Encapsulation of islets of Langerhans
Device design and materials optimization of conformal coating for islets of Langerhans

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Encapsulation of islets of Langerhans may represent a way to transplant islets in the absence of immunosuppression. Traditional methods for encapsulation lead to diffusional limitations imposed by the size of the capsules (600–1,000 μm in diameter), which results in core hypoxia and delayed insulin secretion in response to glucose. Moreover, the large volume of encapsulated cells does not allow implantation in sites that might be more favorable to islet cell engraftment. To address these issues, we have developed an encapsulation method that allows conformal coating of islets through microfluidics and minimizes capsule size and graft volume. In this method, capsule thickness, rather than capsule diameter, is constant and tightly defined by the microdevice geometry and the rheological properties of the immiscible fluids used for encapsulation within the microfluidic system. We have optimized the method both computationally and experimentally, and found that conformal coating allows for complete encapsulation of islets with a thin (a few tens of micrometers) continuous layer of hydrogel. Both in vitro and in vivo in syngeneic murine models of islet transplantation, the function of conformally coated islets was not compromised by encapsulation and was comparable to that of unencapsulated islets. We have further demonstrated that the structural support conferred by the coating materials protected islets from the loss of function experienced by uncoated islets during ex vivo culture.

Significance

Cell encapsulation with biocompatible and permeable hydrogels may allow transplantation without immunosuppression. As an alternative to standard microencapsulation approaches that create single-sized capsules around cell clusters of different sizes, we have designed and optimized a novel approach for conformal coating of islets of Langerhans, resulting in thin, complete, and uniform coatings of similar thickness on differently sized islets. Coated islets exhibited no delay in glucose-stimulated insulin release or loss of function during culture, which is often observed with naked islets. The conformal coating reduces transplant volume relative to traditional encapsulation approaches. When transplanted in syngeneic diabetic mice, conformally coated islets restored and maintained euglycemia for more than 100 d with no foreign body reaction and normal revascularization.


Conflict of interest statement: A.A.T. and J.A.H. are coinventors of intellectual property used in this study. R.D.M. is a stock option holder in Converge Biotech, Inc., and A.P., C.R. and C.L.S. are members of the scientific advisory board and stock option holders in Converge Biotech, Inc., licensee of some of the intellectual property used in this study. R.D.M. is a stock option holder in Converge Biotech, Inc.

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link the precursor into an elastic hydrogel. The method allows for the “shrink-wrapping” of cell clusters with minimal amounts of coating materials by means of microfluidic templating, followed by chemical cross-linking of the liquid hydrogel precursor surrounding the islet to form a stable gel. Computational models were developed to optimize microfluidic chamber design and fluid dynamic variables. The modeling results were then confirmed experimentally with islet-simulating beads and pancreatic islets. In vitro glucose-stimulated insulin release (GSIR) and perifusion assays and syngeneic islet transplants then confirmed experimentally with islet-simulating beads and pancreatic islets. In vitro glucose-stimulated insulin release (GSIR) and perifusion assays and syngeneic islet transplants then confirmed experimentally with islet-simulating beads and pancreatic islets. In vitro glucose-stimulated insulin release (GSIR) and perifusion assays and syngeneic islet transplants then confirmed experimentally with islet-simulating beads and pancreatic islets. In vitro glucose-stimulated insulin release (GSIR) and perifusion assays and syngeneic islet transplants then confirmed experimentally with islet-simulating beads and pancreatic islets.

Results

Flow Focusing for Conformal Coating of Cell Clusters. High-throughput encapsulation of individual cell clusters into nanoliter droplets of PEG gel (conformal coating) was accomplished by exploiting the Raleigh–Plateau instability generated when a water phase is flowing in a coaxial jet within an immiscible oil phase. To generate a stable jet of water in oil, a dripping-to-jetting transition was induced in the water phase by focusing the external oil phase through external physical constraints, with a conical flow of reducing diameter in this study. The Rayleigh–Plateau instability then produces a disruption of the water-phase jet into droplets that have a characteristic size (nanoliters) proportional to the water jet size and dependent on (i) the water- and oil-phase flow rate ratio, (ii) the water- and oil-phase viscosity ratio, and (iii) the interfacial tension between the two phases (37). When particulates, islets of Langerhans in this study, are added to the inner water phase, their entrance in the focusing region induces an upstream rupture of the water jet, resulting in individual islet coating. Irrespective of islet size, the coating thickness is proportional to the size of the nanoliter droplets that are generated in the absence of particulates. Additionally, because the islet diameter is typically bigger than the water jet diameter, islets are kept separate from each other by the elongational component of the flow in the focusing region. Even in cases of elevated cell density, this property individually aligns islets in the center of the jet and maintains their separation, ultimately allowing for the encapsulation of single islets within single droplets, thus achieving conformal coating (Fig. 1A).

A microfluidic chamber and technique were developed to achieve the desired dripping-to-jetting transition of the islet/hydrogel precursor solution [water phase (w)] within the immiscible oil phase (o) and to disrupt the resulting water-phase jet into the desired nanoliter droplets ideal for conformal coating of individual islets. Using previously published work (38–40) and fluid dynamic finite element modeling (Comsol multiphysics), combinations of geometric and hydrodynamic parameter estimates of the oil and water phases were generated and screened to determine optimal settings for water-phase jet generation within the oil phase. Two model flow chamber designs with a total of 10 different combinations of geometric and hydrodynamic parameters, summarized in Table 1, were simulated. Different geometries tested, together with the chosen computational meshes, are shown in Fig. S1A. Three variations of the focusing/injection distance were tested: reduced focusing/standard injection distance of the water phase (a), increased focusing/standard injection distance (b), and reduced focusing and closer injection distance (c).

Based on the model developed by Suryo and Basaran (38), jetting of the water phase was predicted to occur for every combination of parameters tested and is shown in Table 1. This prediction is partly confirmed by the results from our simulations, which show that, depending on the different combinations of geometric and hydrodynamic parameters, jets of water with different characteristics can be generated (Fig. 1B and C; the volume fraction of the water phase is plotted). In particular, when using the less focused jet geometry (Fig. 1B) with a medium viscosity ($\eta_m$) oil phase, jetting of the water phase was achieved when $v_w = v_o$ (model 2), whereas when $v_w = v_o/5$ (model 1),

![Fig. 1. Computational model of the conformal coating procedure for optimization of the fluid dynamic conditions and the design of the encapsulation device. (A) Schematic of conformal coating encapsulation of islets compared with conventional microencapsulation. Consol plots of the water-phase volume fraction for models 1–5 (B; “less focusing”) and 6–10 (C; “more focusing”) and the velocity field for models 1–5 (D) and 6–10 (E) as a function of the z axis and r radius are shown.](image)

Table 1. List of combinations of geometric and hydrodynamic parameters simulated

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<th>$\eta_o$, Pa s</th>
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<th>$v_o$, m/s</th>
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<th>$1/Ca$</th>
<th>$mQ_R$</th>
<th>WIP</th>
<th>FG</th>
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$1/Ca = \mu_1Q_2/\pi R^2$, inverse of capillary number; $\eta_w$, water viscosity; $\eta_o$, oil viscosity; FG, focusing geometry; $\gamma$, interfacial tension; m, viscosity ratio; No., number of simulated model; $Q_w$, water flow rate; $Q_o$, oil flow rate; $Q_o$, $Q_w$; $T_r$, maximum radial stress; $T_z$, maximum z stress; $v_w$, maximum water velocity; $v_o$, maximum oil velocity; WIP, water injection point.
the water phase shifted to dripping. When a higher viscosity oil phase was used, and \( v_o = v_w \), a jet with a higher diameter was achieved (model 3). This result occurs independent of the injection point of the water phase [i.e., with the standard injection point (models 1, 2, 3, and 5) or with the injection point closer to the downstream channel (model 4)]. If, however, \( v_o = 10v_w \) (model 5), the size of the water jet and the stream area from the injection point were increased, resulting in a larger and less focused jet. When using the more focused geometry (Fig. 1C) and a high viscosity oil phase, jetting of the water phase was achieved even when \( v_w = v_o/5 \) (model 6). Increasing \( v_w/v_o \) proportionally increased the diameter of the water-phase jet (models 7–9). The stream area from the injection point was also increased and resulted in water-phase entrainment within the external part of the chamber (model 9). This entrainment could not be avoided even if the viscosity of the water phase was increased 10-fold, representative of the presence of particulates in the water phase (model 10).

In the less focused geometry, the velocity fields were not as affected by parametric variations (Fig. 1D, models 1–5) as they were when the more focused chamber was used, with significantly increased \( v_w/v_o \) ratios (Fig. 1E, models 6–9) and viscosity of the water phase (model 10).

For each simulation, the radial components \( f_r \) and axial components \( f_z \) of the total force per area on the central axis \( (r = 0) \) could be predicted (Fig. S1 B and C). For the less focused geometry (Fig. 1B), \( f_z \) decreased linearly with cylindrical axis \( (z) \), with water jet development occurring at higher velocities \((z = 0)\) for high oil-phase viscosity (Fig. 1B, models 3–5) than for middle oil-phase viscosity (models 1 and 2). At the same time, in middle oil-phase viscosity (model 1 and partially in model 2), \( f_z \) oscillated between positive and negative values, suggesting a dripping profile for the water phase. In the higher oil-phase viscosity (models 3–5), \( f_z \) became more stable with consistently positive values, achieving maximum values with higher \( v_w/v_o \) (model 5). In the more focused geometry (Fig. 1C), \( f_z \) decreased linearly with \( z \), with water jet development starting at both higher values of \( v_w/v_o \) at \( z = 0 \) (Fig. 1C, models 7–9) and higher water-phase velocity (model 10). By varying both \( v_w/v_o \) and the viscosity of the oil and water phases (models 6–10), \( f_z \) was greatly affected. Minimal absolute values were obtained in model 6.

From this computational analysis, we concluded that the desired water-phase jetting from a coaxially flowing oil phase was achieved with geometries of different stream focusing. The position of the water-phase injection tip did not affect the fluidic outcome. Using oil phases of higher viscosity facilitated jet formation, particularly with the less focused geometry. This was the case even with decreased water-phase velocities and resultant minimal pressure drop values.

**Validation of Modeling and Flow Chamber: Conformal Coating of Model Beads.** Following fabrication of the coating device (Fig. 2A and SI Materials and Methods), the next phase focused on the geometric feasibility of conformal coating with consistently complete encapsulation of islets flowing within the water phase (which includes the hydrogel precursor and the cross-linker), break-up of the islet-containing water phase must occur before the initiation of gel polymerization through cross-linking. An eight-arm 10-kDa PEG functionalized with vinyl sulfone (VS) was selected as the gel precursor. The VS-reactive groups can be cross-linked through reduction with DTT. Exploiting the pH sensitivity of this reaction, the PEG-VS was mixed with DTT at a slightly acidic pH of 6 and triethanolamine (TEOA) was placed in the oil phase of the collection vessel downstream encapsulation to drive polymerization. Polypropylene glycol (PPG) was used as the oil phase. An oil-soluble surfactant [10% (vol/vol) Span80] was added to the oil phase to reduce surface tension to the desired value.

Initial encapsulation studies were carried out with idealized spherical beads as models of cell clusters, using sizes typical of islets of Langerhans (i.e., 50–400 \( \mu \)). In preliminary studies with 10% (wt/vol) PEG-VS, it was determined that conformal coating was optimal when the more focused chamber geometry was implemented with a vertical configuration (Fig. 2A) and the following flow rates (Q) were used: \( Q_w = 10 \mu \text{L/min} \) and \( Q_o = 3.5 \text{ mL/min} \) (which gives a velocity ratio, \( V_o/V_w = 3.5 \), with cross...
sectional area of the oil phase ($A_o = 100 \times A_w$ and $Q = V \times A$). TEOA at 0.2% was included in the collection vessel. Resulting conformally coated beads were purified from the oil phase by repeated exposure of the collected exudate to hexane, followed by hexane/HBSS washes with centrifugation, as described in Materials and Methods.

Coating completeness was confirmed in selected procedures by fluorescent labeling (using FITC-labeled PEG-VS) of the coatings (Fig. 2 B and C). As illustrated, the constructed encapsulation system, using parameters designed via modeling, generated the right combination of hydrodynamic conditions to achieve complete conformal (coating thickness of 10–50 μm) encapsulation of model beads with the cross-linked PEG hydrogels.

Optimization of the Conformal Coating Process on Islets. The process for conformal coating was further optimized using multiple sources of islets of Langerhans, examining completeness of coating and the effect of the procedure on islet viability and function in vitro (GSIR).

In initial coating attempts with 10% (wt/vol) PEG-VS, phase-contrast and confocal images of islets coated with fluorescently labeled hydrogels demonstrated that the procedure was readily transferable to coating primary pancreatic islets (Fig. 2 D–F). Islet function in these examples, however, was determined to be compromised.

Step-by-step procedural testing was implemented to determine the effect of each variable of the encapsulation purification process on islet function, with GSIR and coating imaging as the experimental outcomes. The factor with the greatest negative impact on islet viability and function was the concentration of TEOA in the collection vessel. Decreasing the concentration from 0.1 to 0.02% (vol/vol) greatly improved islet function, as indicated by GSIR (Fig. 2G) for both human and rat islets ($P < 0.01$). We further confirmed that proper TEOA concentration was critical to islet viability and function by putting islets through the encapsulation and purification process in the absence of coating polymers and observing that 0.1% TEOA was detrimental (Fig. 2G).

To explore any potential detrimental impact of the residual oil (PPG) phase from the encapsulation process, final processing with or without hexane (which was used to extract PPG completely from the water phase) was carried out. As indicated by GSIR results, purification of encapsulated islets through hexane extraction of PPG did not have an impact on the in vitro function of the coated islets, whereas function was decreased when hexane was not used (Fig. 2 H and I; $P < 0.05$).

These adjustments (utilization of 0.02% TEOA in the oil phase in the collection vessel and final removal of the oil phase using a hexane wash) improved outcomes, as measured by functional assessment, and allowed for reproducible conformal coating of human, NHP, and rat islets when 10% (wt/vol) PEG-VS polymer and DTT cross-linker were used (Fig. 2J; $P > 0.05$).

Optimization of Hydrogel Composition for Conformal Coating of Islets. Following optimization of conditions for generating conformal coatings on islets, the next phase focused on selection of the appropriate biomaterials and cross-linking strategies to achieve stable polymeric capsules of appropriate permeability. Both the composition and degree of cross-linking of coating hydrogels were investigated and were related to changes in islet functional outcomes and resulting coating permeability.

By increasing the concentration of PEG-VS from 10% to 20% (Fig. 3A; trend but $P > 0.05$) or by increasing the thickness of coatings (by making macrocapsules instead of conformal coatings; Fig. 3B), there was a concomitant decrease in islet function, as shown by elevated insulin secretion during the second basal glucose step of the GSIR, a profile indicative of dysfunctional and/or dying beta cells. These results indicate a benefit of thinner, less dense coatings. Decreasing the degree of DTT-mediated cross-linking of 10% PEG-VS by “capping” a portion of the VS functional groups on the eight-arm PEG-VS before DTT exposure did not affect the function of coated islets (Fig. 3 C and D; $P > 0.05$), suggesting that the cross-link density attained with the 10 kDa of PEG-VS was appropriate. Increasing the molecular weight (Mw) and/or branching of the cross-linking agent via linear (1 $M_w$, of Mw) or multiarm (four-arm, 10 $M_w$, and 20 $M_w$, of Mw) PEG multithiol cross-linkers resulted in no statistically significant change in islet GSIR function (Fig. 3E; $P > 0.05$). The addition of 0.8% or 0.4% ALG to 5% PEG-VS improved the function of the encapsulated islets compared with 5% PEG-VS alone, although this improvement was not observed for 10% PEG-VS-ALG (Fig. 3F; $P < 0.01$). Islet functional assessment results positively correlated with the permeability to 10 kDa of FITC-dextran, as a model permeant molecule, of the PEG coatings with different compositions (Fig. 3G). The addition of ALG to the PEG-VS hydrogel precursor increased coating permeability. Based on these results, 5% PEG-VS with 0.8% wt/vol MVG

![Fig. 3. Optimization of hydrogel composition for conformal coating of islets. (A) Effect of percentage of PEG-VS ($P > 0.05$). (B) Effect of coating thickness: conformal coating (CC) vs. macrocapsule (MACRO, diameter: 600–1,000 μm). (C and D) Effect of saturating (CAP) zero, two, or four of the eight functional groups of PEG-VS with CC. (E) Combined effect of cross-linker: DTT vs. 1-kDa linear PEG dithiol (HS-PEG-SH) vs. four-arm 10-kDa or 20-kDa PEG-SH and of PEG-VS percentage: 10% vs. 5%. (F) Effect of ALG (MVG) addition. Shown are mean ± SD with $n = 3$ for each value. (G) Diffusion of 10-kDa FITC-dextran outside of hydrogel microparticles with different PEG compositions as indicated by c/c₀ over time. c, concentration; c₀, concentration of dextran at equilibrium. LG = 2.2 mM and HG = 16.7 mM. *$P < 0.05$; ***$P < 0.01$.](image-url)
[medium viscosity ALG with 60% minimum glucuronate; Pronova] (PEG-ALG), cross-linked with a 4:1 molar ratio (3.1 mg/mL) of DTT, was selected as the optimal hydrogel configuration for further study.

**Conformal Coating of Islets with PEG-ALG Does Not Impair in Vitro and in Vivo Function of Rodent Islets.** Conformal coating with the optimized PEG-ALG formulation allowed for minimal coating thickness (typically a few tens of micrometers) encapsulation on rodent islets (Fig. 4). No delay in insulin secretion during dynamic perfusion assessment was observed in the coated islets relative to uncoated controls (Fig. 4 B and C). Of interest, although uncoated islets gradually lost their GSIR function during extended in vitro culturing (Fig. 4D), this was not observed in PEG-ALG conformally coated islets (Fig. 4 E and F; P < 0.05). Timing of encapsulation after islet isolation was also a critical variable in postprocedural function: Islets encapsulated 48 h after isolation had improved GSIR function relative to those encapsulated after 24 h (Fig. 4 G and H; P < 0.05).

The very small thickness of the PEG-ALG conformally coated islets allowed correspondingly small transplant volumes to be placed in the renal subcapsular space to test function through transplantation of curative numbers of islets. To evaluate this, chemically induced diabetic mice were transplanted with either 700 or 1,500 syngeneic islet equivalents (IEQ) per mouse under the kidney capsule. Euglycemia (nonfasting blood glucose <200 mg/dL) was achieved and maintained for at least 112 d. Function was further confirmed by nephrectomy of the graft-bearing kidney and recipient reversion to hyperglycemia (Fig. 4F, arrows). Biocompatibility of PEG-ALG coatings analyzed with empty PEG-ALG capsules at day 7 and day 21 postimplantation and for PEG-ALG conformally coated syngeneic murine islet transplants at day 56 and day 116 posttransplantation was found to be strong, based on lack of fibrosis (Fig. S2A; H&E staining) and minimal detection of macrophages (Fig. S2B). Intact islets containing insulin<sup>+</sup> beta cells and glucagon<sup>+</sup> alpha cells were found in conformally coated grafts retrieved at day 56 and day 116, and were comparable to those observed in naked islet transplant controls (Fig. S2C). CD3<sup>+</sup> lymphatic vessel endothelial hyaluronan receptor 1 negative (Lyve-1) blood (Fig. S2D) and CD31<sup>+</sup>Lyve-1<sup>+</sup> lymphatic (Fig. S2E) vessels were observed between the implanted capsules but not within them.

**Discussion**

A significant issue limiting the efficacy of correction of type 1 diabetes following the transplantation of encapsulated islets has been the relatively large size of the capsules generated via conventional microencapsulation. In traditional approaches, islets are incorporated within capsules of uniform size, determined by the geometry of a destabilized jet-forming apparatus. As such, the capsule size is adapted for the largest of islets, resulting in thick capsules on the majority of the islets. The thick capsules increase the volume of the total transplant, thus limiting selection of transplant sites; additionally, the thick layer of stagnant water created by the capsule increases transport limitations. To address this issue, we designed and implemented an encapsulation method that has the potential to overcome many of the limitations of prior methods. The presented flow-focusing approach creates a thin film of a water phase upon individual islets suspended within an oil continuous phase. Rather than the total diameter being determined by the encapsulation parameters, the thickness of a conformal coating is determined by the geometry, viscosity ratios, interfacial tension, and flow rate ratios of the process, with the overall diameter being determined by the individual islet diameter. This results in coatings with consistent thickness, independent of islet diameter. In the process we have developed, the conformal water phase contains a hydrogel precursor (PEG-ALG). In this approach, the precursor is converted to a gel upon a pH change initiated by exposure to a diffusible component within the oil phase. Using thiol-mediated (using DTT as a dithiol) cross-linking of a reactive multiarm PEG (using VS moieties), rapid gelation was achieved without cytotoxicity or loss of islet function.

One important challenge facing islet encapsulation therapies is the delayed insulin secretory response to glucose challenge (41, 42). Traditional microencapsulation methods are plagued by such delays, which are the combined result of limited polymer permeability and increased diffusion distances (43). In our hands, minimizing the coating thickness to a few tens of microns in thickness, and thereby the diffusion distance, despite the decreased permeability compared with ALG, resulted in no observable delays in islet insulin secretory response to glucose stimulation. Due to the intrinsic lack of size-limiting factors of the conformal coating encapsulation technology that we have presented here, we were able to transplant conformally coated islets at curative doses in sites not accessible with standard microencapsulation methods, such as the renal subcapsular space. Lack of fibrosis and a necrotic core suggest that conformal coating with the PEG-ALG successfully addressed issues of both biocompatibility and diffusion limitations.
Materials and Methods

Inputs for the computational model in Consol multiphysics were based on the model from Surjo and Basaran (38). Functionalization of eight-arm 10-kDa PEG (Jenken Technology) with VS (Sigma) was achieved by Michael-type addition of VS to PEG-10k. Gelation of PEG-VS was achieved by cross-linking with DTT (Sigma), 1-kDa PEG diithiol (HS-PEG-SH), or a 2:1 molar ratio of four-arm 10-kDa PEG thiol (PEG-SH) or four-arm 20-kDa PEG-SH (CreativePEG- works), adjusting the final pH to 7.4 through addition of TEOA (Sigma) to the collection bath (Fig. 2A, a). Islet-sized polystyrene beads (Polysciences, Inc.) were suspended at 20–40% (wt/vol) and islets at 25,000–75,000 IEQ/mL in a solution of 2.5–20% (wt/vol) eight-arm 10-kDa PEG-VS and cross-linker with 0.4–0.8% (wt/vol) UP-MVG grade ALG (Prontova) in RPMI for selected experiments at pH 6, and were injected through a 16-gauge i.v. catheter (Fig. 2A, a) at constant flow rate of 10 μL/min. An external oil phase of PPG with Mw = 3,500 with 10% (vol/vol) Span80 (Sigma) was flowing coaxially at the constant rate of 3.5 mL/min. Coated beads/islets were separated from the oil phase by hexane extraction. G3IR and perfusion were performed to assess the function of encapsulated islets. Islets were isolated from C57BL/6 mice as described elsewhere (44). Chemically diabetic C57BL/6 mice received 700–1,500 IEQ of C57BL/6 mouse islet grafts under the kidney capsule as described elsewhere (44). Data are presented as mean ± SD (Student t test or one-way ANOVA). Details of these procedures are provided in SI Materials and Methods.

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Supporting Information

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SI Materials and Methods

Determination of Hydrodynamic Jetting Parameters Through Mathematical Modeling. A mathematical model from Suryo and Basaran (1) was used to estimate fluid dynamic values that allow for generation of a water jet phase within a coaxial oil phase. For fixed radial ratio, \( a = R_2/R_1 \), phase 1 (incompressible Newtonian fluid, water in our case) flowing coaxially with phase 2 (incompressible Newtonian fluid, oil in our case) within cylindrical tubes forms a jet when 1/Ca > 0. Here, \( R_1 \) is the extrusion radius of the fluid 1 stream, \( R_2 \) is the radius of the concentrical cylindrical tube containing the fluid 2 stream, Ca = \( \mu_2Q_2/(\pi R_1^2) \) is the capillary number, \( \gamma \) is the interfacial tension, \( m = \mu_2/\mu_1 \) is the dynamic viscosity ratio, and \( Q_2 = Q_2/Q_1 \) is the ratio of the volumetric injection rates. The interfacial tension between the water phase and the oil phase supplemeted with 5% (vol/vol) Span 80 surfactant (Sigma) is estimated to be 5 mN/m. \( R_2 \) is 5 mm and \( R_1 \) is 0.6 mm (16-gauge needle). The value of \( \mu_1 \) is 0.34 Pa s for a moderately viscous silicon oil (Sigma) and 1.301 Pa s for a highly viscous polypropylene glycol [PPG; molecular weight (Mw) = 4,000] oil (Sigma). The value of \( \mu_1 \) is estimated to be 0.01 Pa s for a 10% (wt/vol) 10-kDa PEG solution and 0.1 Pa s for a 10% (wt/vol) 10-kDa PEG solution including model beads.

Comsol Modeling. Comsol multiphysics (two-level set function of the chemical engineering modulus) was used for developing computation models. The effect of different geometric and hydrodynamic parameters on the transition from dripping to jetting of the water phase, the size of the final water jet, and the total stress acting in the center of the water jet (r = 0) were evaluated through the computational model. The geometric parameters were "more focused" and "less focused" flow chambers and different injection points of the water phase into the oil phase. The hydrodynamic parameters were the ratio between the water and oil flow rates, the ratio between the water and oil viscosities, and the interfacial tension between the two phases.

A 2D axisymmetrical geometry was used with two different focusing parts, namely, more focused and less focused, in addition to different water injection points. The used computational mesh had a maximum size in the central axis (r = 0) equal to \( 10^{-5} \) m (Fig. 1B). Velocity values for the oil and water phases used in the model were increased two orders of magnitude to minimize compiling times and memory use. Surface plots of the volume fraction of fluid 1 (water phase) and the velocity profiles at the last time point of compilation, along with boundary plots of the total force per area (stress) in the radial direction (L_r) and in the z direction (L_z) at r = 0, were generated in the post-processing analysis. Conditions compiled for each model (models 1–10) are shown in Table 1.

Design and Fabrication of Flow Chambers for Encapsulation. Flow chambers with variable stream-focusing capability were fabricated, guided by the computational models used to predict optimal settings for conformal coating. Our goal was to test finite element modeling results and determine the effect of different hydrodynamic parameters on the coating of model beads and islets. Flow chambers with two geometries, more focused (38.2°; Fig. 2A) and less focused (68.2°), were designed in SolidWorks (Dassault Systèmes, Waltham, MA) and then fabricated. Based on the results of the modeling, we used the high-viscosity oil phase. Computer-aided design diagrams of the fluid-focusing chambers are shown in Fig. 2A. The main chamber (Fig. 2A, a) was filled with oil through a lateral port (Fig. 2A, b) connected to a peristaltic pump, allowing for a continuous and constant flow rate of the oil phase. The water phase was injected coaxially to the oil phase through a screw cap secured by an O-ring (Fig. 2A, c) into the main chamber. Within the screw cap, a plastic 16-gauge venous catheter was inserted and secured in the position apical to the focusing portion of the chamber (Fig. 2A, c). The outlet of both phases, where jet breakup is expected to occur, was positioned directly after the focusing portion of the main chamber (Fig. 2A, e), exiting into the collection vessel.

Functionalization of PEG with vinyl sulfone, Labeling with Fluoresceinamine and Polymerization. Functionalization of eight-arm 10-kDa PEG (Jenken Technology) with vinyl sulfone (VS; Sigma) (PEG-VS between 75% and 90%) was achieved by Michael-type addition of VS in the presence of equal moles of sodium hydride (NaH; Sigma). The reaction was incubated for 3–5 d. After this period, the NaH was neutralized by slow addition of acetic acid (99%; Sigma). Purification was performed by filtration and consecutive precipitations (3) of functionalized polymer in cold diethyl ether (Sigma) to remove excess VS. 1H NMR was performed to calculate the degree of functionalization. For fluorescence microscopy imaging purposes, PEG-VS was labeled (1.6% functionalization) with fluoresceinamine (Sigma) to yield FITC-PEG-VS, by Michael-type addition in dimethylformamide. Gelation of PEG monomers was achieved by cross-linking eight-arm 10-kDa PEG-VS with DTT (Sigma) or 1-kDa PEG dithiol (HS-PEG-SH) (CreativePEGWorks) at a 4:1 molar ratio or with four-arm 10-kDa PEG-SH or four-arm 20-kDa PEG-SH (CreativePEGWorks) at a 2:1 molar ratio, adjusting the final pH to 7.4 through the addition of triethanolamine (TEOA; Sigma) to the collection bath (Fig. 2A, e).

Validation of Theoretical Models Through Conformal Coating of Model Beads. For cell-free testing and optimization of the encapsulation procedure, islet-sized polystyrene beads (PolySciences, Inc.) ranging from 50 to 400 μm were suspended at 20–40% wt/vol in a solution of 10% vol/vol eight-arm 10-kDa PEG-VS (or FITC-PEG-VS for coating completeness studies) with a 4:1 molar ratio of DTT (6.2 mg/mL; Sigma) in RPMI basal media (Invitrogen). The suspension was made at pH 6 and injected through a 16-gauge iv. catheter at a 10-μL/min constant flow rate. An external oil phase of PEG with Mn = 4,000 with 10% (vol/vol) Span 80 (Sigma) was flowing coaxially at the constant rate of 3.5 mL/min. According to the mathematical model from Suryo and Basaran (1), we have applied the viscosity ratio (m) multiplied by the flow rate ratio (Qr) (mQr) = 45,535, which is greater than 1/Ca_r. For cell-free testing and optimization of the encapsulation process, islet beads were isolated on the results of the modeling, we used the high-viscosity oil phase. Computer-aided design diagrams of the fluid-focusing chambers are shown in Fig. 2A. The main chamber (Fig. 2A, a) was filled with oil through a lateral port (Fig. 2A, b) connected to a peristaltic pump, allowing for a continuous and constant flow rate of the oil phase. The water phase was injected coaxially to the oil phase through a screw cap secured by an O-ring (Fig. 2A, c) into the main chamber. Within the screw cap, a plastic 16-gauge venous catheter was inserted and secured in the position apical to the focusing portion of the chamber (Fig. 2A, d). The outlet of both phases, where jet breakup is expected to occur, was positioned directly after the focusing portion of the main chamber (Fig. 2A, e), exiting into the collection vessel.

Coated beads and PEG bead-free secondary particles were collected in a beaker containing 10% (vol/vol) Span 80 and between 0.01% and 0.1% TEOA. The oil phase was then separated from the water phase by extraction in HBSS (Invitrogen) or hexane (Sigma) and hexane/HBSS and repeated centrifugation. In selected experiments, empty (bead-free) PEG secondary particles were then separated from PEG-coated beads by Percoll density gradient centrifugation: 1.134 g/mL, 1.06 (uncoated beads layer), 1.036 (coated beads layer), and 1.008 (bead-free PEG secondary particles). Process optimization was performed by varying (i) the ratio of water-to-oil velocities, (ii) the bead concentration in the water phase, (iii) the concentration of TEOA in PPG/SPAN-80 in the collection vessel, and (iv) the purification of PEG-coated beads.
Conformal Coating of Pancreatic Islets with PEG-Based Hydrogels. Islets from different sources were suspended at 25,000–75,000 islet equivalents (IEQ)/mL in a solution of 2.5–20% wt/vol eight-arm 10-kDa PEG-VS (or FITC-PEG-VS for coating completeness studies). Cross-linkers were added to the same solution: 4:1 molar ratio of DTT or 1-kDa HS-PEG-SH or 2:1 molar ratio of four-arm 10-kDa PEG-SH or four-arm 20-kDa PEG-SH. In selected experiments, 0.4-0.8% wt/vol of UP-MVG (ultra pure medium viscosity sodium alginate with 60% minimum gluconate; Pronova) in RPMI or HBSS (without Ca²⁺ and Mg²⁺) medium was also added to the solution at pH 6. The islet-containing polymeric solution was then injected within the encapsulation flow chamber (Fig. 2A) through a 16-gauge i.v. catheter at a constant flow rate of 10 μL/min coaxially within an external oil phase made by PPG with 10% (vol/vol) Span80 flowing at a constant rate of 3.5 mL/min. Collection and purification of cell-containing and cell-free secondary particles from the oil phase were carried out as described above for encapsulation of model beads.

Optimization was performed by varying (i) the islet concentration, (ii) the TEOA content in the PPG/Span-80 in the collection vessel, (iii) the purification of PEG-coated islets, and (iv) the composition of the PEG hydrogels. Capping of functional groups was achieved by adding a solution of 2-mercaptoethanol (Sigma) in HBSS to the PEG solution at an n:1 molar ratio to saturate (cap) n-functional groups of the PEG-VS before addition of islets and cross-linker.

Glucose-Stimulated Insulin Release. Glucose-stimulated insulin release (GSIR) was performed to assess in vitro function of encapsulated islets compared with uncoated control islets (naked control). Briefly, islet aliquots (100 IEQ) were loaded within a Sephadex (G-10; GE Healthcare) slurry into microchromatography columns (BioRad) and incubated in low-glucose (LG) Krebs buffer (2.2 mM or 40 mg/dL glucose; Sigma) for 1 h at 37 °C for equilibration and preincubation. This was followed by sequential incubations for 1 h each in low glucose (LG1, 2.2 mM or 40 mg/dL), high glucose (HG, 16.7 mM or 300 mg/dL), and low glucose (LG2, 2.2 mM or 40 mg/dL). At the end of each period, 1 mL of eluate was collected by adding 1 mL of LG Krebs buffer to each column. Insulin concentrations in eluted samples were assessed by ELISA (Mercodia). GSIR was represented as absolute values of insulin concentration in supernatants and as a stimulation index (GSIR Index, namely, the ratio of insulin released after exposure to high glucose over the insulin released in a basal low condition).

Mice. Male C57BL/6 mice (Jackson Laboratories) were housed at five mice per cage in microisolator cages in a temperature-controlled environment with a 12:12-h light/dark cycle with ad libitum access to autoclaved food and water, and they were used at 6–8 wk of age as islet recipients and at 10–12 wk of age as islet donors. Animal studies were performed under protocols reviewed and approved by the Animal Ethics Committee of the University of Miami Institutional Animal Care and Use Committee.

Islet Isolation, Diabetes Induction, and Islet Transplantation. Islets were isolated by collagenase (type V, Sigma) digestion, followed by purification on Eurocollip density gradients (Mediatech), as described elsewhere (3). Diabetes was induced by a single i.v. injection of streptozotocin (200 mg/kg; Sigma–Aldrich). Chemically diabetic C57BL/6 mice received C57BL/6 mouse islet grafts under the kidney capsule as described elsewhere (3). Graft function was monitored by measuring nonfasting glycemic values on whole blood using portable glucometers (OneTouch Ultra 2; LifeScan). Nephrectomy of the kidney with the graft was performed at selected time points to confirm graft function following a return to hyperglycemia.

Graft Histology and Imaging. Formalin-fixed samples of the grafts were embedded in paraffin, sectioned (5 μm), and processed for standard H&E histology or immunofluorescence. Images were acquired with a Leica DMIRB microscope for histology or a Leica SP5 inverted confocal microscope (for fluorescence imaging) and processed with the Leica Application Suite software and ImageJ 3D (National Institutes of Health).

Statistics. Unless otherwise noted, data are presented as mean ± SD. The statistical significance of differences between two groups was determined by a two-tailed standard Student t test using Prism 5.0 for Macintosh software (GraphPad). The statistical significance of differences between more than two experimental groups was determined by one-way ANOVA, followed by Tukey’s post hoc test using Prism software.

Fig. S1. Computational model of the conformal coating procedure for optimization of the fluid dynamic conditions and the design of the encapsulation device. (A) Finite element modeling (using Comsol) geometry and meshing of three flow chamber models: less focusing/standard injection of the water phase (a), more focusing/standard injection (b), and less focusing and closer injection in an axisymmetrical model (z axis vs. radius r) (c). Comsol plots show the radial components ($f_r$) and the axial components ($f_z$) of the total force per area on the central axis ($r = 0$) for models 1–5 in the “less focusing” chamber (B) and models 6–10 in the “more focusing” chamber (C).
Fig. S2. Biocompatibility, islet morphology, and revascularization of cell-free PEG alginate (ALG) (at day 7 and day 21 posttransplantation) and conformally coated syngeneic murine islet grafts (at day 56 and day 116 posttransplantation) transplanted in streptozotocin diabetic C57BL/6 (BL/6) mice under the kidney capsule (KD). Formalin-fixed, paraffin-embedded 5-μm thick tissue sections have been processed. Biocompatibility was assessed by H&E staining (A) and MAC2+ macrophage immunofluorescence (B; green); islet morphology was assessed by insulin (INS, red) and glucagon (GLU, green) immunofluorescence (C), and islet revascularization was assessed by insulin (INS, red) and CD31 (green, arrows) (D) or Lyve-1 (E; green, arrowheads) immunofluorescence. Capsules are outlined. (Scale bars, 50 μm.)