Supporting Information

for Adv. Sci., DOI 10.1002/advs.202203274

Shrinking Fabrication of a Glucose-Responsive Glucagon Microneedle Patch

Zejun Wang, Ruxing Fu, Xiao Han, Di Wen, Yifan Wu, Song Li and Zhen Gu*
Supporting Information

**Shrinking fabrication of a glucose-responsive glucagon microneedle patch**

Zejun Wang, Ruxing Fu, Xiao Han, Di Wen, Yifan Wu, Song Li, Zhen Gu*
Figure S1. PDMS replicating mold (upper panel) of (A) 1.5 cone (6×6, 1.5 mm width × 2.7 mm height), (B) 1.5 pyramid (6×6, 1.5 mm width × 3.75 mm height), and (C) 2 cone (5×5, 2.0 mm width × 3.5 mm height) MN patch generated from the 3D printed MN masters (lower panel).
**Figure S2.** Glucose-dependent swelling evaluation (A) and representative images (B) of the 1.5 cone MN tips post-incubated in PBS supplemented with varying glucose concentrations for 3 h. Data are presented as means ± SD ($n = 5$). Scale bar, 300 µm.
**Figure S3.** The amount of glucagon leached from the GRS glucagon MN patch during different washing time lengths. Data are presented as means ± SD (n = 3).
**Figure S4.** Hematoxylin and eosin (H&E) staining results. GRS glucagon MN patch with or without wash was applied on mice skin for 24 h, respectively. The treated parts of the skin were harvested 3 and 7 days post MN removal. Scale bar, 250 μm.
Figure S5. Representative image of the GRS glucagon MN patch placed on the mouse dorsum skin.
**Figure S6.** Hypoglycemia index of the insulin challenged diabetic mice with or without GRS glucagon MN patch treatment. Data are presented as means ± SD ($n = 5$).
Figure S7. The mass spectrum analysis of the (A) native glucagon and (B) glucagon released from the GRS glucagon MN patch.
Figure S8. Hyperglycemic effects of the non-treated (control) group and groups treated with native glucagon PBS solution after 2 or 4-week storage at 4 °C. PGLs of the hypoglycemia mice were monitored before and 4 hours after the subcutaneous injection, respectively. Data are presented as means ± SD (n = 5).
Supplementary Table 1. Gradient HPLC method for acrylamide, APBA, and MAETAC.

| Time (min) | Mobile phase A (%) | Mobile phase B (%) | Flow (mL/min) |
|-----------|--------------------|--------------------|--------------|
| 0.00      | 95.0               | 5.0                | 1            |
| 4.50      | 70.0               | 30.0               | 1            |
| 5.50      | 70.0               | 30.0               | 1            |
| 8.00      | 50.0               | 50.0               | 1            |
| 8.10      | 5.0                | 95.0               | 1            |
| 9.00      | 5.0                | 95.0               | 1            |
| 9.10      | 95.0               | 5.0                | 1            |
| 10.00     | 95.0               | 5.0                | 1            |

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

B: Acetonitrile with 0.1% formic acid (v/v)
**Supplementary Table 2.** Leachable monomers from the 6×6 GRS glucagon MN patch without the 1-hour washing procedure.

| Monomer  | Leachable amount (µg) | wt% per patch |
|----------|-----------------------|---------------|
| Acrylamide | 703.88 ± 18.29        | 7.38 ± 0.19   |
| APBA     | 168.66 ± 19.37        | 1.77 ± 0.20   |
| MAETAC   | 45.24 ± 5.82          | 0.47 ± 0.06   |
Supplementary Table 3. Leachable monomers from the 6×6 GRS glucagon MN patch with the 1-hour washing procedure.

| Monomer | Leachable amount (µg) | wt% per patch |
|---------|-----------------------|---------------|
| Acrylamide | 44.17 ± 0.82 | 0.463 ± 0.009 |
| APBA | 4.17 ± 0.41 | 0.044 ± 0.004 |
| MAETAC | 1.46 ± 0.02 | <0.015 |