Supporting Information

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SI Methods

Synthesis and Characterizations of HS-HA. HS-HA was synthesized by chemical conjugation with the 6-(2-nitroimidazole) hexylamine through amide formation. First, 6-(2-nitroimidazole) hexylamine was synthesized for reaction with the carboxylic acids of HA. In brief, NI (0.15 g, 1.3 mmol) was dissolved in dimethylformamide (DMF), to which K_2CO_3 (0.28 g, 2.0 mmol) was added. Then, 6-(Boc-amino) hexyl bromide (0.39 g, 1.4 mmol) was added drop-wise into the solution and stirred at 80 °C for 4 h. The solid impurities were removed from the reaction mixture by filter and washed with methanol. The residual solvent was then evaporated to obtain the solid product, which was suspend in de-ionized (DI) water and extracted with ethyl acetate. The organic layer was collected and dried over sodium sulfate, and then concentrated. The product was redissolved in methanol on the ice. Five milliliters of 1.25 M HCl in methanol was added to the solution and stirred for 24 h at room temperature (RT). Afterward, the solvent was removed from the reaction mixture using a rotary evaporator to obtain the amine-functionalized NI. Next, 6-(2-nitroimidazole) hexylamine was conjugated to HA in the presence of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDC) and NHS. Briefly, 0.24 g of HA (molecular mass of ~300 kDa) was dissolved in water, to which EDC (0.56 g, 3.4 mmol) and NHS (0.39 g, 3.4 mmol) were added, and stirred for 15 min at RT. Then, 6-(2-nitroimidazole) hexylamine (0.18 g, 0.85 mmol) was added to the mixture and allowed to react at RT for 24 h. The reaction solution was thoroughly dialyzed against a 1:1 mixture of DI water and methanol for 1 d and against DI water for 2 d. Then, HS-HA was obtained by lyophilization and characterized by ^{1}H NMR (Varian Gemini 2300). The graft degree is calculated as 20% by UV-visible (UV-Vis) absorbance.

6-(2-nitroimidazole) hexylamine: ^{1}H NMR (DMSO-d_6, 300 MHz, δ ppm): 1.30–1.78 [m, 8H, $NH_2CH_2(CH_2)_4$], 2.73 (s, 2H, NH_2CH_2), 4.38 (s, 2H, NCH_2), 7.19 (s, 1H), 7.87 (s, 1H).

HS-HA: ^{1}H NMR (D_2O, 300 MHz, δ ppm): 1.88–2.40 [m, 8H, $NH_2CH_2(CH_2)_4$], 2.87–3.19 (m, 4H, NH_2CH_2, NCH_2), 7.19 (s, 1H), 7.48 (s, 1H).

Synthesis and Characterizations of Methacrylated HA. Methacrylated HA (*m*-HA) was synthesized following the literature (30). Briefly, 1.0 g of HA was dissolved in 50 mL of DI water at 4 °C, to which 0.8 mL of methacrylic anhydride was added drop-wise. The reaction solution was adjusted to pH 8–9 by the addition of 5 N of NaOH and stirred at 4 °C for 24 h. The resulting polymer was obtained by precipitation in acetone, followed by washing with ethanol three times. The product was redissolved in DI water, and the solution was dialyzed against DI water for 2 d. The *m*-HA was achieved by lyophilization, with a yield of 87.5%. The degree of modification was calculated to be 15% by comparing the ratio of the areas under the proton peaks at 5.74 and 6.17 ppm (methacrylate protons) with the peak at 1.99 ppm (*N*-acetyl glucosamine of HA) after performing a standard deconvolution algorithm to separate closely spaced peaks.

m-HA: ^{1}H NMR (D_2O, 300 MHz, δ ppm): 1.85–1.96 [m, 3H, $CH_2 = C(CH_3)CO$], 1.99 (s, 3H, $NHCOCH_3$), 5.74 [s, 1H, $CH^1H^2 = C(CH_3)CO$], 6.17 [s, 1H, $CH^1H^2 = C(CH_3)CO$].

Oxygen Consumption Rate Assay. Oxygen consumption rate was determined using MitoXpress (Cayman Chemical) according to the manufacturer's protocol. Briefly, 200 μL of 5 mg/mL GRV solution with 0, 100, or 400 mg/dL glucose containing 10 μL of MitoXpress probe was placed in a 96-well plate, and the plate was measured on a microplate reader at the excitation/emission wavelength of 380/650 nm at 37 °C. Each sample well was measured repetitively every 5 min by taking two intensity readings at delay times of 30 and 70 μs and a gate time of 30 μs. Obtained time-resolved fluorescence (TR-F) intensity signals for each sample well were converted into phosphorescence lifetime (microsecond) [τ] values as follows: $\tau = (70-30)/\ln(F1/F2)$, where F1 and F2 are the TR-F intensity signals at the delay times of 70 μs and 30 μs, respectively. The resulting increasing lifetime [τ] reflects the sample's oxygen concentration.

In Vitro Release Studies. To evaluate the glucose-responsive capability of GRVs, GRVs were incubated in 600 μL of PBS buffer [137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, 2 mM KH_2PO_4 (pH 7.4)] with 100 μM NADPH and 5 μg/mL cytochrome *c* reductase. Various amounts of glucose were added to the solution to reach a final glucose concentration (0 mg/dL, 100 mg/dL, and 400 mg/dL). The mixture was incubated at 37 °C in a container with an oxygen concentration of 21% by regulation with a mass-flow meter. At predetermined times, the pH value of each mixture was recorded by a pH meter (AB15; Fisher Scientific), and the value was then adjusted to the pI of insulin to isolate the released insulin by centrifugation at 6,200 × *g* for 10 min. The concentration of residual insulin encapsulated in GRVs was examined using a Coomassie Plus protein assay. The A_{595} was detected on an Infinite 200 PRO multimode plate reader (Tecan Group Ltd.), and the insulin content was calibrated with an insulin standard curve. For plotting the UV-Vis absorption of GRV solution, the intensity was measured at an *A* of 330 nm at the set time. To assess the release profile of insulin from GRVs under different pH conditions, GRVs were incubated in PBS buffer solutions with NADPH and cytochrome *c* reductase (pH 4.0 and pH 7.4). The released insulin was measured using the same method mentioned above. The far-UV CD spectra of the native and released insulin from GRVs (0.1 mg/mL) were analyzed by the CD spectrometer (Aviv).

Fabrication of GRV-Loaded MNs. All of the MNs in this study were fabricated using five uniform silicone molds from Blueacre Technology Ltd. Each MN had a 300 × 300-μm round base tapering to a height of 600 μm with a tip radius of around 10 μm. The MNs were arranged in an 11 × 11 array with 600-μm tip-to-tip spacing. Following the preparation of the GRVs, the product was evenly dispersed in 400 μL of DI water. MNs loaded with GRVs were fabricated from silicone molds machined by laser ablation to create 11 × 11 arrays of MN cavities. First, GRV solution was directly deposited by pipette onto the MN mold surface (35 μL per array). Molds were then placed under vacuum (600 mmHg) for 5 min to allow the GRV solution flow into the MN cavities and become more viscose. The covered molds were then transferred to a Hettich Universal 32R centrifuge for 20 min at 2,000 rpm to compact GRVs into MN cavities. The process was repeated three times until the GRV solution layer dried and there was no bubble arising from mold cavities in the vacuum condition. For better MN morphology, a piece of 4 × 9-cm silver adhesive tape was applied around the 2 × 2-cm micromold baseplate. Finally, addition of 3 mL of premixed *N*,*N*'-methylenebisacrylamide (20% wt/vol), photoinitiator (Irgacure 2959; 5% wt/vol), and *m*-HA with solution to the prepared micromold reservoir was followed by centrifugation (2,000 rpm for 20 min) and drying at 25 °C (6–8 h under a vacuum desiccator). After the desiccation was completed, the MN arrays were carefully separated from the

silicone mold and the MNs underwent cross-linking polymerization via UV irradiation (wavelength of 365 nm) for a short period. The needle base can be tailored to fit the injection syringe. The resulting product can be stored in a sealed six-well container for up to 30 d. The morphology of the MNs was characterized on an FEI Verios 460L field-emission scanning electron microscope.

Mechanical Strength Test. The mechanical strength of MNs with a stress–strain gauge was measured by pressing MNs against a stainless-steel plate on an MTS 30G tensile testing machine. The initial gauge was set as 2.00 mm between the MN tips and the stainless-steel plate, with 10.00 N as the load cell capacity. The speed of the top stainless-steel plate movement toward the MN-array patch was set as 0.1 $mm{\cdot}s^{-1}$. The failure force of MNs was recorded as the needle began to buckle.

Skin Penetration Efficiency Test. In separate experiments, treated skin and applied MN arrays were imaged by bright-field microscopy to assess transcutaneous drug delivery and skin penetration efficiency. The MN array was applied to the dorsum of the mouse skin for 30 min. Excited skin was stained with trypan blue for 30 min before imaging for needle penetration. After wiping residual dye from the skin surface with dry tissue paper, the mouse was euthanized and the skin sample was viewed by optical microscopy (Leica EZ4 D stereomicroscope).

In Vivo Studies Using Streptozotocin-Induced Diabetic Mice. The in vivo efficacy of MN-array patches for diabetes treatment was evaluated on streptozotocin-induced adult diabetic mice (male C57B6; Jackson Laboratory). The animal study protocol was approved by the Institutional Animal Care and Use Committee at North Carolina State University and the University of North Carolina at Chapel Hill. The plasma-equivalent glucose was measured from tail vein blood samples (~3 μL) of mice using the Clarity GL2Plus glucose meter (Clarity Diagnostics). Mouse glucose levels were monitored for 2 d before administration, and all mice were fasted overnight before administration. Five mice for each group were selected to be transcutaneously treated with blank MNs containing only cross-linked HA, MNs loaded with human recombinant insulin, MNs loaded with GRV(E + I), MNs loaded with GRV(1/2E + I), or MNs loaded with GRV(I) on the dorsum, with an insulin dose of 10 mg/kg for each mouse. The glucose levels of each mouse were monitored (at 5, 15, 30, and 60 min, and once per hour afterward) until a return to stable hyperglycemia. To measure the plasma insulin concentration in vivo, a blood sample of 25 μL was drawn from the tail vein of mice at indicated time points. The serum was isolated and stored at −20 °C until assay. The plasma insulin concentration was measured using a Human Insulin ELISA kit according to the manufacturer's protocol (Calbiotech). A glucose tolerance test was conducted to confirm the in vivo glucose responsiveness of MNs 1 h postadministration of GRV(E + I)-loaded MNs and insulin-loaded MNs. Briefly, mice were fasted overnight and administered GRV(E + I)-loaded MNs and insulin-loaded MNs with an insulin dose of 10 mg/kg for each mouse, and a glucose solution in PBS was then injected i.p. into all mice at a dose of 1.5 g/kg. The glucose levels were monitored at 5, 10, 15, 20, 30, 40, 50, 60, 80, 100, and 120 min after injection. The glucose tolerance test on healthy mice was used as control. Similarly, the healthy mice used to assess hypoglycemia were administered insulin-loaded MNs, GRV(I)-loaded MNs, or GRV(E + I)-loaded MNs, but were not subjected to a glucose challenge.

Biocompatibility Analysis. The in vitro cytotoxicity of GRVs was measured by a 3-(4, 5)-dimethylthiahiazo(-z-y1)-3,5-di-phenytetrazoliumromide assay in HeLa cells. Briefly, HeLa cells were seeded in a 96-well plate at a density of 6,000 cells per well. After 24 h of incubation in 200 μL of DMEM with 10% FBS, series dilutions of bare GRVs ranging from 0.1 to 1 mg/mL were added into wells. After 24 h of incubation, thiazole blue solution (5 mg/mL) was added into wells and incubated with cells for another 4 h. After removing the culture media, the purple formazan crystal was dissolved in 150 μL of DMSO. The A_{570} of the plates, which is directly proportional to the viable cell number, was measured on a multimode plate reader. To evaluate the biocompatibility of the MN-array patch, on day 2 postadministration, mice were euthanized by CO_2 asphyxiation and the surrounding tissues were excised. The tissues were fixed in 10% formalin and then embedded in paraffin, cut into 50-μm sections, and stained using H&E for histological analysis.

Statistical Analysis. All results presented are mean ± SEM. Statistical analysis was performed using the Student's t test or an ANOVA test. With a P value <0.05, the differences between experimental groups and control groups were considered statistically significant.

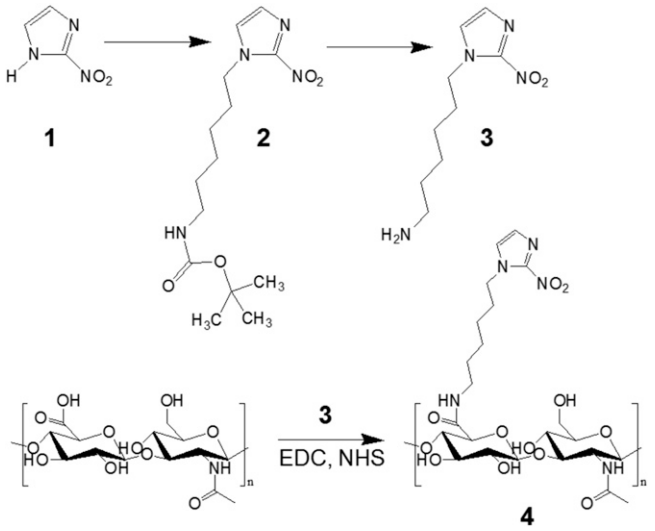

Fig. S1. Synthesis route of HS-HA (4).

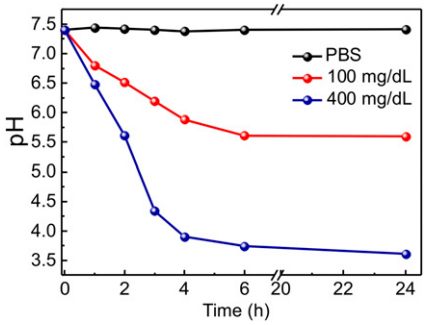

Fig. S2. Relevant pH changes in GRV solutions with different glucose concentrations.

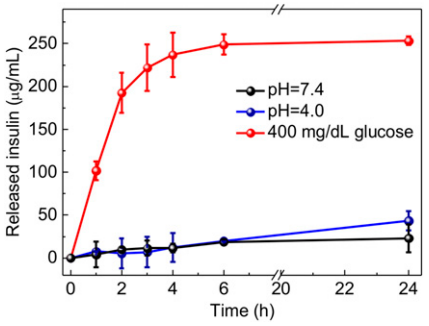

Fig. S3. Accumulated insulin release of GRVs incubated in 400 mg/dL glucose solution or pH 7.4 or pH 4.0 buffer solution. Error bars indicate SD (n = 3).

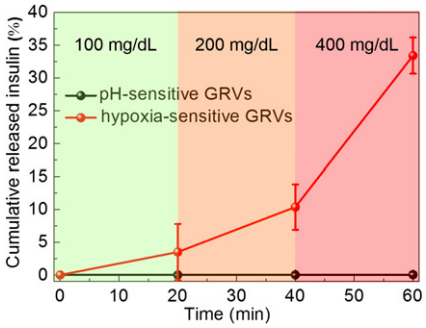

Fig. S4. Self-regulated profile of the hypoxia-sensitive-based GRVs or pH-sensitive–based GRVs presents the rate of insulin release as a function of glucose concentration. Error bars indicate SD (n = 3).

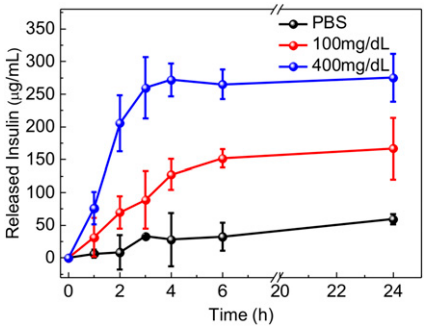

Fig. S5. Accumulated insulin release of the GRVs dissolved from the prepared MNs in different glucose concentrations at 37 °C. Error bars indicate SD (n = 3).

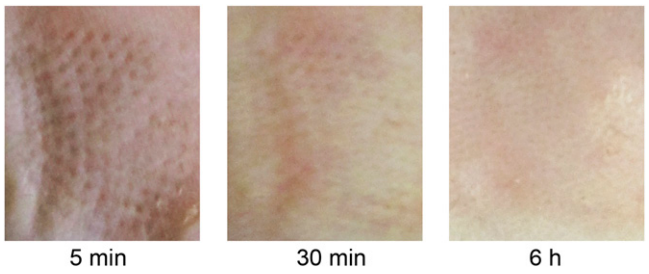

Fig. S6. Skin puncture marks at 5 min, 30 min, and 6 h posttreatment.

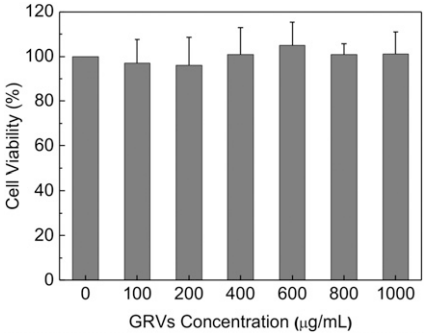

Fig. S7. Cytotoxicity study of empty GRVs after 24 h of incubation with HeLa cells. Error bars indicate SD (n = 6).

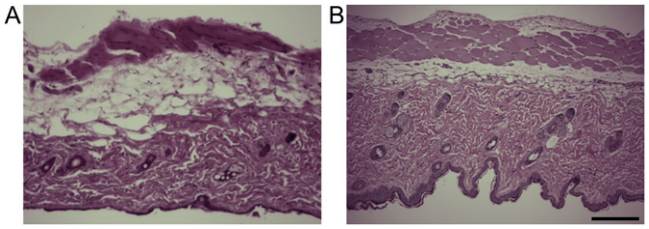

Fig. S8. H&E-stained skin sections administered PBS (*A*) or an MN-array patch (*B*) with surrounding tissues 2 d postadministration of the MN-array patch. (Scale bar: 200 μm.)