SUPPORTING INFORMATION FOR

Visible-Light-Controllable Drug Release from Multilayer-Coated Microneedles

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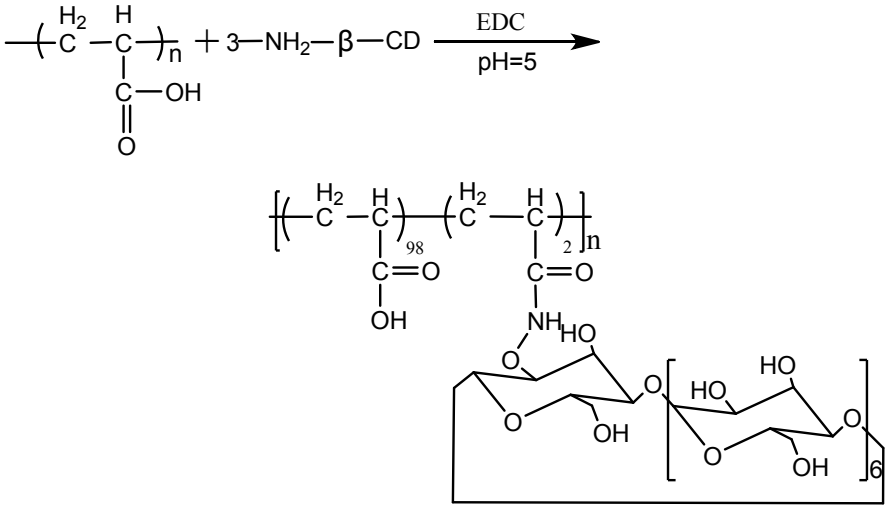
Experimental section

Materials

3-Amino-3-deoxy-α-cyclodextrin was purchased from Tokyo Chemical Industries (TCI). Acryloyl chloride (AC), Poly (acrylic acid) (PAA, Mw: 450 kD), ethylcarbodiimide (EDC), poly (ethylene imine) (PEI, Mn: ~70 kD), neutral red (NR), safranine T (ST), and 3-aminopropyltriethoxysilane (APTES) were obtained from Shanghai Aladin Co., Ltd. (China) and used directly. 1-Azobiscyclo-hexanecarbonitrile (ABCN), N,N′-Dimethylformamide (DMF), 1,4-dioxane and Dimethyl sulfoxide (DMSO) were purchased from Aldrich. Poly (L-lactic acid) (PLLA) was supplied by Jinan Daigang Biological Technology Co., Ltd. (China). AC was distilled under vacuum before use. ABCN was recrystallized from methanol before use. All other chemicals were used without further purification.

Preparation of PAA-β-CD

Poly (acrylic acid) with β-cyclodextrin side groups was synthesized by using a procedure similar to the literature method.[1] Briefly, 0.144 g (2 mmol) PAA and 0.0568 g (0.05 mmol) 3-NH_2-β-CD were dissolved in 30 ml deionized (DI) water in a flask, after the solution's pH value was adjusted to 5, EDC (0.0384 g, 0.20 mmol) was introduced into this solution with stirring for 24 h at room temperature. This solution was purified by dialysis against water in a dialysis tube (MWCO: 12-14 kDa, Isbio Ltd., China) for 72 h and lyophilized for 2 days at room temperature. The degree of modification was determined to be 2 mol% by 1H NMR.



Scheme S1 Synthesis route of PAA-β-CD

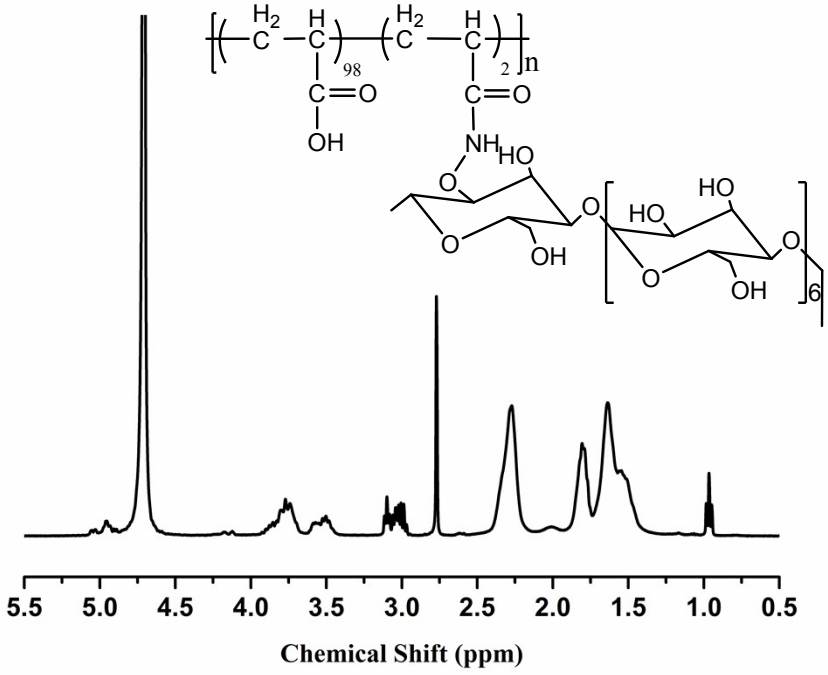
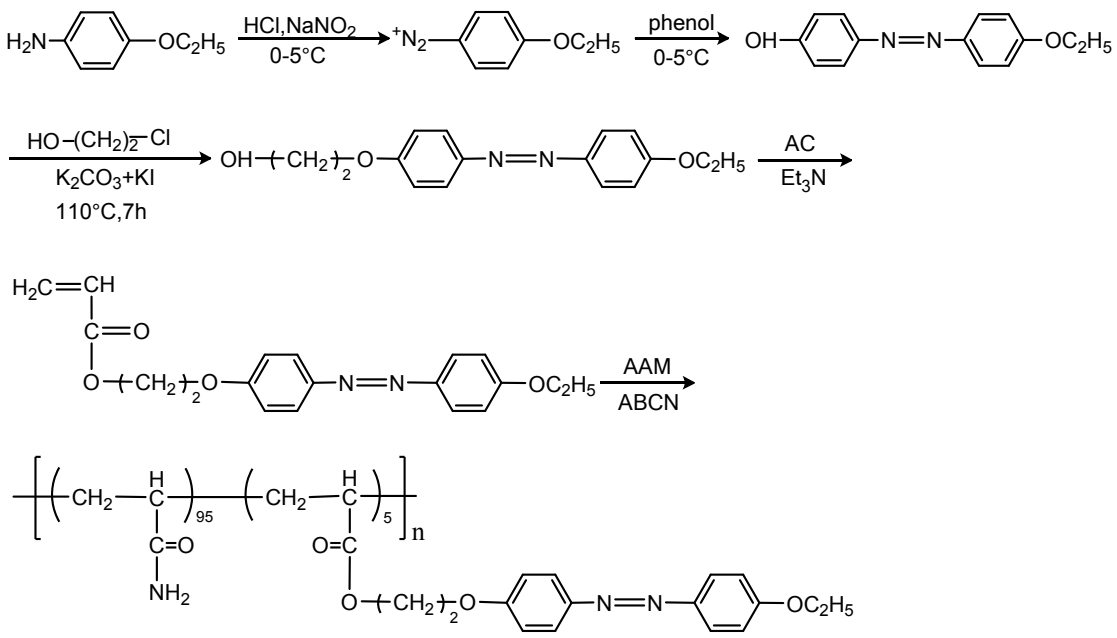


Figure S1 The 1H NMR spectra of PAA-β-CD

Preparation of PAAm-C_2-Azo

PAAm-C_2-Azo were prepared similar to our previous works [1,2]. Briefly talking, AC (0.3 g, 0.0033 mol), triethylamine (0.56 mL, 0.0040 mol), and 2-[4-(4-ethoxyphenylazo)phenoxy]ethanol (whose amount was determined by the required degree of functionalization) were dissolved in anhydrous DMF (33 mL). The mixture was stirred at room temperature for 12 h under N_2 protection. Then suitable amount of water was added into the mixture and stirred for 10 min. The product was precipitated from HCl water solution (0.01 mol/L), collected by filtration, washed several times with water, and dried under vacuum. ABCN (0.5 wt per cent with respect to the monomer) was used as a radical initiator. The polymer was further purified by dissolving in THF and precipitated from petroleum ether, collected by filtration, and washed twice with petroleum ether. The final product was dried at 70 °C under vacuum for 24 h. The degree of modification was determined to be 5 mol% by 1H NMR.



Scheme S2 Synthesis route of PAAm-C_2-Azo

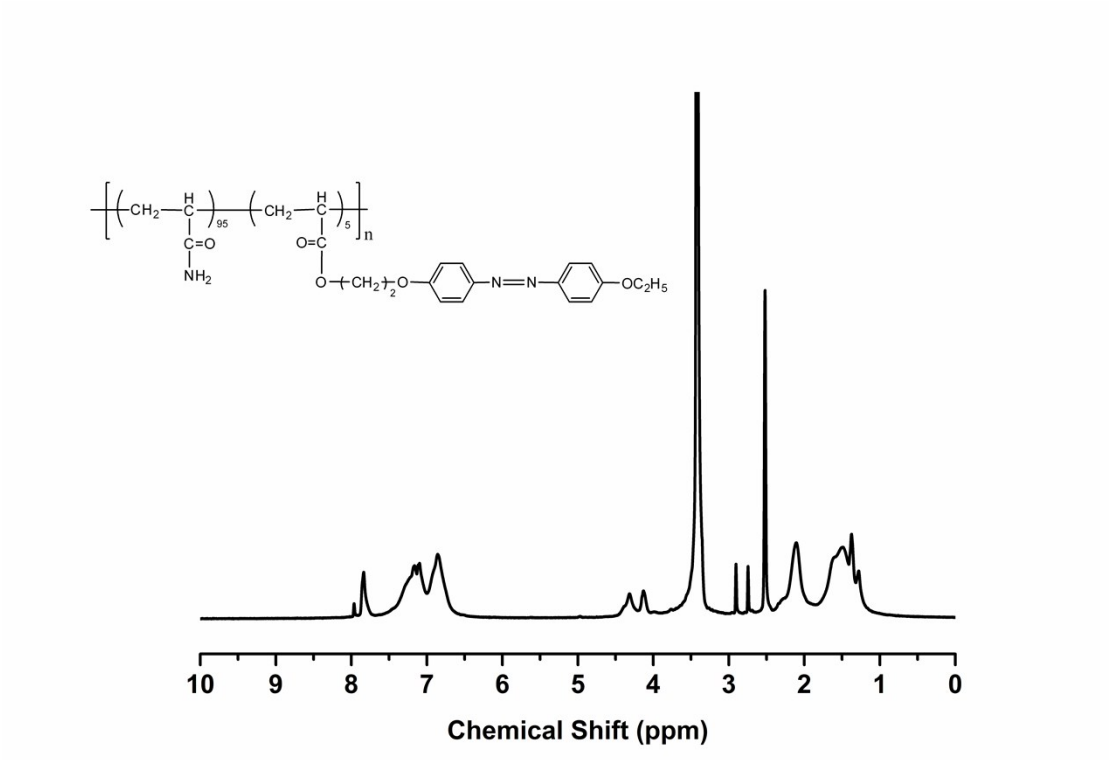


Figure S2 The 1H NMR spectra of PAAm-C_2-Azo

PLLA microneedle fabrication

Fabrication of PLLA microneedle consists of two phases. The first phase was carried out to precisely inverse-replicate master structures by using established soft lithography techniques [3]. In brief, poly(dimethylsiloxane) (PDMS) moulds were created by pouring PDMS solutions over the top of a silicon master with pyramidal microneedle arrays (each 200 μm in diameter at their base and 160 μm in height) and allowing the polymer to cure overnight at room temperature. The cured PDMS moulds were subsequently peeled from the master structures and repeatedly used to make PLLA microneedles (Figure S3). In the second phase of fabrication, a hot-press machine was used to melt-mould PLLA on the PDMS moulds. About 50 mg of PLLA was spread on a PDMS mould and melted over the mould with a proper processing temperature and pressure (170°C, 1 MPa). Then cooled to 25°C before the PLLA microneedle sheet was gently peeled out of the mould. The resulting PLLA microneedles sheet was also presented in Figure S3. The surface morphology of PLLA microneedles was characterized by SEM using a FEI-QUANTA 200-SEM.

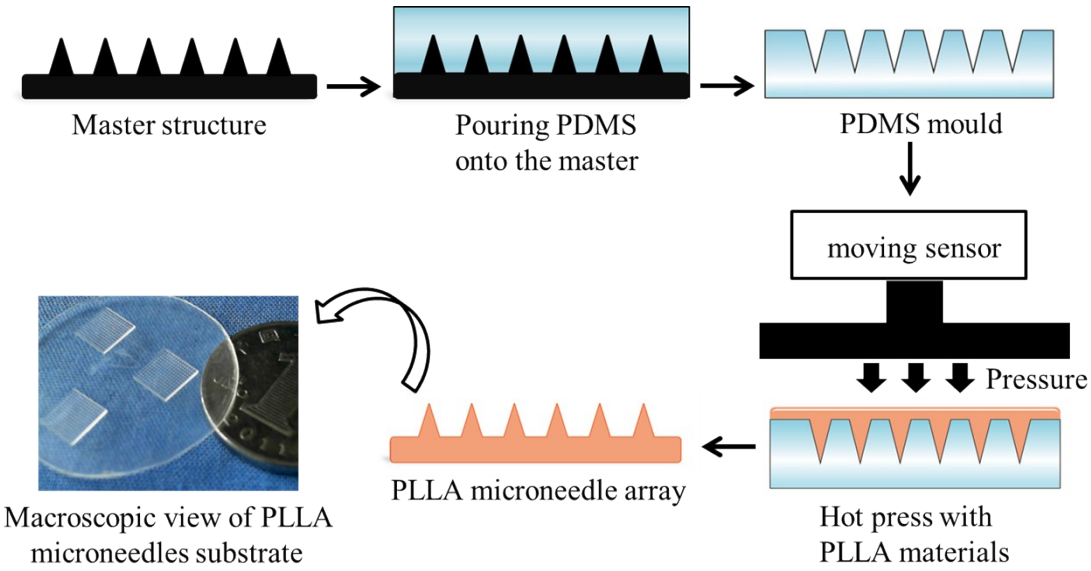


Figure S3 Schematic illustrations of PLLA microneedle fabrication process. The PDMS replicas exhibit the morphology of conical hole arrays.

Substrates treatment.

a. Treatment of quartz slides Quartz slides (approximately 2 cm × 1.2 cm) were first sonicated in acetone, ethanol, and DI water consecutively, each for 10 min. After dried with nitrogen gas, the cleaned quartz substrates were immersed into a fresh piranha solution (30% H_2O_2/98% H_2SO_4, v/v =3:7; CAUTION: Piranha solution can react fiercely with organic chemicals and should be handled with extreme care, including the use of acid-resistant gloves and adequate shielding) and heated until no bubbles were observered. The substrate was rinsed carefully with deionized water and dried with nitrogen. The cleaned quartz slide was treated in 2% (v/v) APTES/95% ethanol solution for 6 h, and subsequently dehydrated at 115°C for 45 min to obtain the amino groups terminated quartz wafer.

b. Treatment of PLLA microneedles

PLLA microneedle sheets need to be modified with a precursor layer of poly (ethyleneimine) (PEI) before LbL deposition. The as-prepared microneedle substrates were orderly immersed in the 5 mg/mL PEI aqueous solution (containing 0.2 mg/mL NaCl) for 20 min, followed by exhaustive cleaning and drying.

Multilayer films preparation on different substrates.

Films were constructed on quartz wafers and PLLA microneedle arrays, respectively. To build (PAA-β-CD/PAAm-C_2-Azo) multilayers on quartz substrates, quartz wafers coated with amine were alternately dipped in the 0.5 mg/mL PAA-β-CD and PAAm-C_2-Azo solutions for 10 min, separated by 2 min rinse in enough DI water until a desired number of layers was achieved. Both solutions were adjusted to pH 2.5 and filtered (0.2 μm) prior to dipping. We used a Shimadzu UV-2550 spectrometer to monitor the film growth behavior, and the whole process was performed under room temperature. Subsequently, cross-linking reaction was accomplished with heating in a vacuum oven at 175°C for 3 h. The same method was

operated on PLLA microneedles substrates, except for a lower temperature and longer time for crosslinking. The substrates successfully coated with multilayer films were kept in glass bottles for following drug delivery experiment. Figure S4 presents the LbL deposition of interacting polymers on the amino-modified substrates.

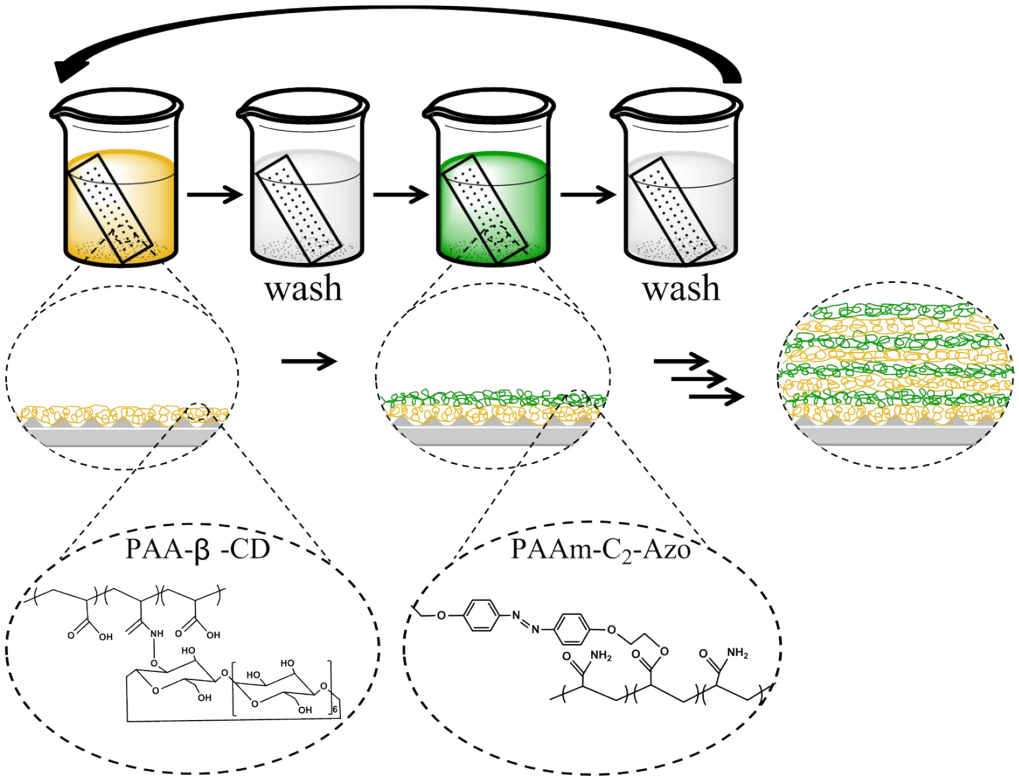


Figure S4 Schematic view of (PAA-β-CD/PAAm-C_2-Azo) multilayers deposited onto PLLA microneedles surfaces. Insets illustrate the structures of the compounds used.

Photoresponsive drug encapsulation and release.

Taking neutral red (NR) as an example, the drug loading procedure is described as follows. 0.2 mg/mL aqueous solutions of NR (pH 6.8) was prepared by dissolving 0.4 g of neutral red in water and diluting to 2000 mL in a 2000 mL standard flask. The pH of aqueous solutions was adjusted to 6.8 with the addition of KH_2PO_4 (0.5 M). An LbL film-modified quartz slide was immersed in a cuvette with 20.0 mL NR solution and irradiated under UV light for 12 minutes. Drug loading of the modified substrate was evaluated every 3 minutes by measuring the absorbance of NR at 500 nm after a brief washing with DI water and drying under a nitrogen stream. Then the NR-adsorbed slide was transferred into 20 mL water and irradiated by visible light for 60

minutes. UV-visible absorption spectrum of the vis-irradiated quartz slide was also recorded at regular intervals of time. The incorporation and release of safranine T (ST) are identical as the above description. Figure S5 shows the photoresponsive ST adsorption/desorption of a multilayer of (PAA-β-CD/PAAm-C_2-Azo) with 10 bilayers. The absorption peak at around 560 nm of the blue line displays the result of drug encapsulation with UV light irradiation, while the green line stands for the removal of the template drug by visible light irradiation. The color of the quartz wafer is red due to the presence of loaded ST dye and the red substrate turns to be colorless after the irradiation of visible light (inset of Figure S4). In addition, the maximum absorbance of the azobenzene moiety (approximately 350 nm) decreased upon UV irradiation for 10 minutes; a slight increase was observed after 60 minutes of visible light irradiation. Agreeing with the previous description [4,5], the spectrum variations evidence the photo-isomerization of the azobenzene chromophores, which contributes entirely to the driving force of the loading and unloading process.

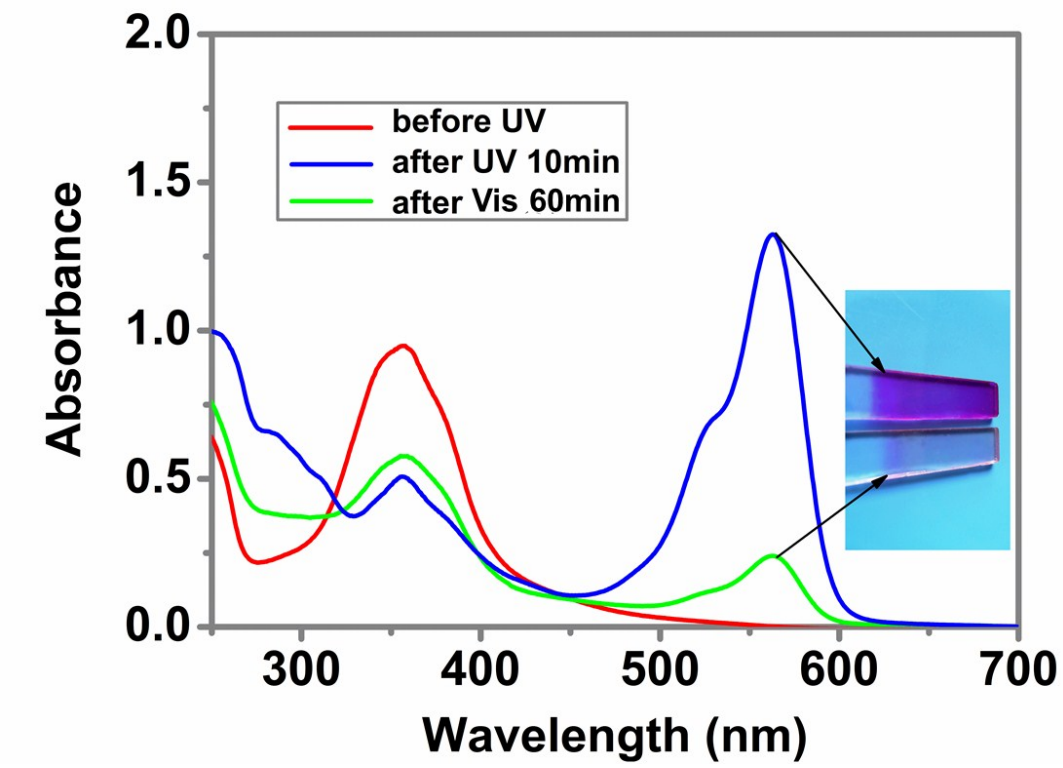


Figure S4 UV-Vis absorption spectra of the ST loading **(**blue line**)** and release **(**green

line**)** of a multilayers-coated quartz substrate. Inset: photograph of the quartz slide with (above) and without (below) ST dye.

The UV and visible light used

The UV light was from a 300 W mercury lamp equipped with 5 in. diameter filter (365 nm). A 100 W incandescent light bulb was used as visible light source. All sample were placed 15 cm away from the lamp. The surrounding temperature of the samples was controlled at 25 ^{o}C using a cold plate.

Release kinetics study of drug-coated microneedles.

For all studies, microneedles sheets were performed in one style (dispalyed in Figure S3) to decrease the variability. Besides, all the photo-regulated encapsulation and release assays were performed with a Shimadzu UV-2550 spectrometer to monitor the concentration of model drug at room temperature.

To quantify the total amount of drug encapsulation of the multilayers-coated microneedles, two groups of 10 mL 0.2 mg/mL dye solution were prepared. One of them was put in a 10-bilayer coated microneedles sheet and the other was taken as control group without any microneedles sheet in it. The two groups were then irradiated by UV light for 10 minutes. Since the molar absorptivity of the model drug was assumed to be almost the same as that of the corresponding drug concentration, the different absorbance of the two solutions was adopted to evaluate the initial amount of model drug incorporated in the microneedle-based multilayers. To investigate the photo-induced controlled release property of such drug-containing microneedles, the sample was placed into a cuvette with 10.0 mL water and then irradiated by visible light. UV spectrum of the released drug in corresponding water was taken at multiple time points. The same procedure was followed for 20-bilayer coated microneedles also. Thus we evaluated the amount of drug released from the multilayers coated microneedles using the calibration curve. The percentage release was calculated using following equation,

$$\frac{C_t}{C_i} \times 100$$

Where C_i and C_t are concentrations of model drugs loaded in multilayers coated microneedles at initial time and drugs released in water after time "t" of incubation, respectively. The intensity of model drug loaded within the microneedle-based multilayers followed a gradual decrease with time. Thus the amount of model drug delivery in water was found to increase as the time increased. Additionally, when these drug-containing microneedles were incubated in the dark at pH 6.5, the release of the model drugs turned to be slow and moderate. As presented in Figure S5 A, under dark environment, the release of the NR was very slowly and only ~13.3% loaded NR could be removed from the microneedle arrays within 200 minutes. This implies that the cis-to-trans isomerization of azobenzene group was very slowly in this situation, and hard to cause the dissociation of the host-guest interaction between model drug and β-CD. In contrast, the back reaction to the trans form occurs upon visible light irradiation is very fast. Therefore, the cumulative released quantity of NR under visible light increased dramatically to 88% within 80 minutes. The similar release profile is also presented for the ST dye. As follows from Figure S5 B, lower than 20% loaded ST was released from the microneedles within 200 minutes in the dark. Whereas, about 89% loaded ST were effectively released under visible light irradiation within the same 80 minutes. From the results, it can be seen that the dye release during this time was still mainly caused by the rate of cis-to-trans isomerization.

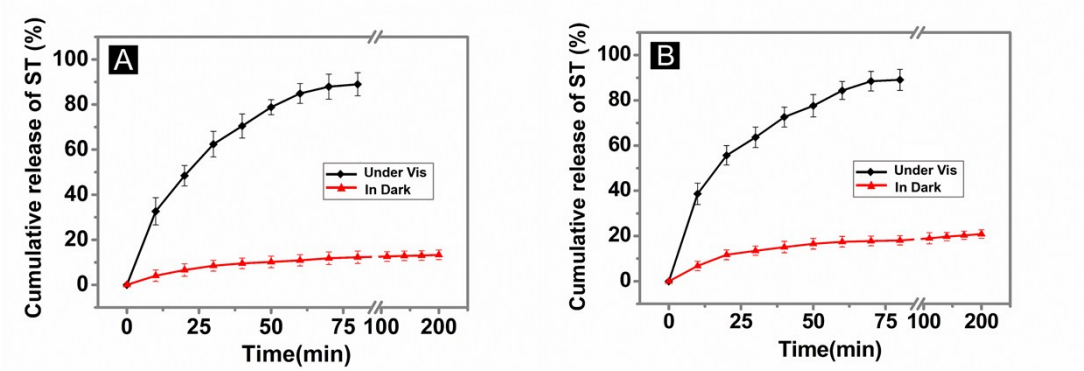


Figure S5 Comparison of release profiles of NR (A) and ST (B) from the 20-bilayer coated microneedls in dark and under visible light irradiation. Vertical axis corresponds to the amount of released model drugs divided by the total amount of model drugs stored in microneedle-based multilayers.

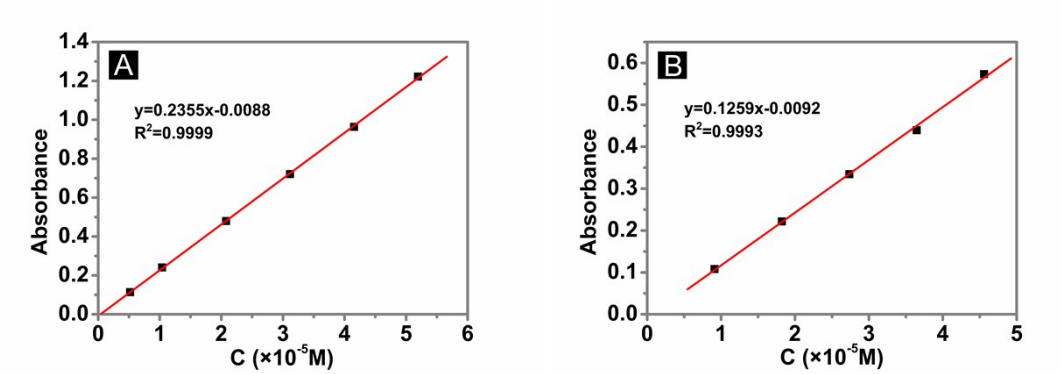


Figure S6 Calibration curves of NR (A) and ST (B) collected from UV-Vis absorption spectra.

Porcine skin tests under dark.

Neonatal porcine skin was obtained from a local slaughterhouse immediately following the stillborn piglet's death (Wuhan, China). The samples of dermatomed skin were packed separately and stored in aluminium foil at −20°C (no longer than 2 months) until use. Porcine skin samples, obtained as described above, were shaved and washed in phosphate buffered saline pH 7.4 (PBS) for 1 h before beginning the experiments.

20-bilayer ST-included patch was inserted into fresh porcine skin and monitored under a dark environment or daylight. After 24 h, the patch was removed from the skin, and Figure S7 showed the En face view of porcine skin.

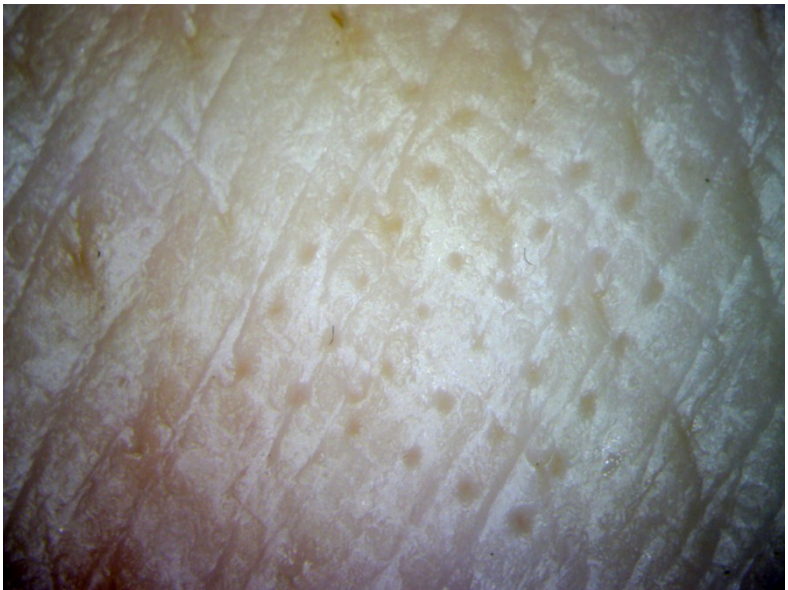


Figure S7 En face view of porcine skin after 24 h storage under daylight, followed by removal of microneedles.

Reference

1. W. Xiao, W.-H. Chen, X.-D. Xu, C. Li, J. Zhang, R.-X. Zhuo and X.-Z. Zhang, *Advanced Materials*, 2011, **23**, 3526-3530.
2. M. Wu, Y. Cao, X. Zhang, Y. Zhang, Y. Chen, L. He and Z. Qian, *Chemical Communications*, 2012, **48**, 9846-9848.
3. W. Chen, R. H. W. Lam and J. Fu, *Lab on a Chip*, 2012, **12**, 391-395.
4. Y. Wang, P. Han, H. Xu, Z. Wang, X. Zhang and A. V. Kabanov, *Langmuir*, 2009, **26**, 709-715.
5. L. Wu, X. Tuo, H. Cheng, Z. Chen and X. Wang, *Macromolecules*, 2001, **34**, 8005-8013.