Supporting information

SPECT Imaging and High Efficient Therapy of Rheumatoid Arthritis Based on Hyperbranched Semiconducting Polymer Nanoparticles

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Keywords: Hyperbranched semiconducting polymer, SPECT imaging, Rheumatoid arthritis, Methotrexate, Targeted drug delivery

Experimental section

1. Fabrication of HSP-NPs

The monomers, 2,2′ -(2,7-dibromo-9H- flfluorene-9,9-diyl) diacetic acid and 1,3,5-tris(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl) benzene, were synthesized according to the methods reported previously.[46−48] 1,3,5-Tris(4,4,5,5-tetramethyl- 1,3,2-dioxaborolan-2-yl)benzene (308.9 mg, 0.76 mmol) and 2,2′ - (2,7-dibromo-9H-flfluorene-9,9-diyl) diacetic acid (500 mg, 1.14 mmol) were dissolved in DMF (50 mL) and then

bubbled with nitrogen for 30 min. Next, Pd(PPh3)4 (50 mg, 0,04 mmol) was added quickly into the mixture followed by injection of degassed 1 M Na2CO3 solution (5 mL). The reaction was conducted under nitrogen with rapid stirring at 90 °C for 48 h and then at 120 °C for 48 h. After being cooled down to room temperature, the suspension was centrifuged at 4000 rpm for 30 min and the precipitate was washed with DMF 5 times to remove unreacted monomers and small molecular impurities. The solid was lyophilized to obtain a light yellow powder, namely HSP (202 mg). Besides, the band gap (Eg) of HSP was estimated to be 3.08 eV on the basis of UV absorption spectrum. Such a wide band gap indicated that HSP was capable of emitting blue fluorescence, which was used for cell and tissue imaging.

2. Animal Model

DBA/1 male mice aged 8 weeks were purchased from Beijing Charles River Company (Beijing, China) and kept under pathogen-free conditions with adequate food and water. All animal experiments were conducted with permission of the ethical committees on laboratory animal welfare of Soochow University. The CIA mouse model was established by a double immunization. For the first immunization, mice were injected subcutaneously at the end of the tail with an emulsion of equal volume bovine type-II collagen solution (2 mg/mL) and complete Freund's adjuvant (4 mg/mL). After 21 days of the first immunization, the boost immunization was given to the mice with bovine type-II collagen solution emulsified in incomplete Freund's adjuvant.

3. Blood Circulation and Biodistribution

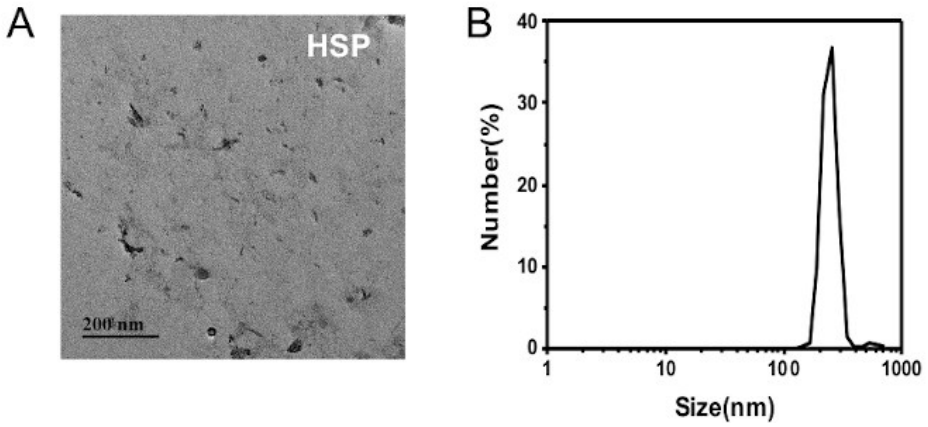
To analyze the blood circulation of HSP-PEG-NPs, blood from mice *i.v.* injected with 200ul ^{99m}Tc-HSP-PEG-NPs (800 μCi per mouse) was collected at appointed time points *p.i.* and then weighed and measured the radioactivity by the gamma counter (LB211, Berthold Technologies Gmbh & Co. KG). Then the mice were sacrificed at 24 h *p.i.* Major organs were collected, weighed, and measured the radioactivity by the gamma counter.

Table S1. Histopathological scores of synovium (HSS)

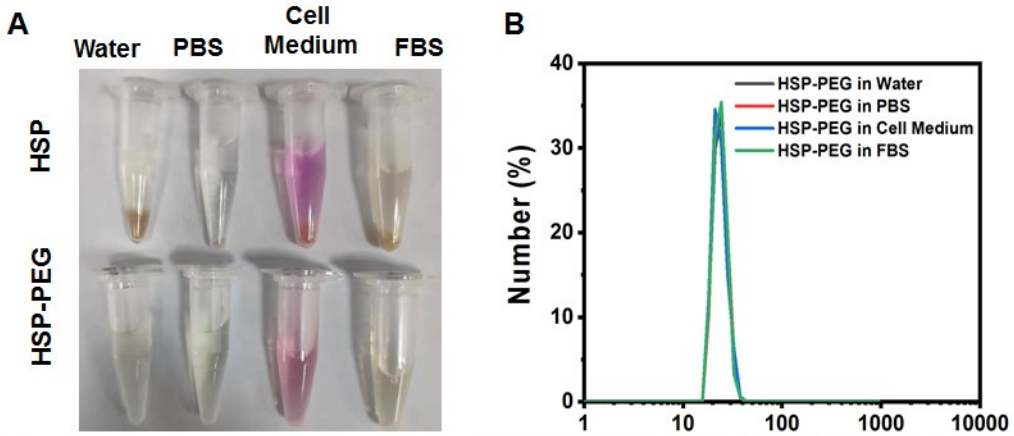
Features		Score
Hyperplasia or enlargement of synovial lining cell layer	Absent	0
	Slight enlargement (two or three cell layers). Giant cells are very rare	1
	Moderate enlargement (four to five cell layers). Some giant cells or lymphocytes	2
	Strong enlargement (more than six cell layers). Giant cells and lymphocytes are frequent	3
Inflammatory infiltration	Absent	0
	Slight inflammatory infiltration (diffusely located single cells and small perivascular aggregates of lymphocytes and/or plasma cells)	1
	Moderate inflammatory infiltration (perivascular and/or superficial lymphatic aggregates, and small sized lymphatic follicles without germinal center may be observed)	2
	Strong inflammatory infiltration (lymphatic follicles with germinal center and/or confluent subsy novial lymphatic infiltration)	3
Activation of synovial stroma/pannus formation	Absent	0
	Slight synovial stroma activation (low cellularity with slight edema, slight fibrosis with some fibroblast, no giant cells)	1
	Moderate synovial stroma activation (moderate cellularity with a moderate density of fibroblasts, endothelial cells, and giant cells may be detected)	2
	Strong synovial stroma activation (high cellularity with dense distribution of fibroblasts and endothelial cells, and giant cells are abundant)	3

Table S2. Modified Osteoarthritis Research Society International (OARSI) scoring system

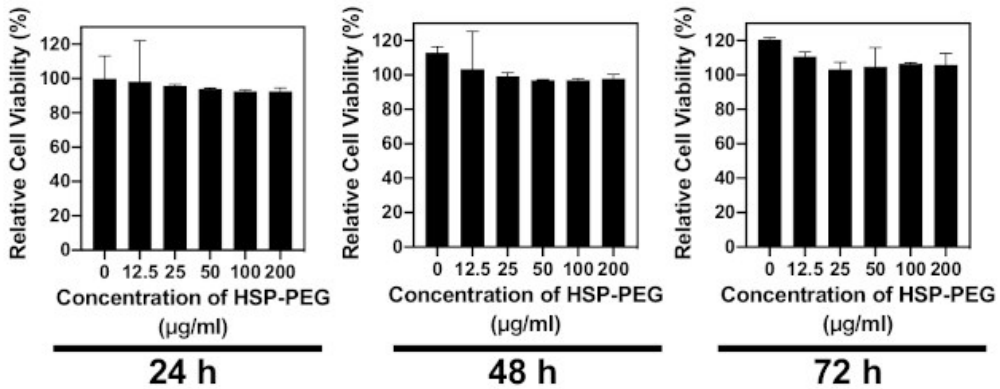
Features		Score
Structure	0. Normal	0
	1. Slight surface irregularities	1
	2. Moderate surface irregularities	2
	3. Severe surface irregularities	3
	4. Clefts/fissures into transitional zone (one-third depth)	4
	5. Clefts/fissures into radial zone (two-thirds depth)	5
	6. Clefts/fissures into calcified zone (full depth)	6
	7. Fibrillation and/or erosion to transitional zone (one-third depth)	7
	8. Fibrillation and/or erosion to radial zone (two-thirds depth)	8
	9. Fibrillation and/or erosion to calcified zone (full depth)	9
	10. Fibrillation and/or erosion to subchondral bone	10
Cellularity	0. Normal	0
	1. Increase or slight decrease	1
	2. Moderate decrease	2
	3. Severe decrease	3
	4. No cells present	4
Chondrocyte cloning	0. Normal	0
	1. Several doublets	1
	2. Many doublets	2
	3. Doublets and triplets	3
	4. Multiple cell nests	4



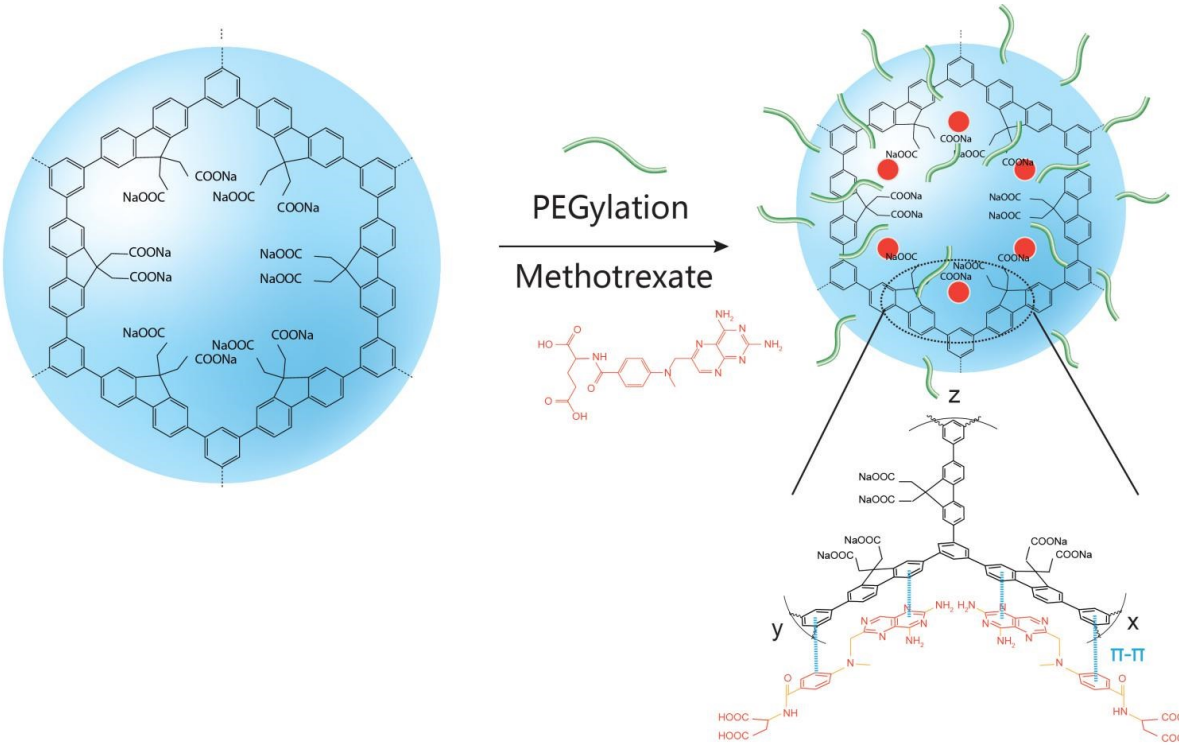
Supporting information Figure S1. A) Representative TEM images of HSP-NPs. B) DLS data of HSP-NPs in PBS.



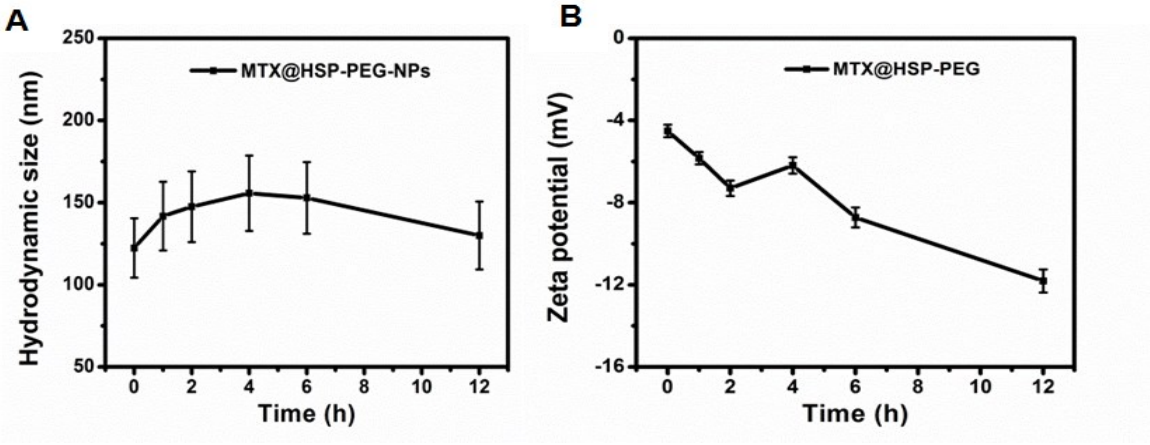
Supporting information Figure S2. A) The photo of HSP-NPs and HSP-PEG-NPs in different solutions including water, PBS, cell medium, and FBS for 24 hours. B) Hydrodynamic diameters of HSP-PEG in different solutions including water, PBS, cell medium, and FBS for 24 hours.



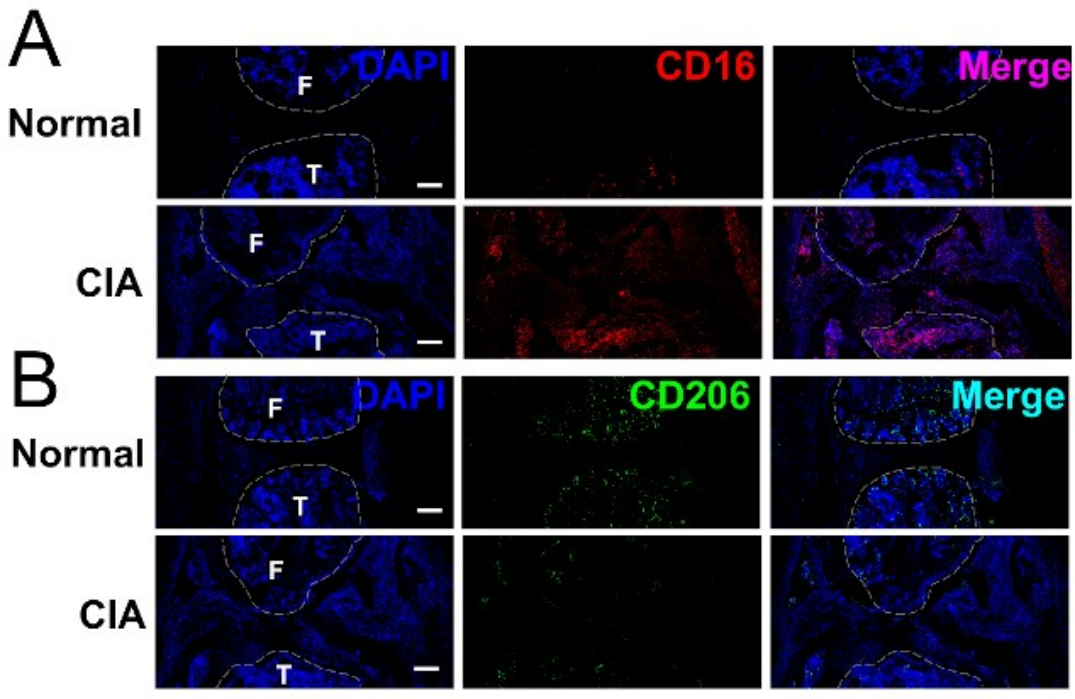
Supporting information Figure S3. The relative cell viabilities of RAW 264.7 cells incubated with different concentrations of HSP-PEG-NPs for 24h, 48h, and 72h. B



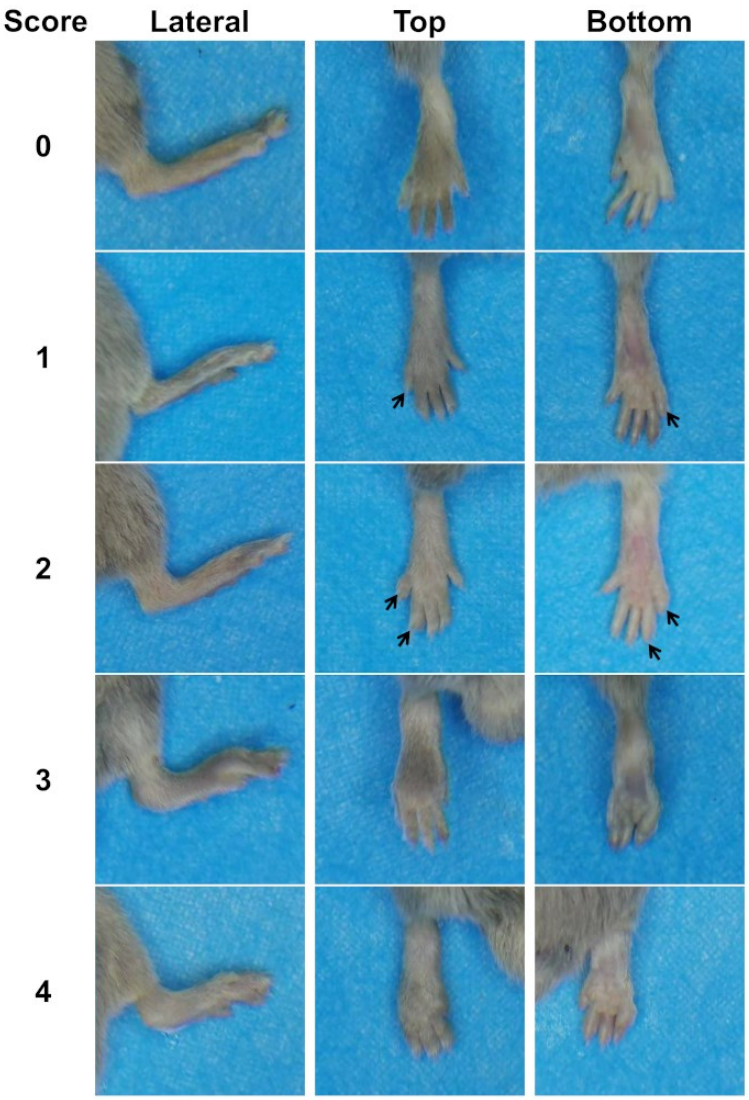
Supporting information Figure S4. Schematic illustration of MTX@HSP-PEG-NPs.



`**Supporting information Figure S5. T**he changes of size (A) and Zeta potential B) of MTX@HSP-PEG-NPs.

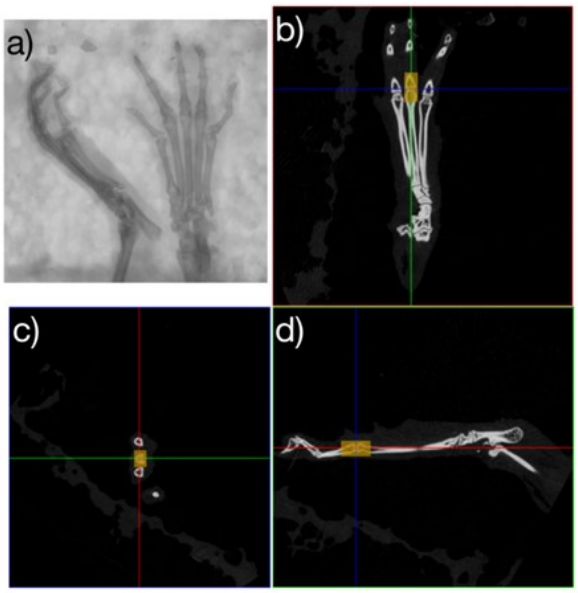


Supporting information Figure S6. (A) CLSM images showing the increase of M1-polarized macrophages labeled with M1 phenotype specific CD16 antibody (red) in the knee joint of CIA mouse 24 hours after injection. Nuclei were stained with DAPI (blue). Scale bars: 100 μm. (B) CLSM images showing the decrease of M2-polarized macrophages labeled with M2 phenotype specific CD206 antibody (green) in the knee joint of CIA mouse 24 hours after injection. Scale bars: 100 μm. F: Femur. T: Tibia.



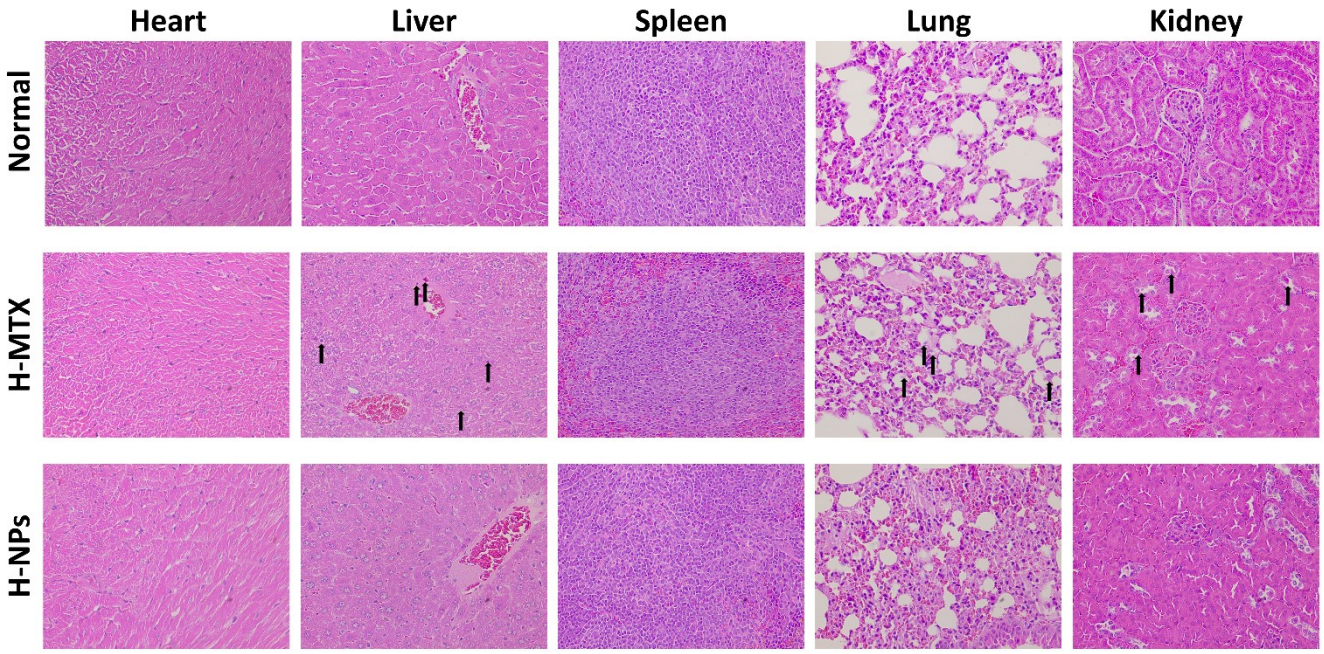
Supporting information Figure S7. Representative images of arthritic paws in CIA mice with clinical scores range from 0 to 4 for each paw. 0): normal paw; 1): One toe with erythema and swollen (black arrow); 2): More than one toe (≥2), but not entire paw with erythema and swollen; 3). Entire paw with erythema and swollen; 4). Very severe erythema and swollen, or ankylosed paw. Each paw is scored in this way, giving a maximum score of 16 per mouse.

Supporting information Figure S8. Representative photo of the means of measuring paw thickness by using a caliper.

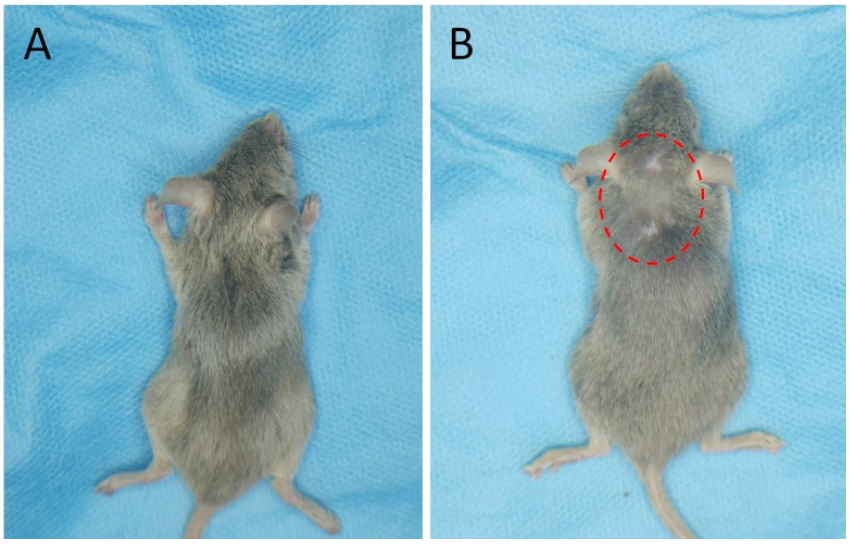


Supporting information Figure S9. a) X-ray transmission image and b-d) 3D navigation view for reference. At each 3D location (shown as cross-hair), three orthogonal views are retrieved from the whole dataset and shown as b) coronal view (the normal images, in x-y plane), c) sagittal view (z-y plane) and d) transaxial view (x-z plane). The volume of interest (VOI) was in an axisymmetric cuboid with a rectangular plane (1.1×0.5 mm) from the top view (c) and a depth of 1.8mm along the longitudinal axis of the 3rd screw (b and d). This volume of interest will be easier to observe and qualitative evaluate the size of bone volume in the 3D reconstructed images.

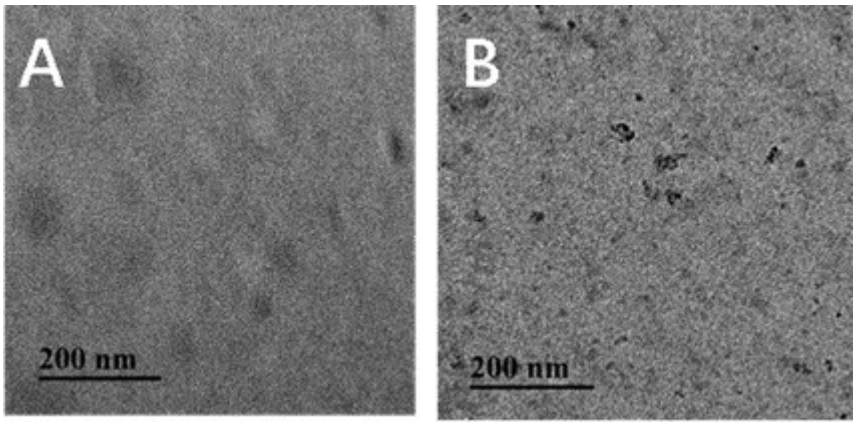
Supporting information Figure S10. The relative cell viabilities of RAW 264.7 cells incubated with different concentrations of free MTX and MTX@HSP-PEG-NPs for 1h, 6h, 12h, and 24h.



Supporting information Figure S11. Representative H&E stained images of major organs (heart, liver, spleen, lung, and kidney) extracted from the mice injected with PBS, 5.0 mg/kg free MTX, or MTX@HSP-PEG-NPs at a dose of 5.0 mg/kg of MTX. Black arrows showed necrotic areas in liver, lung, and kidney. Scale bar: 50 μm.



Supporting information Figure S12. Representative images of CIA mice. A) The mice treated with MTX@HSP-PEG-NPs (5.0 mg/kg of MTX per body weight) exhibited healthy hair throughout entire treatment period. B) The mice treated with free MTX solution (5.0 mg/kg of MTX per body weight) suffered from remarkable hair loss (Red dotted circle).



Supporting information Figure S13. The TEM image of urine collected from CIA mice treated with free MTX (A) and MTX@HSP-PEG-NPs (B) after 6 h.