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Supporting Information

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Title An Automated Process for Layer-by-Layer Assembly of Polyelectrolyte Multilayer Thin Films on Viable Cell Aggregates

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Experimental Section

Automated Coater Design. The automated coater was designed and assembled as shown in **Figure S1**. Syringe pumps (Model 33 Twin, Harvard Bioscience, Holliston, Massachusetts) and three independently controlled 10 mL syringes (Normject, Henke-Sass Wolf GmbH, Tuttlingen, Germany) were used to infuse solutions through 19 gauge 1.5 in needles into the open top of the reactor (standing cell culture insert, 12 μm pore size, 0.6 cm^2 filtration area, Millipore, Billerica, Massachusetts). The reactor-to-Luer Lock fitting was custom fabricated from extruded acrylic polymer by numerically controlled milling. Pinch valves (ValveBank, AutoMate Scientific, Berkeley, California) and reservoirs (glass beakers) were incorporated to purge or refill syringes, as necessary. Clear PVC tubing (0.0625 in inner diameter, 0.0625 in wall thickness, McMaster-Carr, Princeton, New Jersey) and barbed Luer Lock connectors were used to make all necessary connections. A personal computer (PC) running a program written in LabView (National Instruments, Austin, Texas) was used to integrate feedback and control the automated process. All solutions were infused at 15 mL min^{-1} and evacuated at 0.5 mL min^{-1}, the maximum flow rates empirically determined not to cause islet damage. All components except the PC were sterilized with 70% ethanol and housed in a laminar flow hood.

Process Control. Machine Vision (MV) was used to measure fluid levels during each evacuation. Solution was first evacuated for 10 seconds to form a consistently shaped meniscus. Evacuation was then paused for 3 seconds, during which time the LabView program captured 30 still images from a USB webcam (QuickCam 4000, Logitech, Fremont, California) positioned 5 cm from the reactor. As demonstrated in **Figure S2**, the LabView program identified the superior and inferior edges of each fiducial and the bottom of the meniscus using edge detection algorithms (LabView Vision Development Module, National Instruments, Austin, Texas). In each image, the remaining volume in the reactor was calculated based on known geometry. The remaining evacuation time was then adjusted based on an average of 30 images-based measurements so that the desired void volume remained in the reactor.

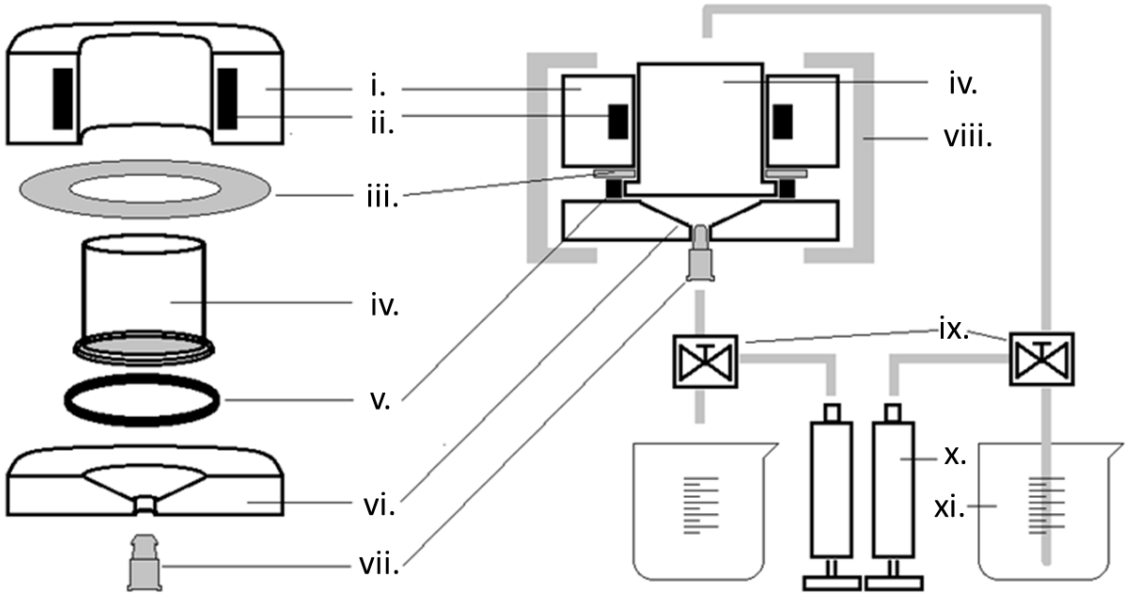

Figure S1. Exploded and assembled view of autocoater system, including superior component of the custom designed reactor adapter (**i**), reference marks for machine vision algorithm (**ii**), metal washer (**iii**), disposable reactor (standing cell culture insert, Millipore, Billerica, Massachusetts) (**iv**), rubber gasket (**v**), cross section of inferior component of the custom designed reactor adapter (**vi**), Luer Lock evacuation adapter (**vii**), clamps (**viii**), computer-controlled valves (**ix**) and syringe pumps (**x**), and reservoirs (**xi**).

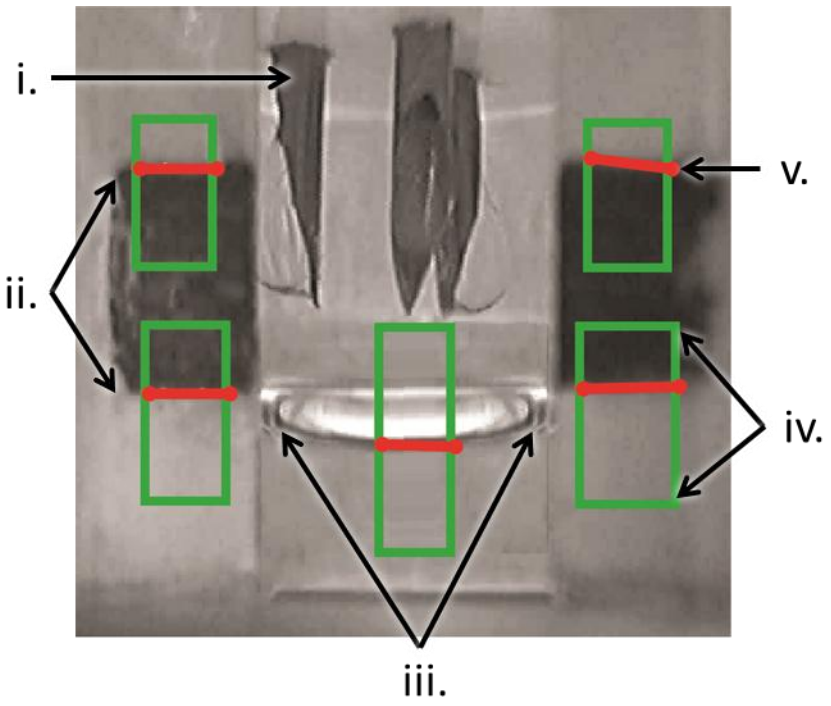

Figure S2. An image taken by USB webcam for reactor volume measurement, including needles (**i**), fiducials on the reactor housing (**ii**), fluid meniscus (**iii**), green search areas for each edge (**iv**), and red edges, as detected by the image analysis algorithm (**v**). Calculations based on the known dimensions of the fiducials and reactor, as well as a 0^{th} order calibration factor, allow measurement of the volume of fluid in the reactor.

Islet Harvest and Culture. Islets were harvested from pancreata of male B10.BR-H2k-T18a/SgSnJ mice between 1.5 and 8 months old, as previously described.[1] Pancreata were distended with collagenase P (1 mg ml^{-1}, Roche, Indianapolis, IN), resected, and digested. Islets were purified by a Ficoll-Histopaque discontinuous gradient (Ficoll: 1.108, 1.096, and 1.037, Mediatech Inc., Manassas, VA). Islets were cultured for at least 48 but not more than 72 hours at 37°C in RPMI 1640 culture media supplemented with 10% heat inactivated fetal calf serum, L-glutaminine (2 mM), penicillin (100 U ml^{-1}), streptomycin (100 mg ml^{-1}), and amphotericin B (0.25 mg ml^{-1}, Mediatech, Inc.) before use in coating experiments. All culture media was changed daily. All animal studies followed local Institutional Animal Care and Use Committee guidelines.

Buffer and Polymer Synthesis. Islet wash buffer consisted of 11 mM glucose (Sigma Aldrich, St. Louis, Missouri) in Dulbecco's Phosphate-Buffered Saline with calcium and magnesium (DPBS, Mediatech, Inc.) that was subsequently passed through a 0.22 μm sterile filter. Poly(L-lysine)MW-g-[D]-poly(ethylene glycol)n copolymers (MW=PLL-HBr molecular weight, D=degree of grafting, n=number of monomeric repeat units) were synthesized, as previously described.[2] All experiments presented here utilized P12P4[45] for polycation solutions. Alginate (UP LVM, NovaMatrix, Sandvika, Norway) was used as the polyanion. In experiments requiring visualization of PEM thin films, fluorescein-labeled Alginate was synthesized, as previously described, through sequential oxidation of urinate residues and reaction with fluorescein-5-thiosemicabazide.[3] For islet coating, 1 mg mL^{-1} polycation solutions and 2 mg mL^{-1} polyanion solutions were prepared in islet wash buffer. In experiments with this polymer system on planar substrates (**Figure S3**), all films were readily disassembled when immersed in 5 M sodium chloride, demonstrating that electrostatic interactions dominate film assembly.

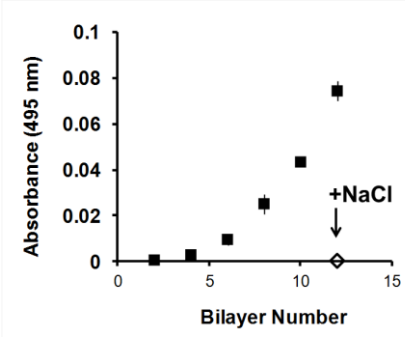

Figure S3. Layer-by-layer PLL-g-PEG/alginate films disintegrate under high salt conditions. Absorbance (mean +/- SD) of AlexaFlour488-labeled P12P4[40] copolymers as a function of bilayer number and upon treatment with 5 M NaCl (5 minutes) measured using solid-state UV-vis spectroscopy.

Coating Protocol. The inferior surface of the reactor's mesh floor, which did not contact islets, was coated with silicone sealant save for a 2 mm square drain area 24 hours before use so that only the small proportion of islets that came to rest on the drain area on a given evacuation would be subject to compression against the mesh by fluid flow. Lighting was adjusted so that the meniscus in the reactor would be consistently illuminated relative to background. The reactor was pretreated to block potential protein binding sites by manually injecting 0.5 mL 1% bovine serum albumin in DPBS (BSA), incubating for 30 minutes at room temperature, then withdrawing the solution through the mesh floor and reactor-to-Luer Lock fitting in one second pulses until empty. Cultured islets were loaded into the empty reactor in 0.6 mL BSA and the LabView computer program was initiated. Each polyelectrolyte bilayer was assembled as follows: 3 washes with islet wash buffer, 5 minute incubation in 1 mg mL^{-1} polycation solution, 3 additional washes with islet wash buffer, and 5 minute incubation in 2 mg mL^{-1} polyanion solution. After a total of 8 bilayers were assembled, islets were washed 3 times with islet wash buffer, incubated in BSA for 5 minutes, and manually retrieved by pipette. All protocols were executed at room temperature in a laminar flow hood. All reagents were passed through a sterile 0.22 μm filter prior to use. A new reactor and syringes were used for each coating protocol. Connection tubing and other

components were filled with 70% ethanol between coating protocols and replaced every three weeks.

Microscopic Analysis. After retrieval from the reactor, islets were placed in islet wash buffer on a sterile untreated culture dish and imaged with an inverted light microscope at 40X magnification for process yield and circularity determinations. Circularity was determined for individual islets using particle detection and analysis algorithms in the ImageJ computer program (National Institutes of Health, http://rsbweb.nih.gov/ij/). For viability assessment, islets were stained with 4 μm calcein AM (live) and 8 μm ethidium homodimer (dead, Molecular Probes, Eugene, Oregon) for 1 hour, followed by Laser-Scanning Confocal Microscopy (LSCM) to probe for both fluorescent markers at each islet's equatorial plane, as previously described.[2] Image processing algorithms were used to compute the percent of islet area stained with calcein AM (live) as a fraction of total area using MATLAB (The MathWorks, Natick, MA).[2] LSCM was also used to image PEM films formulated with fluorescein-labeled alginate.

Insulin Secretion. For insulin secretion analysis, islets were autocoated and subsequently incubated in RPMI 1640 culture media at 37°C for 12 hours. Islets were then pre-incubated at 37°C in 3.3 mM glucose in DPBS (basal glucose) for 1 hour. Islets were switched to fresh basal glucose for 1 hour, then 16.7 mM glucose for 1 hour. ELISA for mouse insulin (Mercodia, Inc., Winston Salem, NC) was used to quantify insulin concentrations in glucose solution samples after the latter two incubations.

Statistical Analysis. Unless otherwise indicated, all data are presented as mean ± standard deviation. Student's two tailed t-test for two samples with equal variance was used to compare sample means.

References for Supporting Information

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[3] T. Boontheekul, H. J. Kong, D. J. Mooney, *Biomaterials* **2005**, *26*, 2455-65.