Supporting Information

Microneedle Patches Loaded with Nanovesicles for Glucose Transporter-mediated Insulin Delivery

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Methods

Synthesis of Glu-Insulin. Glu-Insulin conjugate was synthesized in a two-step process. In brief, insulin was thiolated by reacting with Traut's Reagent (2-iminothiolane, Pierce) in PBS (pH=8.0) at a molar ratio of 1:5 for 2 h at 4 °C. Thereafter, excess Traut's reagent was removed using a centrifugal filter device (molecular weight cutoff MWCO =3 kDa) to purify the SH-insulin. D-(+)-Glucosamine was mixed with sulfosuccinimidyl-4-(*N*-maleimidomethyl)-cyclohexane-1-carboxylate (Sulfo-SMCC, Pierce) in PBS (pH=7.4) at a molar ratio of 1:1 for 2 h at room temperature. Finally, SMCC-activated glucosamine and insulin-SH were mixed in PBS (pH=8.0) at a molar ratio of insulin: glucosamine=1:10. After a 24 h reaction at 4 °C, the excess glucosamine was removed using a centrifugal filter device (MWCO =3 kDa). The obtained Glu-Insulin was stored at 4 °C until use.

GLUT1 expression and purification. The gene encoding GLUT1 from rat was amplified from the plasmid prGT3 (Addgene 15993)[1] and subcloned into the pET-28a vector (Novagen) with primers rGLUT1-s (5*'*-GCAAATGGGTCGCGGATCCATGGAGCCCAGCAGC-3*'*, BamHI underlined) and rGLUT-a (5*'*-CGAGTGCGGCCGCAAGCTTTCACACTTGGGAGTCAG-3*'*, Hind III underlined). The obtained pET28a-rGLUT1 was transformed into *Escherichia coli* Rosetta (DE3) pLysS cells for rGLUT1 expression. Briefly, a fresh E. coli colony was inoculated into 5 mL of LB medium (supplemented with 10 μg/mL kanamycin and 34 μg/mL chloromycetin) at 37 °C overnight. The cell culture was then diluted 100-fold with fresh LB medium and cultured for another 2-3 h (OD600 0.6-0.8). Afterward, 0.5 mM isopropyl β-D-1-thiogalactopyranoside (IPTG) was added to induce rGLUT1 expression at 20 °C for 8 h. The cells were collected by centrifugation at 4000 ×g for 15 min and resuspended in Buffer A (20 mM Tris-HCl pH 8.0, 0.15 M NaCl, 10 mM imidazole, 5% glycerol, 1 mM PMSF, 0.5 mg/mL

lysozyme, 0.4 mg/mL DNase I, and 2% DDM). The suspension was incubated at room temperature for 30 min on ice for another 30 min. The cells were then lysed by sonication, and cell debris was removed by centrifugation (20000×g, 10 min). The clear lysate was added to a column containing 1 mL Ni-NTA resin (Qiagen). After washing the column with Buffer B (20 mM Tris-HCl pH 8.0, 0.15 M NaCl, 25 mM imidazole, 5% glycerol, and 0.05% DDM), rGLUT1 was eluted with Buffer C (20 mM Tris-HCl pH 8.0, 0.15 M NaCl and 500 mM imidazole, 5% glycerol and 0.05% DDM). The purified rGLUT1 was quantified by the Bradford assay (Bio–Rad) and analyzed by SDS–PAGE.

Mechanical strength test. The mechanical strength of MNs was measured by pressing MNs against a stainless steel plate. 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 MNs was 0.1 mm/s. The failure force of MNs was recorded as the needle began to buckle.

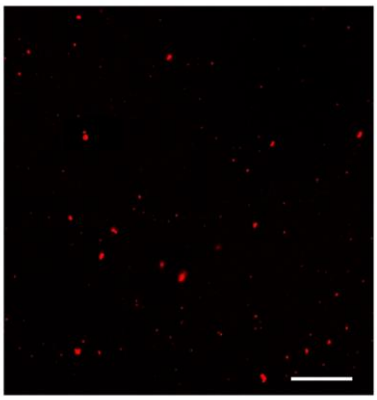
***In vitro* skin penetration test.** To evaluate the *in vitro* skin penetrating ability of MNs, the MNs were inserted into the skin of mice for 10 min. The skin was stained with trypan blue for 10 min before imaging by optical microscopy (Leica EZ4 D stereomicroscope).

Intraperitoneal glucose tolerance tests (IPGTTs). A glucose tolerance test was conducted to confirm the *in vivo* glucose responsiveness of MNs 1 h post-administration of RBC-insulin-MN or liposome(GLUT1)-insulin-MN and free insulin. Briefly, mice were fasted overnight and administrated with RBC-insulin-MN or liposome(GLUT1)-insulin-MN and free insulin, and then a glucose solution in PBS was intraperitoneally injected into all mice at a dose of 1.5 g/kg. The glucose levels were monitored over time after injection. The glucose tolerance test on healthy mice was used as the control.

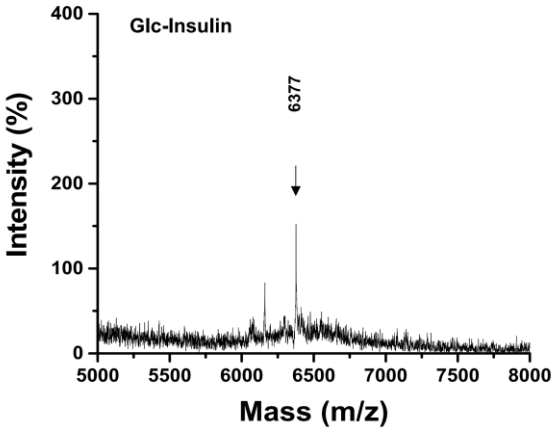
Reference

1. Birnbaum, M. J.; Haspel, H. C.; Rosen, O. M., Cloning and characterization of a cDNA encoding the rat brain glucose-transporter protein. *Proc. Natl. Acad. Sci.* **1986,** *83* (16), 5784-

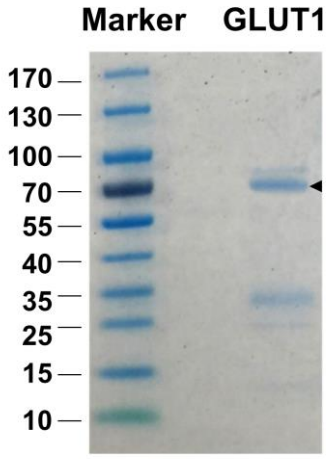
5788.



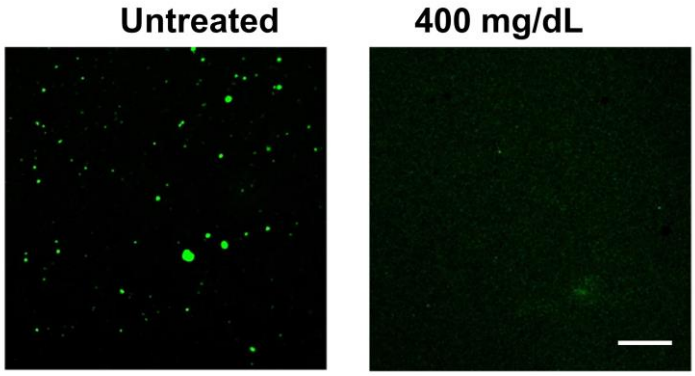
Supplementary Figure 1. Immunofluorescence imaging of GLUT4 in RBC vesicles indicated that GLUT4 was reserved in RBC vesicles (scale bar = 10 μm).



Supplementary Figure 2. Matrix-assisted laser desorption/ionization mass spectrometry (MALDI MS) assay of glucose-insulin conjugates. The molecular weight of Glc-Insulin conjugates was ~6377.



Supplementary Figure 3. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) analysis of purified GLUT1.



Supplementary Figure 4. Confocal microscopy images of Glu-Insulin-attached liposome-GLUT1 untreated or treated with 400 mg/dL glucose solution (scale bar = 10 μm).