Supporting Information

Visible light-initiated interfacial thiol-ene photopolymerization for forming islet surface conformal coating

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Experimental Section

Materials: Eosin-Y disodium salt and DTT were purchased from Fisher Scientific. 8-arm PEG-hydroxyl (MW: 20 kDa) and 8-arm PEG-amine (MW: 20 kDa) were purchased from JenKem Technology USA. PEG-monoacrylate (MW: 2 kDa) was purchased from Monomer-Polymer. Linear raw PEG-hydroxyl (MW: 2, 3.4 or 10 kDa) and all other chemicals were obtained from Sigma-Aldrich unless noted otherwise. CD1 mice (7- to 8-week old) were obtained from Charles River as islet donors. Permission for animal studies was approved by Indiana University School of Medicine IACUC (protocol #10235-MD/R).

Synthesis of Poly(ethylene glycol)-ester-norbornene (PEGNB) macromer: Linear or 8-arm PEGNB was synthesized by reacting linear or 8-arm hydroxyl-terminated PEG with 5-norbornene-2-carboxylic acid (5-fold excess to hydroxyl group) in anhydrous dichloromethane (DCM) using N,N'-dicyclohexylcarbodiimide (DCC, 2.5-fold excess) as the coupling reagent. Norbornene acid was mixed with DCC and stirred at room temperature for 1 hour. The resulting norbornene anhydride was filtered into an addition funnel and added drop wise into a flask

containing linear (10 kDa) or 8-arm PEG (20 kDa), 4-(dimethylamino) pyridine (DMAP, 0.5-fold excess), and pyridine (5-fold excess) pre-dissolved in anhydrous DCM. The flask was kept in an ice bath and the mixture was reacted for overnight in dark. The product was filtered and precipitated in cold ethyl ether. After drying *in vacuuo*, PEGNB product was re-dissolved in water and dialyzed for 2 days (in pH 6 ddH_2O). ^1H NMR (Bruker 500) was used to confirm the degree of PEG functionalization (> 90 %).

Synthesis of Poly(ethylene glycol)-amide-octa-norbornene (PEGa8NB) macromer: Norbornene acid (5-fold excess to amine groups) was activated by HBTU/HOBT (5.5-fold excess to amine group) in dimethylformamide (DMF) for 3 minutes. With nitrogen gas purging, N,N-Diisopropylethylamine (DIEA, 6-fold excess to amine group) was added to the activated norbornene acid solution and stirred for 5 minutes. The solution was added drop wise to a two-neck flask containing PEG-octa-amine in DMF. After overnight reaction at room temperature, the product was precipitated in cold ethyl ether, dried *in vacuuo*, followed by dialysis for 2 days. ^1H NMR (Bruker 500) was used to confirm the degree of PEG functionalization (> 90 %).

Synthesis of PEGdSH macromer: Linear PEG (2, 3.4 or 10 kDa) was dissolved in anhydrous toluene and dried by rotary evaporation. Dried PEG was re-dissolved in anhydrous tetrahydrofuran (THF), to which sodium hydride (1.5-fold excess of hydroxyl group) was added slowly and the mixture was stirred until no hydrogen gas bubbles were visible. The set up was purged with nitrogen and kept in an oil bath at 40 °C. Allyl bromide (6-fold excess of hydroxyl group) was added drop wise to the PEG solution and the reaction was allowed to continue overnight in dark. Next, sodium bromide salt was filtered off to obtain a clear solution containing PEG-allylether (PEGdAE), which was precipitated in cold ethyl ether, filtered, and dried *in vacuuo*. Next, thiolacetic acid (2-fold excess to allylether group) was added slowly to

dichloromethane (DCM) solution containing PEGdAE and photoinitiator Irgacure I-2959 (0.5 wt%). Photo-conjugation was initiated by UV-light exposure (Omicure S1000, 365 nm and 10 mW/cm^2) for 15 minutes and continued for another 30 minutes after supplementing with another portion (0.5 wt%) of I-2959. Linear PEG-thiolacetate was precipitated in cold ethyl ether, filtered, and dried *in vacuuo*. After re-dissolving PEG-thiolacetate in ddH_2O (30 mL), 15 mL of sodium hydroxide (2N) was added to the solution for 5 minutes to hydrolyze the thiolacetate group. The solution was neutralized by adding equal volume of hydrochloride acid (2N) solution, followed by dialysis for 2 days at room temperature. The product PEGSH was obtained from lyophilization. The purity (> 90 %) was characterized with H^1NMR Bruker 500).

Cytocompatibility of interfacial thiol-ene photopolymerization on β-cell aggregates or islets: MIN6 β-cells were suspended in non-treated tissue culture plate and placed on an orbital shaker to obtain multi-cell aggregates (10 days). The aggregates (average aggregate diameter ~100 μm) were maintained in high glucose DMEM supplemented with 50 μM β-mercaptoethanol, 10 % fetal bovine serum (FBS, Gibco), and 1× antibiotic-antimycotic (Invitrogen). To perform coating experiments, cell aggregates were washed with HBSS and stained with 10 mM of eosin-Y for 5 minutes. After staining, aggregates were washed 3 times with HBSS to remove excess eosin-Y and were transferred to non-gelling precursor solutions containing: (1) 16 wt% $PEGMA_{2kDa}$ (80 mM of acrylate), 50 mM of TEA, and 9 mM of NVP, or (2) 20 wt% $PEGdNB_{10kDa}$ (40 mM of norbornene) and 20 mM of DTT (40 mM of thiol) to make up a unity functional group molar ratio ($R_{[thiol]/[ene]} = 1$). These solutions were exposed to visible light using a fiber optic microscope illuminator (wavelength: 400 to 700 nm, AmScope) for 1 minute. Aggregates were allowed to recover in culture media for 1 hour, followed by staining with 0.25 μL/mL Calcein AM and 2 μL/mL Ethidium homodimer-3 for 1 hour. The stained

aggregates were imaged with a confocal microscope (Olympus Fluoview, FV1000). Pancreatic islets were isolated from CD1 mice following established protocol.[1] These islets were cultured in RPMI media supplemented with 10 % fetal bovine serum, and 1 × antibiotic-antimycotic. In a similar manner as described above, islets were stained with Calcein AM and Ethidium homodimer dyes for evaluating their viability.

Coating of β-cell aggregates or pancreatic islets: MIN6 β-cell aggregates or isolated CD1 islets were stained with 10 mM of eosin-Y for 5 minutes. For the one-step coating method, aggregates were washed three times with HBSS (centrifugation for 3 minutes at 500 rpm each) and suspended in 50 μL of macromer solution contained 20 wt% PEG8NB with stoichiometric ratio of thiol (from DTT) (**Figure S1a**). For the two-step coating method, stained aggregates/islets were first incubated in 20 wt% of PEGdSH for 5 minutes, and washed three times with HBSS before suspension in macromer solution containing PEG8NB or PEGa8NB with DTT (**Figure S1b**). The mixture was exposed to visible light (between 20 to 60 seconds). Aggregate/islet density was fixed at approximately 500 aggregates/mL. The coated aggregates or islets were imaged with an inverted microscope (Nikkon Eclipse *Ti*) and coating thickness was defined as the length difference between the estimated circular diameter of gel coated aggregates/islets and the diameter of the aggregates/islets (analyzed by NIS-Elements BR 3.2 software).

Glucose stimulated insulin secretion (GSIS) and ELISA: Glucose-containing (2.5 or 25 mM) Krebs-Ringer bicarbonate *(*KRB) buffer (23 mM NaCl, 1 mM KCl, 4.8 mM sodium bicarbonate, 0.2 mM magnesium chloride hexahydrate, 0.2 mM calcium chloride dehydrate, 0.5 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid and 0.1 vol% bovine serum albumin) were used for GSIS test. Islets (30 islets, N = 4 tubes per sample group) were washed with HBSS

and primed with 500 μL of low glucose KRB for 1 hour at 37 °C and 5 % CO_2. Then, the islets were incubated with 500 μL of low or high glucose KRB for 2 hours each. The buffer solution was collected after each glucose treatment and the islets were lysed with acid ethanol extraction buffer (75 vol% of ethanol, 1.5 vol% 1N hydrochloric acid and 23.5 vol% ddH_2O).[2] The samples were diluted with KRB and the concentrations of insulin were quantified by mouse insulin ELISA kit (Mercodia) and normalized to their corresponding total insulin content (i.e., the sum of intracellular and secreted insulin).

Hydrogel fabrication and swelling: Step-growth thiol-norbornene hydrogels were formed by radical-mediated photopolymerization using 0.1 mM of eosin-Y under visible light exposure at an intensity of 10 mW/cm^2 (at 555 nm) using a fiber optic microscope illuminator (AmScope) for 5 minutes. To quantify hydrogel swelling, circular hydrogel discs were prepared from 50 μL precursor solution. Immediately after gelation, hydrogels were incubated in ddH_2O at 37 °C on an orbital shaker for 24 hours to remove sol fractions. Gels were then dried and weighed to obtain dried polymer weights (W_{Dry}). The dried polymers were incubated in 5 mL of buffer solution (pH 7.4 PBS) at 37 °C on an orbital shaker. At specific time points, hydrogels were removed from the medium, blotted dry with Kimwipe tissue, and weighed to obtain swollen weights ($W_{Swollen}$). Hydrogel mass swelling ratios (q_{exp}) were determined by a ratio of $W_{Swollen}$ to W_{Dry}.

Determination of hydrogel mesh size: For an ideal step-growth hydrogel network, the mesh size was determined with polymer and crosslinker information. First, the average molecular weight between crosslinks ($\bar{M}_c$) is defined as: [3]

$$\bar{M}_c = 2\left(\frac{MW_A}{f_A} + \frac{MW_B}{f_B}\right) \quad \text{(S1)}$$

Here, MW_A and MW_B represent the molecular weight of PEG8NB and cross-linker DTT, respectively. f_A and f_B are the number of reactive functionality for PEG8NB and DTT, which were 8 and 2, respectively. With a known $\bar{M}_c$, the ideal network cross-linking density or density of elastically active chains (v_c) and polymer volume fraction (v_2) can be calculated based on the Flory-Rehner theory:[4]

$$v_c = \frac{V_1}{\bar{M}_c \bar{v}_2} = \frac{-[\ln(1-v_2) + v_2 + \chi_{12} v_2^2]}{v_2^{1/3} - \frac{2v_2}{f_A}} \quad \text{(S2)}$$

Here, $\bar{v}_2$ is the specific volume of PEG (0.92 cm^3/g at 37 °C), V_1 is the molar volume of water (18 cm^3/mole) and χ_{12} is the Flory-Huggins interaction parameter for a PEG-H_2O system (0.45). After obtaining v_2, ideal hydrogel mass swelling ratio q_{ideal} can be obtained using the following equation:

$$v_2 = \frac{\bar{v}_2}{(q_{ideal} - 1)\bar{v}_1 + \bar{v}_2} = \frac{\frac{1}{\rho_2}}{\frac{q}{\rho_1} + \frac{1}{\rho_2}} \quad \text{(S3)}$$

where $\bar{v}_1$ is the specific volume of water (1.006 cm^3/g at 37 °C), ρ_1 and ρ_2 are the densities of the solvent and polymer, respectively. Here, q was either q_{ideal} or q_{exp}.

The root-mean-squared end-to-end distance of network chains between two cross-links in the unperturbed state or $(\bar{r}_0^2)^{1/2}$ was determined based on:

$$(\bar{r}_0^2)^{\frac{1}{2}} = l\left(C_n \frac{n\bar{M}_c}{M_r}\right)^{\frac{1}{2}} \quad \text{(S5)}$$

Here, C_n is the Flory characteristic ratio, l is the average bond length along the polymer backbone, and n is the number of chemical bonds within a repeating unit (e.g., n equals to 3 for PEG). M_r is the molecular weight of the repeating units of the polymer chain.

With calculated v_2 and $(\overline{r_0^2})^{1/2}$, mesh size of the hydrogel (ξ) was obtained as the product of the two parameters:

$$\xi = v_2^{-\frac{1}{3}}(\overline{r_0^2})^{\frac{1}{2}} \quad \text{(S6)}$$

Estimation of insulin diffusivity in thiol-norbornene hydrogel coating: To estimate the diffusivity of insulin in a swollen thiol-norbornene hydrogel, a diffusivity model developed by Lustig and Peppas was used:[5]

$$\frac{D_g}{D_0} = (1 - \frac{R_{H,ins}}{\xi})exp[(-Y\left(\frac{v_2}{1-v_2}\right)]$$

where D_g is the insulin diffusion coefficient in the swollen thiol-norbornene hydrogel, D_0 is the diffusion coefficient of insulin in buffer (1500 μm^2/second)[6], and $R_{H,ins}$ is the hydrodynamic radius of insulin (2 nm).[7] Y is the ratio of the critical volume required for a successful movement of insulin and the average free volume per molecule of solvent. A good estimation of Y is 1.[5]

Data Analysis: Data analysis was performed on Prism 5 software. Unless otherwise noted, all experiments were conducted independently for three times. For coating thickness characterizations, at least 200 aggregates were analyzed per experimental condition. The results were reported as mean ± SEM. Student's t-test was conducted for statistical analysis where significance was reported for $p < 0.05$.

References

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[2] A. Amrani, S. Durant, M. Throsby, J. Coulaud, M. Dardenne, F. Homo-Delarche, *Endocrinology*, 1998, 139, 1115.
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[7] V. Banerjee, R. K. Kar, A. Datta, K. Parthasarathi, S. Chatterjee, K. P. Das, A. Bhunia, *PLoS ONE,* 2013, 8, e72318.

Supporting Figures

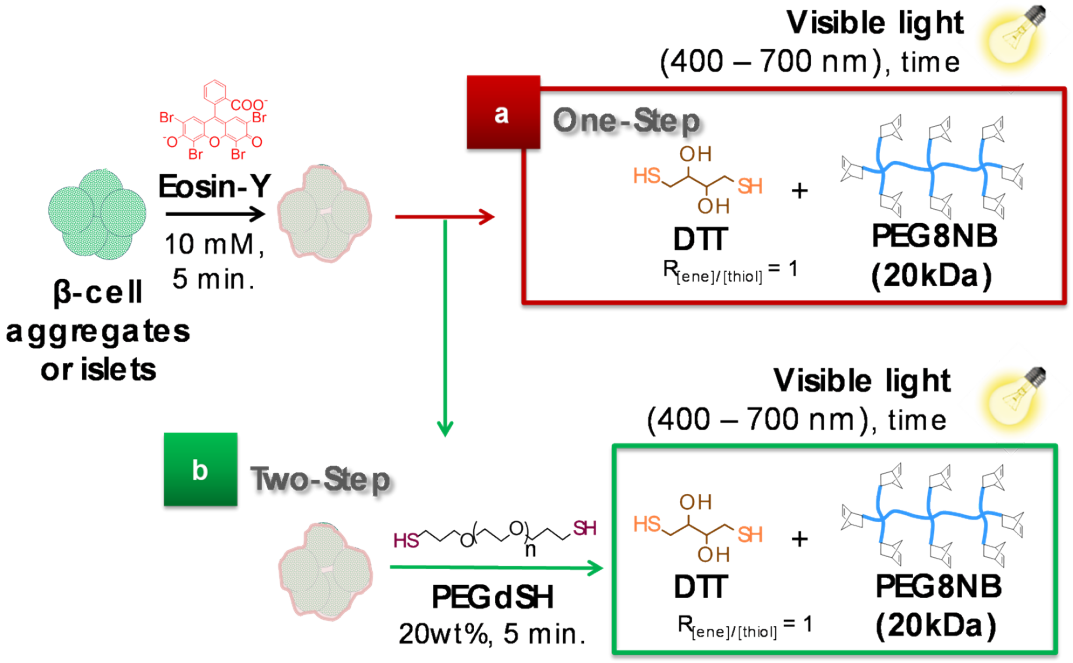

Figure S1. Schematic of visible light-mediated interfacial thiol-norbornene photopolymerization and conformal coating on β-cell aggregates or islets: (a) one-step or (b) two-step coating method.

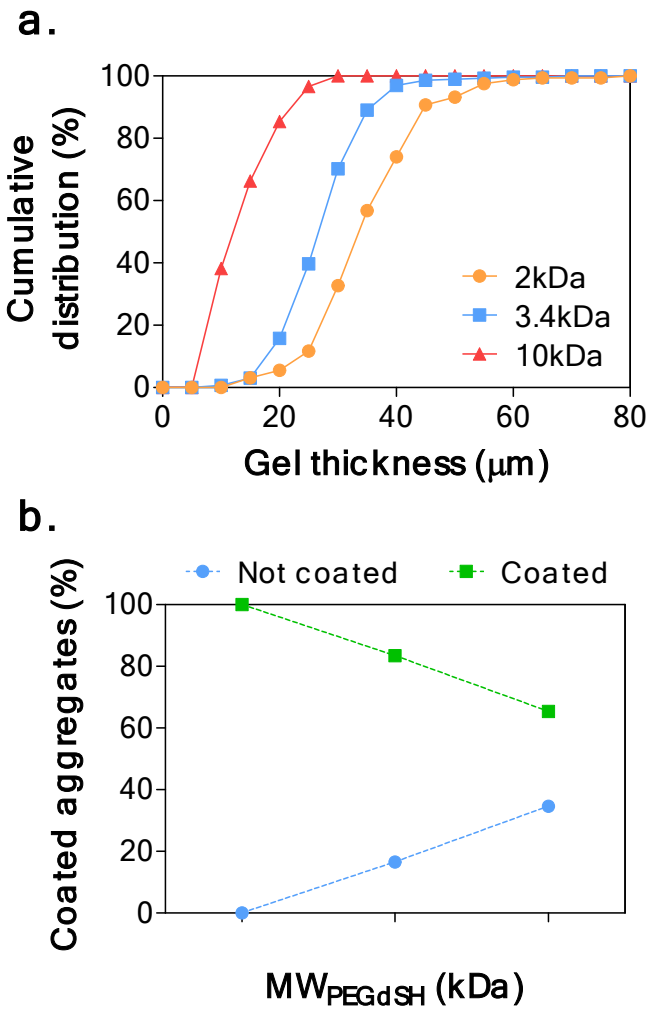

Figure S2. Effects of molecular weight of PEGdSH on (a) cumulative distribution of conformal gel coating thickness, and (b) percent of coated aggregates. (20 wt% of PEG8NB-DTT, $R_{[PEG8NB]:[DTT]}$ = 1, 30 seconds of light exposure)

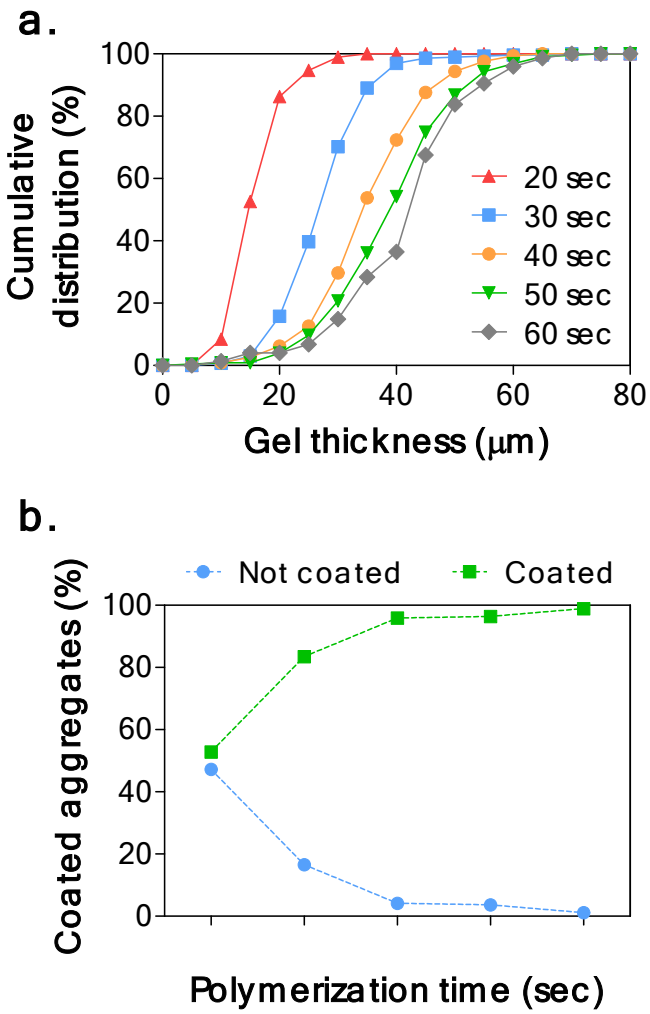

Figure S3. Effects of polymerization time on (a) cumulative distribution of conformal gel coating thickness, and (b) percent of coated aggregates. (MW of PEGSH = 3.4 kDa, 20 wt% of PEG8NB-DTT, $R_{[PEG8NB]:[DTT]} = 1$)

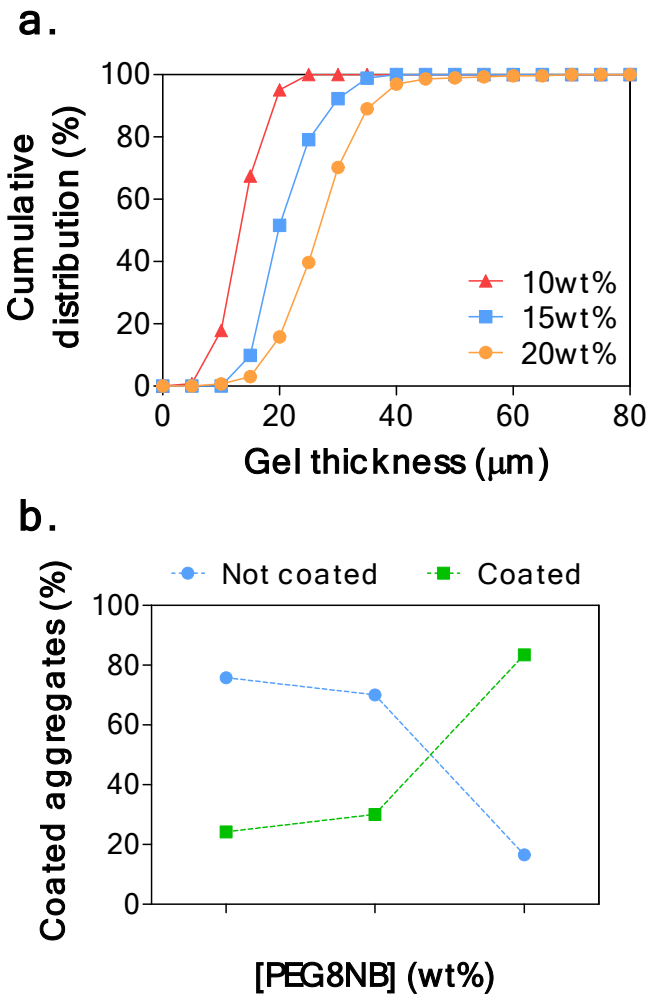

Figure S4. Effects of PEG8NB concentration in the precursor solution on (a) cumulative distribution of conformal gel coating thickness, and (b) percent of coated aggregates. (MW of PEGSH = 3.4 kDa, $R_{[PEG8NB]:[DTT]}$ = 1, 30 seconds of light exposure)

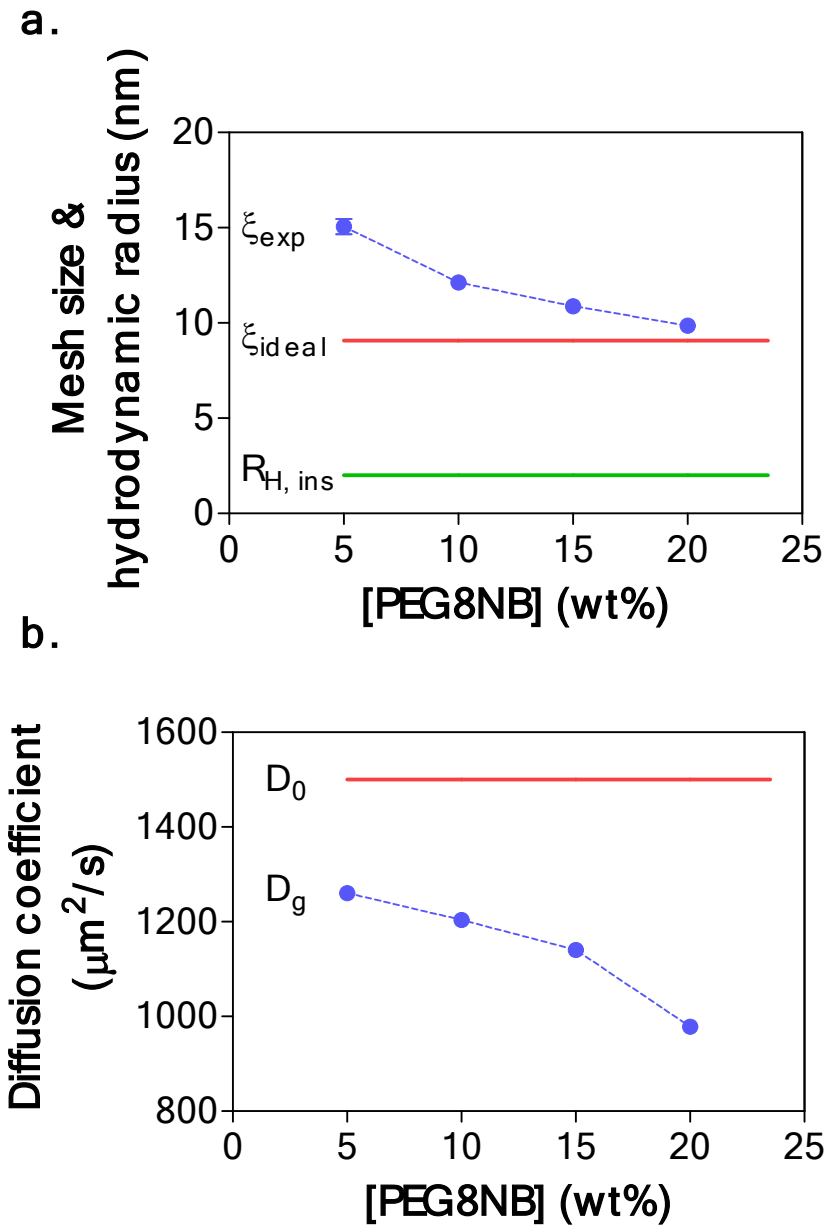

Figure S5. (a) Effect of PEG8NB concentration on the mesh size (ξ) of hydrogels. The mesh size of an ideal network (ξ_{ideal}) was determined by Flory-Rehner equations (**Equation S1 to S6**). The experimental mesh size (ξ_{exp}) was calculated from the swelling ratio of PEG8NB-DTT hydrogels at different macromer contents (**Equation S4, S5 & S6**). Hydrodynamic radius ($R_{H,ins}$) of insulin was obtained from reference [7]. (b) Effect of PEG8NB concentration on the diffusion coefficient (D_g) of insulin in swollen hydrogel. The diffusion coefficient of insulin in pure solvent is obtained from reference [6] and the calculation of D_g was based on Lustig-Peppes model in reference [5]. (PEG8NB-DTT, 0.1 mM of eosin-Y, 5 minutes of light exposure, N = 5)

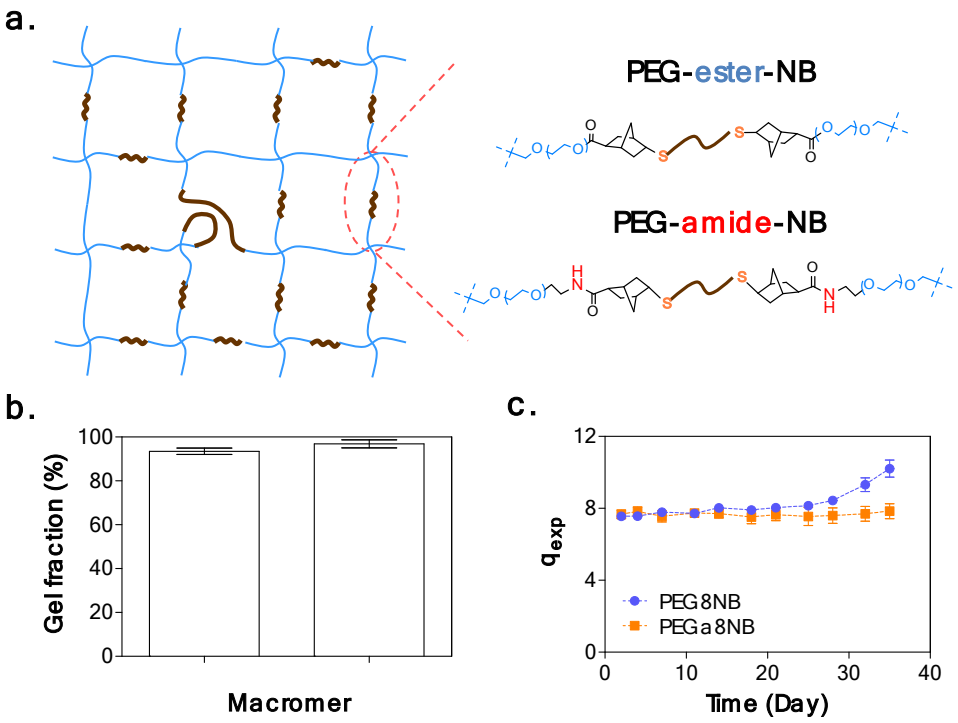

Figure S6. (a) Schematic representation of thiol-ene hydrogels formed by hydrolytically degradable PEG8NB and non-degradable PEGa8NB. Effects of PEG macromer on (b) gel fraction, and (c) hydrolytic degradability of hydrogels. (20 wt% PEG8NB or PEGa8NB-DTT, 0.1 mM of eosin-Y, 5 minutes of light exposure, N = 5)

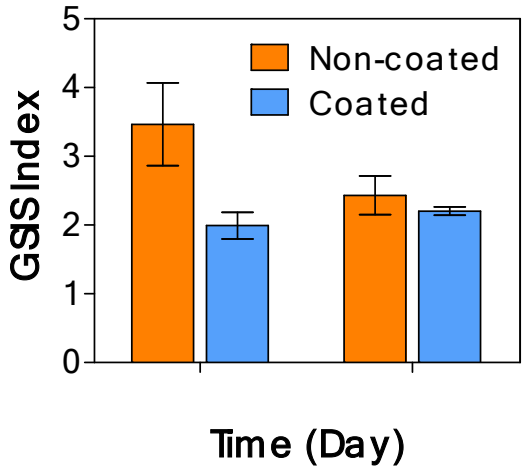

Figure S7. *In vitro* glucose stimulated insulin secretion (GSIS) index of CD1 murine islets. GSIS index is the normalization of insulin secreted at high glucose to low glucose. (Coating conditions: 3.4 kDa PEGdSH, 20 wt% PEGa8NB-DTT and 25 seconds light exposure)