Supporting Information

Lin and Anseth 10.1073/pnas.1014026108

SI Materials and Methods

Cell Viability Assays and Confocal Microscopy. Viability of encapsulated cells was determined quantitatively by measuring (*i*) intracellular ATP concentration using Celltiter Glo® regeant; and (*ii*) reduction of resazurin fluorescent dye using Almarblue® reagent, as well as qualitatively by live/dead staining and confocal microscope imaging. For ATP measurements, cell-laden hydrogels were transferred into a 48-well plate containing 300 μL, 50% CellTitler Glo® reagent in HBSS. Following incubation on a shaker for 45 min, 200 μL of the solutions were transferred into a 96-well white plate for luminescence quantification using a microplate reader. Solutions of known concentrations of ATP disodium salt hydrate were used to generate standard curves for calibration. Viability of encapsulated cells was also quantified by Almarblue® reagent, which measures nonspecific intracellular redox activity. Briefly, each cell-laden hydrogel (approximately 30 μL) was placed in 0.8 mL cell culture media containing 10% Almarblue® reagent and incubated for 15 hr to allow for the reduction of resazurin dye by viable cells. Following incubation, 180 μL of media were transferred into a black 96-well plate. Fluorescence of the media was determined using a microplate reader (excitation: 560 nm, emission: 590 nm). Qualitative cell viability was determined using a LIVE/DEAD® viability kit. Cell-laden hydrogels were placed in PBS containing calcium AM and ethidium homodimer according to manufacturer's staining protocol for at least 1 hour. Gels were then washed in fresh PBS to remove excess dyes, following by imaging with a Zeiss LSM 5 pascal confocal microscope and at least three images per gel (300 μm depth) were obtained.

Glucose Stimulated Insulin Secretion from Encapsulated β-Cells. Cell-laden hydrogels were cultured in RPMI-1640 media containing 1% or 10% FBS for 10 days. Prior to static glucose treatment, hydrogels were primed in Kerbs–Ringer buffer solution containing 2 mM glucose for 45 min, followed by sequential incubation in KRB solutions containing 2 mM and 25 mM glucose for 1 hour each. The amount of insulin secreted by the encapsulated MIN6 cells was quantified using a mouse insulin ELISA kit (Mercodia Inc.) immediately following sample collection. The amount of insulin secretion in 25 mM glucose containing KRB was normalized to insulin secreted in 2 mM glucose containing KRB and expressed as insulin secretion index.

Mouse Islet Isolation and Dissociation. Adult Balb/c mouse islets were isolated at the Diabetes and Endocrinology Research Center at the Barbara Davis Center for Childhood Diabetes, Denver. Briefly, isolated islets were digested by type V collagenase, followed by purification in a Histopaque density gradient. To obtain single islet cells, isolated whole islets were suspended in the digestion medium (0.005% trypsin, 0.76 mg/mL EGTA, 0.053 mM EDTA, 2 μg/mL DNase in MEM) for 4 min at 37 °C, followed by briefly and vigorously vortexing. This process was repeated once again to obtain single islet cells. The viability of the dissociated islet cells immediately following digestion was between 70–85% as determined by trypan blue staining. Isolated whole islets or dissociated islet cells were encapsulated as previously described. Encapsulated islets or single cells were cultured in RMPI 1640 culture media on an orbital shaker. Culture media was changed every other day.

Statistics. All results presented are Mean ± SEM. Statistical analysis was performed using a two-tailed, unpaired Student's *t*-test. With a p value of less than 0.05, the differences between selective experimental groups were considered statistically significant.

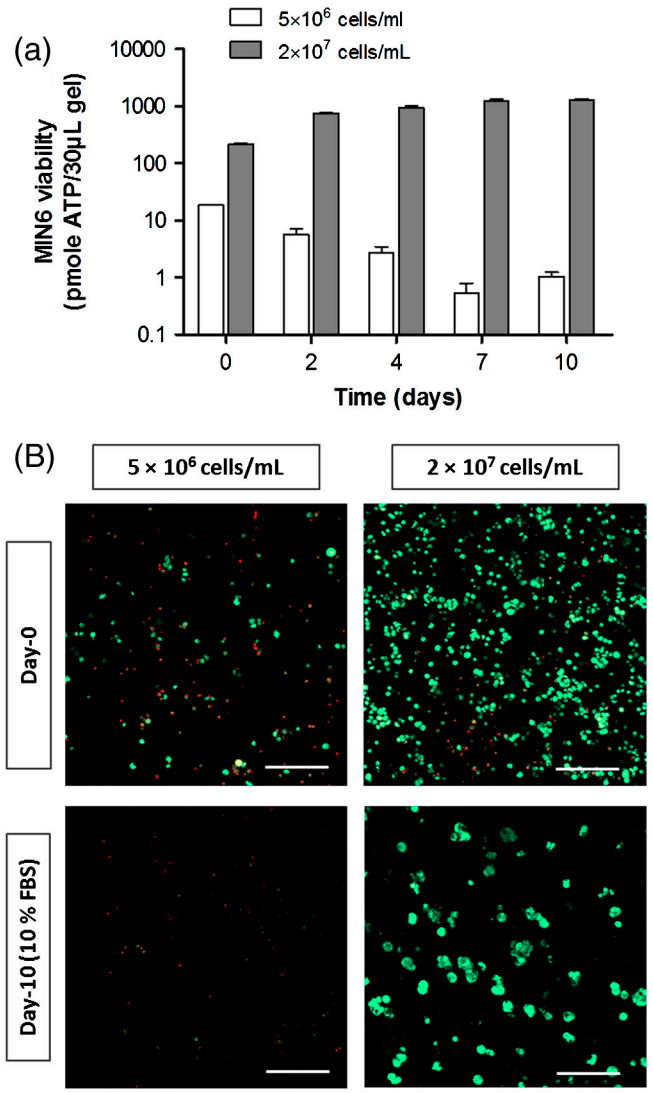

Fig. S1. Effects of cell packing density on the survival of MIN6 cells in PEG hydrogels maintained in RPMI-1640 medium containing 10% FBS. MIN6 cells were dispersed into single cells and encapsulated at different cell densities as indicated. (*A*) Intracellular ATP of encapsulated MIN6 cells was measured at the indicated time after encapsulation and was used to represent cell metabolic activity and viability (Mean ± SEM, $n = 3$, note: log scale). (*B*) Representative confocal Z-stack (300 μm) images of encapsulated MIN6 cells stained with a live/dead viability staining kit at day-0 and day-10. Live cells were stained green while dead cells were stained red (scale: 200 μM).

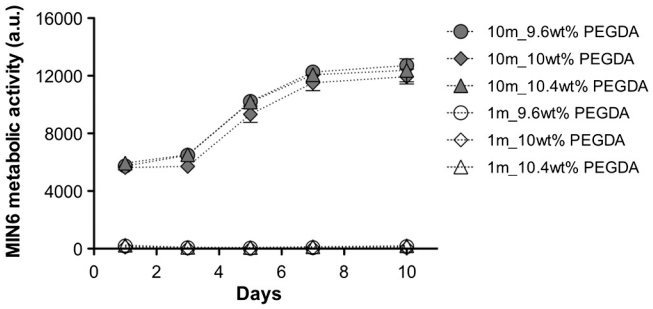

Fig. S2. Effect of PEGDA wt% on MIN6 β-cell viability in photopolymerized PEG hydrogels as determined by Alamarblue reagent. No statistical differences were found between different PEGDA concentrations at the same cell packing density. (10 m: 10^7 cells/mL; 1 m: 10^6 cells/mL).

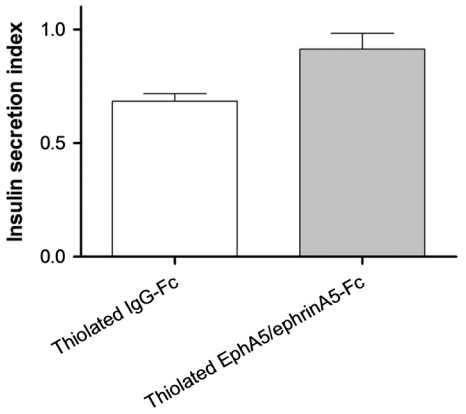

Fig. S3. Effects of biomimetic hydrogels in enhancing glucose-responsive insulin secretion. MIN6 cells were dispersed into single cells and encapsulated at 6.7×10^6 cells/mL. Hydrogels were functionalized with either 200 nM thiolated IgG–Fc (control) or EphA5–Fc/ephrinA5–Fc (1:1). Cell-laden hydrogels were maintained in RPMI-1640 medium containing 10% FBS for 10 days before insulin secretion tests were conducted in Krebs-Ringer buffer solutions (Mean ± SEM, $n = 4$).

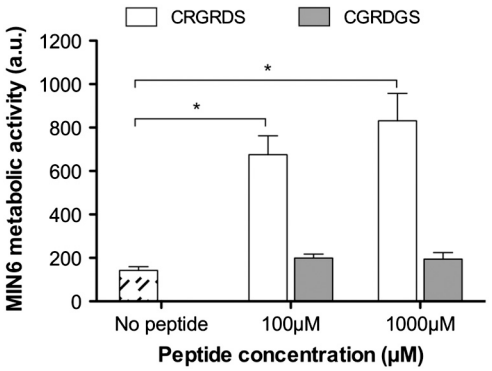

Fig. S4. Effect of cysteine-containing peptides on MIN6 cell viability in PEG hydrogels 24 hr after photoencapsulation. MIN6 cells were encapsulated at 2×10^6 cells/mL.

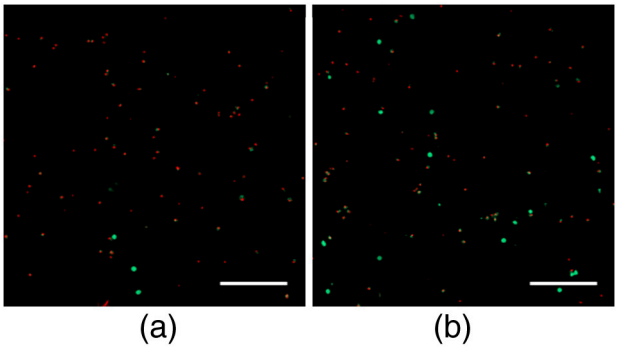

Fig. S5. Representative confocal Z-stack (300 μm) images of dissociated islet cells stained with live/dead viability staining kit in PEG hydrogels functionalized with 200 nM of (*B*) thiolated IgG-Fc, or (*C*) thiolated EphA5–ephrinA5–Fc (1:1). Live cells were stained green while dead cells were stained red. Cell-packing density: 2×10^6 cells/mL. (scale: 200 μM).

Table S1. Estimated cell volume and effective PEGDA concentration at various cell-packing densities

Cell density (cells/mL)	0	1×10^6	1×10^7	2×10^7
Estimated cell volume (μL) *	0	0.06	0.6	1.2
Solution volume (μL)	30	29.94	29.4	28.8
Volume fraction of cells in gel (%)	0	0.2	2	4
Effective [PEGDA] (mM)	10	10.02	10.2	10.4

*Estimated MIN6 cell volume: approximately 2 μL per 1 million cells.