Supporting Information

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

Di Shen¹, Haojie Yu¹*, Li Wang¹, Xiang Chen¹, Jingyi Feng², Qian Zhang², Wei Xiong², Jin Pan², Yin Han³, Xiaowei Liu¹

¹ State Key Laboratory of Chemical Engineering, College of Chemical and Biological Engineering, Zhejiang University, Hangzhou 310027, P.R. China
² The First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou 310003, P.R. China
³ Zhejiang Institute of Medical Device Testing, Hangzhou 310018, P.R. China

* Correspondence to

E-mail: hjyu@zju.edu.cn (Haojie Yu)
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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 procedure$^3$. 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 assay$^4$. 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.
The skin sections were stained according to the hematoxylin and eosin (H & E) staining protocol and observed by the microscope.

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

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

6 MTT assay

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

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

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

The extract-containing groups, negative control and positive control were included in the hemolysis test. Each extract-containing sample was prepared by mixing 0.8 mL extract with 0.2 mL RBC dispersion. The final glucose concentration was adjusted to the same level as that of the corresponding extract. The extract-containing groups were named as “0 g/L”, “1 g/L” and “4 g/L”, respectively. The samples of the negative controls included 0.8 mL PBS and 0.2 mL RBC dispersion, while the positive controls adopted DI water instead of PBS under different glucose concentrations. All the prepared samples were incubated at 37 °C for 1 hours followed by centrifugation (1500 rpm, 5 min). The values of absorbance for all the samples were given by a microplate reader at 545 nm. The hemolysis value for each sample was calculated according to the following equation. Here, H\textsubscript{s}, H\textsubscript{n} and H\textsubscript{p} respectively represented the values of absorbance for the extract-containing samples, negative control and positive control.

\[
\text{Hemolysis} = \frac{H_s - H_n}{H_p - H_n} \times 100\%
\]
Scheme S1. The synthetic procedure of CFPBA-g-PL.

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

<table>
<thead>
<tr>
<th>Name</th>
<th>PL mmol units</th>
<th>CFPBA mg</th>
<th>EDC mmol</th>
<th>NHS mmol</th>
<th>DMSO mg</th>
<th>DI water mL</th>
<th>CFPBA/PL (mmol/mmol units)</th>
<th>Graft ratio %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFPBA-g-PL-52</td>
<td>1.17</td>
<td>148.0</td>
<td>0.70</td>
<td>128.7</td>
<td>0.70</td>
<td>141.6</td>
<td>0.70</td>
<td>60</td>
</tr>
<tr>
<td>CFPBA-g-PL-65</td>
<td>1.17</td>
<td>150.3</td>
<td>0.88</td>
<td>162.1</td>
<td>0.88</td>
<td>172.2</td>
<td>0.88</td>
<td>75</td>
</tr>
<tr>
<td>CFPBA-g-PL-79</td>
<td>1.17</td>
<td>149.1</td>
<td>1.05</td>
<td>194.2</td>
<td>1.05</td>
<td>209.0</td>
<td>1.05</td>
<td>90</td>
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</table>
Fig S1. $^1$H 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 D$_2$O. The solvents of (b)-(d) were D$_2$O containing Na$_2$CO$_3$ (10 mg Na$_2$CO$_3$/mL D$_2$O).

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 μm.

Fig S4. Size distributions of CFPBA-g-PLs in water.
Fig S5. The SEM images of the blank microneedle patch at (a) low magnification (scale bar: 500 μm) and (b) high magnification (scale bar: 10 μm).

Fig S6. Cumulative FITC-INS release profiles of the FITC-INS/GRP-12.8-containing microneedle patches in PBS of different glucose concentrations (0, 1 or 4 g/L) at 37 °C (n=3).
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 μm).

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

<table>
<thead>
<tr>
<th>Group name</th>
<th>AAC (h·mg/dL)</th>
<th>Dose of insulin (μg/kg)</th>
<th>RPA (%)</th>
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<tr>
<td>INS</td>
<td>3293.4±111.4</td>
<td>250</td>
<td>100.0±3.4</td>
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<tr>
<td>INS/GRP</td>
<td>3200.0±186.8</td>
<td>250</td>
<td>97.2±5.8</td>
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<tr>
<td>MNP</td>
<td>948.8±300.1</td>
<td>3000</td>
<td>2.4±0.8</td>
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Table S3. Details for the hemolysis test.

<table>
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<tr>
<th>C_{glucose} (g/L)</th>
<th>Hemolysis (%)</th>
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<tr>
<td></td>
<td>Positive control</td>
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<tr>
<td>0</td>
<td>100.0±7.5</td>
</tr>
<tr>
<td>1</td>
<td>100.0±0.9</td>
</tr>
<tr>
<td>4</td>
<td>100.0±2.7</td>
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References


