

Supporting Information

Hyperthermia triggered UK release nanoverctors for deep venous thrombosis therapy

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EXPERIMENTAL SECTION

Materials:

Cetyltrimethyl ammonium bromide (CTAB), tetraethylorthosilicate (TEOS), etrachloroauricacid ($\text{HAuCl}_4 \cdot 4\text{H}_2\text{O}$), formaldehyde solution (37.0 wt%), sodium hydroxide (96.0 wt%), absolute anhydrous ethanol (99.7 wt%), fluorescence isothiocyanate (FITC), UK ($5\text{-}6 \times 10^4$ IU/mg), thrombin, Lauric acid (96.0 wt%) and formalin solution were purchased from Macklin Co. (Shanghai, China). Stearic acid (96.0 wt%) was purchased from Sigma Co. (St.Louis, MO, USA). Methanol (99.5 wt%), N, N-dimethylformamide (DMF, ≥ 99.5 wt%) XiLong Chemical (Guangdong, China). Presto blue cell viability reagent were purchased from ThermoFisher Co. (USA). RPMI 1640 medium, fetal bovine serum, and penicillin streptomycin were supplied by Gibco CO (USA). NO, PGI_2 , LDH ELISA Kit was purchased from Jiang lai biological Co. (Shanghai, China).

Characterization

The size and feature of the synthesized nanoparticles was measured by Transmission electron microscope (TEM, JEM-2010 type; Japan Electronics Co.), scanning electron microscopy (SEM, Zeiss/Sigma 300). Brunauer-Emmett-Teller (JW-BK132F, China). Fourier transform infrared spectra (FTIR, model 6300, Bio-Rad Co. Ltd, USA) were employed to analyze the physical properties of Au@MSNs, UK-FA@Au@MSNs, respectively. Ultrasonic diagnostic machine, Phillip (USA) NIR laser (808 nm) was

supplied by Hi-Tech Optoelectronics Co. Ltd, China. UK and the cytocompatibility of materials was measured by enzyme-linked immunosorbent assay (Spectra Max M5 type, Tianjin Science and Technology Development Co.) H&E staining were observed by fluorescence microscope.

Preparation of Au@MSNs

All glassware was cleaned in a bath of prepared aqua regia (HCl: HNO₃, 3:1 by volume) and rinsed thoroughly in water prior to use. 0.4 g of CTAB was dissolved in a solution containing 240 mL of distilled water and 4.8 mL of 0.5 M NaOH. After stirring the reaction for 15 minutes in an environment of 80°C, 8 mL of 37% acetaldehyde was added, and 3.2 mL of 0.029 M HAuCl₄ was stirred for 10 minutes. A solution containing 2 g TEOS and 4 g ethanol was added. The products were obtained by centrifugation at 11000 RCF for 20 min after reaction for 90 min, followed by washed four times with water and once with absolute ethanol, and then dried at 55 °C overnight. Removal of the surfactant template was achieved by calcination in air from room temperature to 550 °C for 6 h at a rate of 1 °C min⁻¹.

Fabrication of PCM nanoparticles

The PCM nanoparticles were fabricated derived from nanoprecipitation via a method. Briefly, 228 mg lauric acid and 72 mg stearic acid (76% : 24% by weight) were first dissolved in methanol at a concentration of 10 mg mL⁻¹. For the fabrication of PCM nanoparticles made of single-component fatty acids, the concentration of lauric acid or stearic acid was fixed at 10 mg mL⁻¹. The preparation process involved pouring the mixture samples into 100mL beakers separately, and finally using a magnetic stirrer at 400 rpm to stir the mixture at 70°C for 45 min. Added 30 mL of methanol, then store the PCM in 4 °C.

Preparation of UK-FA@Au@MSNs particles

2 mg UK was dissolved in 10 mL methanol, following 6 mL PCM was added to the

above solution. 15 mg Au@MSNs were added to 30 mL DMF, sonicated for 20 min to dissolve the particles in the solvent, then added the above solution dropwise, the temperature rose from room temperature to 80 °C and stirred for 90 min. Centrifuge at 4 °C, 11000 rpm for 15 minutes, washed off the excessed UK. The UK-loaded products were freeze-dried at -65 °C.

Drug encapsulation efficiency and drug loading efficiency

The amount of UK-loaded in UK-FA@Au@MSNs was tested by an enzyme-linked immunosorbent assay (ELISA). The protein content in the supernatant was detected after high-speed centrifugation (11,000*g). Finally, the protein content could be measured from the supernatant. The amount of UK-loaded was calculated from total UK by subtracting the measured. The drug encapsulation efficiency was defined as the ratio of the drug amount in the ultimately product to the total drug amount added in the process. The drug loading efficiency was defined as the ratio of the drug amount in the final product to the NP amount, which could be determined by lyophilizing the purified NPs. The experiments were performed three times independently for each sample.

Blood collection and clot formation

The blood clot was prepared via adult healthy rabbit. 5 mL blood was collected from healthy adult rabbit ear vein extraction then divided into several tubes. Which contained 40 U/mL thrombin solution, the test tube was placed at 4°C for 3 days to obtain stable plaque.

Thrombolysis Assessment In Vitro

The prepared blood clot was placed into a transparent bottle containing 5 mL saline, respectively. The experiment was divided into the presence or absence of the NIR group, and with or without drug-add group. The blood clots were photographed at setting time. The NIR groups were treated via NIR laser (0.66 W/cm²) for the indicated time points.

Thrombolysis Assessment In Vivo

All animal procedures were performed according to the protocol approved by the Institutional Animal Care and Use Committee of China. 10 % chloral hydrate, 2 mL/kg rabbit ear vein injection anesthesia, disinfection, along the proximal left leg along the

femoral vein, to make a 4 cm long oblique skin incision, free femoral vein about 2 cm. Near heart end clamped with a blood vessel clip, and the distal vessel wall was clamped 10 times with a smooth forcep. The femoral vein was parallel inserted into a 1 mL syringe at a distance about 1 cm from the distal end of the vessel clamp, and 0.1 mL of thrombin was injected (final concentration 150 U/mL), quickly withdraw the needle and pressed the needle hole for a few minutes. The blood vessel clamp was kept for 2 h, and saline was added dropwise to the tissue from time to time to keep it moist. After 2 h, the vessel clamps were slowly removed and the muscles and skin were layered suture. The limbs of the control side were not treated. Twelve rabbits were divided randomly into four groups, respectively treated with untreated, UK, NIR and UK-FA@Au@MSNs (Inner thigh of rabbit thigh was irradiated by NIR laser for 30 min) via intravenous injection after emerging blood clots. The dissolution of the thrombus was observed on the Day 1, 2, 3 with B-ultrasound. After the rabbit was sacrificed, the major organs and the vascular tissue at the thrombus was taken for H&E staining. Three stained sections were chosen randomly to image and analysis in each slice.

Cell Culture and Cytotoxicity Evaluation

Human umbilical vein endothelial cell (HUVEC) were seeded at 2×10^3 cells for CCK8 and 5×10^3 for ELISA per well in a 96-well microtiter plate and cultured with RPMI 1640 medium containing 10% fetal bovine serum (FBS, Gibco), 100 U mL^{-1} of penicillin and $100 \text{ } \mu\text{g mL}^{-1}$ streptomycin at 37°C under 5% CO_2 atmosphere. The culture medium was replaced with fresh media containing UK-FA@Au@MSNs at a concentration of $100 \text{ } \mu\text{g mL}^{-1}$. The cells were further incubated at 37°C for another 1d, 2d, 3 d, 4d for CCK8, respectively. Then, the cells were washed and incubated with PrestoBlue (PB) reagent. The absorbance was recorded at 450 nm after 2 h incubation of endothelial cells with PB reagent. Another part of the cell culture 24 hours, to measure the release of LDH, PGI_2 and NO by ELISA. The cell viability and release was expressed as a percentage relative to the cells untreated with plant compounds.

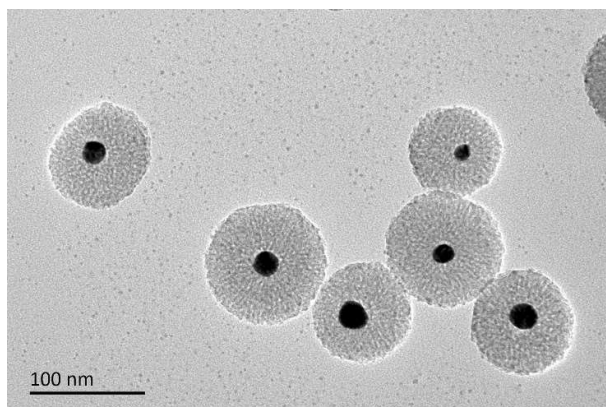


Figure S1. TEM image of synthesized Au@MSNs.

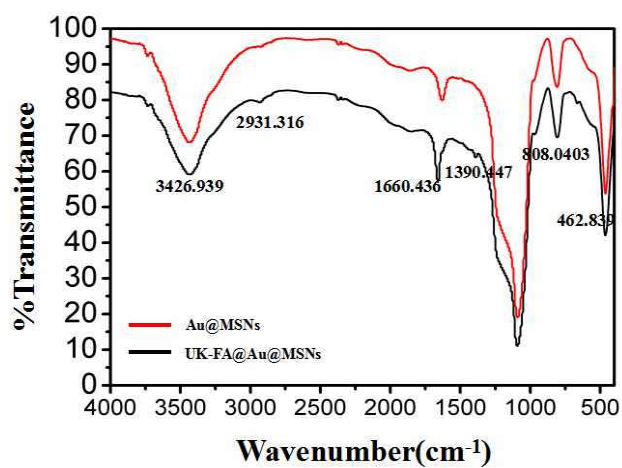


Figure S2. FTIR spectra of Au@MSNs and UK-FA@Au@MSNs.