Supporting Information

Core-Shell Microneedle Gel for Self-Regulated Insulin Delivery

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METHODS

1. Materials. 4-(Bromomethyl) phenylboronic acid and 4-(hydroxymethyl)phenylboronic acid pinacol ester were purchased from Boron Molecular. *N,N,N',N'*-tetramethyl-1,3-propanediamine (TMPA), 4-nitrobenzoyl chloride, triethylamine, PVA (89-98KDa, 99% hydrolysis) were purchased from Sigma-Aldrich. 4-nitrophenyl-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl carbonate (NBC) was synthesized according to literature[1]. TSPBA was synthesized from TMPA and 4-(bromomethyl) phenylboronic acid. PVA methacrylate was synthesized from the esterification reaction between PVA and methacrylic anhydride. Insulin-NBC was synthesized from insulin and NBC in a mixed solvent of DMSO and $NaHCO_3$ aqueous solution.

2. Synthesis

2.1 Synthesis of TSPBA. 4-(Bromomethyl) phenylboronic acid (1 g, 4.6 mmol) and *N*, *N*, *N*', *N*'-tetramethyl-1,3-propanediamine (0.2 g, 1.5 mmol) mixed in DMF (40 mL) was stirred at 60 ^{o}C for 24 h. Then, the clear solution was precipitated in THF (100 mL) and filtrated, and the white solid was further washed with THF (3×20 mL). After placed under vacuum overnight, pure TSPBA (0.6 g, yield 70%) was obtained as a white solid. ^{1}H-NMR (300 MHz, D_2O, δ): 7.677 (d, 4H), 7.395 (d, 4H), 4.409 (s, 4H), 3.232 (t, 4H), 2.936 (s, 6H), 2.81 (m, 2H).

2.2 Synthesis of insulin-NBC. Insulin (100 mg) was dissolved in 0.1 M $NaHCO_3$ buffer solution (5 mL, pH=8.5) under stirring. Then, DMSO (5 mL) solution containing NBC (20 mg) was added to the above solution. The reaction mixtures were then stirred at room temperature overnight, followed by pH adjustment to precipitate insulin-NBC. The product was purified using preparative scale high performance liquid chromatography (HPLC, Agilent).

2.3 Synthesis of PVA methacrylate. PVA (1 g) and methacrylic anhydride (1 g) were dissolved in DMSO (20 mL) containing triethylamine (1 g). The solution was stirred overnight at room temperature. PVA methacrylate was precipitated upon addition of THF and washed three times. The product was dried under vacuum.

2.4 Rhodamine B or FITC labeled insulin or CAT. Rhodamine B isothiocyanate (0.5 mg) dissolved in DMSO (1 mL) was added to insulin (20 mg) dissolved in $NaHCO_3$ (10 mM, 1 mL). The mixture was stirred

overnight and dialysis against DI H_2O (3×2 L). The resultant solution was lyophilized to obtain rhodamine B labeled insulin. Other fluorescence-labeled proteins were obtained with the same methods. The fluorescently labeled insulin or CAT were used in the same way as the one not labeled, and the fluorescence images were taken on a fluorescence microscopy (Olympus, IX71).

3. Insulin release from PVA methacrylate gel. Insulin or insulin-NBC was dissolved in H_2O containing PVA methacrylate, and photoinitiator (Irgacure 2959; 5% wt/vol) was added. This solution was assigned to three tubes, and exposed to UV light (360 nm) for 30 s for gelation. Then, another 1 mL PBS and predetermined amount of H_2O_2 was added. At predetermined time intervals, solution (10 μL each tube) was withdrawn and stained with Coomassie blue (200 μL). The absorbance at 595 nm was detected on an Infinite 200 PRO multimode plate reader (TecanGroup Ltd.). The insulin content was calibrated using a standard curve.

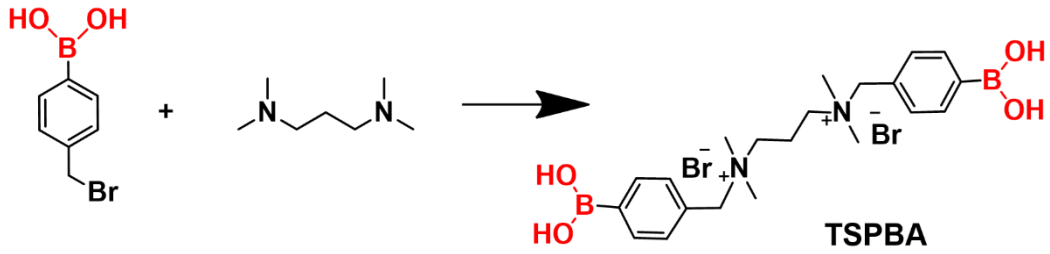
4. GOx-NG release from PVA methacrylate gel. Native GOx or GOx-NG was dissolved in H_2O containing PVA methacrylate, and initiator (Irgacure 2959; 5% wt/vol) was added. This solution was assigned to one of three tubes, and exposed to UV light (360 nm) for 30 s. Then, another 1 mL PBS was added. At predetermined time intervals, solution (10 μL each tube) was withdrawn, and added to Coomassie blue (200 μL). The absorbance at 595 nm was detected on an Infinite 200 PRO multimode plate reader (TecanGroup Ltd.). The GOx concentration was calibrated by a standard curve.

5. The mechanical strength test. The mechanical strength of microneedles with a stress-strain gauge was determined by pressing a stainless steel plate against microneedles on an MTS 30G tensile testing machine. The initial gauge was 2.00 mm between the tips of microneedle and the plate, with 10.00 N as the load cell capacity. The speed of the plate approaching microneedles was set as 0.1 mm/s. The failure force of microneedles was recorded as the force at which the needle began to buckle.

6. *In vitro* skin penetration test. To evaluate the *in vitro* skin penetrating ability of MNs, the MNs were inserted into the skin of the mouse for 10 min. The skin was stained with trypan blue for 10 min before imaging by optical microscopy (Leica EZ4 D stereomicroscope).

7. Animal experiment

The sample size calculated by power analysis: G*power 3.1. The experiments were not use a method of randomization. The investigators were not blinded to allocation during experiments and outcome assessment.



Scheme S1. Synthesis route of TSPBA.

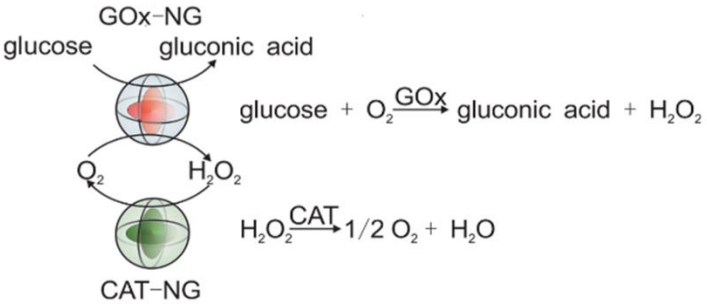


Figure S1. Schematic illustration of H_2O_2 generation by GOx-NG and elimination by CAT-NG.

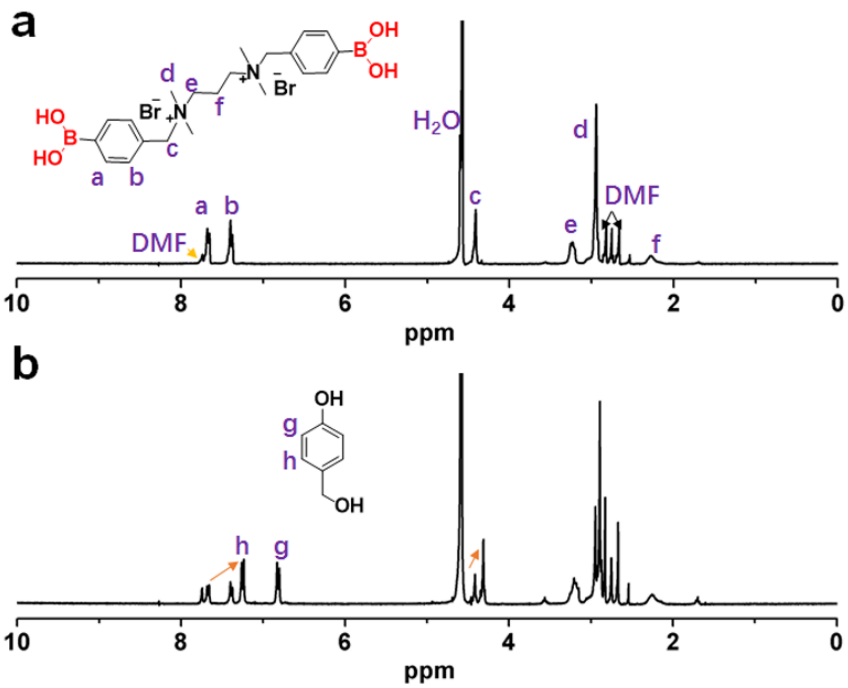


Figure S2. ^{1}H-NMR (300 MHz, in D_2O) of TSPBA before and after oxidization in PBS. **a,** before oxidation; **b**, after oxidation in 10 mM H_2O_2 for 1 h.

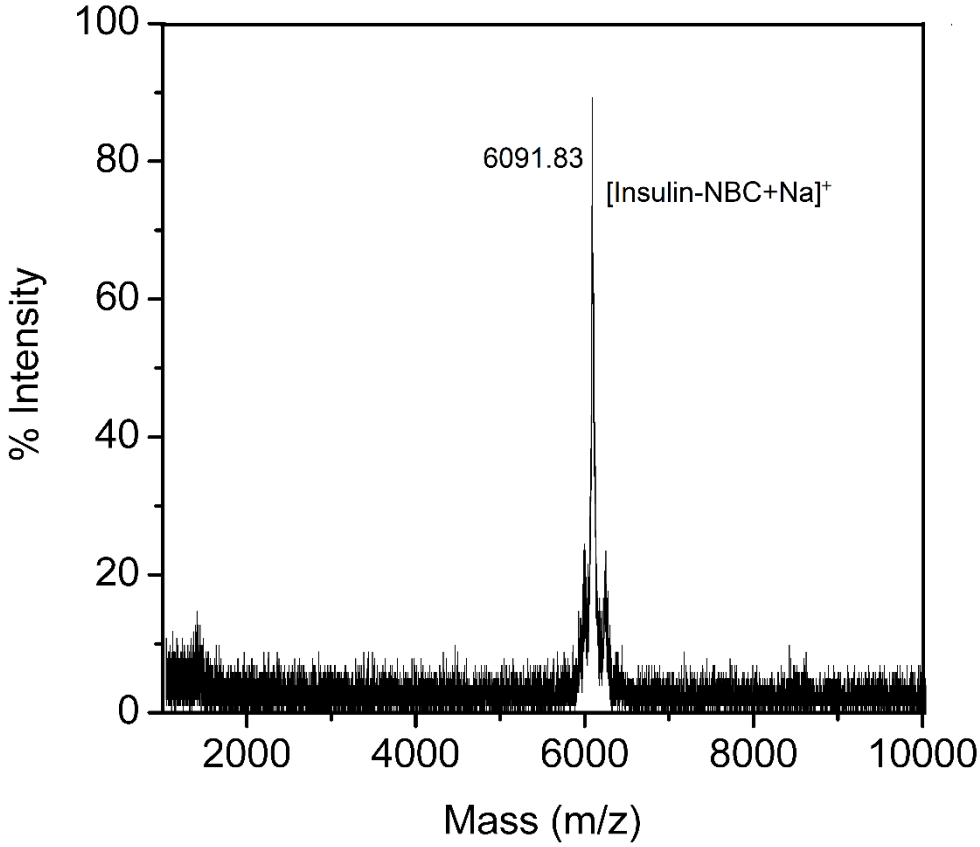


Figure S3. MALDI-TOF mass spectrum of the purified insulin-NBC.

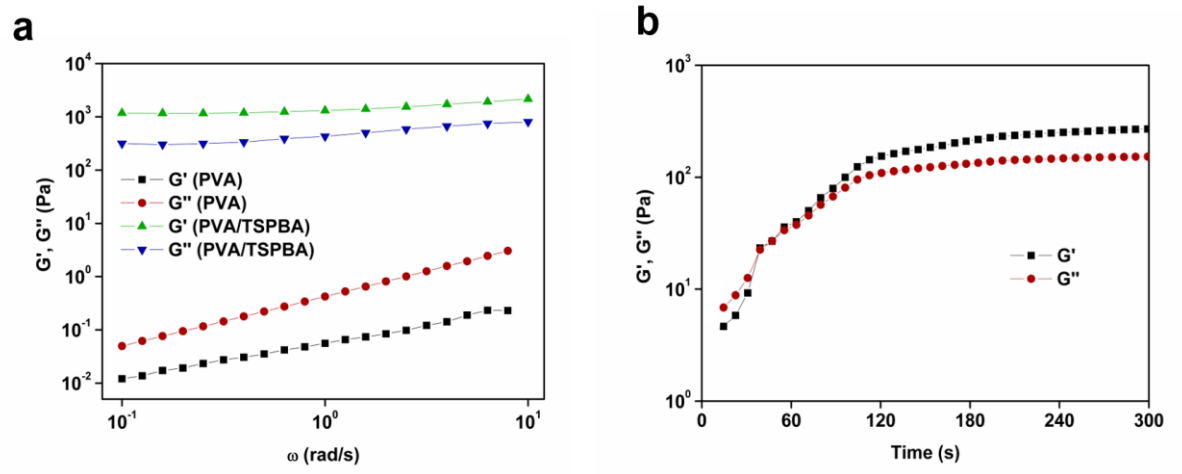


Figure S4. Dynamic rheological behavior of PVA before and after gelation at 25 °C measured using a TA Instruments AR-2000 stress-controlled rheometer with 25 mm aluminum cross-hatched parallel plates. All experiments were conducted in the linear viscoelastic regime with a 500 µm gap between the plates. **a**) Frequency spectra of the elastic (G') and viscous (G'') moduli of PVA and PVA-TSPBA samples, with the former exhibiting solution-like characteristics and the latter a gel-like behavior. **b**) Evolution of G' and G'' as a function of time of the PVA-TSPBA sample showing sol-gel transition. Experiments were at a constant frequency of 5 rad/s. Measurements were started after pre-shear the sample for 10 s at a shear rate of 10 s^{-1}.

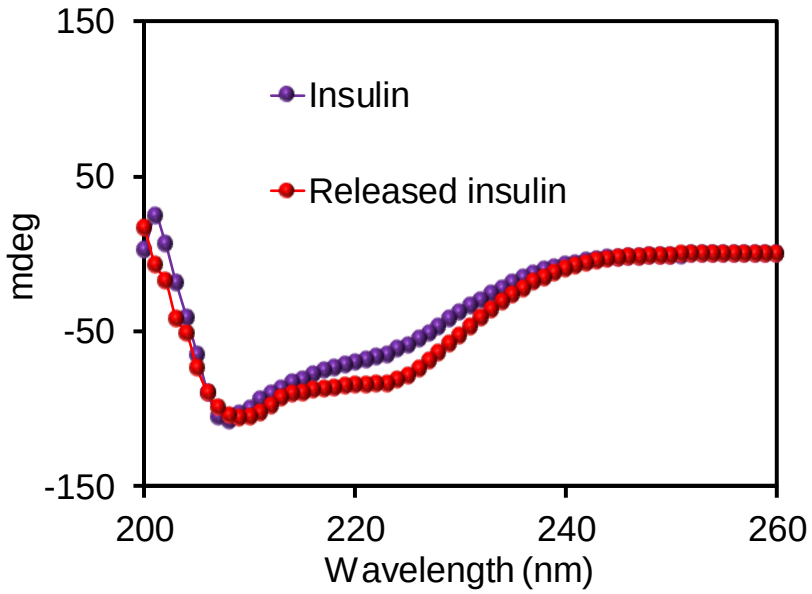


Figure S5. CD spectra of native insulin solution and insulin released from the gels incubated with 400 mg/dL glucose.

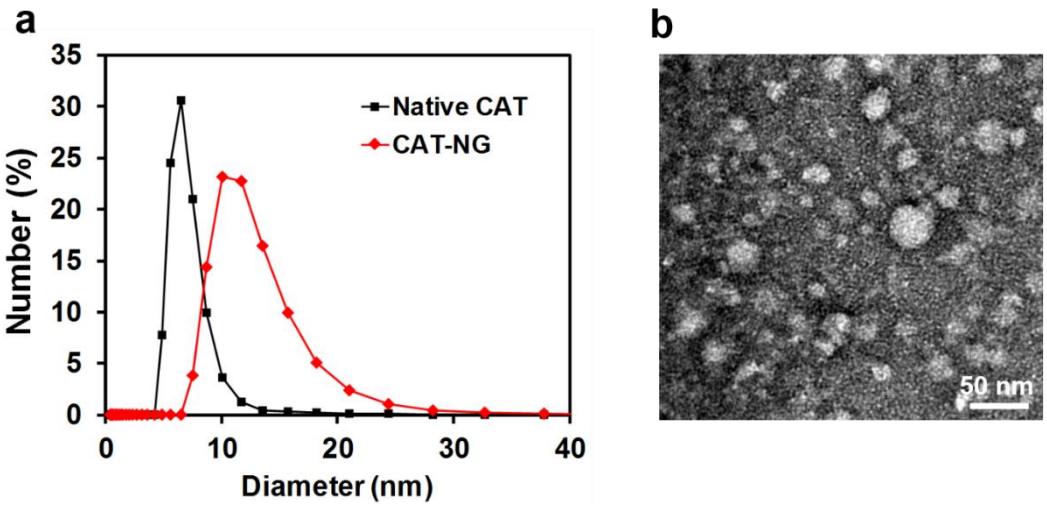


Figure S6. Characterization of CAT-NG. **a)** The size distribution of CAT and CAT-NG measured by dynamic laser scattering. **b**) The representative TEM images of CAT-NG.

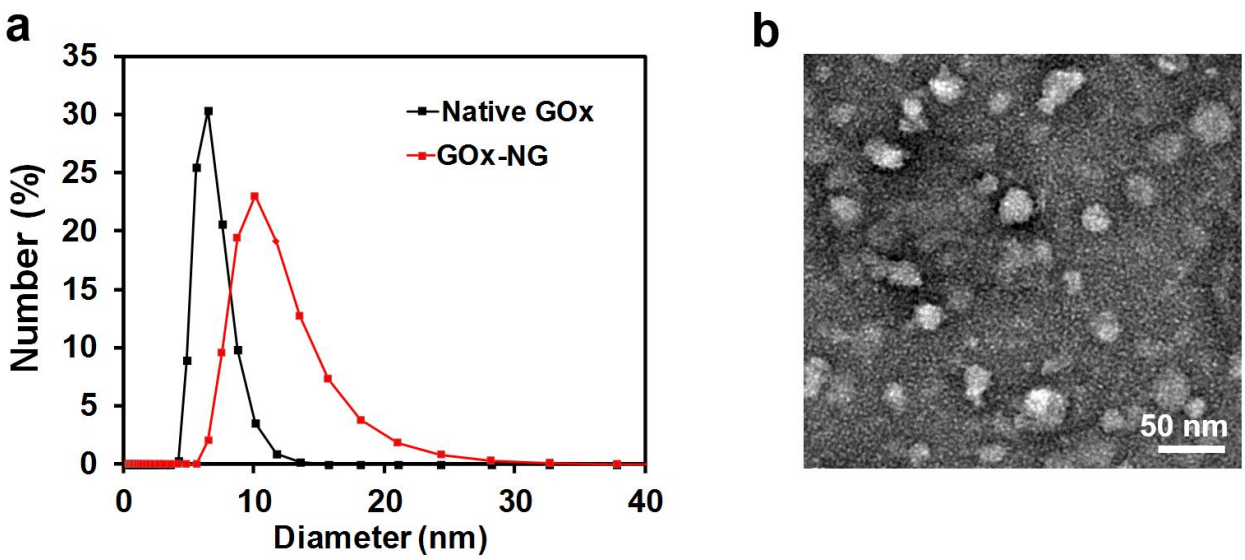


Figure S7. Characterization of GOx-NG. **a)** The size distribution of GOx and GOx-NG measured by dynamic laser scattering. **b**) The representative TEM image of GOx-NG.

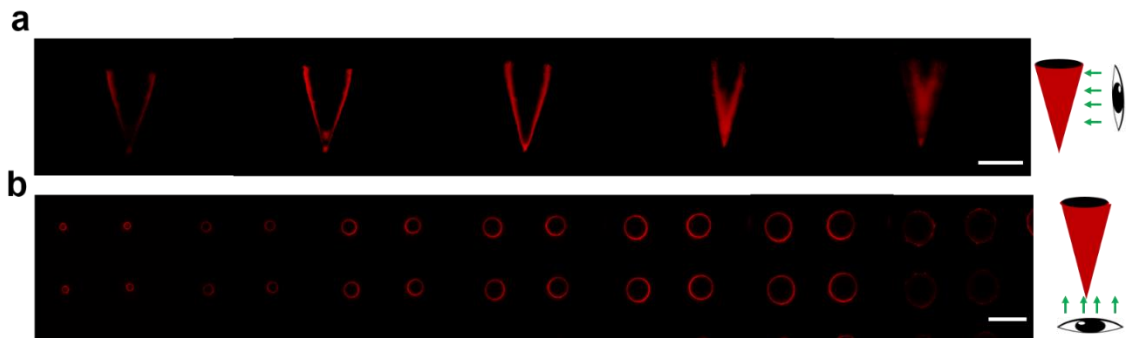


Figure S8. Representative images of hollow CAT loaded MNs: side view (**a**) and overhead view (**b**). The intervals for **b** was 80 μm at direction from bottom to top of microneedle.

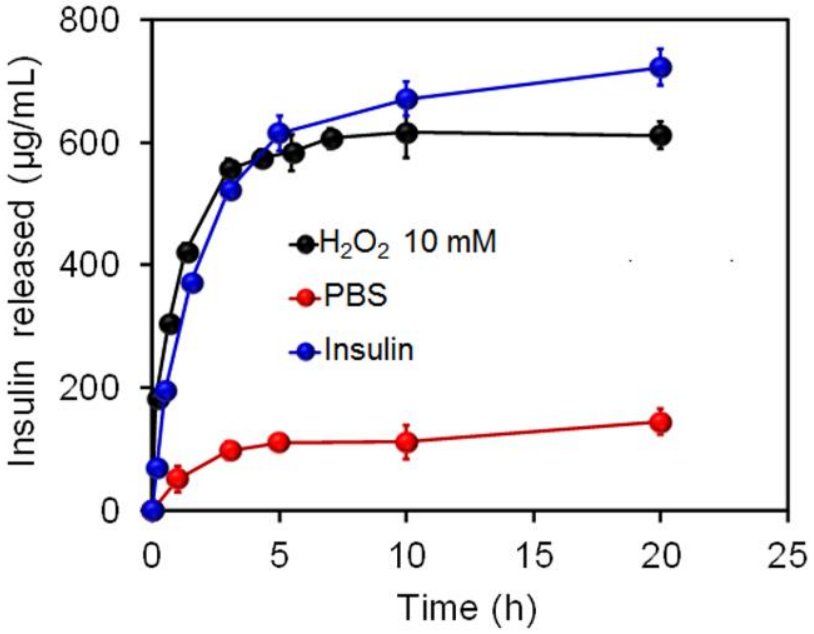


Figure S9. The H_2O_2 dependent release of insulin from insulin-NBC loaded in PVA methacrylate gel. Native insulin was used as control. Data points represent mean ± SD (n = 3). Error bars indicate SD.

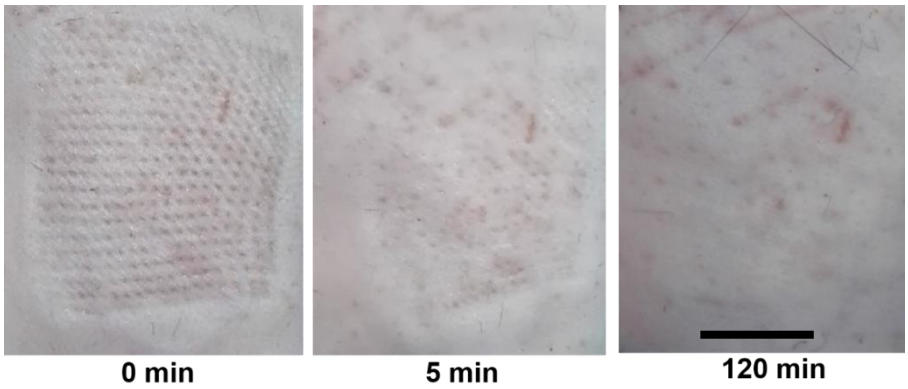


Figure S10. Skin puncture marks at 0 min, 5 min and 120 min post-treatment of MNs. Scale bar, 0.5 cm.

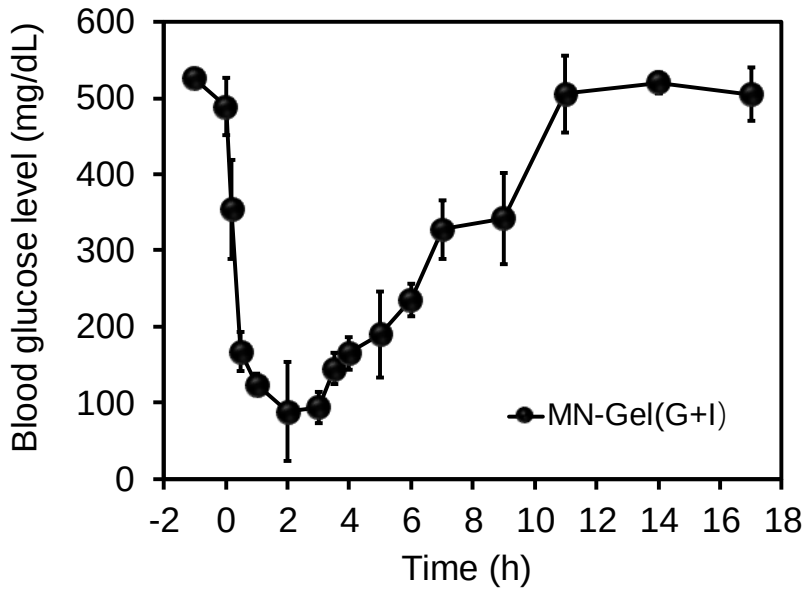


Figure S11. Blood glucose level of type 1 diabetic mice treated by MN-Gel (G+I). Data points represent mean ± SD (n = 5). Error bars indicate SD.

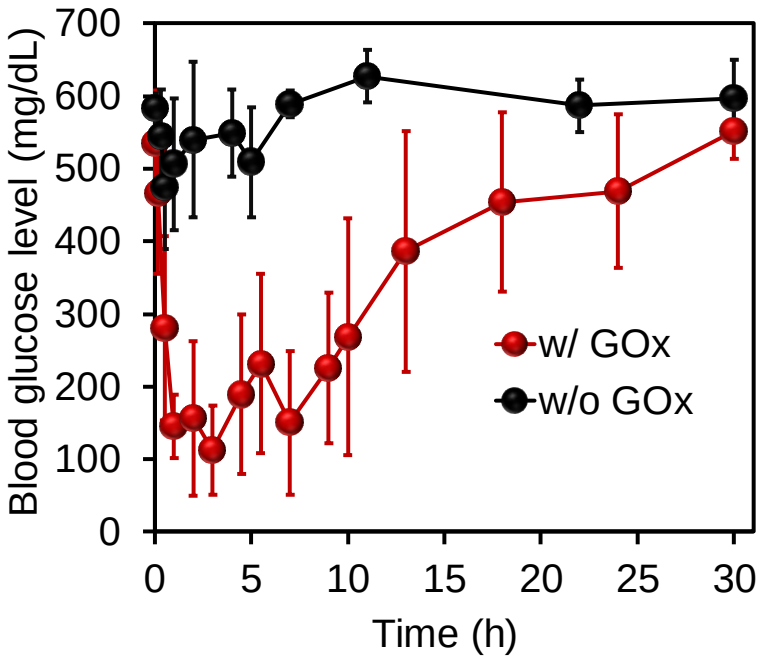


Figure S12. Blood glucose levels in STZ-induced diabetic mice after treatment with insulin-NBC loaded PVA-TSPBA gel with or without GOx. Data points represent mean ± SD (n = 4). Error bars indicate SD.

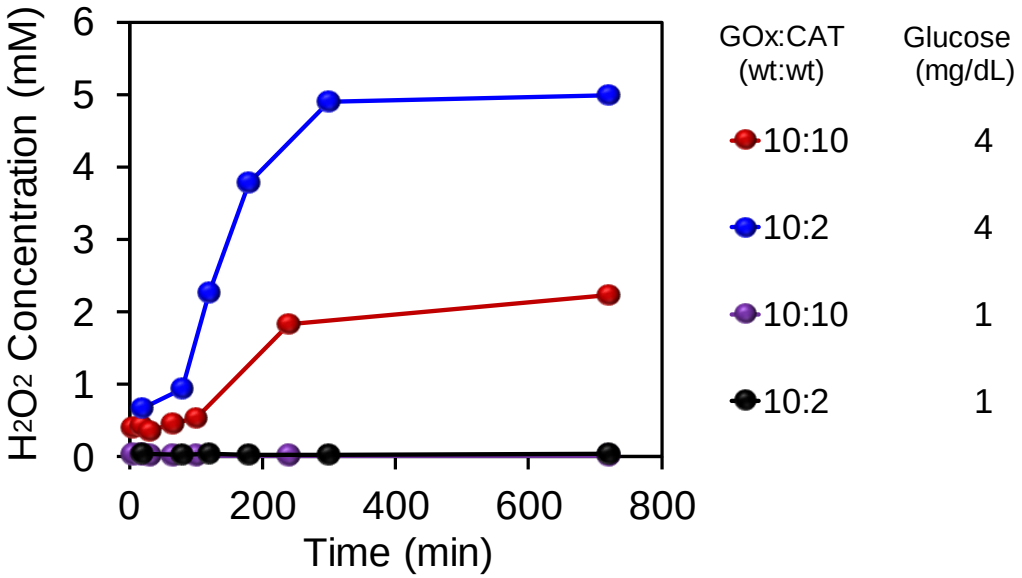


Figure S13. The H_2O_2 generation rate through oxidation of glucose by GOx in the presence of CAT of different ratio in glucose solution (100 or 400 mg/dL) in PBS with an initial pH at 7.4. The concentration of GOx was set as 0.2 mg/mL.

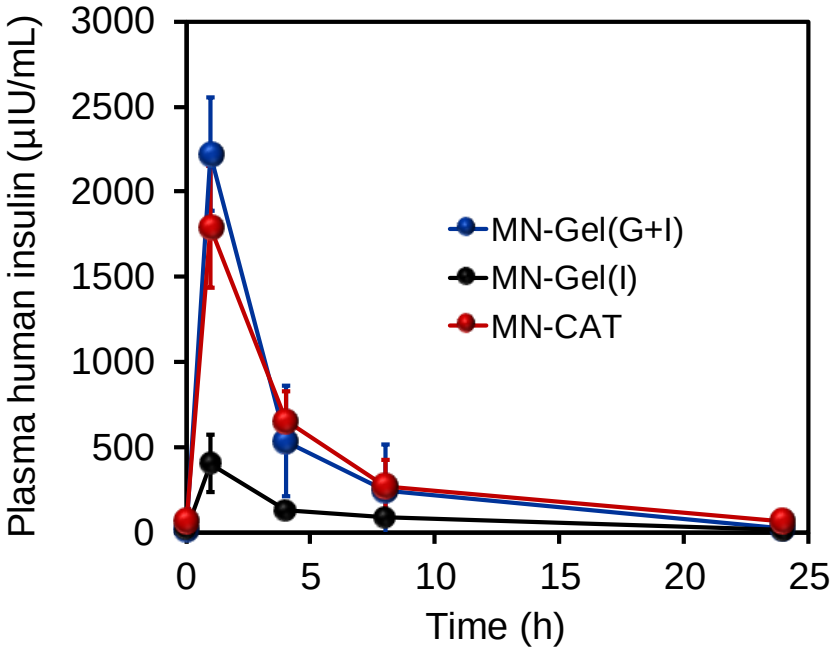


Figure S14. The plasma human insulin levels in mice treated with MN-CAT, MN-Gel(I)，or MN-Gel(G+I). Data points represent mean ± SD (n = 3). Error bars indicate SD.

Figure S15. Skin bubbling induced by subcutaneously injected Gel-(G+I). **left**: the site of gel inoculation; **right**: skin swelling observed 1 h post-inoculation. Scale bar, 1 cm.

1. Major Jourden, J.L. & Cohen, S.M. Hydrogen Peroxide Activated Matrix Metalloproteinase Inhibitors: a Prodrug Approach. *Angew. Chem. Int. Ed.* **2010**, *49*, 6795-6797.