Electronic Supplementary Information (ESI)

Near-infrared light triggered and separable microneedles for transdermal delivery of metformin on diabetic rats

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Experimental Section

Synthesis and characterization of PB NPs

The PB NPs was synthesized according to a previously published method [1]. In detail, 132 mg $K_3[Fe(CN)_6]\cdot 3H_2O$ (99%, Alladin) and 3 g polyvinylpyrrolidone (PVP, 58 kDa, Alladin) were dissolved in 40 mL 0.01 M HCl solution by stirring for 30 min at room temperature. The formed yellow solution was loaded in sealed vials and treated with a 20 h bake in an 80 °C oven. The resultant products were centrifuged and washed with distilled water for several times to remove the PVP. Finally, the obtained solution was freeze-dried to collect PB NPs powdery. XRD (ARL XTRA, Thermo ARL, Switzerland) and SEAD was used to confirm the preparation of PB NPs. The particle size distribution, absorption spectra and morphology of PB NPs were characterized by DLS (Nano-ZSZEN3600, Marvin, UK), UV−vis absorption spectrometry (U3900H, Hitachi, Japan), TEM (JEM-2100, JEOL, Japan) and SEM (Vltra55, Zeiss, German) (Fig. S1).

Preparation of MNs patches

Polymethyl methacrylate (PMMA) MNs templates were purchased from Micropoint Technologies Pte Ltd. The templates consisted of 100 (10*10) pyramids-shaped tiny needles. Each tiny needle tapered from a square base of ~300 μm to a tip which measured ~10 μm in width. Both the height and pitch of needles are ~600 μm. PDMS female molds were inverse-replicated from templates according to a published literature.[2] PDMS solution (Sylgard 184, Dow Corning) was casted over templates and then cured at 80 ^{0}C for 1 h. At last, they were peeled off from templates.

The PDMS female molds were used to fabricate MNs in two steps, as shown in Fig.S2. In the first step, 10% (w/v) PCL solution was prepared by dissolving 1 g PCL in 5 mL dichloromethane (DCM) and 5mL methanol (ME), then PB NPs and metformin hydrochloride (97%, Alladin) were added into PCL solution stirring for 20 min to form a homogeneous PB NPs/PCL/metformin solution. After using a qualitative filter paper (pore diameter: 80-120 μm) covered on surface of the PDMS mold, 150 μL PB NPs/PCL/metformin solution was cast on the filter paper by a pipette. Then the mold was centrifuged at 12000 rpm for 15 min at 10 ^{0}C to push the solution into caves. At

last, the filter paper was removed and the mold was placed in the ambient environment to form drug-loaded needle tips. In the second step, a 50% (w/v) PVP (130 kDa, Alladin) solution and a 50% (w/v) PVA (1788, Alladin) solution were mixed at a volume ratio of 1:1. Half a milliliter of the mixture was filled in the needle tips loaded mold with another centrifugation at 8000 rpm for 5 min to form a supporting substrate and before this step the PDMS mold with needle tips loaded was baked in a 60^{o}C oven for 5 min to remove the residual DCM and ME solvents. The filled mold was desiccated in the room temperature overnight. The obtained MNs were then peeled from PDMS molds and characterized by SEM and macroscopic bright-field images.

To facilitate the measurement of metformin remain in the MNs in ex vivo drug release, an inseparable MNs system that can be extracted from puncture sites was prepared. The MNs used a PCL substrate to replace the PVA/PVP water soluble substrate. In detail, after finishing the first step of MNs fabrication, 0.1 g PCL pellets were placed on the surface of the tip loaded mold and the mold was then put into oven at 60 ^{0}C for 5 min. After the PCL melt, the mold was centrifuged at 6000 rpm for 3 min at 10 ^{0}C. The fabricated MNs can be peeled off from the mold immediately.

MNs with Rhodanmine 6G (R6G) included in the needle tips were prepared for skin penetration studies. To fabricate MNs, R6G at a loading of 1 mg/mL was added into the PB NPs/PCL/metformin solution in the first step of MNs fabrication. An inseparable MNs system with R6G loaded was prepared for observing the melting behavior of needle tips under 0, 1, 3 cycles of NIR irradiation after extracting from the puncture sites.

NIR triggered melting behavior of MNs

To determine the best addition of PB NPs in the MNs fabrication, MNs made from four concentrations (0, 0.1, 1, 4 mg/mL) of PB NPs in PB NPs/PCL/metformin solution (The weight proportion of PB NPs in the needle tips account for 0, 0.1%, 1%, 4%) were evaluated under a NIR light (808 nm, 1600 mW, 0.4 W/cm^2) continuously to record the temperature and observe the melting behavior. For the MNs made from 0.1 wt% PB NPs in needle tips, 5 cycles of NIR irradiation were applied to evaluate the repeated NIR triggered heating stability. During each irradiation cycle, the NIR light was applied

on the MNs for 40 s to heat the needle tips and switched off for 30 s to lower the temperature. The temperature and NIR thermal images were recorded by a NIR thermal imager (Ti400, Fluke, America). The brightfield macroscopic images and video were recorded by a macroscope (BST500xUSB, BAISITE, China).

Ex vivo studies on skin samples

To evaluate the skin penetration ability, Rhodanmine 6G loaded MNs were applied on the rat skin samples for 30 s, then the samples were used for histological sections analysis. All skin samples were cleaned using 75% alcohol before use.

In the experiments of NIR triggered melting properties of R6G loaded MNs ex vivo, the prepared separable and inseparable MNs were applied on the rat skin samples respectively with NIR irradiation for 0, 1, 3 cycles. During each cycle, the NIR laser was applied on the MNs for 1 min and switched off for 30 s. Following the inseparable MNs extracted from the puncture sites were imaged by a macroscope to observe the melting behavior of needle tips. And the skin samples after separable MNs application were characterized under a confocal laser scanning microscope (CLSM) to reconstruct the 3D images of R6G diffusion and then used for histological sections analysis to observe the melting behavior of needle tips in the skin.

To evaluate the ex vivo NIR triggered release of metformin, inseparable MNs with 20 mg metformin loaded were applied on the skin samples, the release content was calculated by subtracting the content of residual metformin in the MNs from initial loading content in the MNs. These samples were then exposed to NIR irradiation in a short period (1 min) or a long period (2 min), after NIR irradiation, the NIR light was switched off for 1.5 h. The NIR irradiation cycles were applied in succession. Each time before and after NIR irradiation, the MNs were extracted from the puncture sites and the needles tips were cut off and dissolved in 1 mL DCM/ME solution. The solutions were analyzed by a UV-Vis spectrophotometer (U3900H, Hitachi, Japan) to determine the content of metformin according to the standard calibration curve (Figure. S3).

In vivo NIR triggered release of metformin on diabetic rats

The experiments on animals were carried out according to the guide for the care and use of laboratory animals, provided by Experimental Animal Center of Zhejiang

Academy of Medical Sciences, China. The procedure was approved by the Animal Ethics Committee of Zhejiang SciTech University. SD rats, 200 ± 20 g, were used in this experiment. The type 2 diabetic animal model induced by streptozotocin(STZ) was built according to a published literature.[3] The rats were divided into 5 groups: 1) Control group, diabetic rats applied with blank MNs under four cycles of long period NIR irradiation, 2) Healthy group, healthy rats with no treatment, 3) Injection group, diabetic rats treated with intraperitoneal injection of 20 mg metformin, 4) MNs group, diabetic rats applied with 10 mg metformin loaded MNs under four cycles of long period NIR irradiation, and 5) MNs group, diabetic rats applied with 20 mg metformin loaded MNs under four cycles of long period NIR irradiation. The blood glucose levels (BGLs) were monitored every 0.5 h for 6 h using a glucose meter (Sinocare Inc., Changsha, China).

3.2. MTT analysis

The in vitro cell proliferation analysis was measured using a colorimetric methyl thiazolyl tetrazolium (MTT) assay in Hela cells. For the MTT assay, the MNs patches were autoclaved and were incubated with complete medium at 37^{o}C for 24 h with an extraction ratio of 6 cm^2/mL. Hela cells were seeded in a 96-well plate at a density of 8000 cells per well and cultivated in 200 μL of DMEM. The wells are grouped according to the difference in time (0, 3, 6, 9, 12, 24 and 48h, respectively) of extraction solution added, each group including three wells. The plates were incubated at 37 °C in a CO^2 (5%) incubator for 24 h before addition of extraction solution. After incubation for 48 h, 20 μL 3- (4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium solution (MTT solution, 5 mg/mL) was added. The plates were incubated for an additional 4 h. After that, the culture media was removed with caution and then followed by the additional of 150 μL dimethyl sulfoxide (DMSO). The absorbance of the plates was read at a reference wavelength of 490 nm using a microplate reader (Multiskan MK3, Thermo Electron Corporation) within 10 min.

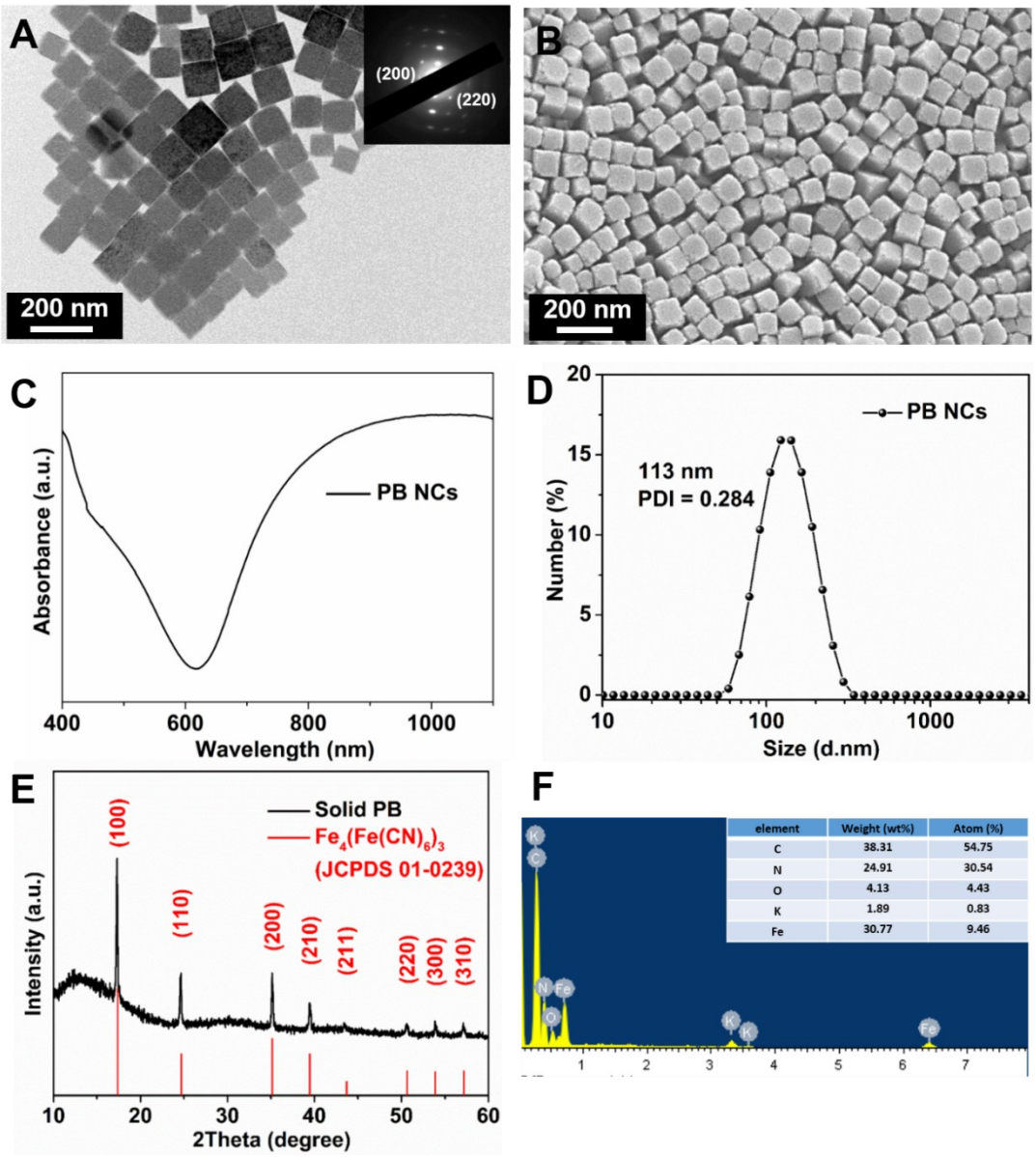

Fig. S1 TEM image of as-prepared PB NPs (A, inset shows the SEAD pattern). SEM image of as-prepared PB NPs (B). UV-Vis spectroscopy of PB NPs shows a strong absorption in the NIR region (C). Dynamic light scattering result of PB NPs dispersed in water (D). X-ray diffraction (E) and EDS (F) of PB NPs.

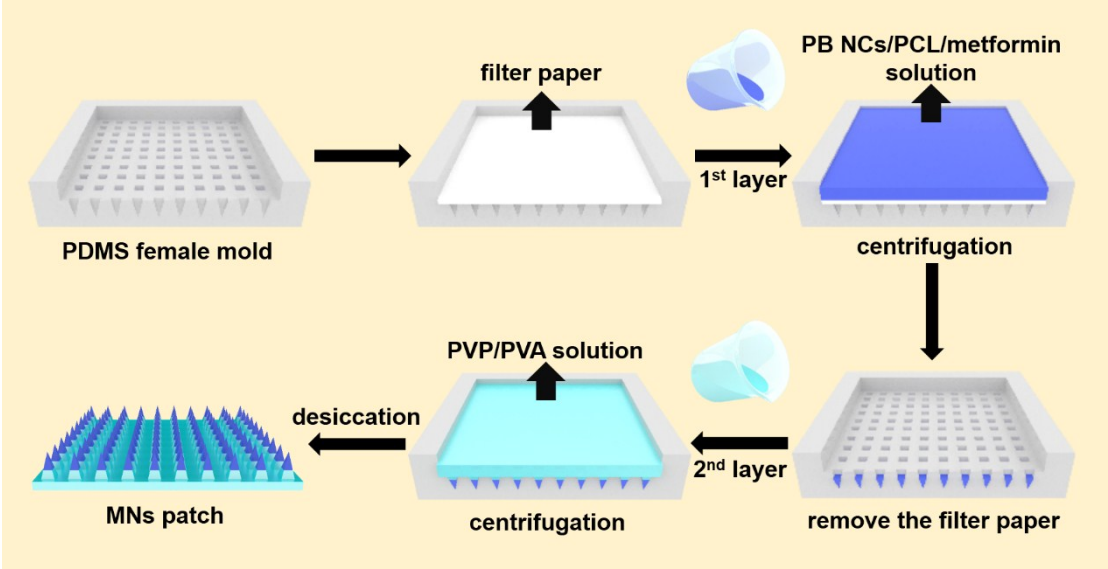

Fig. S2 Schematic illustrations of the process to fabricate separable MNs.

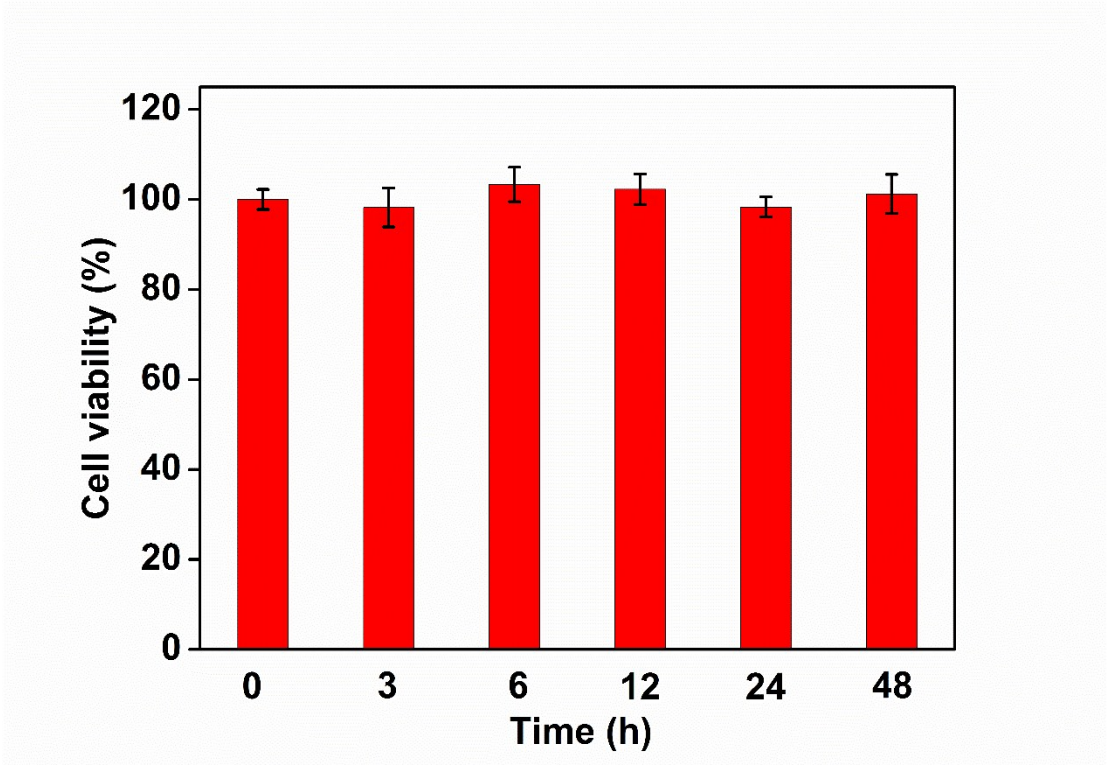

Fig. S3 Viability of Hela cells against MNs patches with varied culture time by MTT assay, data are presented as average ± standard deviation (n = 3).

References:

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