Supporting Information

Supersensitive oxidation-responsive biodegradable PEG hydrogels for glucose-triggered insulin delivery

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Experimental Section

^{1}H NMR and GPC Measurements. ^{1}H NMR spectra of the macromonomers in $CDCl_3$ were measured on a Bruker ARX 400 MHz spectrometer at ambient temperature. The same NMR equipment was also applied to get the time-dependent ^{1}H NMR spectra of **4-arm-PEG$_{20k}$-A** in deuterated PB solution, using DSS as the internal reference. Gel permeation chromatography (GPC) measurements were carried out on the equipment consisting of a Waters 1525 binary HPLC pump, a Waters 2414 refractive index detector, and three Waters Styragel columns (HT2-HT4) at 35 ^{o}C. THF was used as eluent with a rate of 1.0 mL/min. The narrowly dispersed polystyrenes were applied for calibration.

FT-IR. Fourier transform infrared (FT-IR) spectra of the hydrogels were recorded on a Bruker Vector22 spectrometer. Briefly, the dry hydrogel was powdered, ground with KBr powder, pressed into a pellet, and measured on the spectrometer with a resolution of 4.0 cm^{-1} in wavenumber. A blank KBr pellet was used as the reference.

Rheological Measurements. Dynamic rheological measurements were conducted on a Physica MCR 301 Rheometer (Anton Paar) equipped with a parallel plate geometry (8 mm in diameter) at a gap of 1 mm. After being loaded on the measuring plate, the superfluous edge of the gel samples was removed by a razor blade. Oscillatory frequency sweeps were performed from 10 to 0.1 rad/s at a constant strain of 1%. Oscillatory strain sweeps were performed from 0.01%-1000% at 1 rad/s. For the H_2O_2- or glucose-triggered degradation monitored by the rheological method, a batch of hydrogels (**A3**, **B1-B5**) with 20 wt% of the macromonomers in feed were prepared. A piece of the hydrogels was measured by oscillatory frequency sweep in the range of 10-0.1 rad/s at a constant strain of 1%, and the obtained data were applied for 0 time point. Then, all the hydrogels were soaked in 1 mL PB solution (200 mM) containing a desired concentration of H_2O_2 or glucose and incubated at 37 ^{o}C. At the desired time points, one of the residual hydrogels was taken out carefully and the oscillatory frequency sweep was conducted.

Transmission Electron Microscopy (TEM). The morphology and size of the nanogels

were measured on a TEM equipment (JEOL JEM-100CXII). The acceleration voltage was 100 kV. Briefly, 10 μL of nanogel solution (0.1 mg/mL) in 200 mM PB was placed onto a copper mesh. After 20 s, the solution was blotted using a filter paper, the residual liquid on the mesh was dried at room temperature overnight prior to TEM observation. For H_2O_2-initiated degradation of the nanogels, the solution was incubated with H_2O_2 for 24 h at 37 ^oC. Then, 10 μL of the solution was taken out and placed on a copper mesh. TEM specimen was fabricated by the aforementioned procedure.

Laser Light Scattering (LLS). The laser light scattering equipment (Brookhaven Inc., Holtsville, NY) with a BI-200SM goniometer, a BI-TurboCorr digital correlator, and a vertically polarized He-Ne laser (R-30995, 633 nm, 17 mW, Newport, USA) as the light source was used for both dynamic light scattering (DLS) and static light scattering (SLS) measurements. The z-averaged root-mean square radius of gyration (R_g) and hydrodynamic radius (R_h) were measured by SLS and DLS, respectively. Prior to LLS measurement, the nanogel solution (0.1 mg/mL) in PB (200 mM, pH 7.4) was filtered into a dust free vial through a Millipore 0.45 μm PVDF membrane, heated to 37 ^oC quickly and maintained at this temperature. For the H_2O_2-triggered degradation, the nanogel solution (0.1 mg/mL, 2.0 mL) was first measured by LLS at 37 ^oC, the obtained data were used for 0 min time point. Afterwards, 0.2 mL of H_2O_2 in PB with a specific concentration was added to the nanogel solution and mixed thoroughly by shaking quickly. At the desired time points, the LLS measurements were conducted at 37 ^oC.

Modification of Insulin by FITC. 50 mg (~ 0.01 mmol) of insulin was dissolved in 10 mL of PB solution (100 mM, pH 8.4), to which 15 mg (0.039 mmol) of FITC in 1.5 mL of DMSO was added dropwise at room temperature. After being stirred in dark for 24 h, the reaction mixture was transferred into a dialysis tubing (MWCO: 3000 Da) and dialyzed against PB solution (pH 7.4, 50 mM) for 2 days at room temperature in dark. During this period, the dialysis medium was changed 6 times. Finally, the product (**FITC-insulin**) was obtained as an orange solid after lyophilization.

Activity Assays of GOx and Insulin. To evaluate the effect of the loading procedure on

the activity of GOx or insulin, the redox-initiated polymerization was carried out under the similar condition for the preparation of **A3** hydrogel (50 mM PB solution, pH 7.4, 20 ^{o}C, 48 h), but using poly(ethylene glycol) monomethyl ether methacrylate (Mw = 2500, PEGMA) as the monomer instead of **4-arm-PEG$_{20k}$-A**. In this case, we obtained a poly(PEGMA) aqueous solution with insulin or GOx, which was used directly for the activity assay. Far-UV circular dichroism (CD) spectra of native insulin and the insulin treated by the above polymerization procedure were recorded on an Aviv Model 202 Circular Dichroism Spectrometer. For GOx, native or treated by the above polymerization procedure, the time-dependent pH of the PB solution (10 mM, initial pH = 7.4) with 1000 mg/dL glucose at 25 ^{o}C was monitored by a Mettler Toledo pH meter. The final concentrations of insulin and GOx for measurements were 0.03 mg/mL and 0.05 mg/mL, respectively.

Cytotoxicity Assay. For the degradation products, **4-arm-PEG$_{20k}$-A** (10 mg, 0.5 μmol) was dissolved in PB solution (pH 7.4, 200 mM), to which 5 μL H_2O_2 (10 M) was added. After 12 h at room temperature, the solution was mixed with MnO_2 and incubated for additional 12 h. Then, MnO_2 was removed by centrifugation, the upper aqueous solution was used for cytotoxicity assay. mPEG$_{113}$ and branched polyethyleneimine (PEI, Aldrich, average molecular weight ~25000 as measured by LS) dissolved in the same buffer solution were used as the negative and positive controls, respectively. CCK-8 assay was used to assess the cytotoxicity of the samples to LO2 cells. The cells were seeded in 96-well plates and incubated at 37 ^{o}C with 5% CO_2 humidified atmosphere for 24 h. Then, 10 μL of the sample solution with a specific concentration was added to each well, the cells were cultured for another 24 h and subjected to CCK-8 assay. The absorbance of the solution in each well was detected on a PerkinElmer EnSpire multimode microplate reader at 450 nm. Cell viability (%) was defined as ($A_{sample}/A_{control}$) × 100. The data were obtained in triplicate. The cytotoxicity assay of the nanogel was performed by the same procedure.

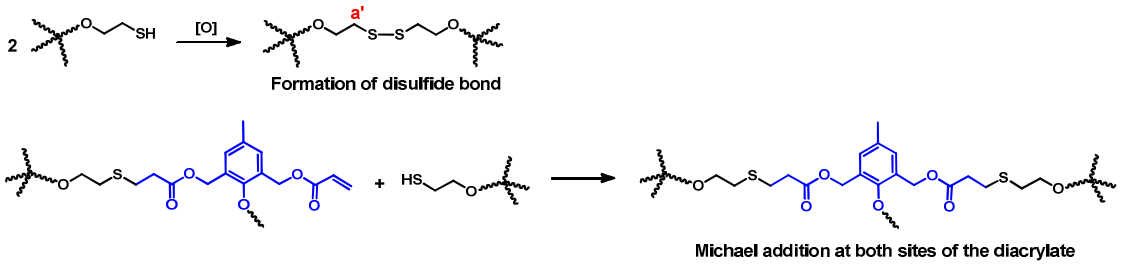
For the cytocompatibility assay of the macroscopic hydrogels, the gel samples were sterilized by ultraviolet radiation overnight prior to the cytotoxicity assay. Then, each of the hydrogels with a volume of 50 μL was dipped in 0.6 mL DMEM nutrient solution and

incubated at 37 ^{o}C with 5% CO_2 humidified atmosphere for 24 h. At the same time, LO2 cells were seeded in the 96-well plates and also incubated at 37 ^{o}C with 5% CO_2 humidified atmosphere for 24 h. Afterwards, 10 μL of the extracting solution was added to each well, the cells were cultured for additional 24 h and subjected to CCK-8 assay. The absorbance of the solution in each well was detected on a PerkinElmer EnSpire multimode microplate reader at 450 nm. Cell viability (%) was defined as $(A_{sample}/A_{control}) \times 100$. The data were obtained in triplicate. The cytotoxicity of the macroscopic hydrogels was also evaluated by directly incubation the sterilized gels with cells.

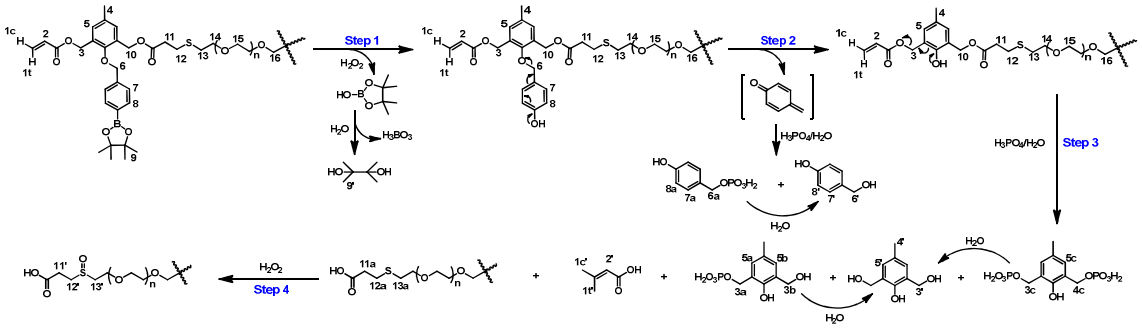
Table S1. Characterization of Hydrogels with Different Feed Ratios of the Two Macromonomers [a]

Hydrogel	A/B [b]	SR [c]	G_0' [d] (kPa)	LC (wt%) [e]		LE (wt%) [f]		G' [g] (kPa)
				Insulin	GOx	Insulin	GOx	
A3	100/0	11.1	8.53	2.9 ± 0.01	0.48 ± 0.007	98 ± 0.34	96 ± 1.39	---
B1	80/20	11.0	9.14	2.9 ± 0.02	0.48 ± 0.006	97 ± 0.71	96 ± 1.26	0.95
B2	60/40	11.2	9.89	3.0 ± 0.02	0.48 ± 0.006	97 ± 0.61	96 ± 1.16	2.24
B3	40/60	11.1	9.26	3.0 ± 0.04	0.48 ± 0.007	98 ± 0.18	96 ± 1.30	5.07
B4	20/80	11.5	9.51	3.1 ± 0.01	0.48 ± 0.007	97 ± 0.44	96 ± 1.48	6.75
B5	0/100	11.6	9.52	3.1 ± 0.03	0.48 ± 0.006	97 ± 0.42	96 ± 1.27	9.60

[a] Polymerization conditions: 48 h, 4 °C, [4-arm-PEG_{20k}-A+4-arm-PEG_{20k}-B]:[KPS]:[TMEDA]=1/3.7/7.4 in molar ratio. [b] Molar feed ratio of **4-arm-PEG_{20k}-A** to **4-arm-PEG_{20k}-B**. [c] The swelling ratios were calculated by SR = $(W_s-W_d)/W_d$, where W_s and W_d are the weights of swollen and dry gels, respectively. [d] The G_0' value was defined as the storage shear modulus of the hydrogels at 1 rad/s angular frequency measured by the oscillatory shear tests with a fixed strain of 1%. [e] Loading capacity (measured in triplicate) defined as FITC-insulin (or GOx) in hydrogel/dry hydrogel (x 100%). [f] Loading efficiency defined as FITC-insulin (or GOx) in hydrogel /FITC-insulin (or GOx) in feed (x 100%). [g] The G' value was defined as the storage modulus of the hydrogels with 0.5 wt% GOx incubated for 12 h in the presence of 100 mg/dL glucose, measured by the oscillatory shear tests with a fixed strain of 1% and 1 rad/s angular frequency.



Scheme S1. Plausible reactions that result in the side products with large molecular weights.



Scheme S2. Oxidative decomposition mechanism of **4-arm-PEG_{20k}-A.**

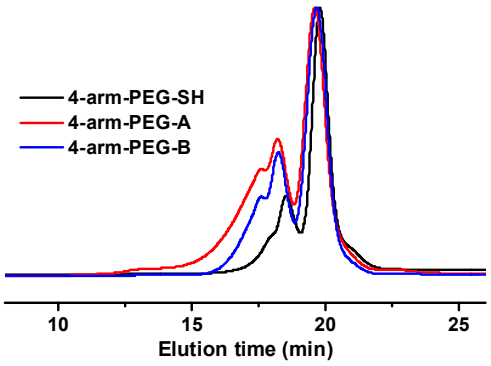


Figure S1. GPC curves of **4-arm-PEG$_{20k}$-SH**, **4-arm-PEG$_{20k}$-B** and **4-arm-PEG$_{20k}$-A** with THF as the eluent.

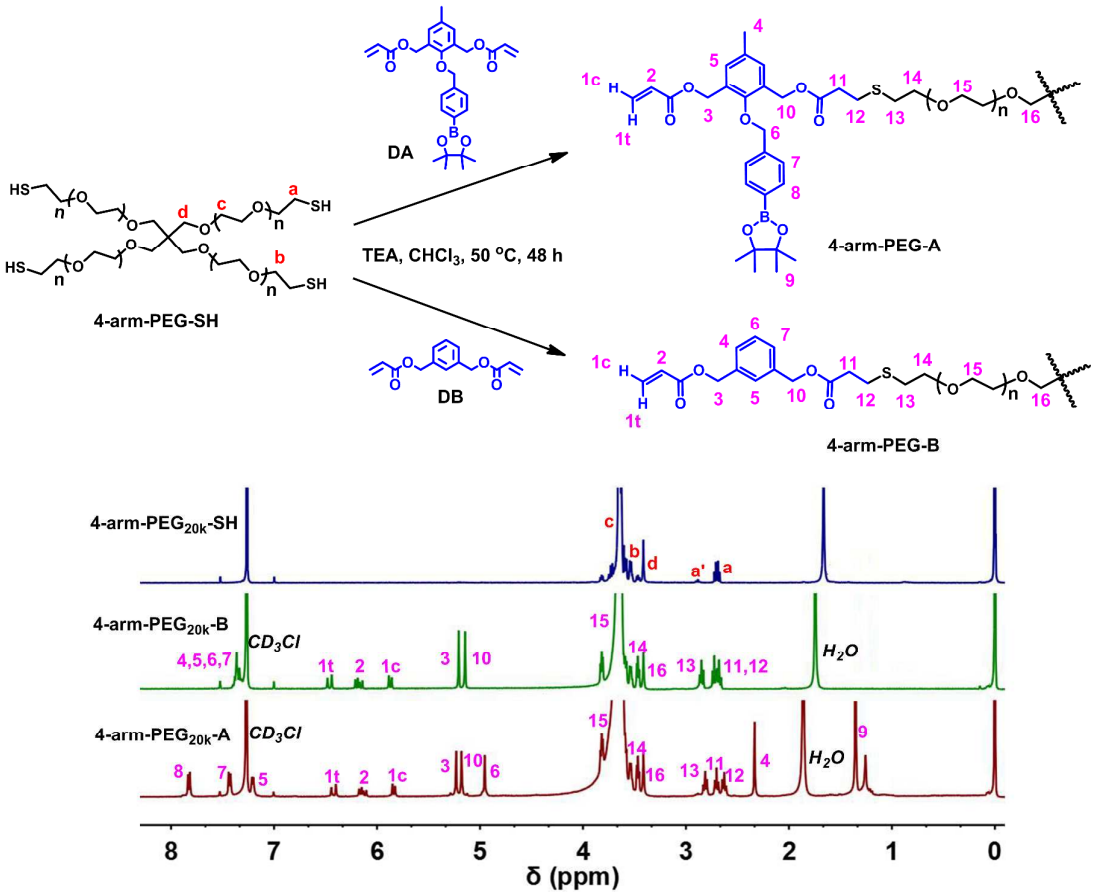


Figure S2. ^{1}H NMR spectra of **4-arm-PEG$_{20k}$-SH**, **4-arm-PEG$_{20k}$-B** and **4-arm-PEG$_{20k}$-A** in $CDCl_3$. The ratios of I_{16}:I_{10}:I_3 are 1.25:1.13:1.0 for **4-arm-PEG$_{20k}$-A** and 1.31:1.19:1.0 for **4-arm-PEG$_{20k}$-B**, respectively. I_{16} (I_{10}, I_3) denotes peak intensity of the corresponding proton signal.

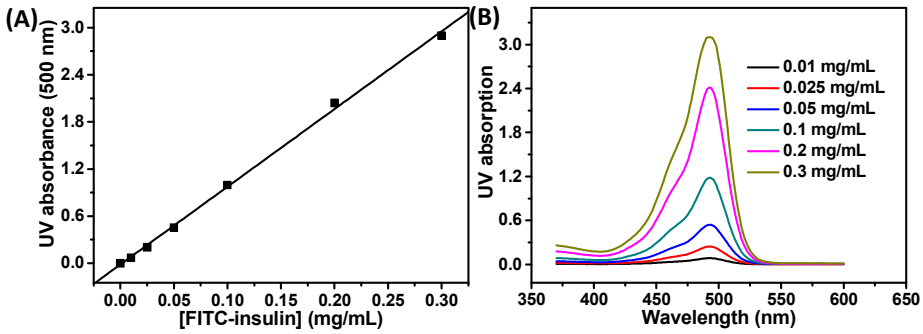


Figure S3. (A) The calibration curve and (B) UV absorption spectra of FITC-insulin with different concentrations in 200 mM PB solution (pH 7.4).

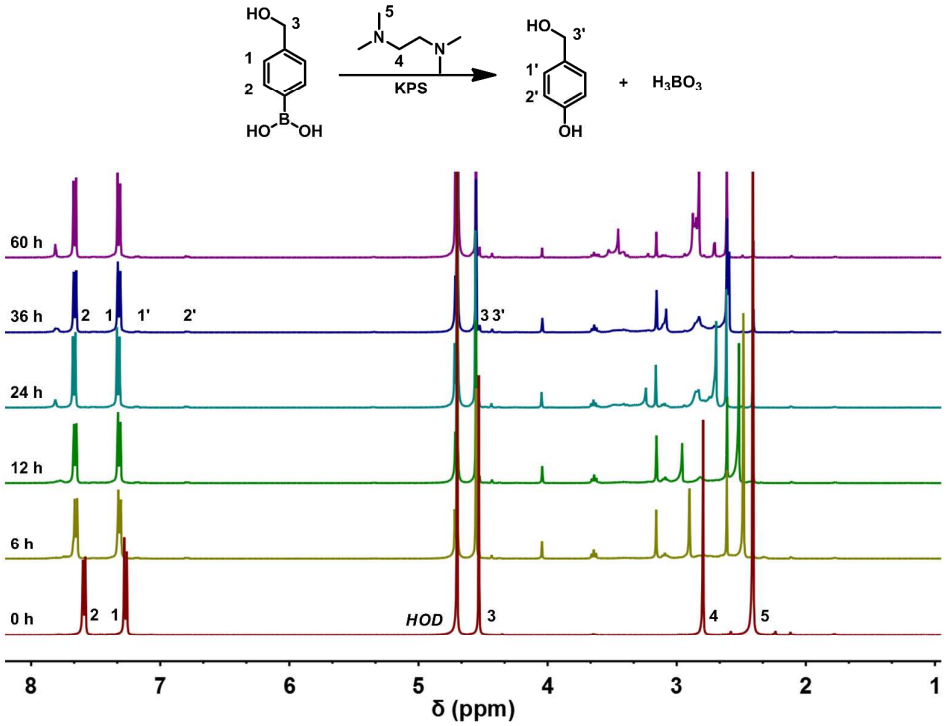


Figure S4. ^{1}H NMR spectra of a model compound (4-hydroxymethylphenylboronic acid, 50 mg/mL) in PB (pH 7.4, 50 mM) incubated with 18.5 mM KPS and 37 mM TMEDA at 4 ^{o}C and different incubation times. The result indicates that the phenylboronic acid moiety was stable enough against the redox initiation system.

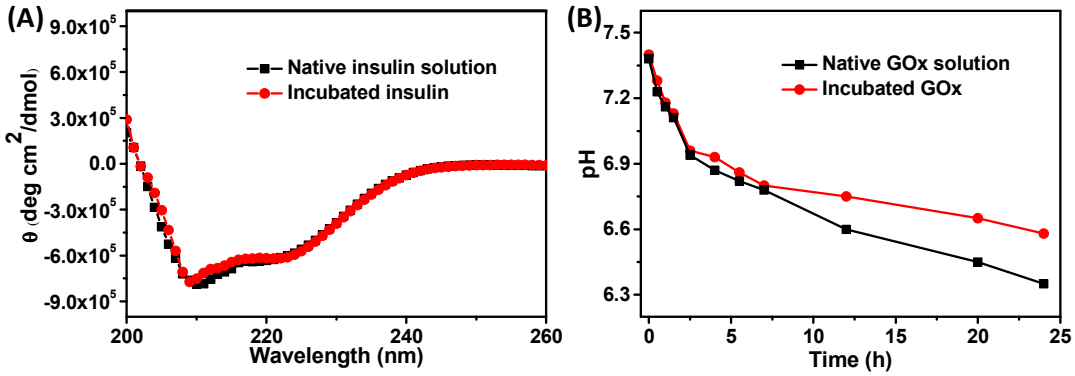


Figure S5. (A) CD spectra of native insulin and the insulin treated by the polymerization procedure. Concentration of insulin: 0.03 mg/mL. (B) pH change vs time plots of native GOx and the treated GOx by the polymerization procedure, incubated with 1.0 g/dL glucose in 10 mM PB solution (initial pH = 7.4) at 25 ^{o}C.

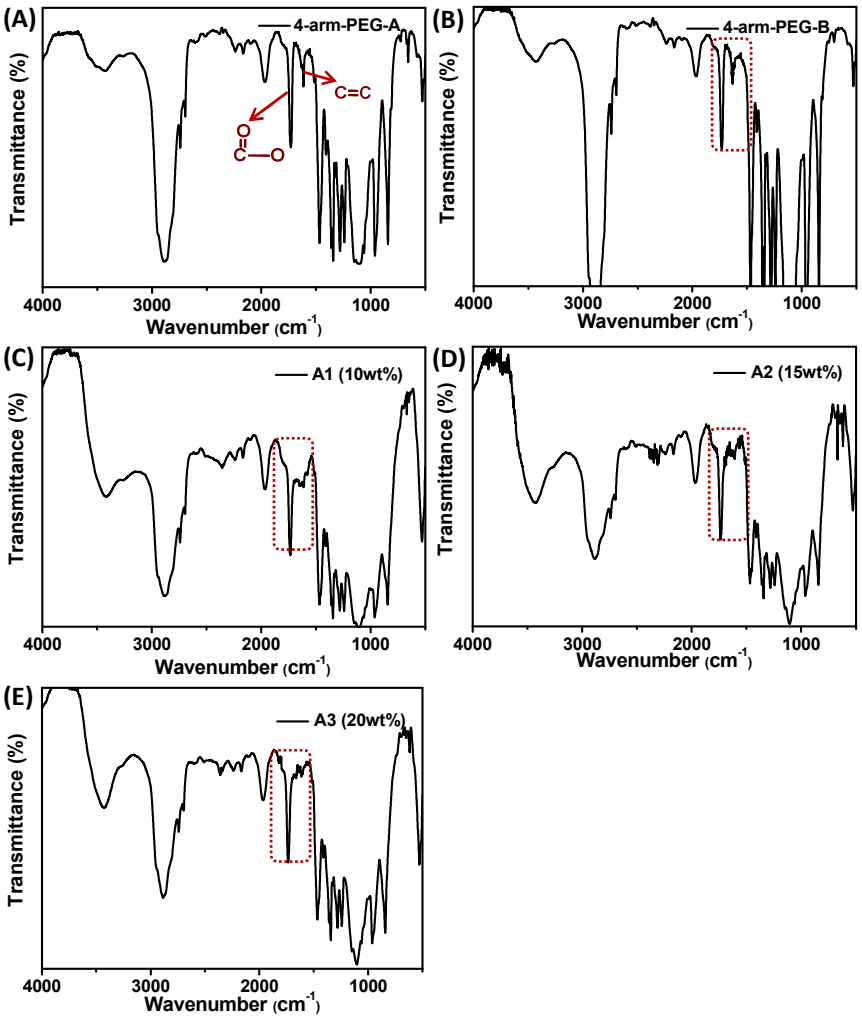


Figure S6. FT-IR spectra of (A) **4-arm-PEG$_{20k}$-A**, (B) **4-arm-PEG$_{20k}$-B**, the lyophilized hydrogels (C) **A1**, (D) **A2**, and (E) **A3**.

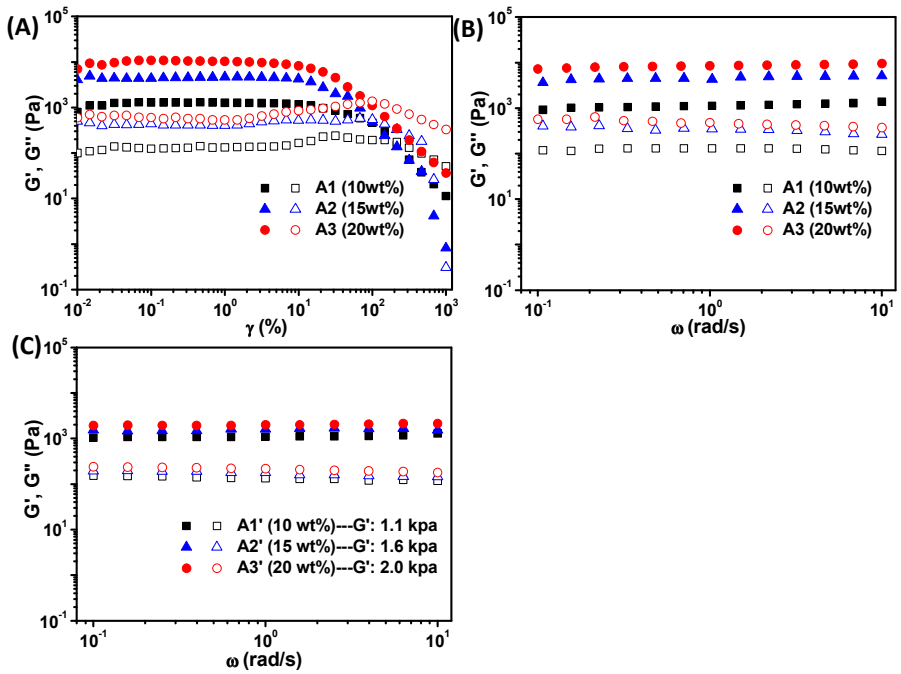


Figure S7. (A) Strain sweep (1 rad/s angular frequency) and (B) frequency sweep (1% strain amplitude) rheological properties of hydrogels with different macromonomer concentrations (**A1**, **A2**, **A3**) at 37 ^{o}C. (C) Frequency sweep (1% strain amplitude) rheological properties of hydrogels (**A1'**, **A2'**, **A3'**) with the same macromonomer **4-arm-PEG$_{20k}$-A** concentration (10 wt%). For **A2'** and **A3',** 5 wt% and 10 wt% of the monofunctional monomer PEGMA were added into the polymerization mixture, besides 10 wt% of **4-arm-PEG$_{20k}$-A.** The solid and empty symbols denote G' and G", respectively. **A3** hydrogel has a G' of 8.5 kPa, which is much higher than that (G' = 2.0 kPa) of **A3'** hydrogel, demonstrating the key role of the crosslinking degree on the mechanical strength of the hydrogels.

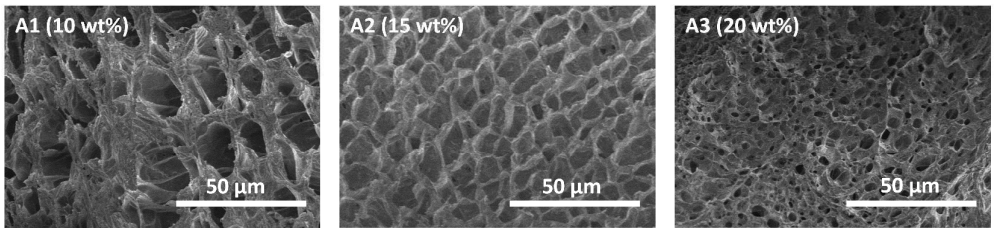


Figure S8. Amplified SEM images of hydrogels with different macromonomer concentration (**A1**, **A2**, **A3**).

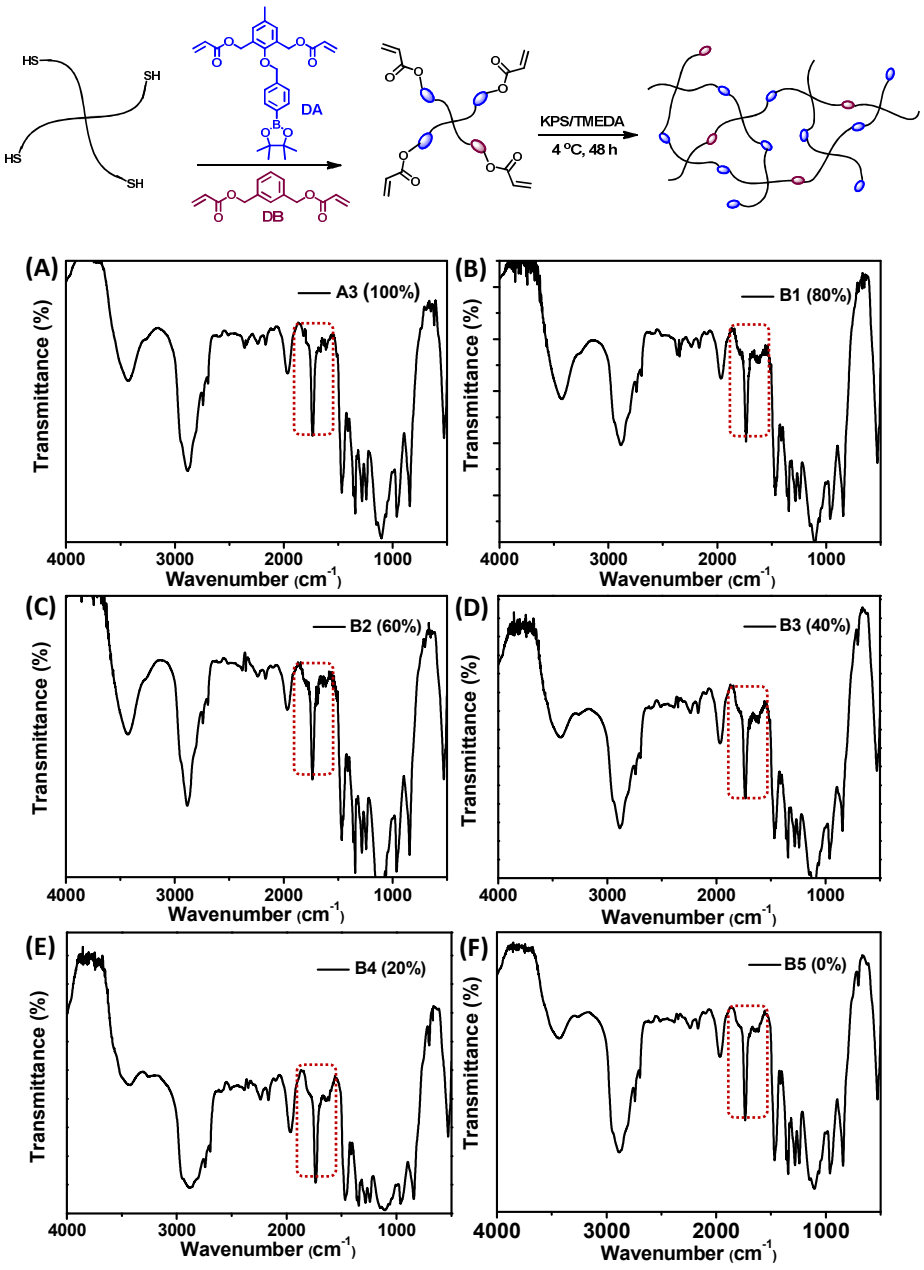


Figure S9. FT-IR spectra of the lyophilized hydrogels (A) **A3**, (B) **B1**, (C) **B2**, (D) **B3**, (E) **B4** and (F)**B5**.

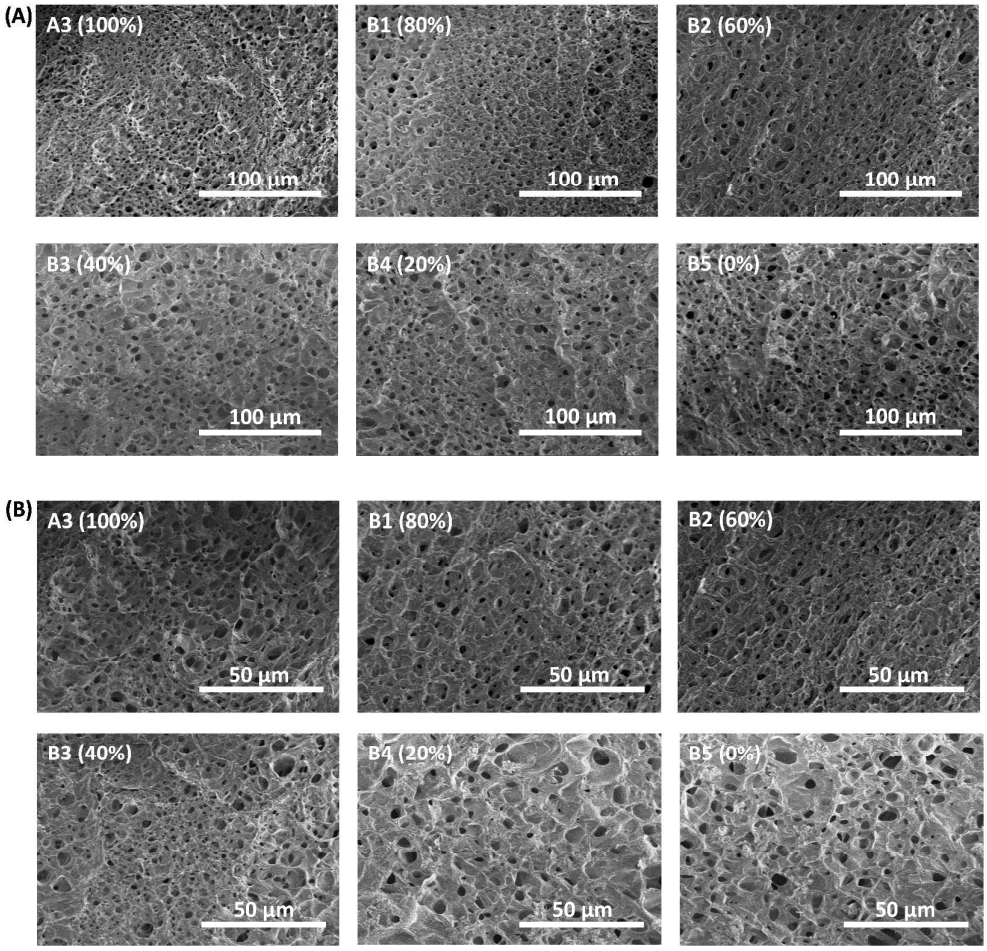


Figure S10. (A) SEM images and (B) amplified SEM images of hydrogels **A3**, **B1**, **B2**, **B3**, **B4** and **B5**.

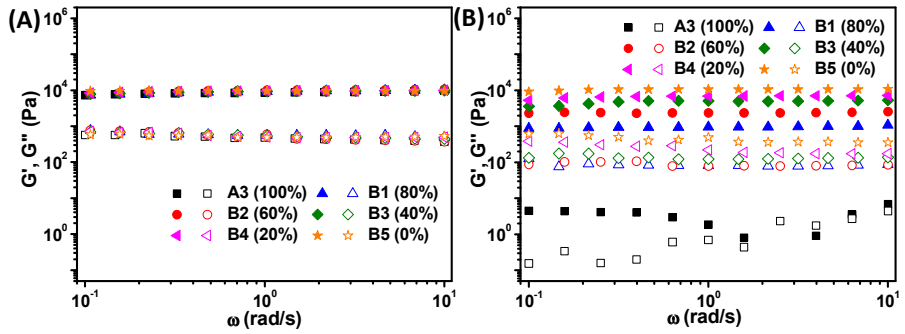
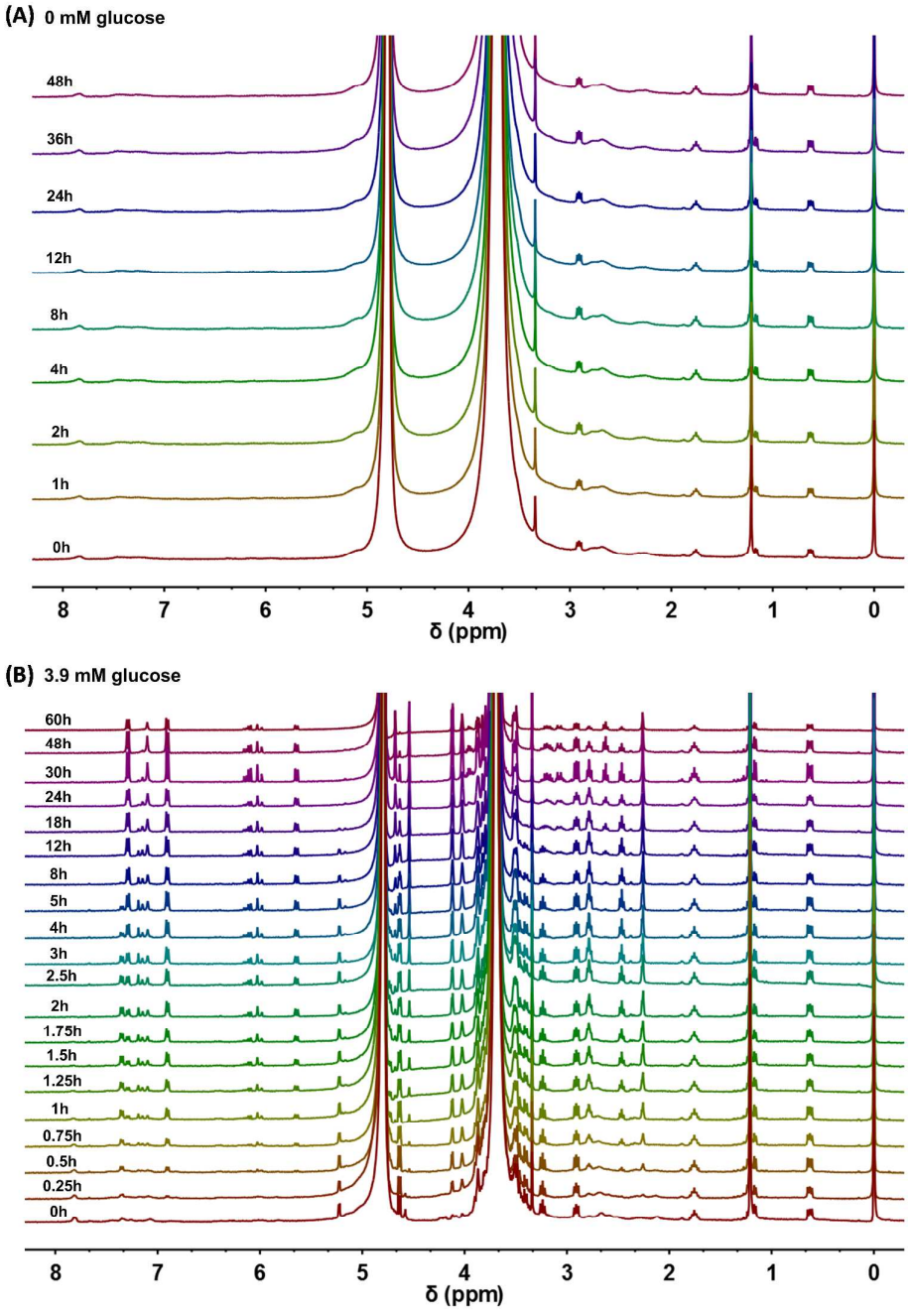


Figure S11. Frequency sweep (1% strain amplitude) of the 0.5 wt% GOx-loaded swollen hydrogels with varying feed ratios of **4-arm-PEG$_{20k}$-A** to **4-arm-PEG$_{20k}$-B** in PB solution (pH 7.4, 200 mM), (A) without and (B) with glucose (100 mg/dL) after 12 h incubation at 37 ^{o}C. The solid and empty symbols denote G' and G", respectively.


(A) 0 mM glucose
48h
36h
24h
12h
8h
4h
2h
1h
0h
8
7
6
5
4
3
2
1
0
δ (ppm)
(B) 3.9 mM glucose
60h
48h
30h
24h
18h
12h
8h
5h
4h
3h
2.5h
2h
1.75h
1.5h
1.25h
1h
0.75h
0.5h
0.25h
0h
8
7
6
5
4
3
2
1
0
δ (ppm)

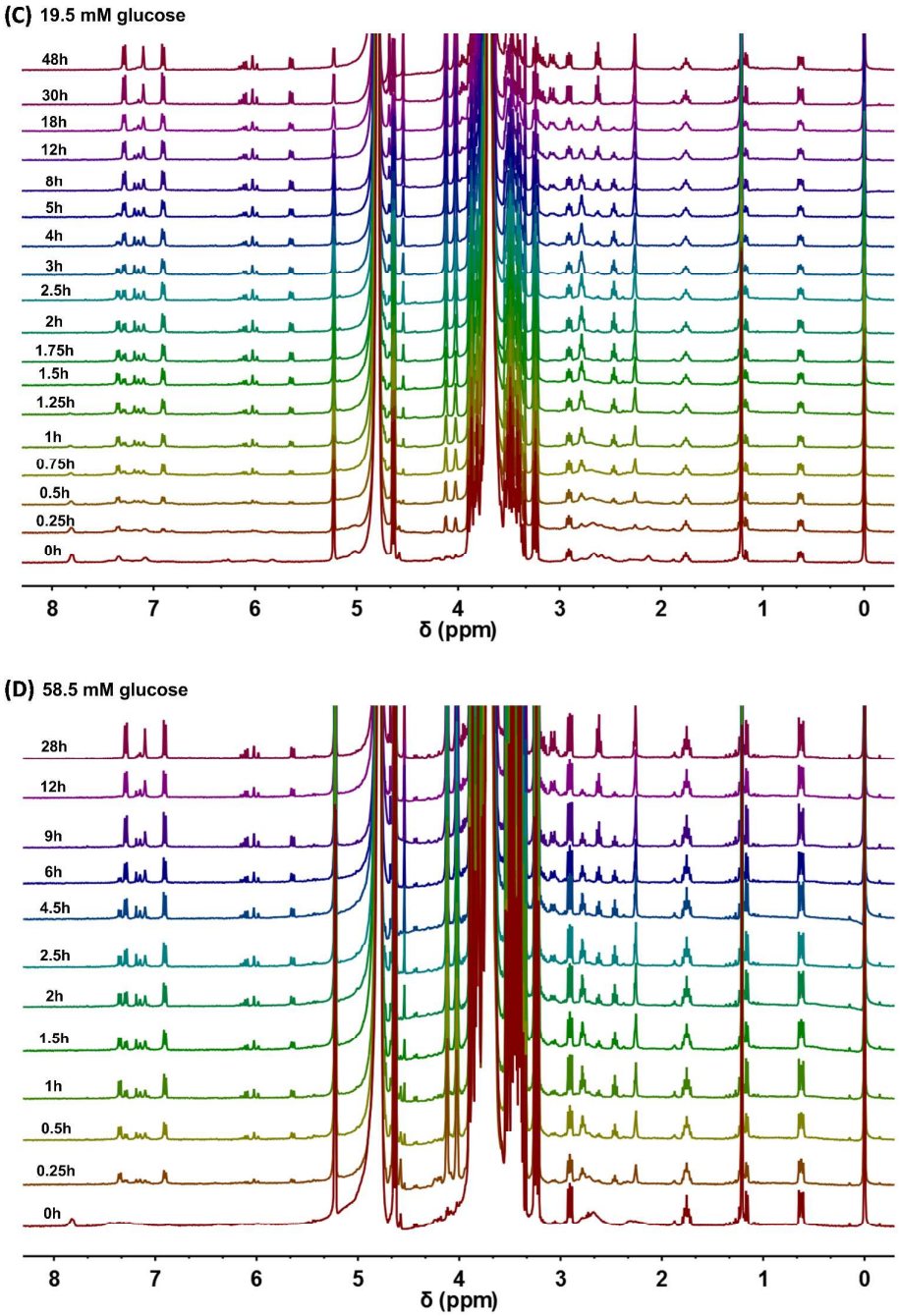


Figure S12. Time-dependent ^{1}H NMR spectra of **4-arm-PEG$_{20k}$-A** (10 mg/mL) in 200 mM deuterated PB solution (pH 7.4) (A) without glucose and with (B) 3.9 mM, (C) 19.5 mM and (D) 58.5 mM glucose in the presence of 3.1 μM GOx at 37 ^{o}C.

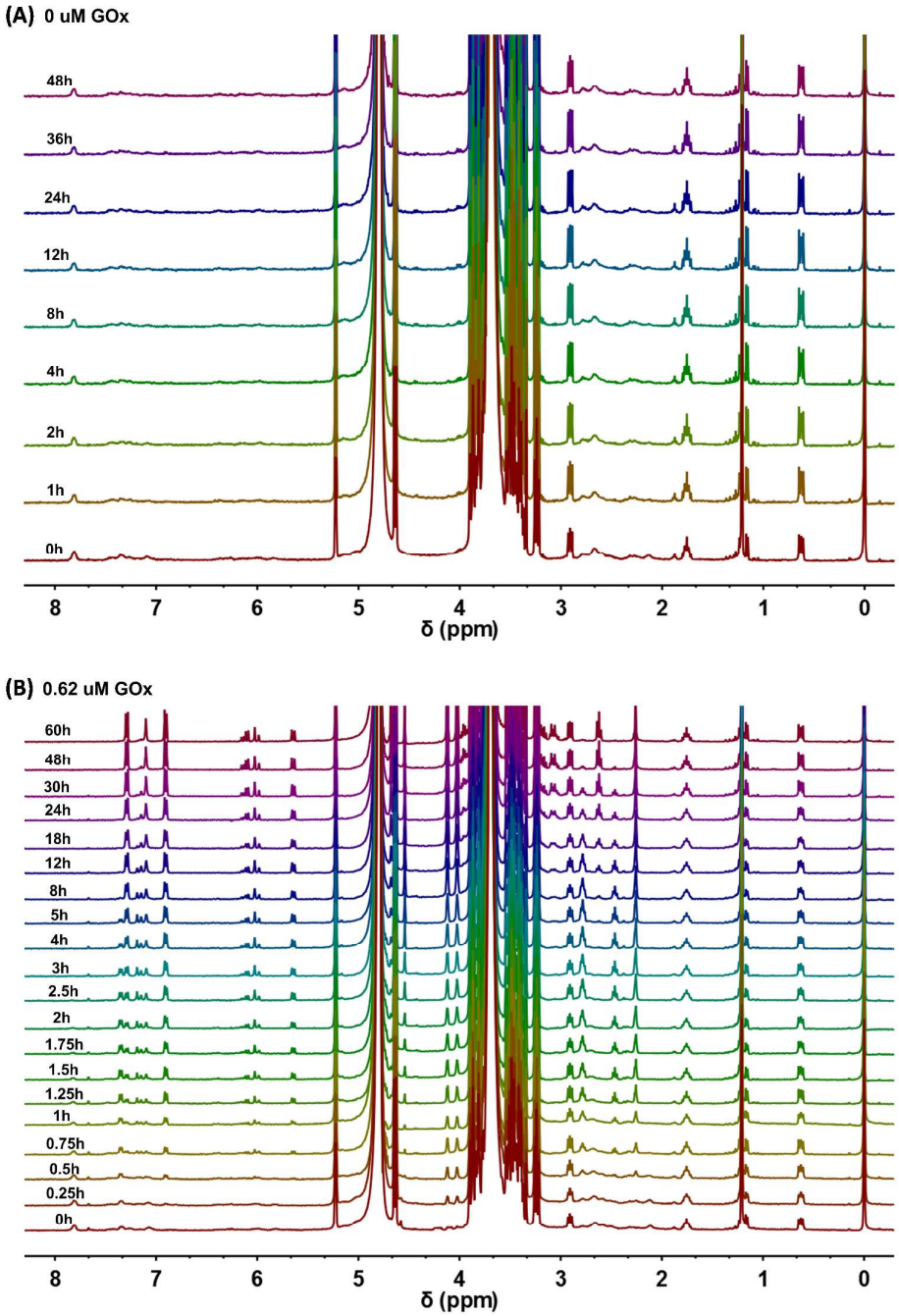


Figure S13. Time-dependent ^{1}H NMR spectra of **4-arm-PEG$_{20k}$-A** (10 mg/mL) in 200 mM deuterated PB solution (pH 7.4) (A) without and (B) with 0.62 μM GOx in the presence of 19.5 mM glucose at 37 ^{o}C.

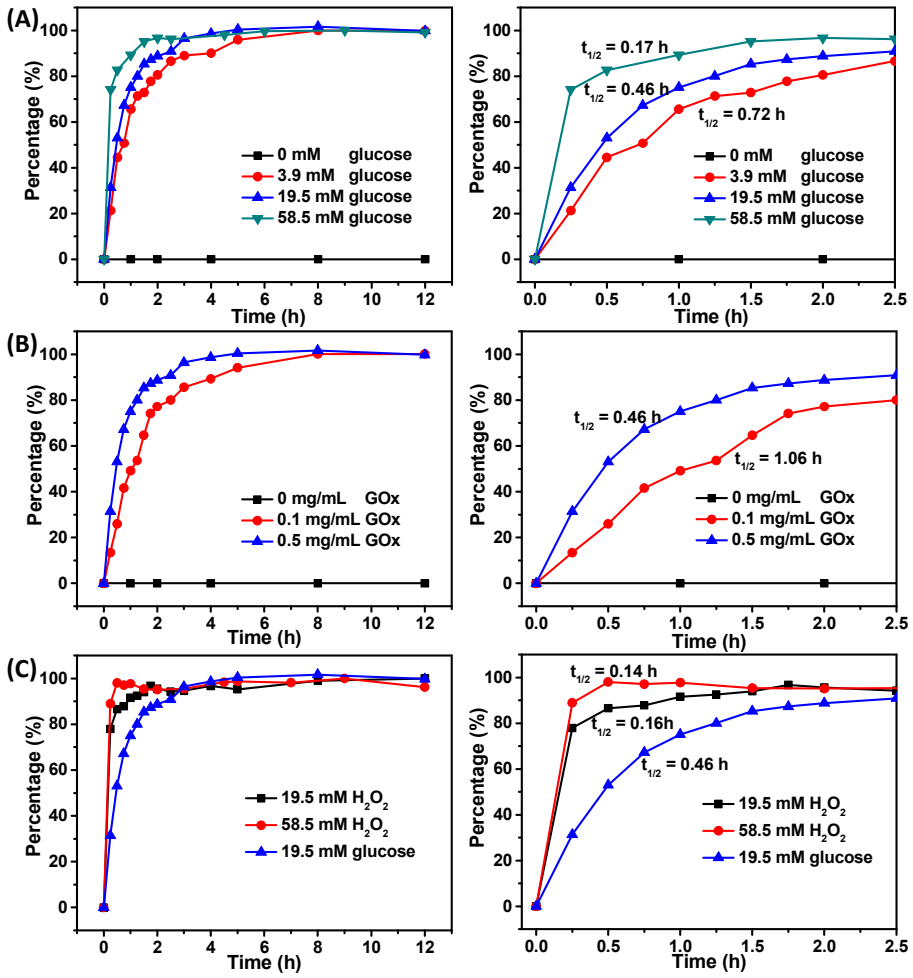


Figure S14. Oxidative decomposition kinetics of **4-arm-PEG$_{20k}$-A** (10 mg/mL) (A) at different concentration of glucose with 3.1 μM GOx, (B) at varying amounts of GOx in the presence of 19.5 mM glucose, and (C) at various H_2O_2 concentration in 200 mM deuterated PB solution (pH 7.4) at 37 ^{o}C. The curves were obtained by calculating the normalized intensity of proton signals of 4' + 4a + 4b (~2.26 ppm, **Figure 2**) using DSS as the internal reference. The $t_{1/2}$ was defined as the time when 50% **4-arm-PEG$_{20k}$-A** decomposition occurred upon oxidation.

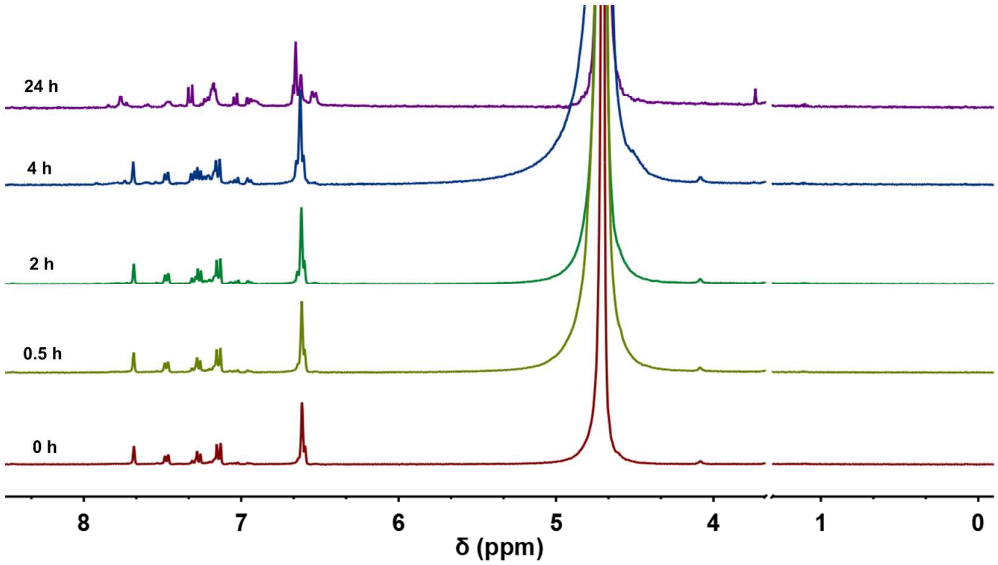


Figure S15. Time-dependent ^{1}H NMR spectra of FITC (5 mg/mL) in 200 mM deuterated PB solution (pH 7.4) with 10 mM H_2O_2 at 37 ^{o}C. The proton signals of FITC changed drastically with time upon oxidation by H_2O_2.

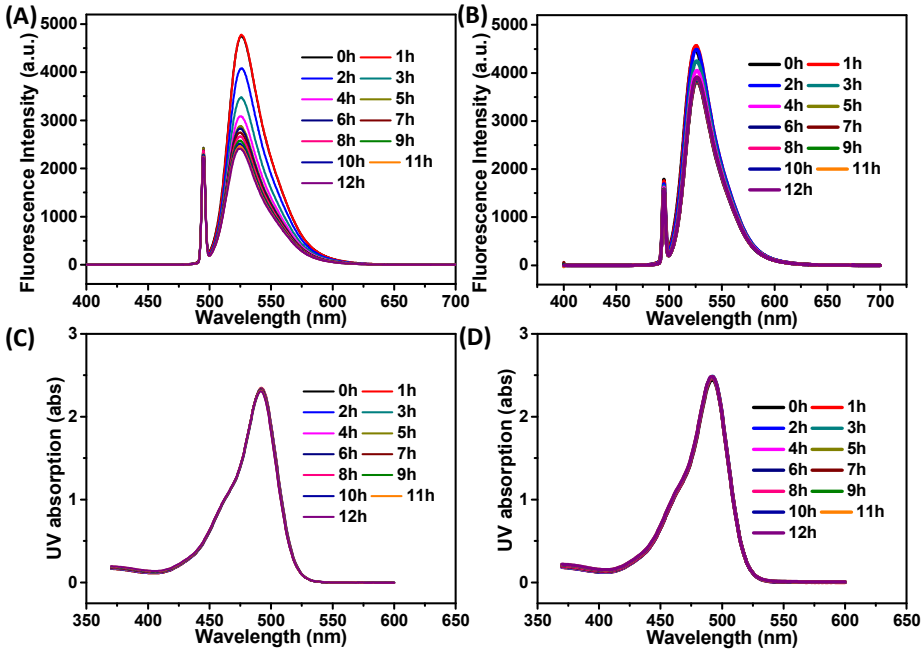


Figure S16. (A, B) Time-dependent fluorescence spectral changes and (C, D) time-dependent UV-Vis absorption spectral changes of FITC-insulin (0.3 mg/mL) in PB solution (200 mM, pH 7.4) in the presence of (A, C) 0.05 mg/mL GOx and 1000 mg/dL glucose or (B, D) 0.3 mg/mL GOx and 100 mg/dL glucose at 37 ^{o}C.

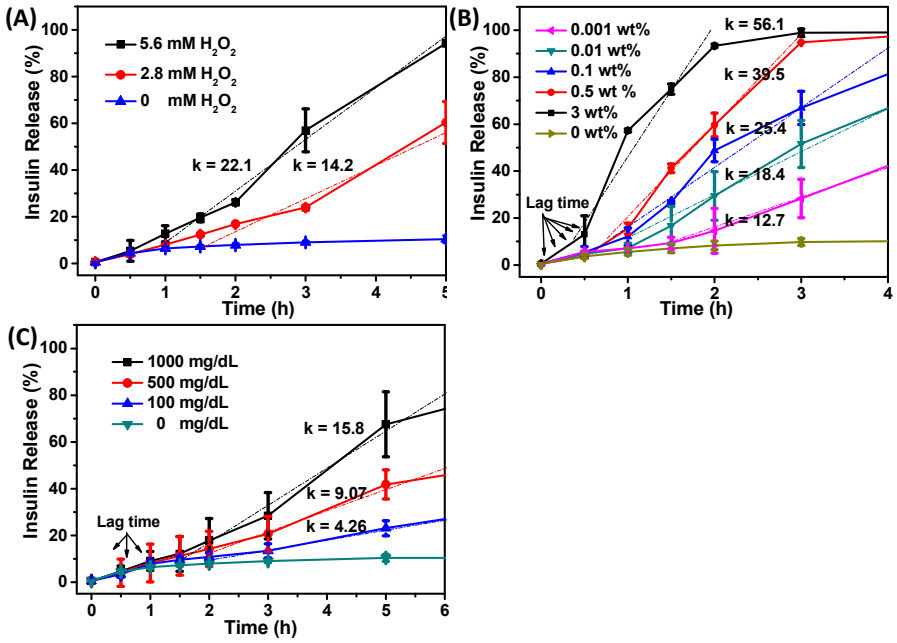


Figure S17. The magnified graphs of (A) **Fig. 3A**, (B) **5A** and (C) **5B**.The apparent rate constants of insulin release were obtained from the slopes of the dashed lines.

Figure S18. Time-dependent photographs of FITC-insulin-loaded hydrogel **A3** in the presence of H_2O_2 with different concentrations.

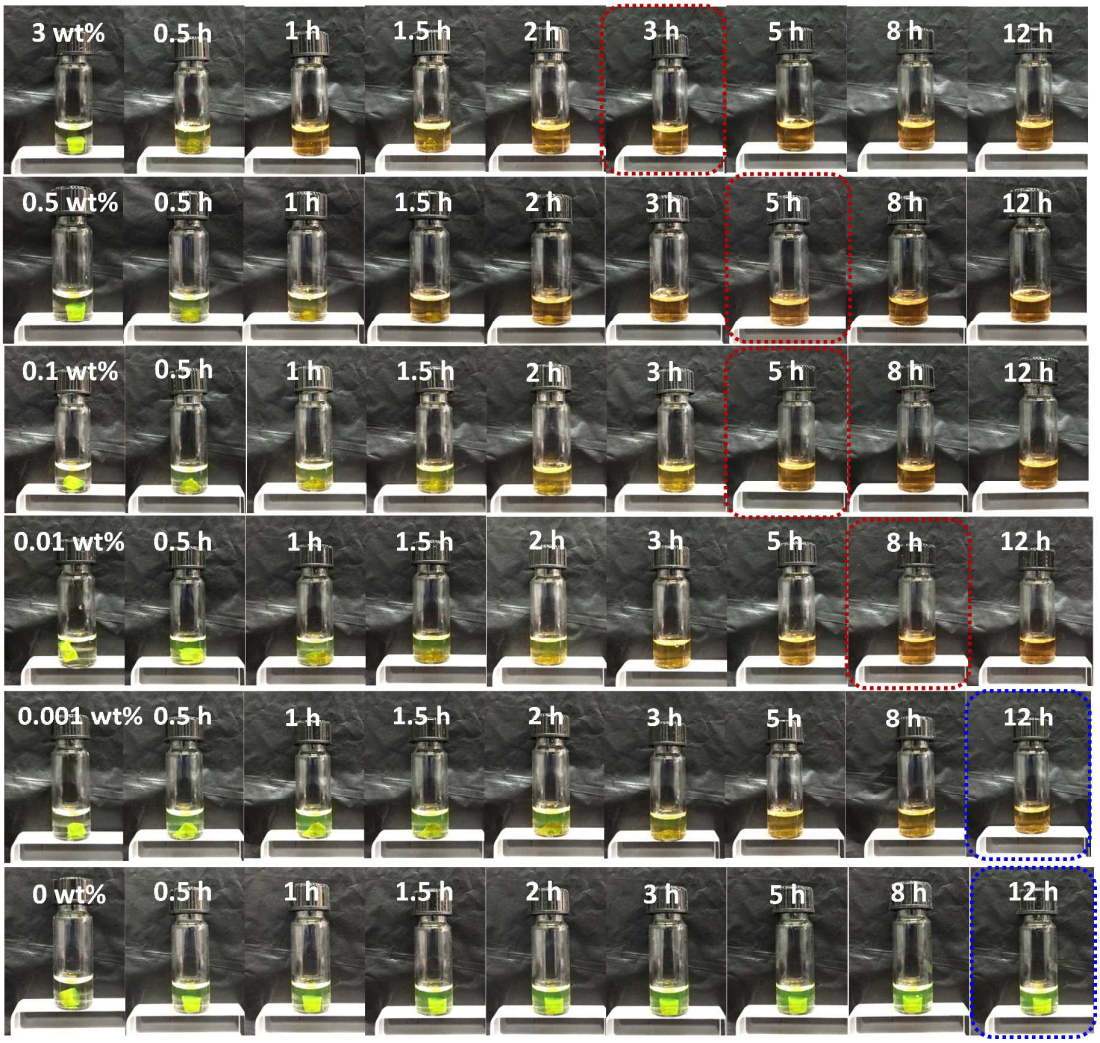


Figure S19. Time-dependent photographs of hydrogel **A3** loaded with FITC-insulin and different contents of GOx in PB solution (pH 7.4, 200 mM) at 37 ^{o}C upon addition of 100 mg/dL glucose.

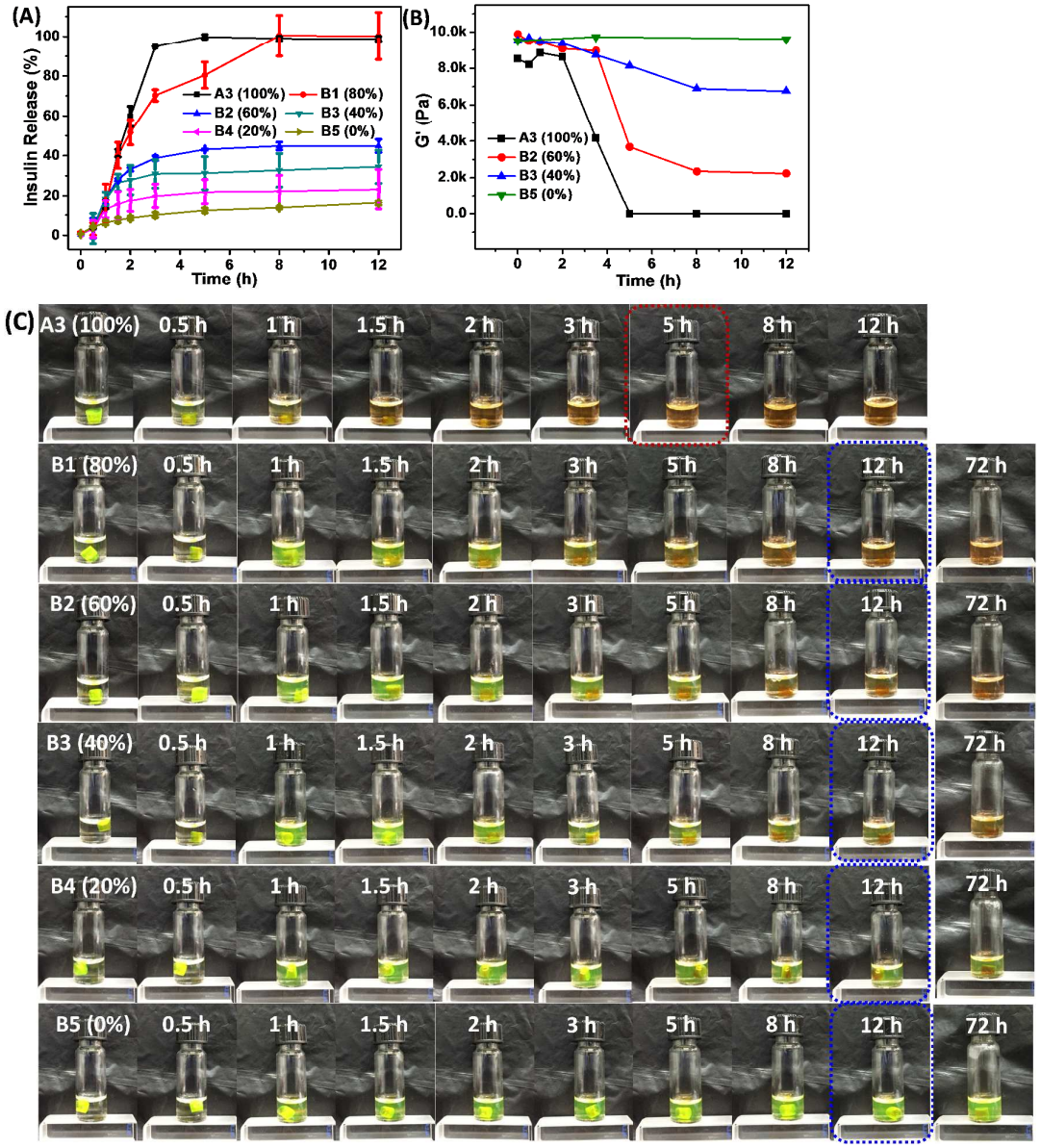


Figure S20. (A) Cumulative percent release of FITC-insulin from the 0.5 wt% GOX-loaded copolymer hydrogels triggered by 100 mg/dL glucose. (B) Storage modulus as a function of degradation time for 0.5 wt% GOx-loaded copolymer hydrogels in PB solution (pH 7.4, 200 mM) at 37 ^{o}C upon addition of 100 mg/dL glucose. (C) Time-dependent photographs of copolymer hydrogels loaded with FITC-insulin and 0.5 wt% GOx in PB solution (pH 7.4, 200 mM) at 37 ^{o}C upon addition of 100 mg/dL glucose.

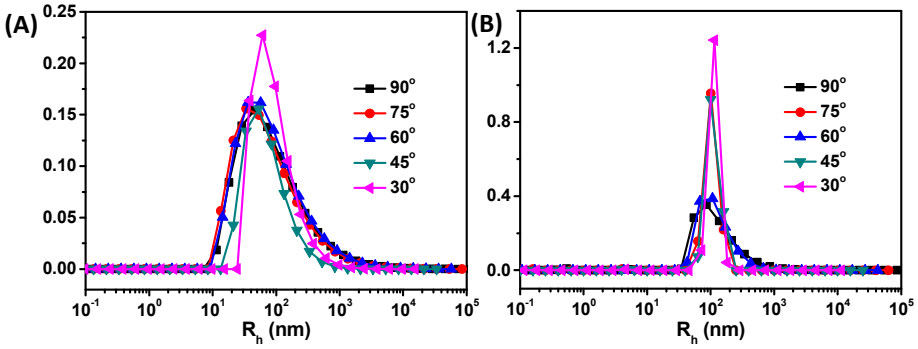


Figure S21. Size distribution of (A) the naked nanogel (0.1 mg/mL) and (B) the protein-loaded nanogel (0.1 mg/mL) in 200 mM PB solution (pH 7.4) at 37 °C.

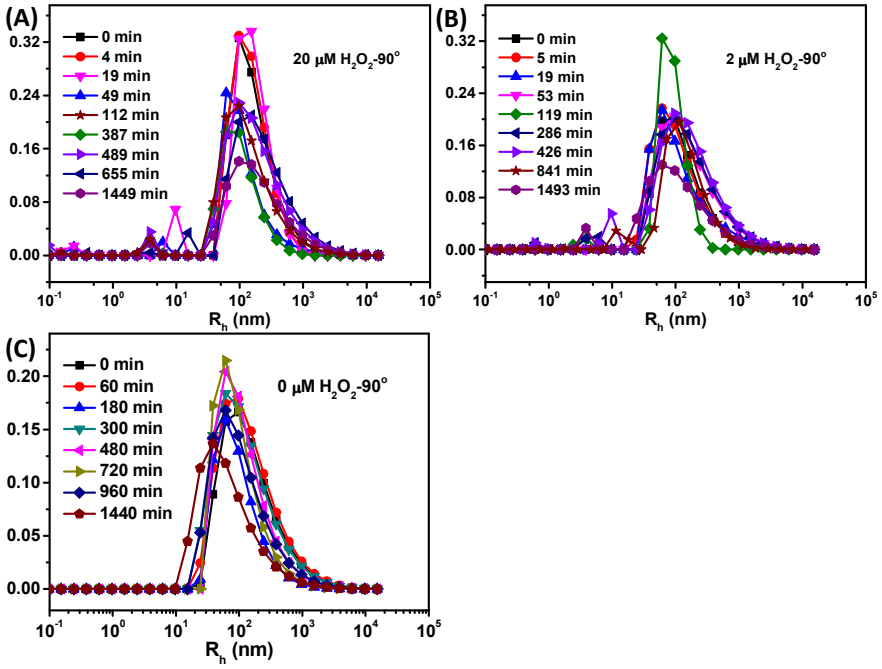


Figure S22. CONTIN analyses of the naked nanogel (0.1 mg/mL) in 200 mM PB solution (pH 7.4) incubated with (A) 20 μM, (B) 2.0 μM and (C) 0 μM of H_2O_2 for different times at 37 °C. Detection angle: 90°.

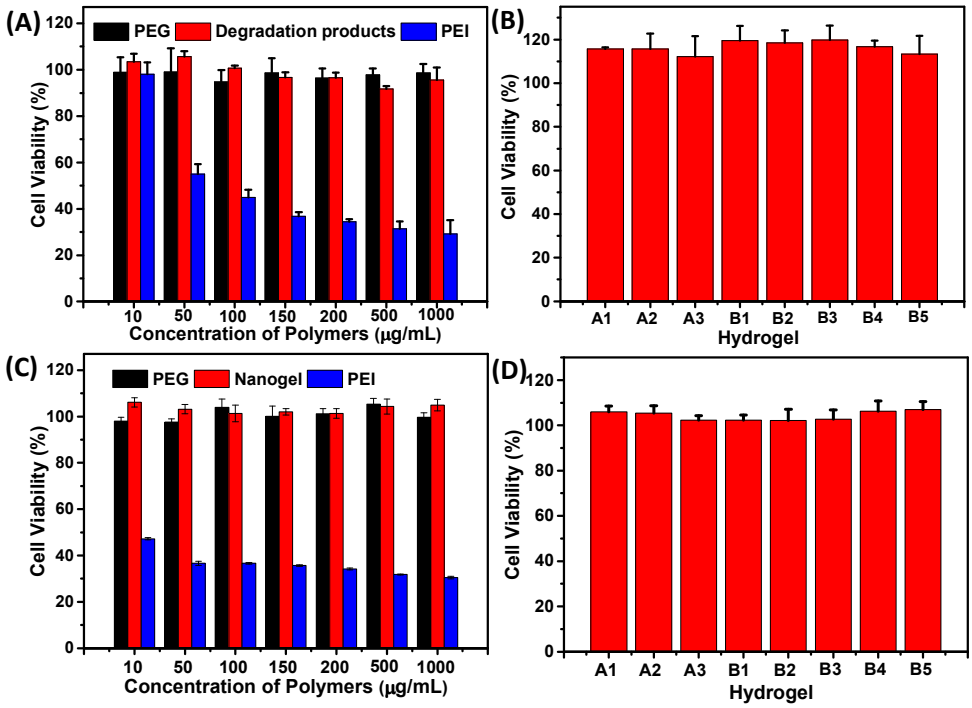


Figure S23. Cell viability of (A) the degradation products of **4-arm-PEG$_{20k}$-A**, (B) the nanogel, (C, D) the macroscopic hydrogels by (C) extraction and (D) co-incubation method. LO2 cells, CCK-8 assay, 37 ^{o}C.