Supporting Information

H_2O_2-Responsive Vesicles Integrated with Transcutaneous Patches for Glucose-Mediated Insulin Delivery

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MATERIALS. All chemicals were purchased from Sigma–Aldrich unless otherwise specified and were used as received. Sodium HA (molecular mass of 300 kDa) was purchased from Freda Biochem Co., Ltd. (Shandong, China). Human recombinant insulin (27.5 IU/mg of Zn salt) was purchased from Life Technology (U.S.A.). Poly(ethylene glycol) amine (PEG2000-NH_2) was purchased from Laysan Bio, Inc. (U.S.A.). The deionized water was obtained by a Millipore NanoPure purification system (resistivity > 18.2 MΩ cm^{-1}). All the organic solvents for synthesis and analysis were ordered from Fisher Scientific Inc. and used as received.

METHODS

Synthesis and Characterizations of Acrylate Modified HA (*m*-HA). *m*-HA was synthesized according to the literature.[1] Briefly, 2.0 g of HA was dissolved in 100 mL of DI water at 4 ^oC, to which 1.6 mL of methacrylic anhydride (MA) was added drop-wise. The reaction solution was adjusted to pH 8-9 by the addition of 5 N NaOH and stirred at 4 ^oC for 24 h. The resulting polymer was obtained by precipitation in acetone. The product was re-dissolved in DI water and the solution was dialyzed against DI water for 2 days. *m*-HA was obtained by lyophilization (Yield: 86%). 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) to the peak at 1.99 ppm (*N*-acetyl glucosamine of HA). *m*-HA: ^1H 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).

Preparation of Cross-Linked Microneedle. The matrix of the microneedles (MNs) were fabricated by adding *m*-HA, the cross-linker *N, N*′-methylenebis (acrylamide) (MBA), and a

photo initiator (Irgacure2959, 0.05 wt %) into the mold and cross-linked through *in situ* polymerization upon exposure to UV light (365 nm at intensity of 9 mW/cm^2 for 30s).

Mechanical Strength Test. The mechanical strength of MNs was tested by pressing arrays of MNs against a stainless steel plate on an MTS 30G tensile testing machine. The initial gauge was set at 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 towards the MN-array patch was 0.1 mm/s. The failure force of MNs was recorded as the needle began to buckle.

***In Vitro* Insulin Release Studies.** Polymeric vesicles of 8 mg was added to PBS (1 mL) with different glucose concentrations (0, 100, or 400 mg/dL) and incubated at 37 °C on an orbital shaker to evaluate the release of insulin. At predetermined time points, 50 μL of the sample was taken out for analysis and 50 μL of fresh media was then added to the well to maintain a constant volume and placed back to the incubator. Insulin content in the withdrawn sample was determined by absorbance measurement at 595 nm and by virtue of the insulin standard curve. To access the responsiveness of PVs to changes in glucose levels, PVs were first incubated in 100 mg/dL glucose solution for 10 min then were separated by a centrifugal filter (100,000 Da molecular mass cutoff, Millipore), and then incubated in 400 mg/dL glucose for another 10 min. This cycle was repeated several times and the released insulin was measured.

The H_2O_2-responsive capability of vesicles were tested by adding 8 mg of polymeric vesicles to PBS (1 mL) with different H_2O_2 concentrations (0, 50, 200μm) and incubated at 37 °C on an orbital shaker to evaluate the release of insulin. The released insulin was tested as the above method.

Biocompatibility Analysis. The *in vitro* cytotoxicity of PVs was tested using MTT assay towards HeLa cells. Briefly, HeLa cells were seeded in 96-well plate at a density of 6000 cells per well. After 24 h incubation in 200 μL of Dulbecco's Modified Eagle Medium (DMEM) with 10 % fetal bovine growth serum (FBS), series dilutions of PVs ranging from 0.1 to 1 mg/mL were added into wells. After 24 h incubation, thiazolyl 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 absorbance of the plates at 570 nm, which is directly proportional to the viable cell number, was measured on multimode plate reader.

The *in vivo* biocompatibility of MN patch was evaluated using histological analysis. Briefly, blank HA MN, MN[PV(E+I)], and MN[I] were transcutaneously pierced into the back of the mice for 10 h. After 24 h, the 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 5 μm sections, and stained using hematoxylin and eosin (H&E) and fluorescent TUNEL staining 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.

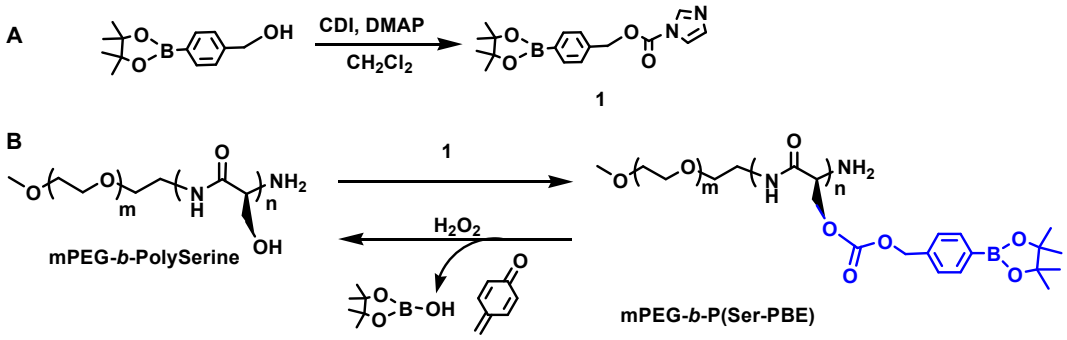

Figure S1. Synthetic route of mPEG-*b*-P(Ser-PBE).

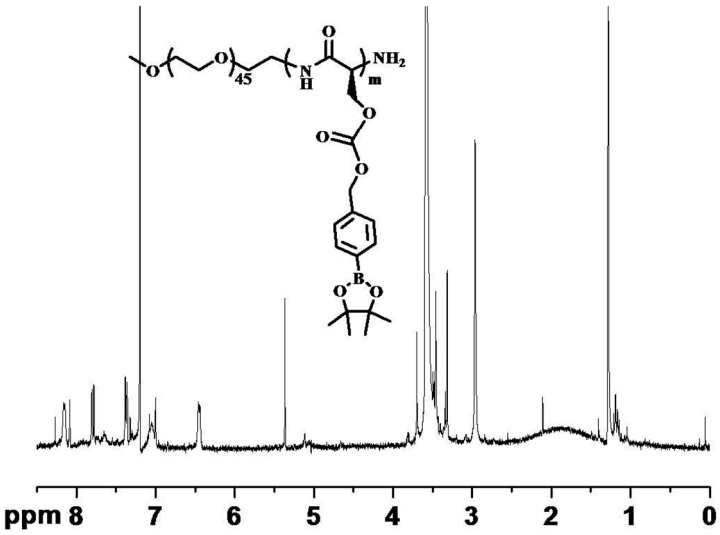

Figure S2. ^{1}H NMR spectra of mPEG-*b*-P(Ser-PBE)

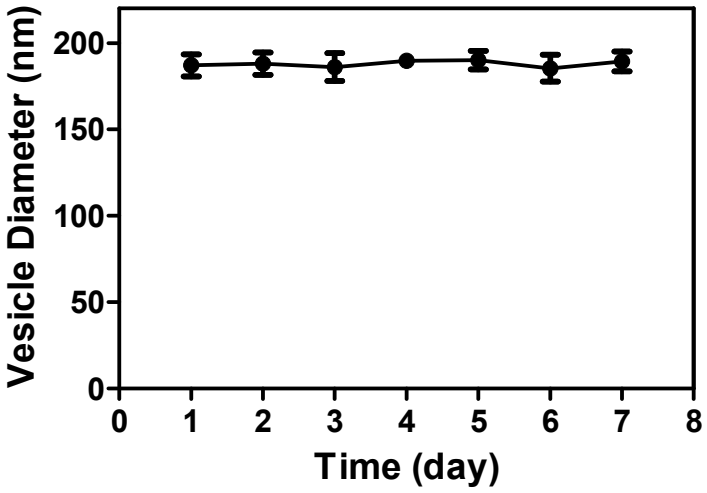

Figure S3. Stability of PVs in PBS at room temperature.

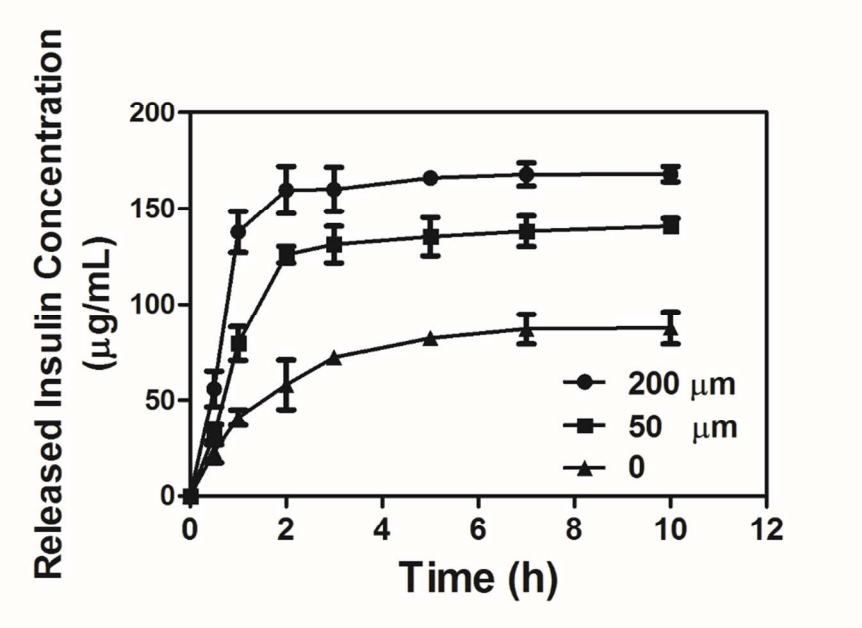

Figure S4. *In vitro* released insulin concentration from PVs(E+I) at different H_2O_2 concentrations at 37^{o}C;

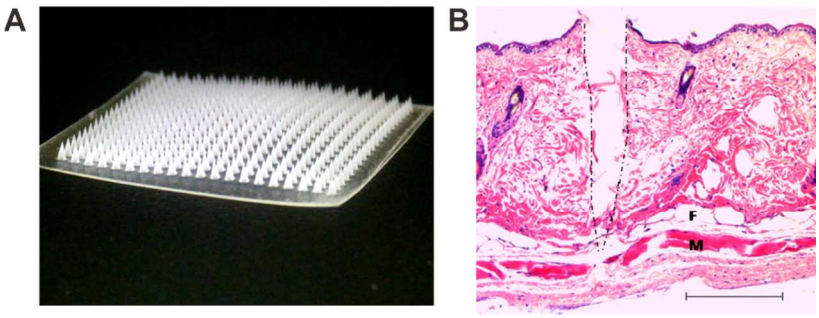

Figure S5. A: Photo of an MN patch; B: H&E-stained section of mouse skin penetrated by MN-array patch. The skin muscle and fat tissue regions are indicated by M and F, respectively. The region where MN patch insertion took place is indicated by the black dashed line.

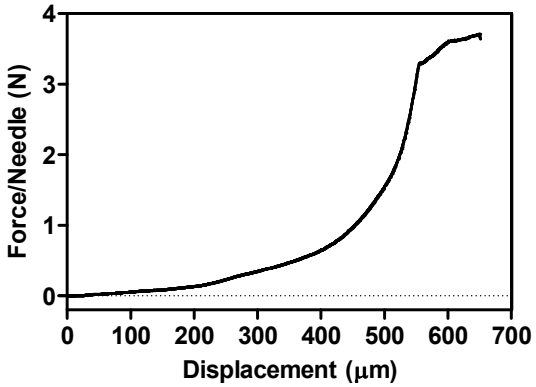

Figure S6. Mechanical behavior of a MN patch.

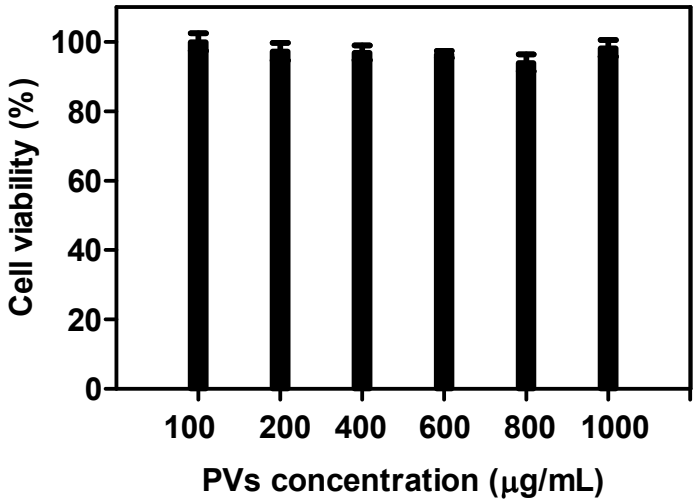

Figure S7. Cytotoxicity of empty PVs after 24 h of incubation with HeLa cells. Error bars indicate SD (n = 4).

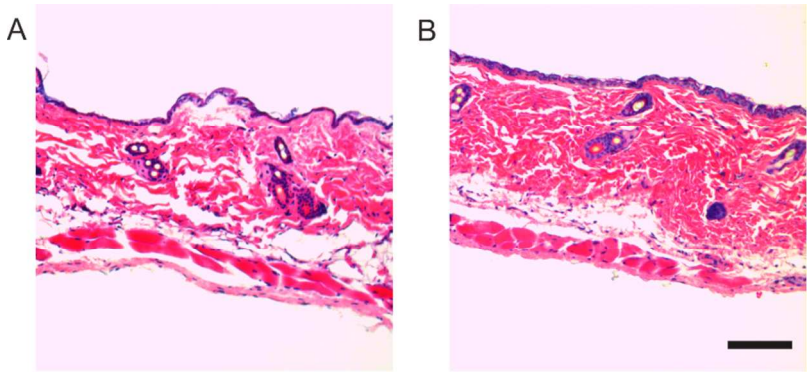

Figure S8. H&E-stained skin sections administered an MN patch (A) and surrounding tissues (B) 2 days post administration. (Scale bar: 100 μm).

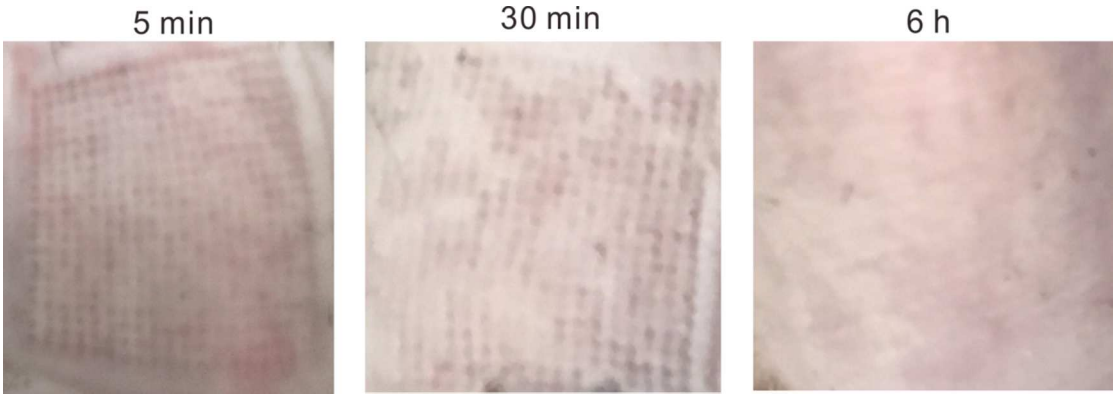

Figure S9. Skin puncture marks at 5 min, 30 min, and 6 h post treatment.

REFERENCE

(1) Wang, C.; Ye, Y.; Hochu, G. M.; Sadeghifar, H.; Gu, Z. Enhanced Cancer Immunotherapy by Microneedle Patch-Assisted Delivery of Anti-PD1 Antibody. *Nano Lett.* **2016**, *16*, 2334－2340.