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SUPPLEMENTARY INFORMATION

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Glucose-responsive insulin patch for the regulation of blood glucose in mice and minipigs

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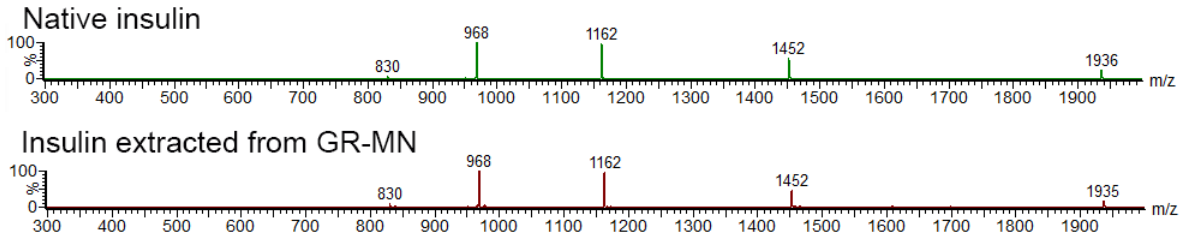
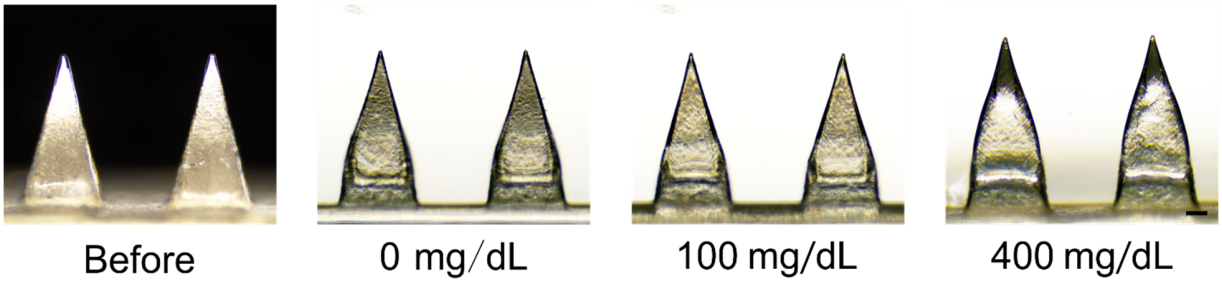
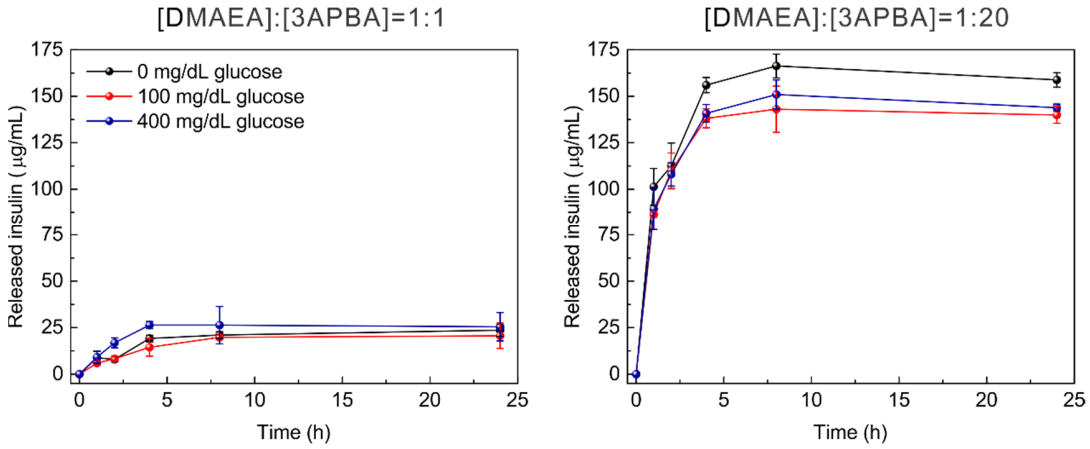


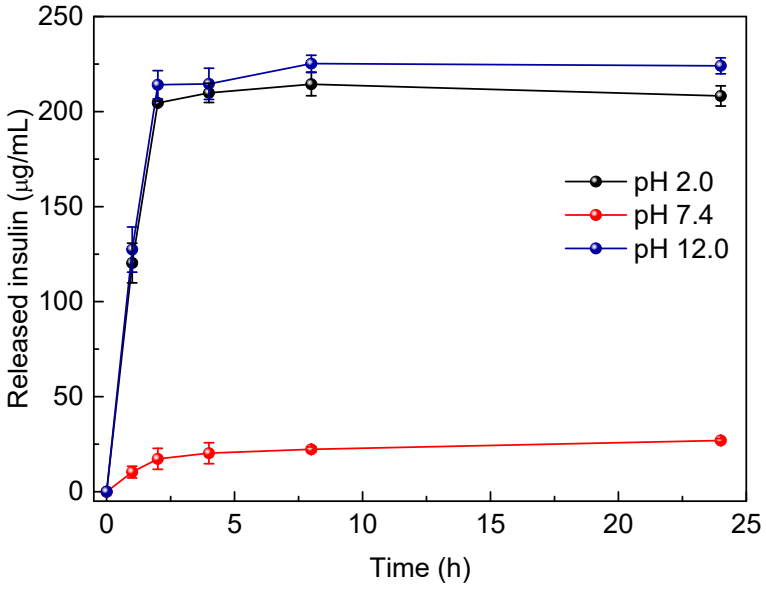
Supplementary Figure 1. Mass spectrum analysis of the native insulin and insulin extracted from GR-MN.



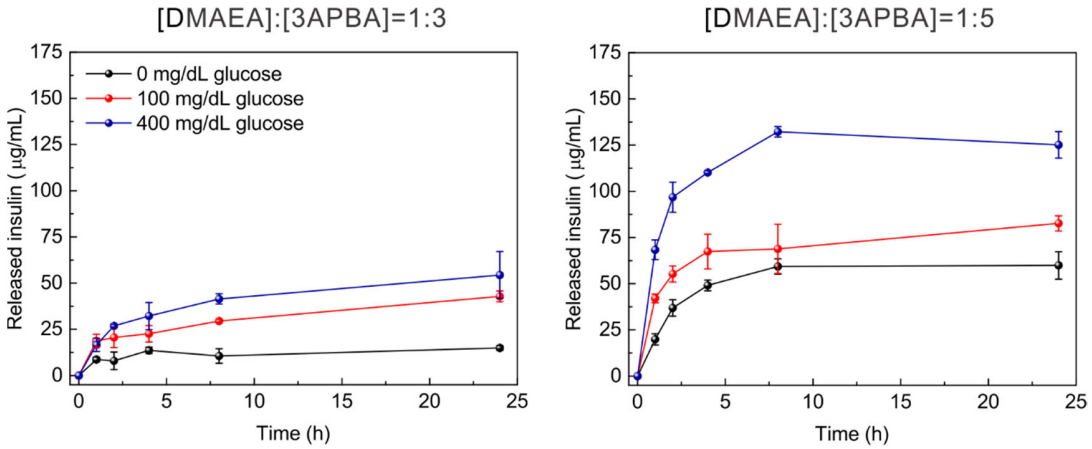
Supplementary Figure 2. Photographs of GR-MN with a 1:4 ratio of DMAEA to 3APBA pre-incubated and post-incubated in PBS with varying glucose concentrations for 1 h. Scale bar: 100 μm.



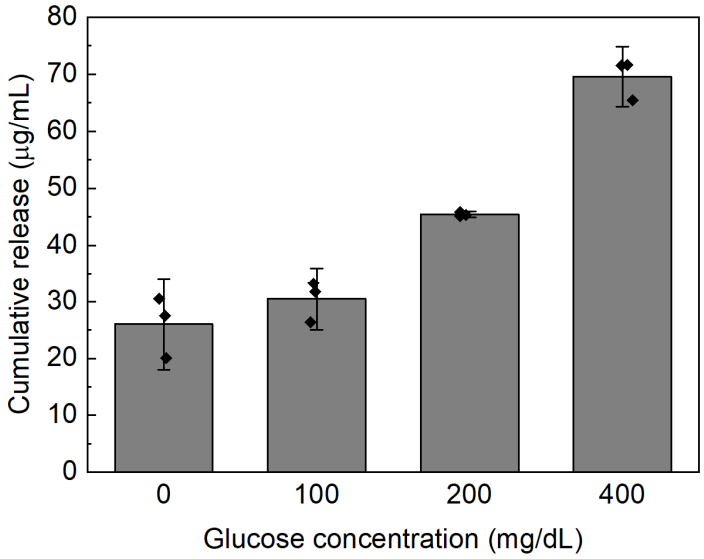
Supplementary Figure 3. *In vitro* accumulated insulin release from the polymeric matrix with a ratio of DMAEA to 3APBA at a 1:1 ratio (left) or a 1:20 ratio (right) across different glucose concentrations at 37 °C. Data are presented as mean ± s.d. (*n*=3).



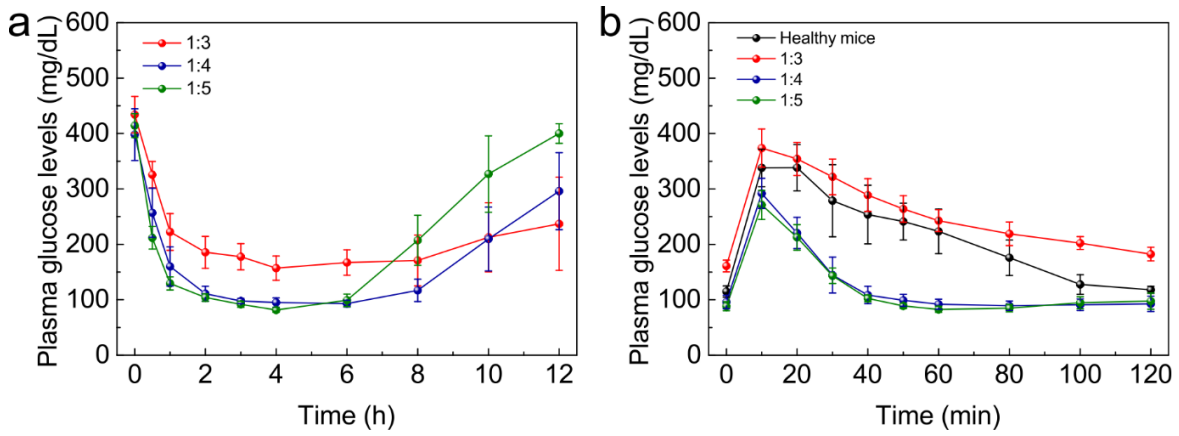
Supplementary Figure 4. Insulin release from the polymeric matrix with a 1:4 ratio of DMAEA to 3APBA under different pH values at 37 °C. Data are presented as mean ± s.d. (*n*=3).



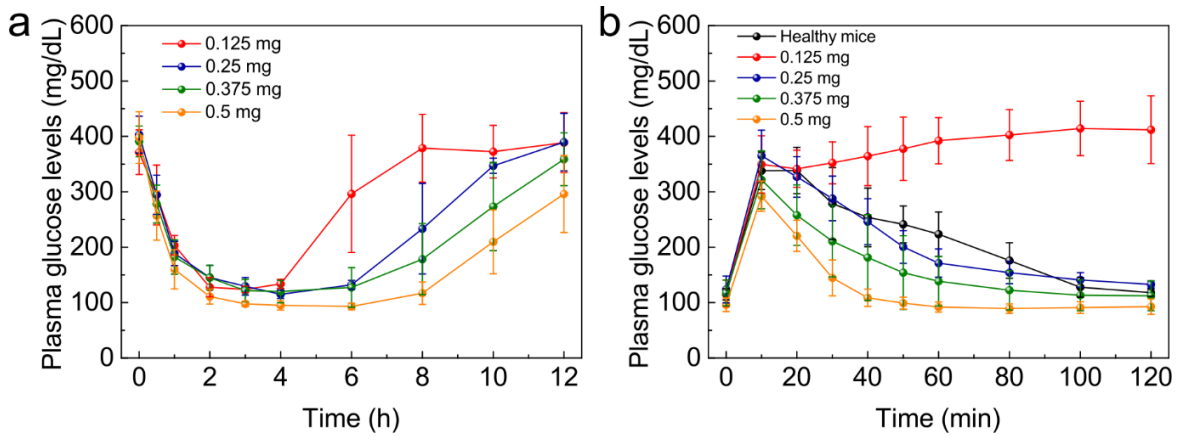
Supplementary Figure 5. *In vitro* accumulated insulin release from the polymeric matrix with a ratio of DMAEA to 3APBA at a 1:3 ratio (left) or a 1:5 ratio (right) across different glucose concentrations at 37 °C. Data are presented as mean ± s.d. (*n*=3).



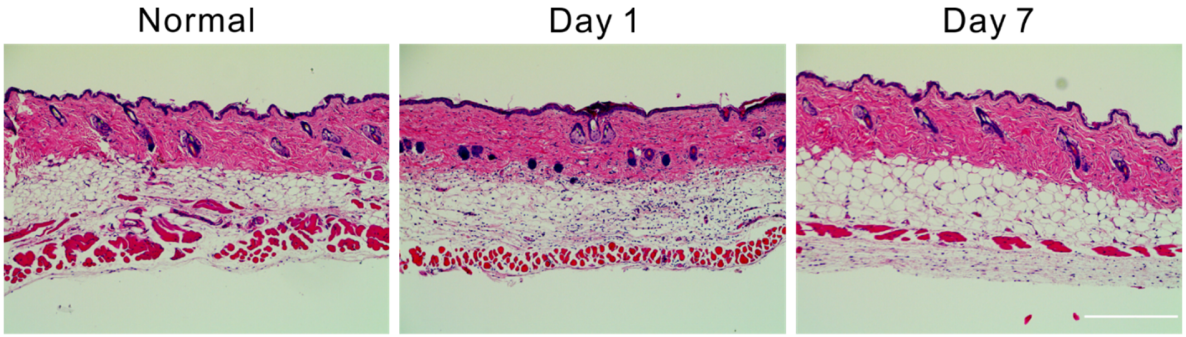
Supplementary Figure 6. Insulin release of the polymeric matrix with a 1:4 ratio of DMAEA to 3APBA as a function of glucose concentrations. Data are presented as mean ± s.d. (*n*=3).



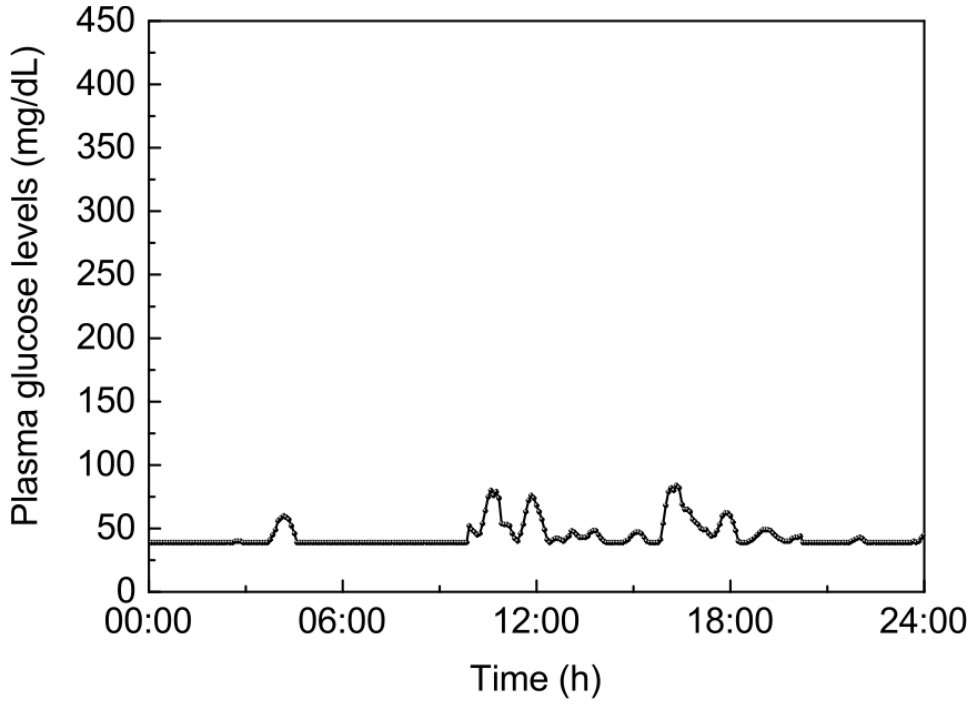
Supplementary Figure 7. (**a**) PGLs in STZ-induced diabetic mice after treatment with GR-MN with a ratio of DMAEA to 3APBA at 1:3, 1:4 or 1:5. Insulin dose: 0.5 mg. Data are presented as mean ± s.d. (*n*=5). (**b**) *In vivo* intraperitoneal glucose tolerance test in diabetic mice at 4 h post-administration of GR-MN with a ratio of DMAEA to 3APBA at 1:3, 1:4 or 1:5 in comparison with the healthy control mice. Glucose dose: 1.5 g/kg. Data are presented as mean ± s.d. (*n*=5).



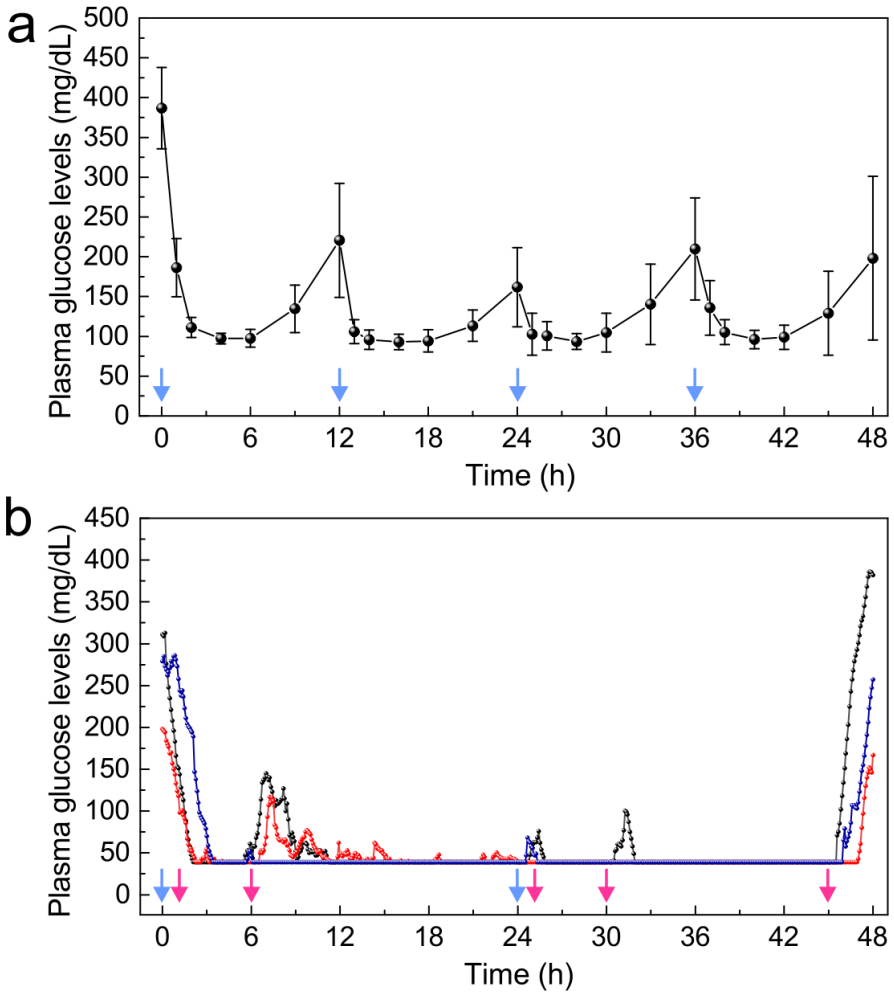
Supplementary Figure 8. (a) PGLs in the STZ-induced diabetic mice after treatment with GR-MN with different insulin doses. Data are presented as mean ± s.d. (*n*=5). (**b**) *In vivo* intraperitoneal glucose tolerance test in diabetic mice at 4 h post-administration of GR-MN with different insulin doses in comparison with healthy control mice. Glucose dose: 1.5 g/kg. Data are presented as mean ± s.d. (*n*=5).



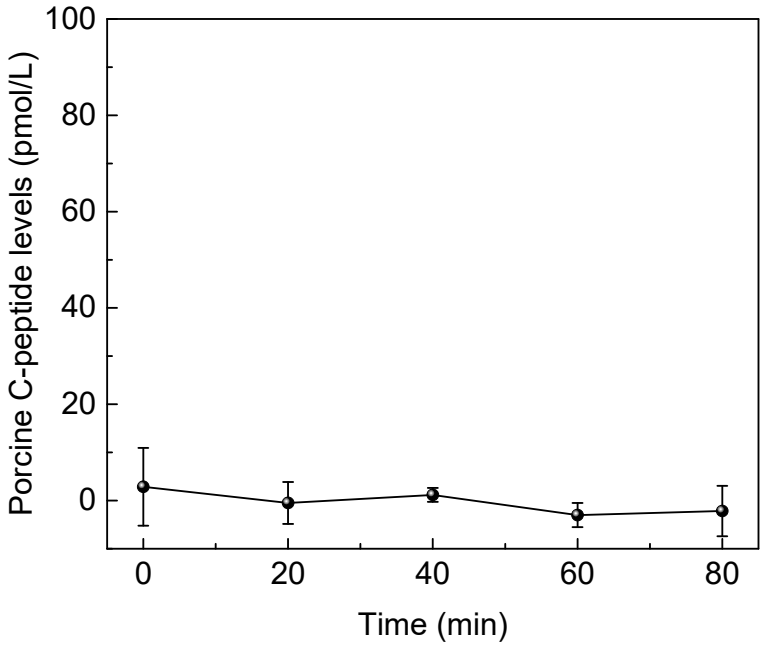
Supplementary Figure 9. Hematoxylin and eosin (H&E)-stained sections of mouse skin pre- and post-treatment of GR-MN. Scale bar: 400 μm.



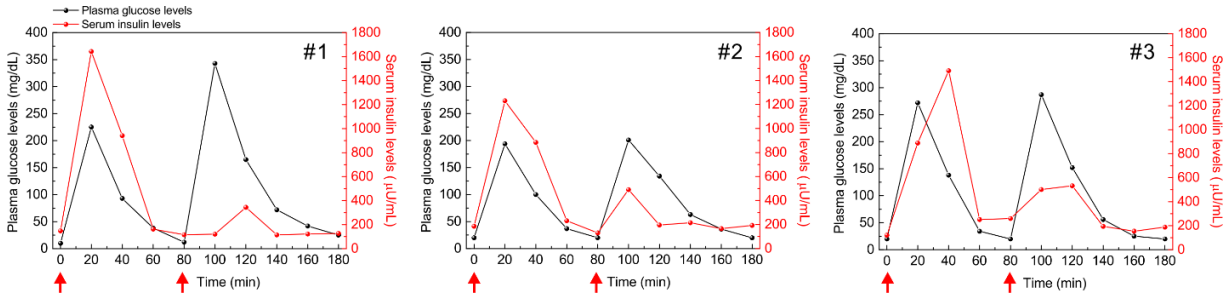
Supplementary Figure 10. The daily glucose pattern of the healthy Göttingen minipig recorded by calibrated CGMS.



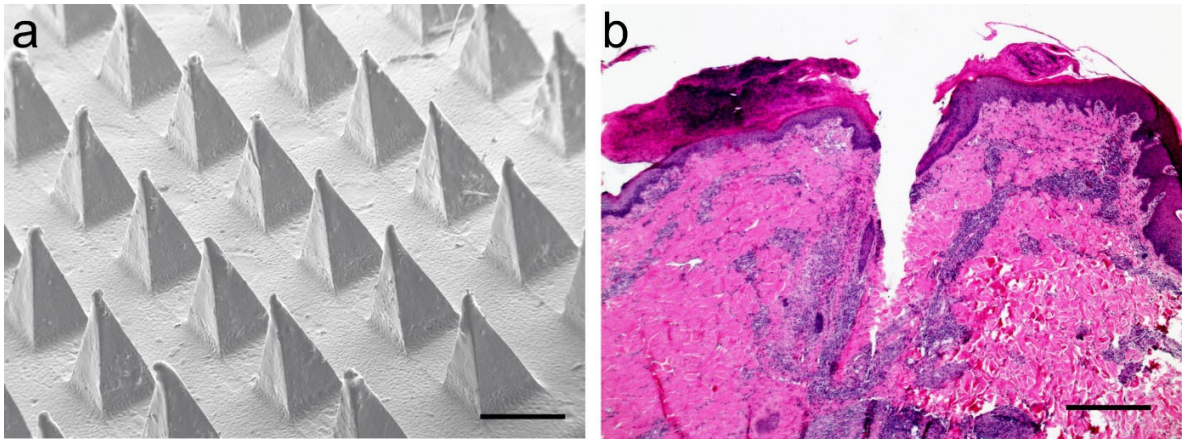
Supplementary Figure 11. (a) PGLs in STZ-induced diabetic mice during a 48-hour consecutive administration of GR-MN. Insulin dose: 0.5 mg. The blue arrows indicate the time points of MN administration. Data are presented as mean ± s.d. (*n*=5). **(b)** PGLs in three STZ-induced diabetic minipigs during a 48-hour consecutive administration of GR-MN. Insulin dose: 7 mg. The blue arrows indicate the time points of MN administration, and the pink arrows indicate the time points of feeding.



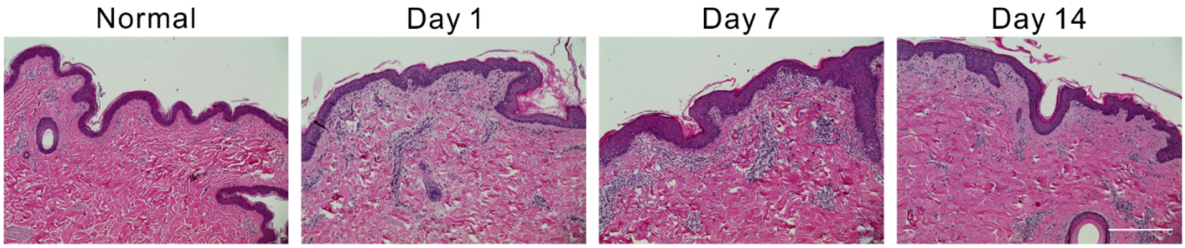
Supplementary Figure 12. Porcine C-peptide levels in diabetic minipigs after an intravenous glucose challenge at 4 h post-administration of GR-MN. Data are presented as mean ± s.d. (*n*=3).



Supplementary Figure 13. The *in vivo* glucose-responsive insulin release promoted by intravenous glucose challenge at 4 h post-administration of GR-MN for multiple rounds in three individual experiments. Glucose dose for each round: 0.7 g/kg. The red arrows indicate the time points of glucose administration.



Supplementary Figure 14. (a) Representative SEM image of the MN array after removal from the skin. Scale bar: 500 µm. **(b)** H&E-stained section indicates that insignificant fragments were found in the skin tissue after the removal of GR-MN. Scale bar: 200 µm.



Supplementary Figure 15. H&E-stained sections of minipig skin pre- and post-treatment of GR-MN. Scale bar: 400 µm.

Supplementary Table 1. Leachable monomers from the GR-MN patch with 20 × 20 array of microneedles.

Monomer	Leachable monomer amount (mg)	% per patch
NVP	0.082 ± 0.020	0.24 ± 0.05
DMAEA	<0.001	<0.003
3APBA	0.055 ± 0.010	0.16 ± 0.04
EGDMA	<0.020	<0.06

Supplementary Table 2. Pharmacokinetic parameters of insulin after administration of GR-MN (insulin dose: 0.5 mg), CR-MN (insulin dose: 0.5 mg), and subcutaneous injection (insulin dose: 0.05 mg) in diabetic mice. Data are presented as mean ± s.d. (*n*=5).

Group	$AUC_{0\rightarrow12}$ (μIU h^{-1} mL^{-1})	RBA (%)
GR-MN	3051.6 ± 429.9	11.6 ± 1.9
CR-MN	2966.2 ± 489.0	11.9 ± 1.6
SC insulin	2554.4 ± 376.1	100

$AUC_{0\rightarrow12}$, area under the plasma insulin concentration over time; RBA, relative bioavailability compared with subcutaneous injection.

Supplementary Table 3. The plasma glucose levels (PGLs) of diabetic pigs.

PGL (mg/dL) / Time (h)	GR-MN			CR-MN		
	1	**2**	**3**	**1**	**2**	**3**
3	35	37	38	36	72	35
6	38	121	37	213	154	39
9	49	51	48	296	241	172
12	35	37	36	271	299	181
24	155	307	367	253	252	364

Supplementary Table 4. Gradient HPLC method for NVP, 3APBA and EGDMA.

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Flow (mL/min)
0.00	95.0	5.0	0.3
4.50	70.0	30.0	0.3
5.50	70.0	30.0	0.3
8.00	50.0	50.0	0.3
8.10	5.0	95.0	0.3
9.00	5.0	95.0	0.3
9.10	95.0	5.0	0.3

A: water with 0.1% formic acid (v/v)

B: acetonitrile with 0.1% formic acid (v/v)

Injection volume was set to 10 μL.

Supplementary Table 5. Gradient HPLC method for DMAEA.

Time (min)	Mobile phase A (%)	Mobile phase B (%)	Mobile phase C (%)	Flow (mL/min)
0.00	0.0	20.0	80.0	1.0
2.00	0.0	20.0	80.0	1.0
12.00	25.0	20.0	55.0	1.0
15.00	25.0	20.0	55.0	1.0
15.10	0.0	20.0	80.0	1.0
22.00	0.0	20.0	80.0	1.0

A: acetonitrile

B: 50 mM perchloric acid

C: water

Injection volume was set to 10 μL.