

Supporting Information

Biodegradable phenylboronic acid-modified ϵ -polylysine for glucose-responsive
insulin delivery *via* transdermal microneedles

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Experimental

1 Insulin activity test

The activities of the native insulin and insulin released from the INS-loaded GRPs were studied. Herein, the sample of native insulin was freshly prepared under the concentration of 0.2 mg/mL in DI water. The sample of released insulin was the supernatant after the incubation of INS-loaded GRPs in glucose-containing PBS ($C_{\text{glucose}} = 4 \text{ g/L}$). These two samples were tested *via* a circular dichroism spectroscope (J-1500-150ST)^{1, 2}.

2 Compression test

The mechanical strength of the microneedles was pre-studied *via* the universal material testing machine (Zwick/Roell Z020) according to Xu's procedure³. Briefly, a piece of microneedle patch was placed between two parallel stainless steel plates and the microneedles were perpendicular to the plates. The initial distance between these two plates was set as 2.5 mm. The compression speed was set as 0.5 mm/min.

3 Trypan-blue-staining test

The skin penetration test was carried out according to the trypan blue stain assay⁴. Briefly, the cadaver skin was obtained from the SD rat and incubated in PBS at 37 °C for 1 hour. Then, a piece of microneedle patch was pressed on the skin by the human's thumb. The microneedle patch was removed after 30 seconds and the trypan blue stain solution was poured on the cadaver skin. The stain process lasted for 2 minutes.

4 Histological analysis

The skin section was prepared after the skin was treated by the microneedles.

56 The skin sections were stained according to the hematoxylin and eosin (H & E)
57 staining protocol and observed by the microscope⁴.

58 **5 Glucose-responsive insulin release of INS/GRP-12.8-containing microneedle** 59 **patches *in vitro***

60 The FITC-INS/GRP-12.8-containing microneedle patches were selected to
61 evaluate glucose-responsive insulin release *in vitro*. Each patch was incubated in PBS
62 of different glucose concentrations (0, 1 or 4 g/L) at 37 °C. The released amount of
63 FITC-INS for each sample was calculated according to the fluorescence intensity of
64 the incubation solution above.

65 **6 MTT assay**

66 The MTT assay followed the previous report¹. Briefly, the extract of CFPBA-g-
67 PL was obtained by incubating 100 mg CFPBA-g-PL in 10 mL MEM medium
68 (containing 10% fetal bovine serum, 100 IU/mL penicillin, 100 IU/mL streptomycin
69 and 2 mmol/L glutamine) at 37 °C for 24 hours. The concentration of the extract was
70 defined as 100%. Then the extract was diluted to the concentrations of 75%, 50% and
71 25%, respectively. L929 cells were then co-incubated with the extracts in a 96-well
72 plate at 37 °C for 24 hours followed by another 2-hour incubation with MTT at 37 °C.
73 Next, the liquid was removed and isopropanol was added in each well (100 µL/well).
74 Finally, the values of absorbance for the wells were given by a microplate reader at
75 570 nm (reference wavelength: 650 nm). The cell viability was calculated according
76 to the following equation. Here, A_s and A_b respectively represented the values of
77 absorbance for the extract-containing samples and the blank control.

$$Cell\ viability = \frac{A_s}{A_b} \times 100\%$$

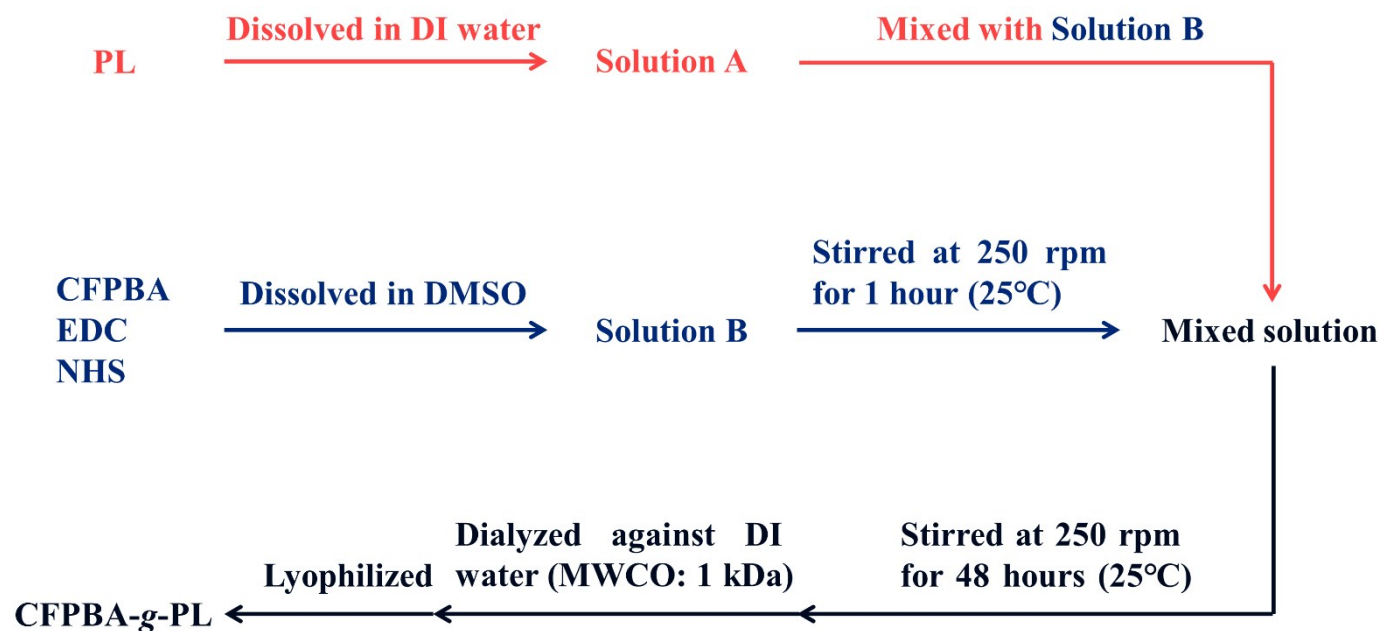
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79 7 Hemolysis test

80 The extract of CFPBA-g-PL was obtained by incubating 10 mg CFPBA-g-PL in
81 10 mL PBS ($C_{\text{glucose}} = 0, 1 \text{ or } 4 \text{ g/L}$, respectively) at 37 °C for 12 hours. The red blood
82 cell (RBC) dispersion (5%) was prepared according to the previous report⁵. Briefly,
83 the fresh rat blood was treated by the ACD anticoagulant and washed by PBS. The
84 RBCs were then collected *via* centrifugation (1500 rpm, 5 min) from the washed
85 sample above. Finally, the RBC dispersion (5%) in PBS was prepared.

86 The extract-containing groups, negative control and positive control were
87 included in the hemolysis test. Each extract-containing sample was prepared by
88 mixing 0.8 mL extract with 0.2 mL RBC dispersion. The final glucose concentration
89 was adjusted to the same level as that of the corresponding extract. The extract-
90 containing groups were named as “0 g/L”, “1 g/L” and “4 g/L”, respectively. The
91 samples of the negative controls included 0.8 mL PBS and 0.2 mL RBC dispersion,
92 while the positive controls adopted DI water instead of PBS under different glucose
93 concentrations. All the prepared samples were incubated at 37 °C for 1 hours
94 followed by centrifugation (1500 rpm, 5 min). The values of absorbance for all the
95 samples were given by a microplate reader at 545 nm. The hemolysis value for each
96 sample was calculated according to the following equation. Here, H_s , H_n and H_p
97 respectively represented the values of absorbance for the extract-containing samples,
98 negative control and positive control.

$$Hemolysis = \frac{H_s - H_n}{H_p - H_n} \times 100\%$$



101 Scheme S1. The synthetic procedure of CFPBA-g-PL.

102 Table S1. Details for the synthetic procedure of CFPBA-g-PLs.

Name	PL		CFPBA		EDC		NHS		DMSO	DI water	CFPBA/PL	Graft ratio
	mmol units	mg	mmol	mg	mmol	mg	mmol	mg	mL	mL	(mmol/mmol units) %	%
CFPBA-g-PL-52	1.17	148.0	0.70	128.7	0.70	141.6	0.70	83.2	20	1.5	60	52
CFPBA-g-PL-65	1.17	150.3	0.88	162.1	0.88	172.2	0.88	105.6	20	1.5	75	65
CFPBA-g-PL-79	1.17	149.1	1.05	194.2	1.05	209.0	1.05	121.7	20	1.5	90	79

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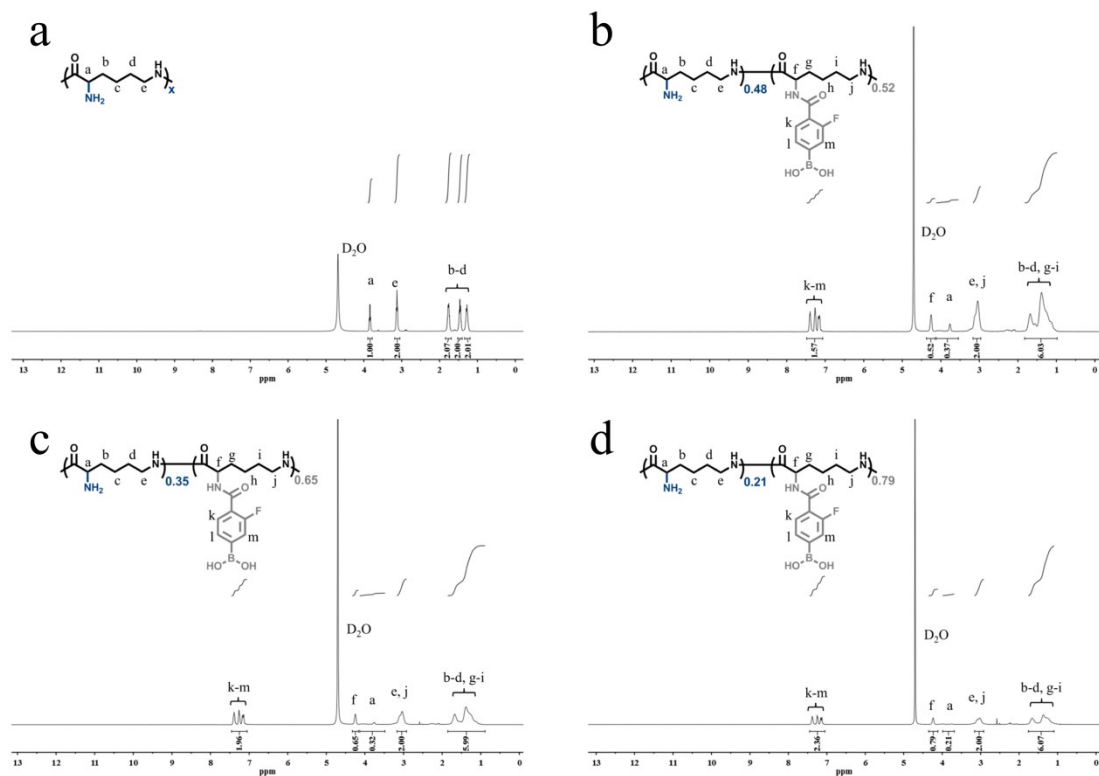


Fig S1. ¹H NMR spectra of PL (a), CFPBA-g-PL-52 (b), CFPBA-g-PL-65 (c) and CFPBA-g-PL-79 (d). The solvent of (a) was D₂O. The solvents of (b)-(d) were D₂O containing Na₂CO₃ (10 mg Na₂CO₃/mL D₂O).

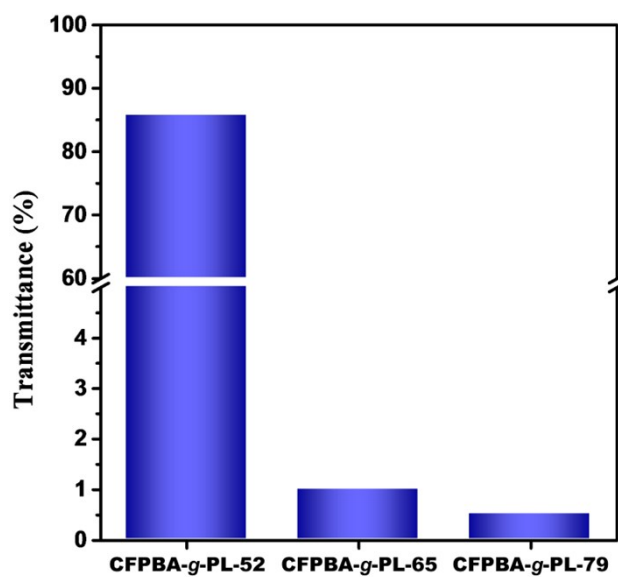


Fig S2. Transmittances of 1 mg/mL CFPBA-g-PL dispersions in water at the wavelength of 540 nm.

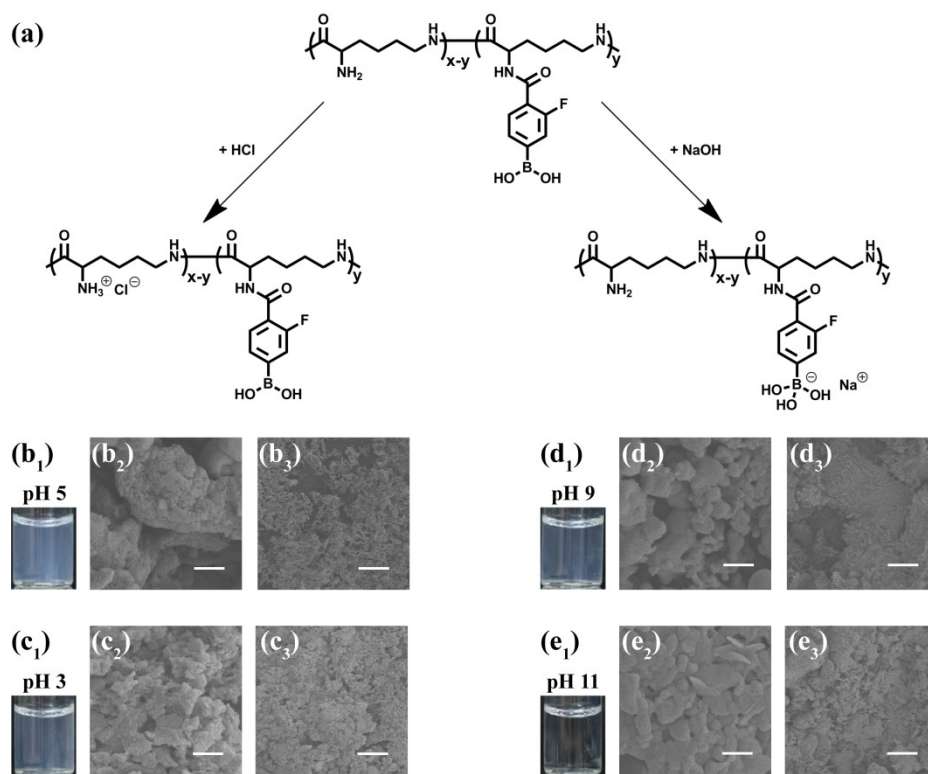


Fig S3. 1 mg/mL CFPBA-g-PL-65 in water of different pHs. (a) Different structures of CFPBA-g-PL-65 at the acidic condition and basic condition. The optical photos and SEM images for CFPBA-g-PL-65 at (b) pH 5, (c) pH 3, (d) pH 9 and (e) pH 11. The scale bars for (b₂), (c₂), (d₂) and (e₂) were 500 nm. The scale bars for (b₃), (c₃), (d₃) and (e₃) were 20 μ m.

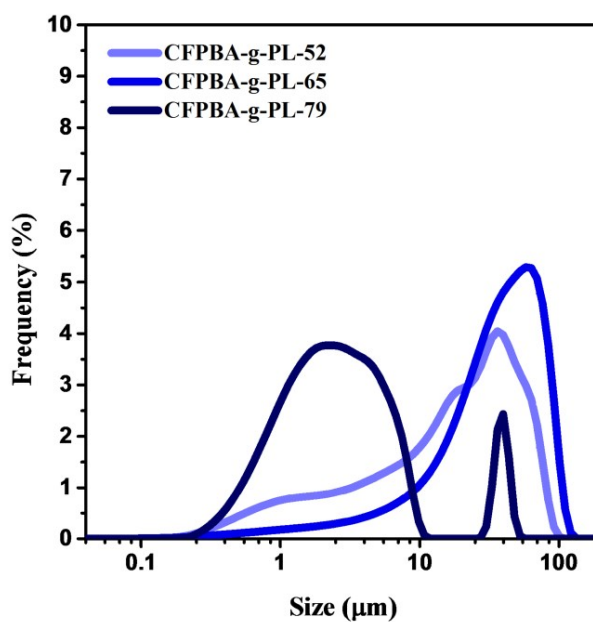
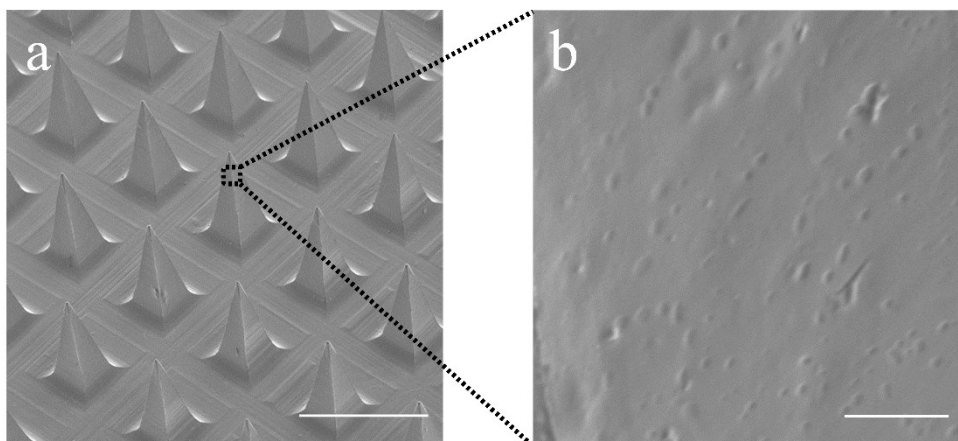
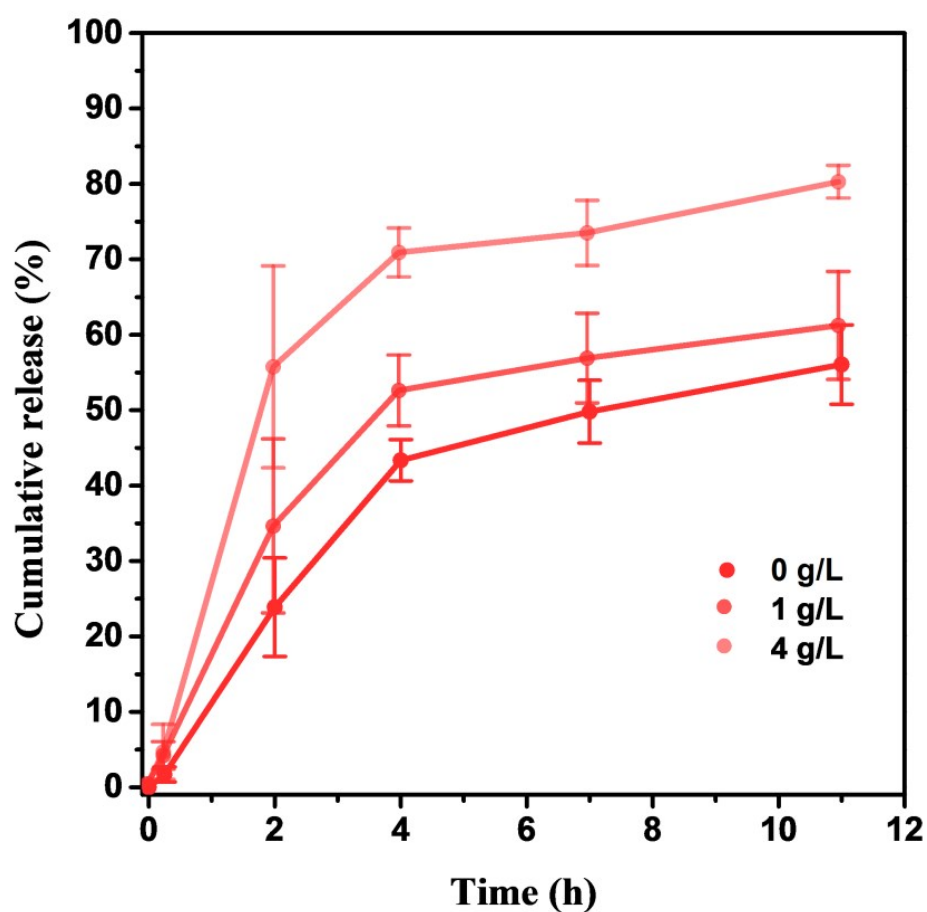


Fig S4. Size distributions of CFPBA-g-PLs in water.



115 Fig S5. The SEM images of the blank microneedle patch at (a) low magnification (scale bar:
116 500 μm) and (b) high magnification (scale bar: 10 μm).



117 Fig S6. Cumulative FITC-INS release profiles of the FITC-INS/GRP-12.8-containing
118 microneedle patches in PBS of different glucose concentrations (0, 1 or 4 g/L) at 37 $^{\circ}\text{C}$ (n=3).

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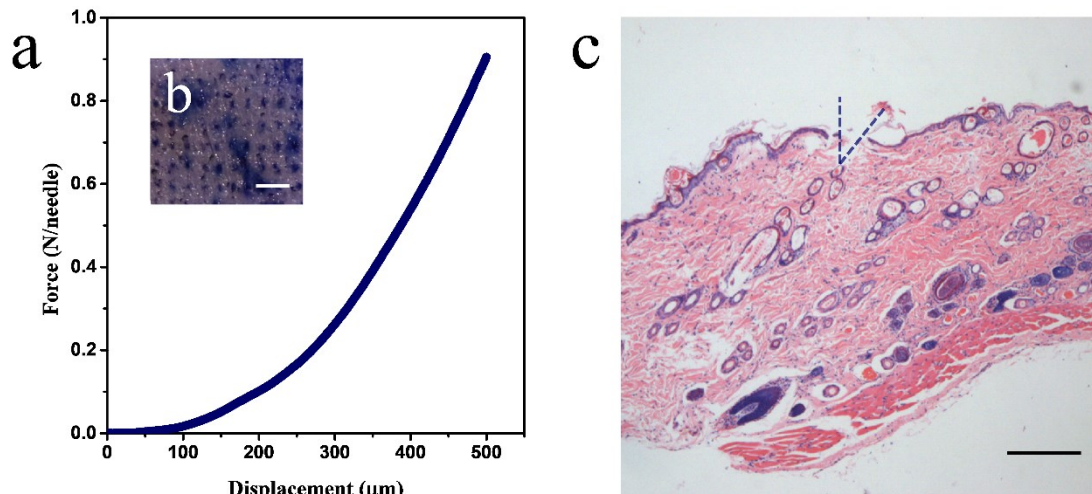


Fig S7. (a) The compression test of an INS/GRP-12.8-containing microneedle patch. (b) The trypan-blue-staining test of the skin treated by an INS/GRP-12.8-containing microneedle patch (scale bar: 1 mm). (c) Histological analysis of the skin treated by an INS/GRP-12.8-containing microneedle patch (scale bar: 250 μ m).

Table S2. The calculation results for the study of pharmacodynamics.

Group name	AAC (h·mg/dL)	Dose of insulin (μ g/kg)	RPA (%)
INS	3293.4 \pm 111.4	250	100.0 \pm 3.4
INS/GRP	3200.0 \pm 186.8	250	97.2 \pm 5.8
MNP	948.8 \pm 300.1	3000	2.4 \pm 0.8

Table S3. Details for the hemolysis test.

C_{glucose} (g/L)	Hemolysis (%)		
	Positive control	Negative control	CFPBA-g-PL-65
0	100.0 \pm 7.5	0.0 \pm 1.9	1.7 \pm 0.9
1	100.0 \pm 0.9	0.0 \pm 0.2	1.5 \pm 0.4
4	100.0 \pm 2.7	0.0 \pm 1.0	0.7 \pm 0.1

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