Supporting information

Hyalase-mediated Cascade Degradation of Matrix Barrier and Immune Cell Penetration by Photothermal Microneedle for Efficient Anticancer Therapy

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Fabrication of NPs.

PCPDTBT (0.25 mg) and F127 (50.0 mg) or F127-NH_2 (50.0 mg) were dissolved in 0.5 mL of THF respectively, then the fully mixed solution was dropped into a 10.0 mL 10% THF solution (water and THF, v/v = 9:1) under ultrasonic for 2 min. Then the THF was removed by a nitrogen flow and filtered products through a 0.22 μm PVDF filter. Concentrated the SPN or SPN-NH_2 through ultrafiltration (50 KD, 3500 rpm, 25 min) and the concentration was determined by a UV-vis spectrophotometer. For the preparation of HSPN, 0.1 mg hyaluronidase was stirred with EDC (1.8 μmol, 0.35 mg) and NHS (1.8 μmol, 0.21 mg) in 10 mL DI water for 2 h, then above obtained SPN-NH_2 was added to the solution and stir for another 8 h. Then ultrafiltration (50 KD, 3500 rpm, 25 min) was carried out to concentrate the obtained HSPN. For Fluorescent labeling, 5 mg FITC was dissolved in 0.5 mL DMSO, then 20 μL solution was added into obtained SPN-NH_2 and HSPN and stir for 1 h. Add 10% FITC (W/W) in the PCPDTBT during the process of SPN synthesis is another alternative labeling way.

Characterization of NPs.

Morphology of SPN and HSPN were observed by transmission electron microscopy (TEM). ζ-Potential and size distribution of SPN and HSPN were measured with dynamic laser scattering (DLS). UV-vis absorption measurement and fluorescence absorption were performed on a UV-vis spectrophotometer and a fluorescence spectrophotometer. After that, 1mL hyaluronidase, SPN, and HSPN were mixed with 1 mL BCA reagent respectively, incubated at 37 °C in an oven for 2 h, and then tested by a UV-vis spectrophotometer. The ER (encapsulated ratio) % and DL (drug loading) % were calculated as follows:

$$ER\ \% = \frac{\text{Weight of encapsulated PCPDTBT}}{\text{Weight of the feeding PCPDTBT}} \times 100\%;$$

$$DL\% = \frac{\text{Weight of encapsulated PCPDTBT}}{\text{Weight of the feeding polymer and PCPDTBT}} \times 100\%.$$

200 μL SPN solutions were put into a 96-well plate and irradiated using an 808 nm laser for 6 min (1.0 W/cm^2). Then the laser was turned off, and samples were naturally

cooled. An IR (infrared ray) thermal camera was used to monitor the temperature change. Five cycles of laser on/off were carried out to study the photothermal stability of SPN.

Penetration Assay in Multicellular Tumor Spheroid (MCTS).

50 μL of hot 1.5% agarose solution (w/v) was quickly placed into a 96-well plate and then naturally cooled. Then the plate was irradiated under UV light for 2 h for sterilization and then washed three times by 10% penicillin/streptomycin with PBS buffer. 4-T1 cells were seeded into the agarose coated plate at a density of 6×10^3 cells per well in 100 μL of RPMI 1640 and cultured for 2 weeks with the medium changed every two days. Then the formed MCTS were co-incubated with FITC labeled SPN and HSPN for 4 h. After the incubation, MCTS was transferred to a confocal dish. fluorescence images were captured using Z-stack imaging from the top of the MCTS at 20 μm intervals using CLSM.

Preparation of MN model.

PDMS was used to fabricate MN mold as illustrated in Figure S20. PDMS and its curing agent were mixed at a mass ratio of 10:1 and then stirred well. To remove bubbles in the mixture solution, a vacuum was performed at -0.08 MPa for 2 h. the PDMS solution was cast on a steel MN master model and then dried at 70 °C for 1 h to form PDMS flexible female mold. The PDMS mold was then separated from the steel master mold and was applied as a mold to prepare MN.

Table S1. Diameter and PDI of SPN and HSPN.

Sample	Diameter (nm)	PDI
SPN	94.66 ± 1.21	0.40 ± 0.02
HSPN	102.13 ± 0.60	0.34 ± 0.08

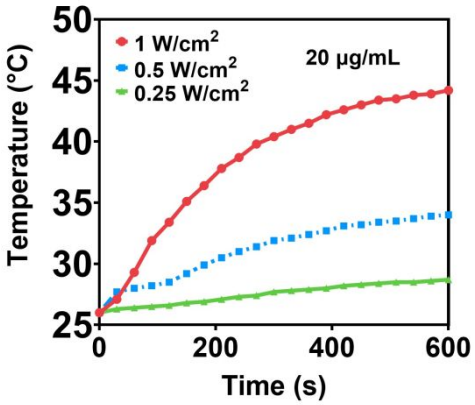

Figure S1. Photothermal curves of SPN (20 μg/mL) at different power.

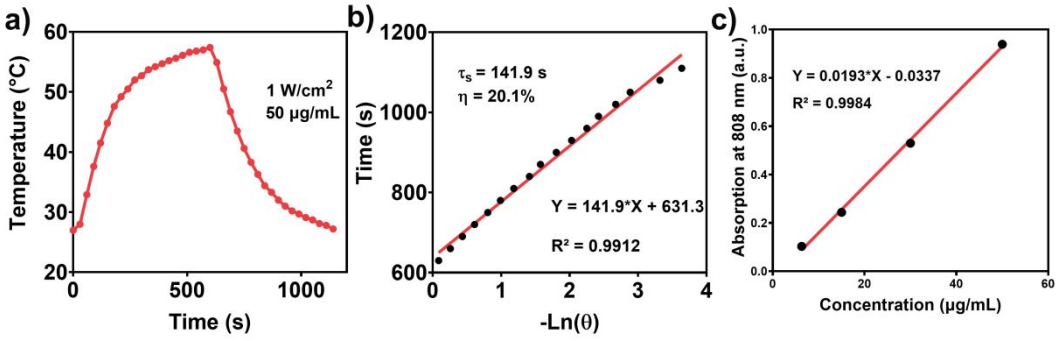

Figure S2. The photothermal effect of the SPN aqueous suspensions under irradiation and then the laser was shut off and calculation of the photothermal-conversion efficiency according to the (a) Photothermal curve, (b) Calculation of the time constant (τ_s), (c) Absorption of SPN at 808 nm at different concentration.

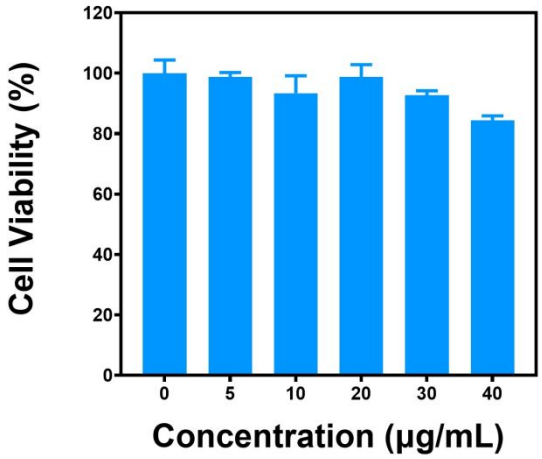

Figure S3. The cell viability of SPN to NIH3T3 cells after 24 h incubation.

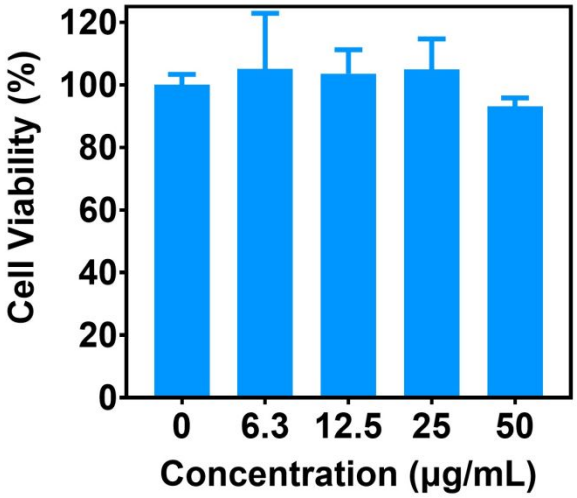

Figure S4. Cell viability of Melanoma cells after incubation with PIC at different concentrations after 24 h.

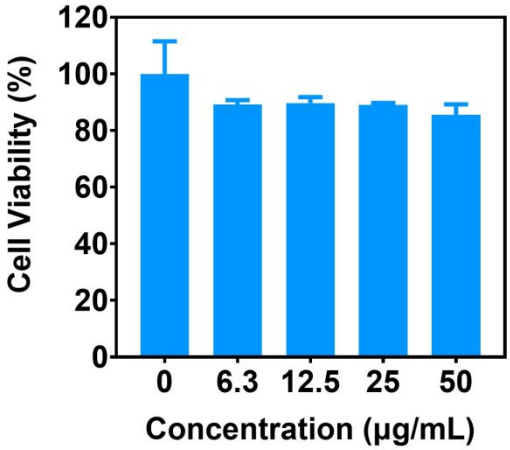

Figure S5. Cell viability of macrophages after incubation with PIC at different concentrations after 24 h.

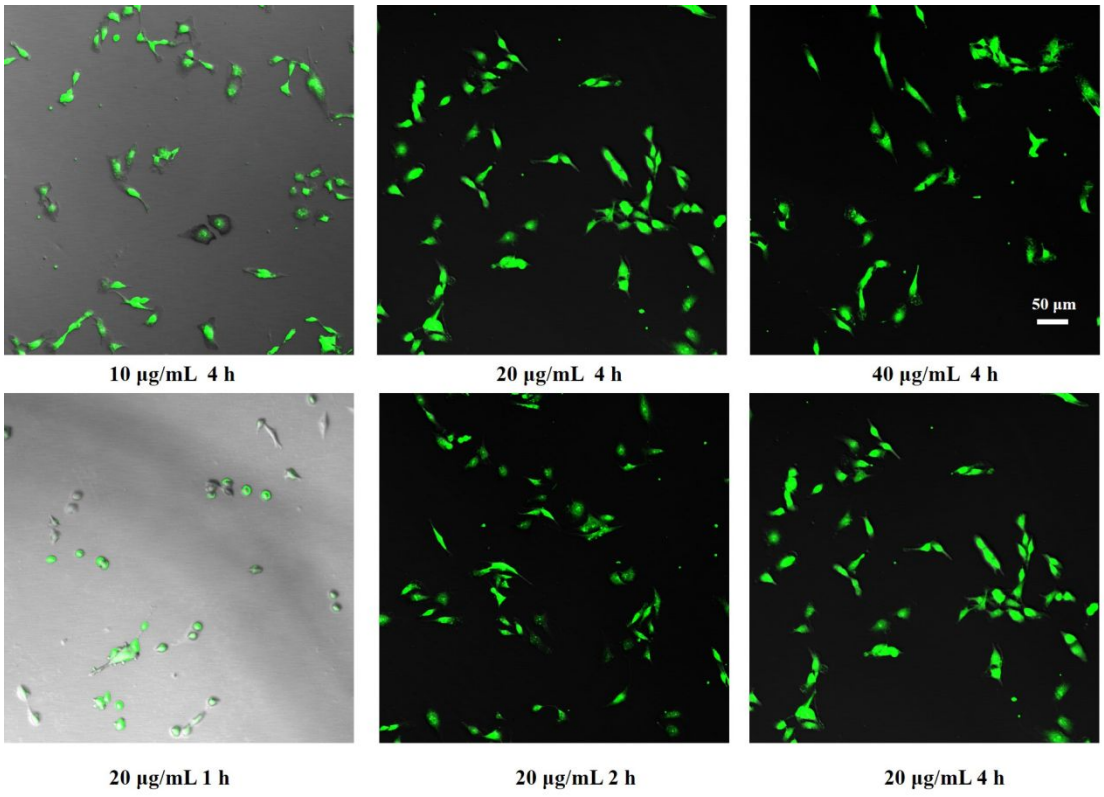

Figure S6. Fluorescence microscopy merged images of FITC labeled SPN uptake by B16-F10 melanoma cells in vitro, scale bar is 50 μm.

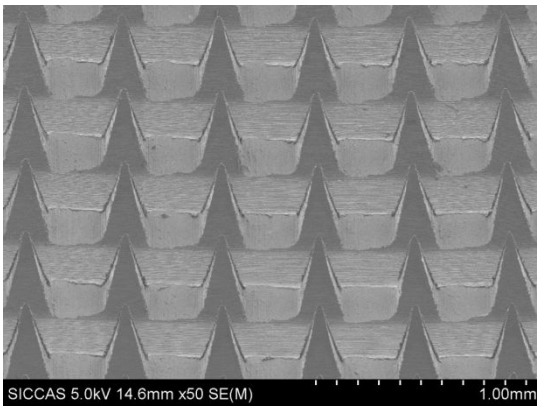

Figure S7. SEM images of MN in drying state (100×).

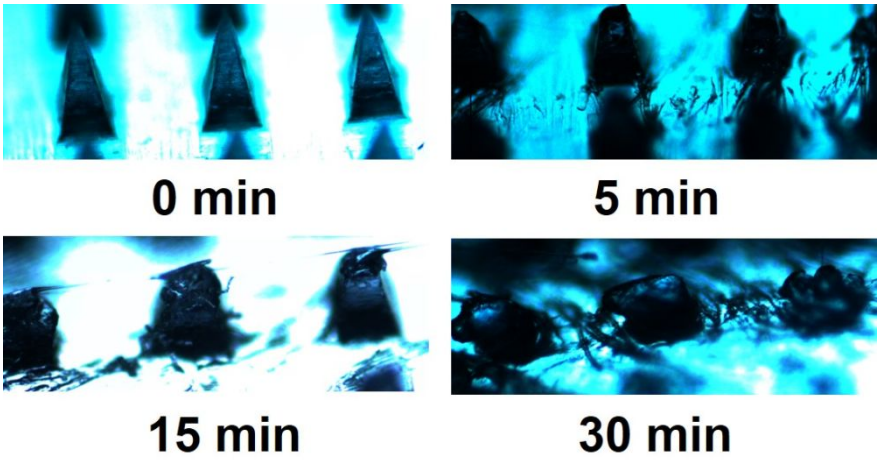

Figure S8. The time-dependent dissolving of MN.

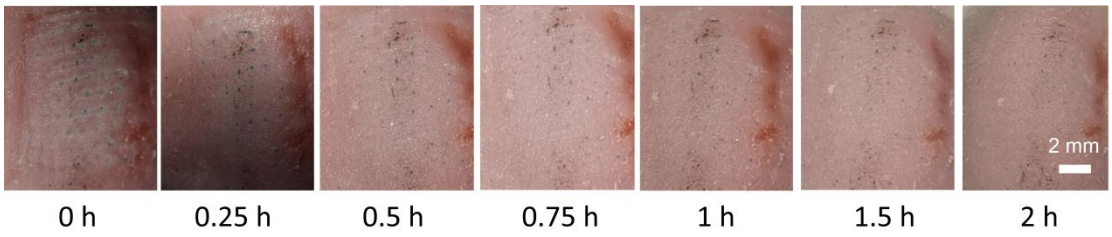

Figure S9. Optical images of skin recovery after penetration at 0.25 h, 0.5 h, 0.75 h, 1 h, 1.5 h, 2 h.

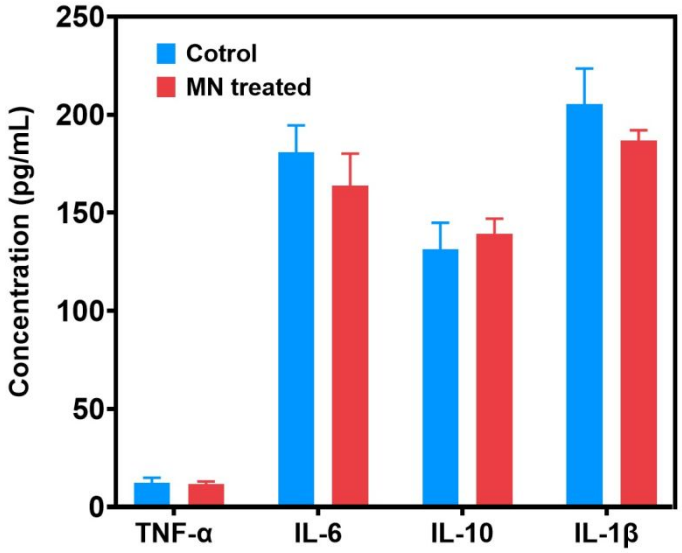

Figure S10. The changes of inflammatory cytokines of skin tissue after the insertion of MN for 2 h.

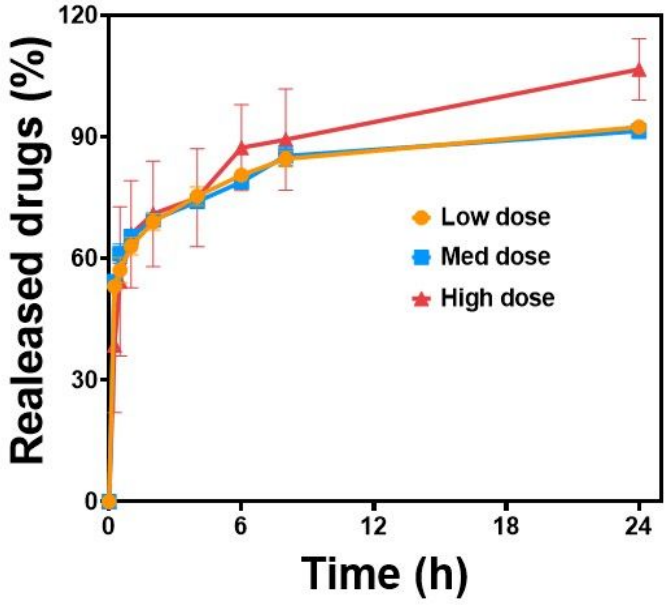

Figure S11. Accumulated percentage of PCPDTBT released from low dose, med dose and high dose (3 μg, 6 μg, 12 μg) MN incubated in PBS (n = 3).

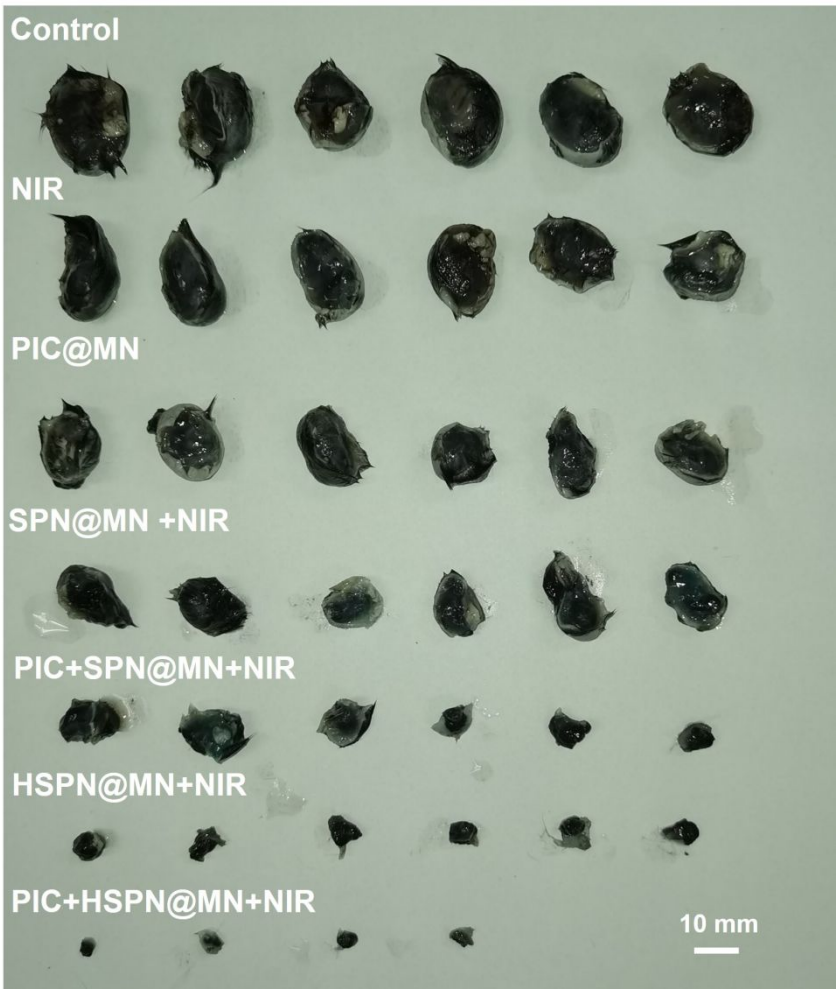

Figure S12. Photographs of C57B16 mice bearing transplanted B16-F10 tumors on the 12th day.

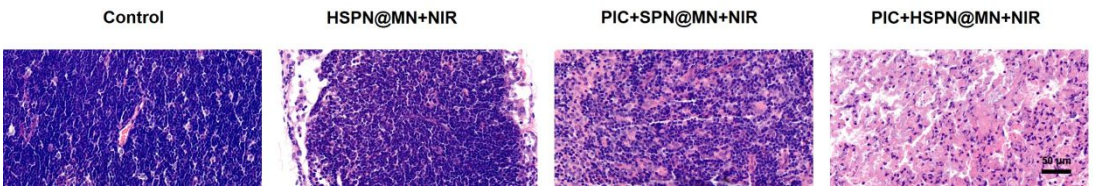

Figure S13. Histopathologic analysis of H&E-stained tissue sections of the lung in tumor-bearing mice after treatment for natural metastatic, scale bar is 50 μm.

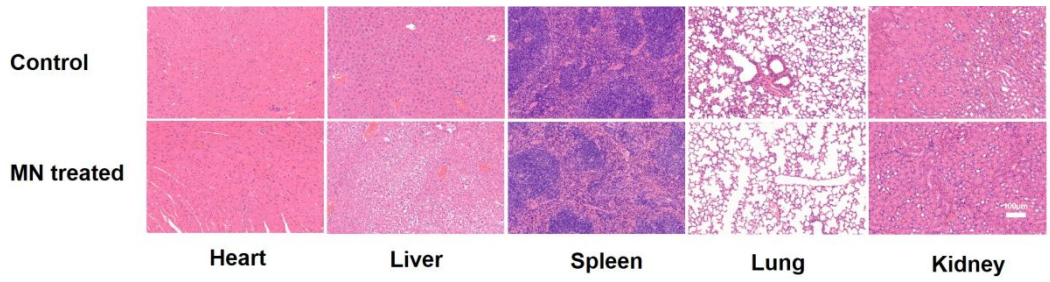

Figure S14. Pathological H&E stained images of the major organs sections of mice treated with PIC+HSPN@MN after 21 days, scale bar is 200 μm.

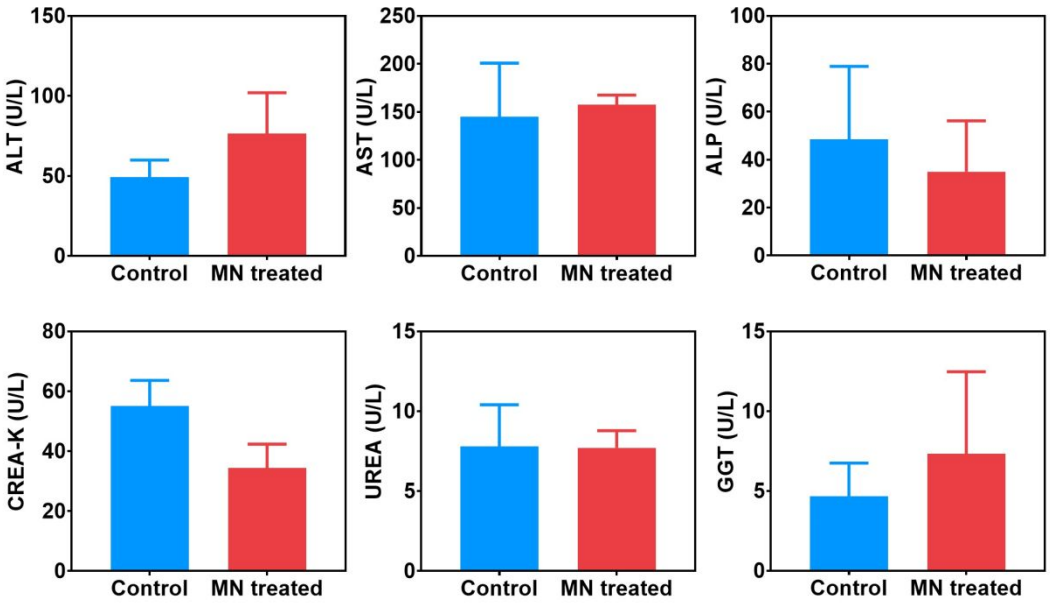

Figure S15. Routine blood test was analyzed in mice treated with PIC+HSPN@MN after 21 days. Data are expressed as SD ± SEM (n=3).

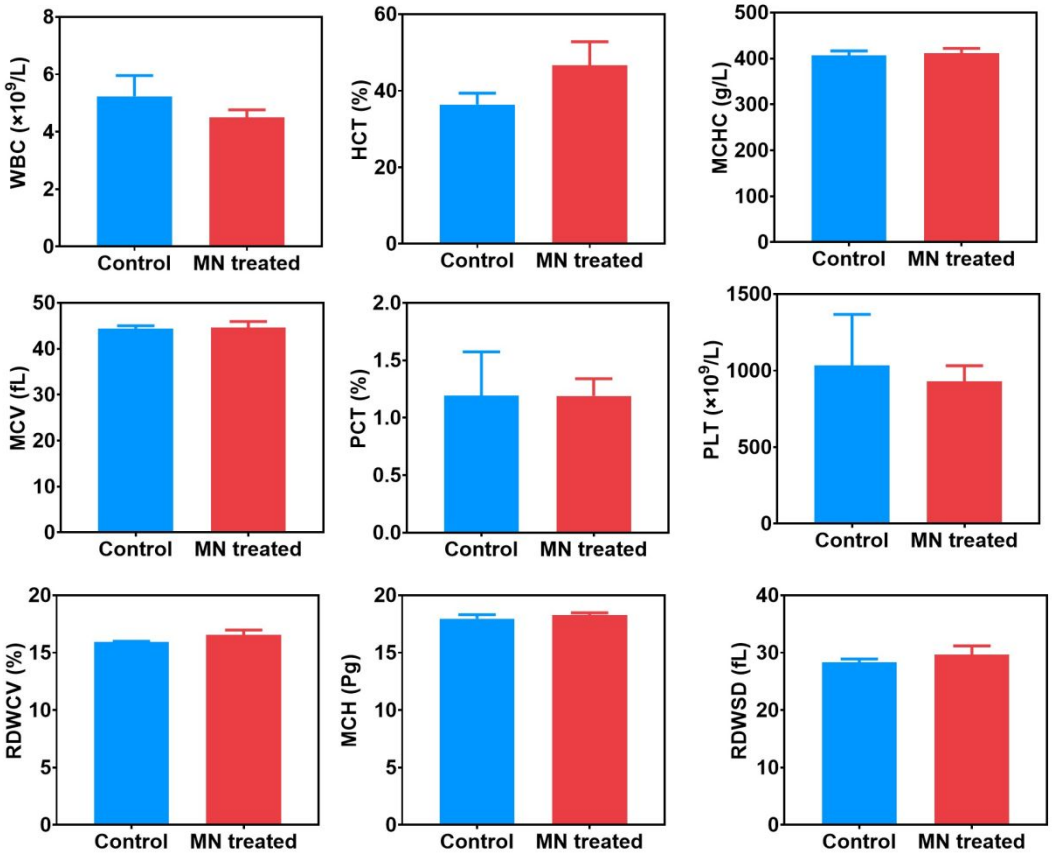

Figure S16. Comprehensive serum chemistry profiles were analyzed in mice treated with PIC+HSPN@MN after 21 days. Data are expressed as SD ± SEM (n=3).

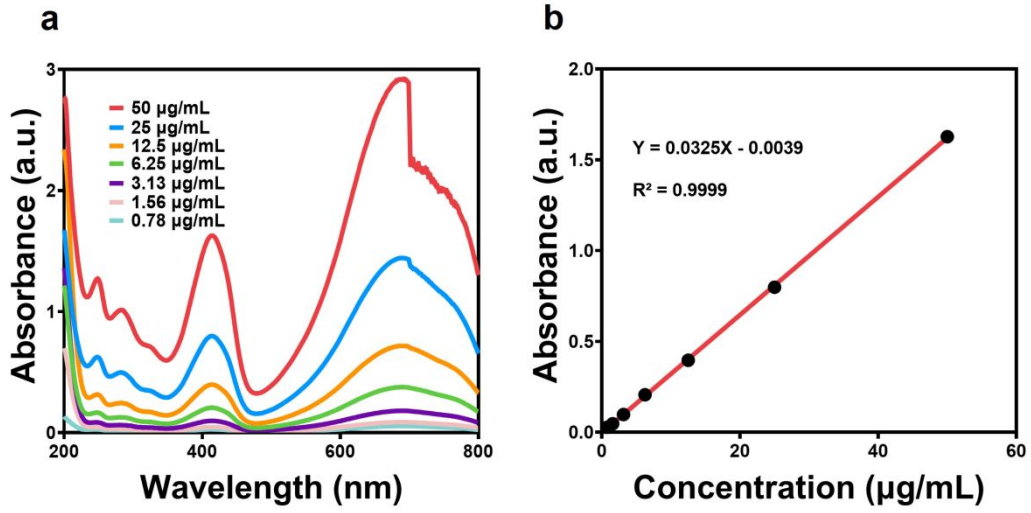

Figure S17. (a) UV-vis spectra of PCPDTBT and (b) Standard curve of PCPDTBT at 414 nm.

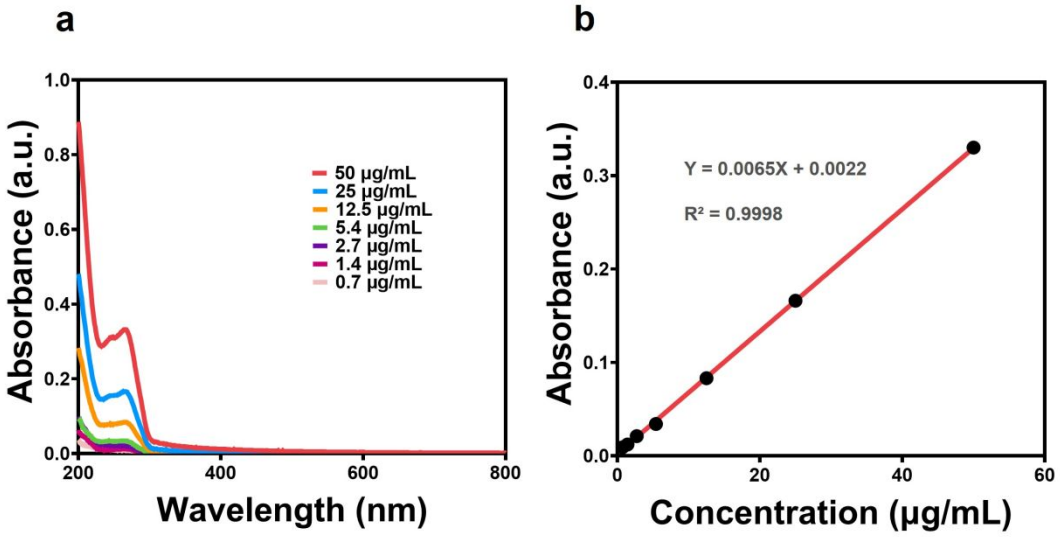

Figure S18. (a) UV-vis spectra of PIC and (b) Standard curve of PIC at 263 nm.

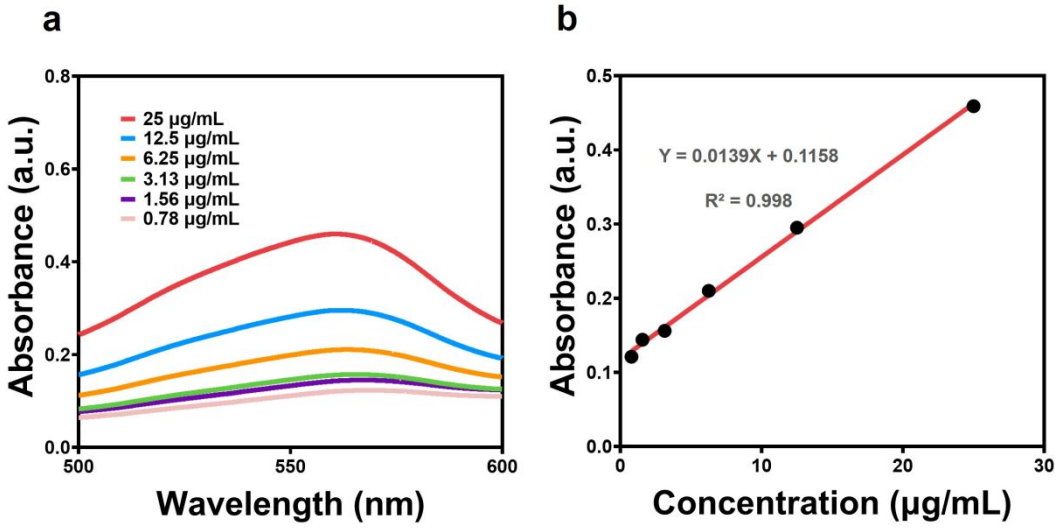

Figure S19. (a) UV-vis spectra of hyaluronidase after reacting with BCA assay and (b) Standard curve of hyaluronidase at 562 nm.

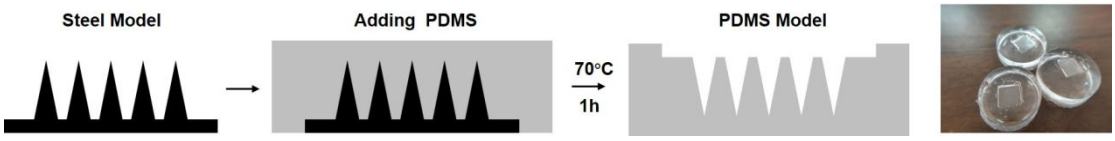

Figure S20. Schematic illustration showing the preparation of MN mold.