotechnology present a Janus face. *Acc. Chem. Res* 2007;40(5):335–342. [PubMed: 17474708]
56. Avgoustiniatos ES, Colton CK. Effect of external oxygen mass transfer resistances on viability of immunoisolated tissue. *Ann. N.Y. Acad. Sci* 1997;831:145–167. [PubMed: 9616709]
57. Trivedi N, Keegan M, Steil GM, Hollister-Lock J, Hasenkamp WM, Colton CK, Bonner-Weir S, Weir GC. Islets in alginate macrobeads reverse diabetes despite minimal acute insulin secretory responses. *Transplantation* 2001;71(2):203–211. [PubMed: 11213060]

58. Wang T, Lacik I, Brissova M, Anilkumar AV, Prokop A, Hunkeler D, Green R, Shahrokhi K, Powers AC. An encapsulation system for the immunoisolation of pancreatic islets. *Nat. Biotechnol* 1997;15(4):358–362. [PubMed: 9094138]
59. Huang NP, Michel R, Voros J, Textor M, Hofer R, Rossi A, Elbert DL, Hubbell JA, Spencer ND. Poly(l-lysine)-g-poly(ethylene glycol) layers on metal oxide surfaces: Surface-analytical characterization and resistance to serum and fibrinogen adsorption. *Langmuir* 2001;17(2):489–498.
60. Contreras JL, Eckstein C, Smyth CA, Bilbao G, Vilatoba M, Ringland SE, Young C, Thompson JA, Fernandez JA, Griffin JH, Eckhoff DE. Activated protein C preserves functional islet mass after intraportal transplantation: a novel link between endothelial cell activation, thrombosis, inflammation, and islet cell death. *Diabetes* 2004;53(11):2804–2814. [PubMed: 15504960]
61. Kaufman DB, Platt JL, Rabe FL, Dunn DL, Bach FH, Sutherland DE. Differential roles of Mac-1+ cells, and CD4+ and CD8+ T lymphocytes in primary nonfunction and classic rejection of islet allografts. *J. Exp. Med* 1990;172(1):291–302. [PubMed: 2113565]
62. Xie D, Smyth CA, Eckstein C, Bilbao G, Mays J, Eckhoff DE, Contreras JL. Cytoprotection of PEG-modified adult porcine pancreatic islets for improved xenotransplantation. *Biomaterials* 2005;26(4):403–412. [PubMed: 15275814]
63. Yun Lee D, Hee Nam J, Byun Y. Functional and histological evaluation of transplanted pancreatic islets immunoprotected by PEGylation and cyclosporine for 1 year. *Biomaterials* 2007;28(11):1957–1966. [PubMed: 17188350]
64. Contreras JL, Xie D, Mays J, Smyth CA, Eckstein C, Rahemtulla FG, Young CJ, Anthony Thompson J, Bilbao G, Curiel DT, Eckhoff DE. A novel approach to xenotransplantation combining surface engineering and genetic modification of isolated adult porcine islets. *Surgery* 2004;136(3):537–547. [PubMed: 15349100]
65. Cabric S, Sanchez J, Lundgren T, Foss A, Felldin M, Kallen R, Salmela K, Tibell A, Tufveson G, Larsson R, Korsgren O, Nilsson B. Islet surface heparinization prevents the instant blood-mediated inflammatory reaction in islet transplantation. *Diabetes* 2007;56(8):2008–2015. [PubMed: 17540953]
66. Yolcu ES, Askenasy N, Singh NP, Cherradi SE, Shirwan H. Cell membrane modification for rapid display of proteins as a novel means of immunomodulation: FasL-decorated cells prevent islet graft rejection. *Immunity* 2002;17(6):795–808. [PubMed: 12479825]



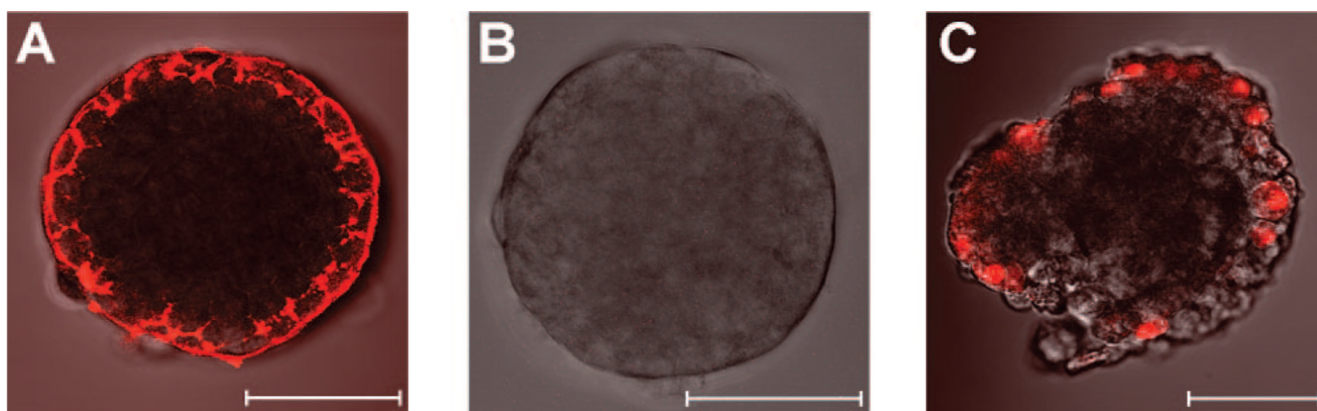
**Figure 1.**

(A) Islets were incubated with PLL, PPB[5], and PPB[2.5] at 1 mg/mL, and viability was assessed after various incubation times (mean  $\pm$  standard deviation,  $*p < 0.05$  compared to untreated controls). PLL exerted significant toxicity after only 15 min, while incubation with PPB[5] decreased islet viability slightly, but significantly, after 4 h. PPB[2.5] did not reduce islet viability even after a 12 h incubation ( $p > 0.05$ ). (B) Representative confocal micrographs of islets stained with calcein AM (green, viable) and ethidium homodimer (red, nonviable) overlaid on bright field micrographs demonstrate changes in islet morphology associated with polycation-mediated cell death (from left to right: PLL, PPB[5], PPB[2.5]).



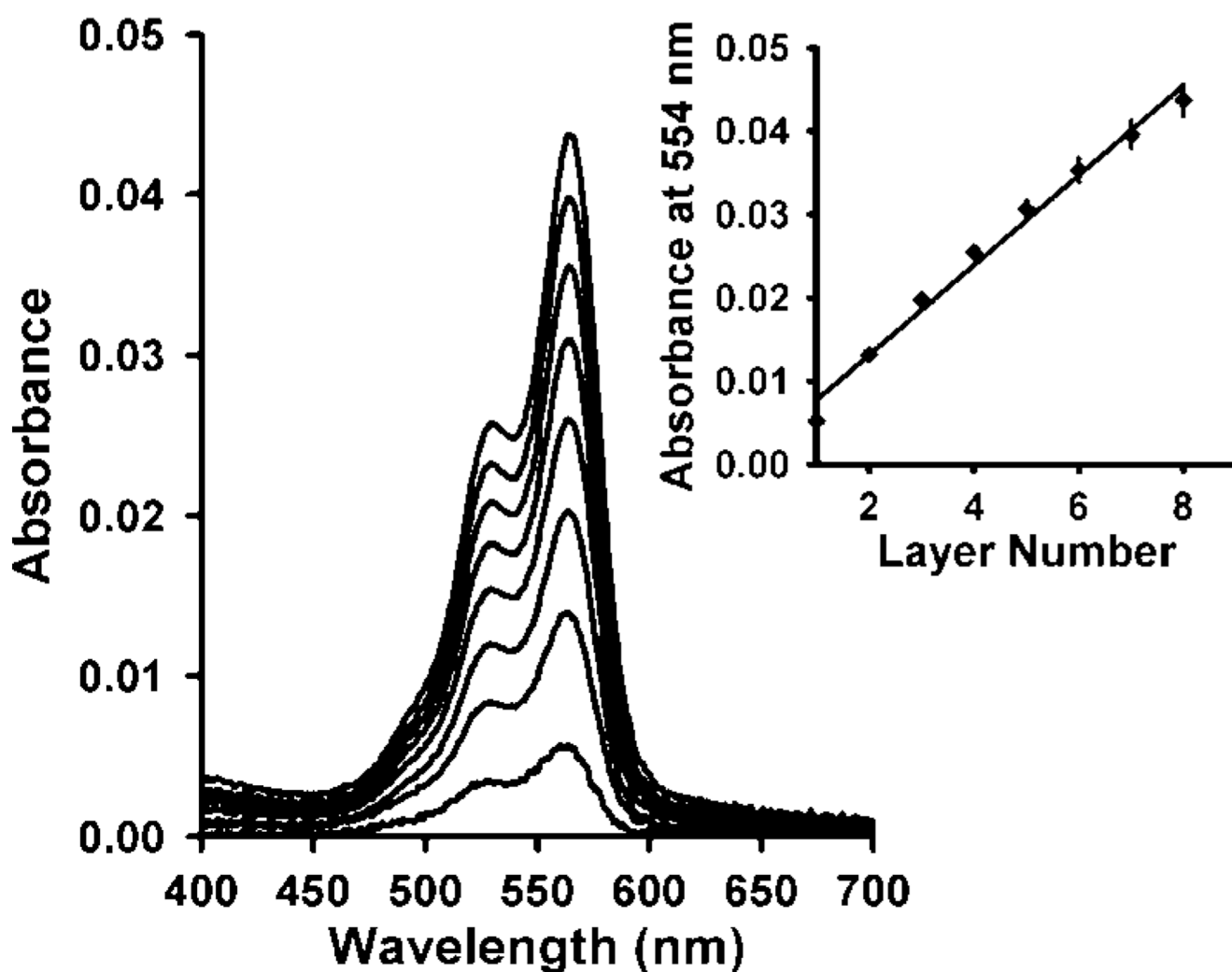
**Figure 2.**

PAH/PSS/PAH film assembly is toxic to human pancreatic islets. Representative confocal micrographs of (A) untreated and (B) PAH/PSS/PAH coated human islets stained with calcein AM (green, viable) and ethidium homodimer-1 (red, nonviable) overlaid on bright field micrographs (scale bar = 50  $\mu\text{m}$ ). (C) In a subpopulation of islets, coating with a PAH/PSS/PAH film resulted in considerable peripheral cell death, but a viable islet core (scale bar = 50  $\mu\text{m}$ ). (D) Fluorescent emission associated with ethidium homodimer-1 staining demonstrates a punctate distribution consistent with binding to nucleic acids within islet cell nuclei (scale bar = 20  $\mu\text{m}$ ). (E) Image analysis of confocal micrographs (live/dead) as well as viability assessment by MTS assay revealed a significant difference ( $*p < 0.01$ ) in viability between untreated (black bar) and PAH/PSS/PAH coated (gray bar) islets. (F) Lactose dehydrogenase (LDH) release from islets during deposition of the initial PAH layer (first layer), as well as after formation of a PAH/PSS/PAH film (gray bars) was significantly greater ( $*p < 0.01$ ) than untreated controls (black bars), indicating that islet cell membranes are compromised as a result of PAH/PSS/PAH coating.



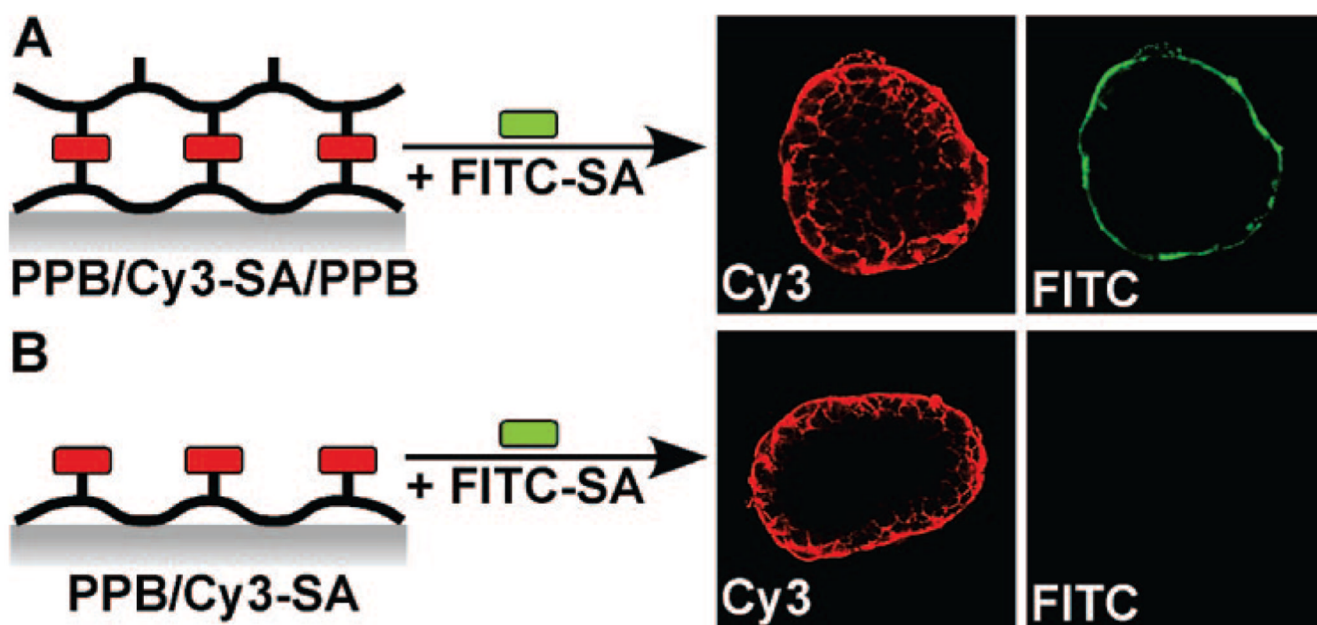
**Figure 3.** PPB facilitates specific binding of streptavidin to the surface of pancreatic islets. (A) Islets incubated with PPB for 15 min and subsequently with Cy3-labeled streptavidin (Cy3-SA) demonstrated fluorescent emission around the islet periphery. Islets incubated in only Cy3-SA demonstrated no fluorescent signal (B), and treatment of islets with nonmodified PLL prior to Cy3-SA resulted in discontinuous, concentrated domains of fluorescent emission (C) (scale bar = 50  $\mu\text{m}$ ).





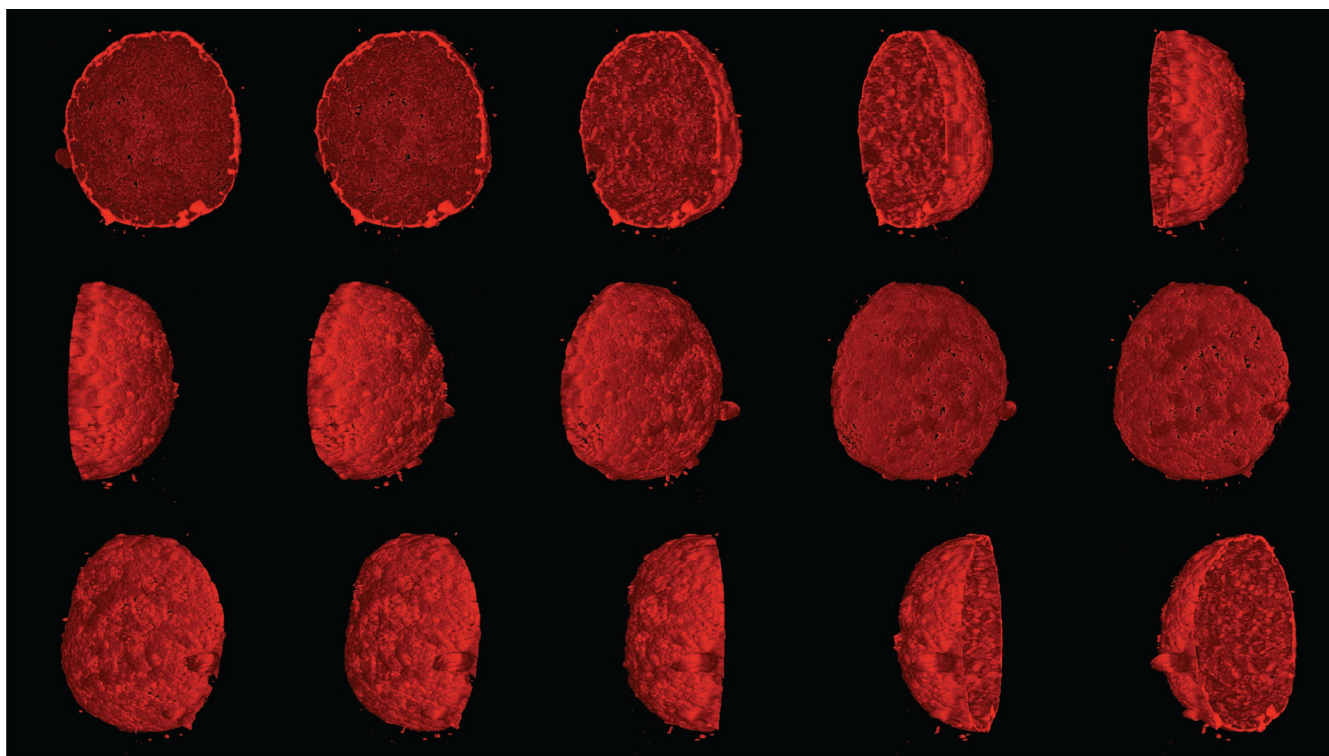
**Figure 4.**

PPB/SA multilayer thin films can be assembled on planar substrates. Solid-state UV-vis spectroscopy was used to monitor film growth on quartz slides. Absorbance spectra recorded after each PPB/Cy3-SA bilayer deposition demonstrates a regular layer-by-layer growth pattern. Inset: absorbance at 554 nm (Cy3; mean  $\pm$  standard deviation) increases linearly with layer number through at least eight bilayers.



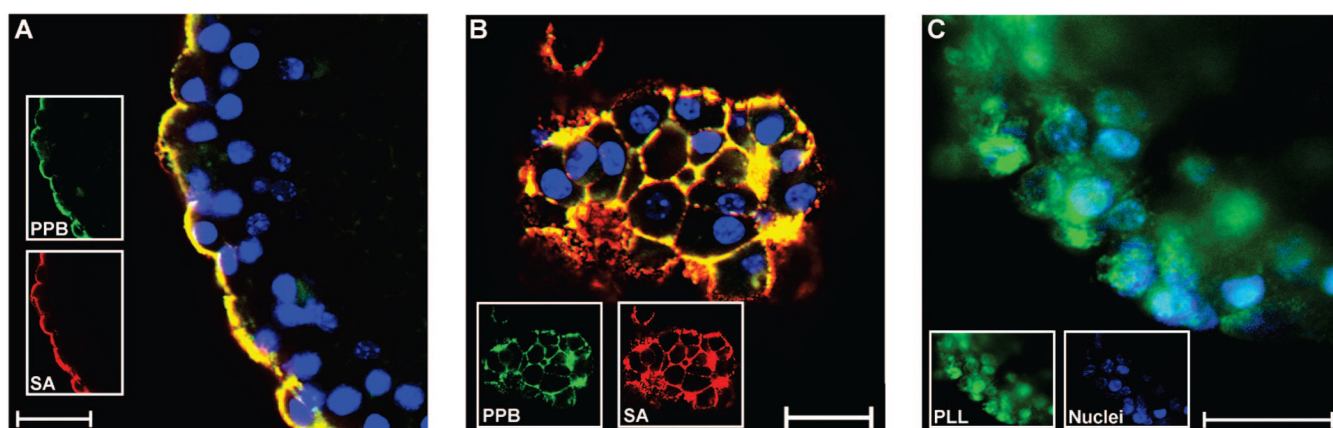
**Figure 5.**

PPB/SA multilayer films can be assembled on individual pancreatic islets. After formation of a PPB/Cy3-SA bilayer, islets were either incubated with a second layer of PPB (A) or placed into cell culture media (B). Both groups were then incubated with FITC-labeled streptavidin (FITC-SA) for 5 min. Only islets incubated with a second layer of PPB (A) demonstrated fluorescence emission from FITC-SA due to regeneration of accessible biotin groups on the islet surface.



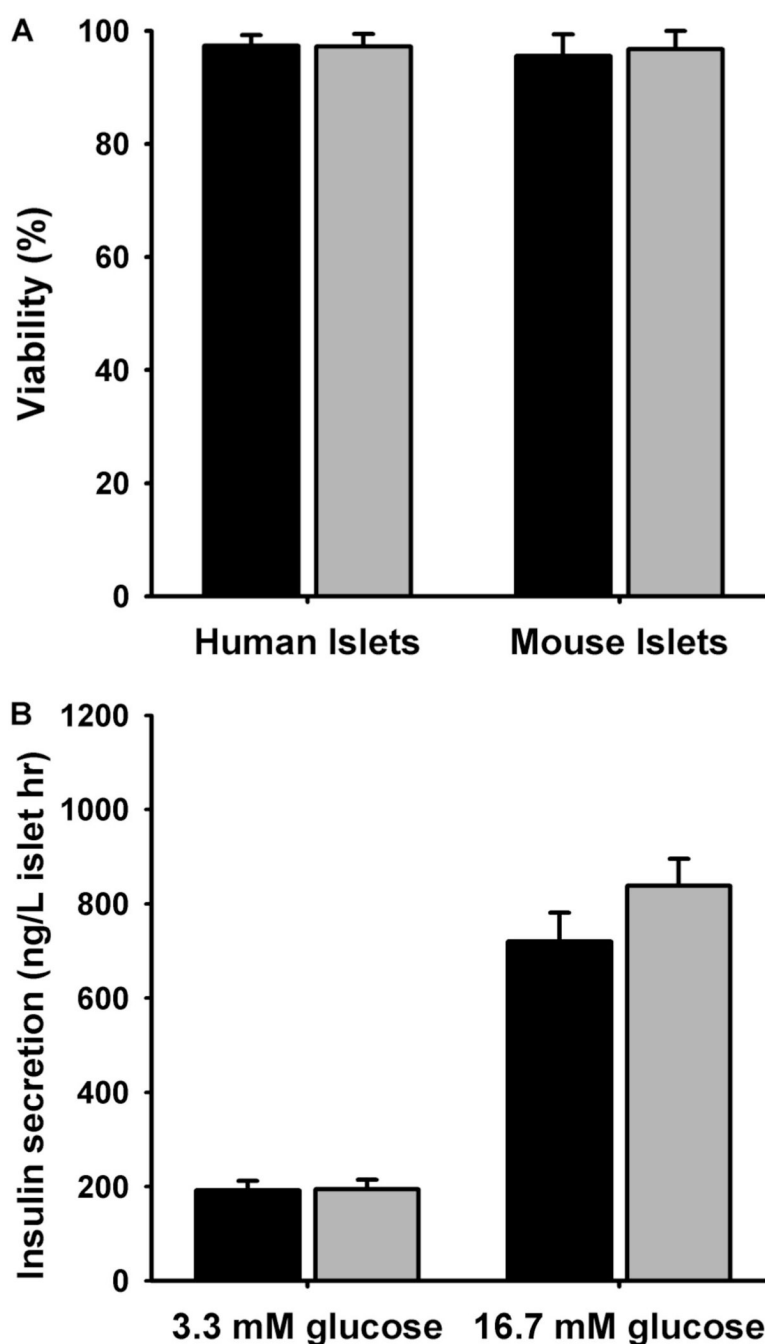
**Figure 6.**

Three-dimensional reconstruction of optical confocal microscope sections ( $0.5\ \mu\text{m}$ ) of the lower half of an islet coated with a  $(\text{PPB}/\text{Cy3-SA})_4$  multilayer film. Each image is rotated  $\sim 24^\circ$  from the previous (left to right, top to bottom). The film is grossly uniform and conforms to protrusions and indentations of the islet surface.



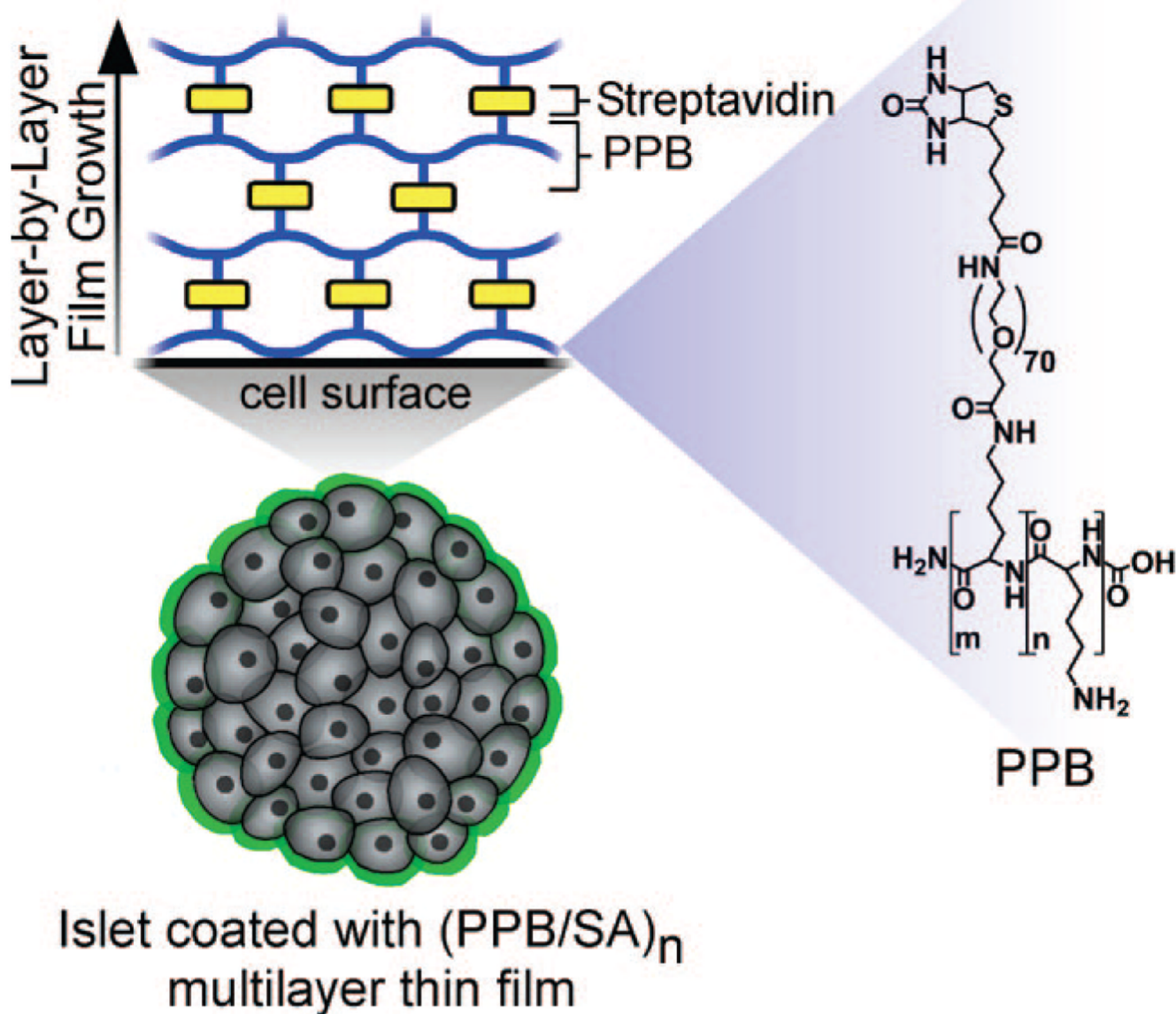
**Figure 7.**

PPB/SA multilayer films assemble extracellularly. Islet cell nuclei were stained with Hoechst (blue) to identify individual cells within islets. Islets were coated with a (FITC-PPB/Cy3-SA)<sub>4</sub> multilayer film, and confocal microscopy was used to identify film components. PPB and SA were colocalized on the surface of cells on the islet periphery (A) as well as in the interstitial space between individual cells within the core of the islet (B). Conversely, FITC-PLL was observed throughout the cytoplasm of cells and often colocalized with cell nuclei (C).



**Figure 8.**

Islet viability and function are preserved after formation of a (PPB[2.5]/SA)<sub>8</sub> multilayer film. (A) Viability (mean  $\pm$  standard deviation) was assessed after film formation via calcein AM and ethidium homodimer staining. Image analysis of confocal micrographs revealed no statistical difference ( $p > 0.05$ ) in islet viability between untreated (black bar) and coated islets (gray bar) for both mouse and human islets. (B) Untreated (black bar) and coated islets (gray bar) secrete statistically similar ( $p > 0.05$ ) amounts of insulin at both 3.3 and 16.7 mM glucose, indicating that islet function is not influenced by film formation. Data points represent mean  $\pm$  standard error, for a minimum of seven independent measurements.

**Scheme 1.**

Assembly of PEG-Rich, Nanothin Conformal Islet Coatings via Layer-by-Layer Deposition of Poly(L-lysine)-g-Poly(ethylene glycol) (PPB) and Streptavidin (SA)<sup>a</sup>

<sup>a</sup> PPB interacts electrostatically with negatively charged cell surfaces, facilitating the binding of SA. Unoccupied biotin binding sites of immobilized SA allow a second layer of PPB to be added, thereby enabling incorporation of a second SA layer. This process may be repeated to generate thin films assembled via alternating deposition of PPB and SA.