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Supporting Information

Melanin nanoparticles as an endogenous drug for efficient iron overload therapy in living mice

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Biodistribution of PEG_{2000}-MNP in ICR mice

ICR mice were injected intravenously with 100 μl 3.7 MBq ^{89}Zr-PEG_{2000}-MNP and then anesthetized with isoflurane (n = 6 per group). PET images were acquired for 10 min at 2 and 24 h after injection with the microPET (Inveon, Siemens). The image was reconstructed by a three-dimensional ordered subset expectation maximum (OSEM) algorithm. For each microPET scan, regions of interest (ROIs) over the major organs were drawn on decay-corrected whole-body coronal images using vendor software ASI Pro 6.7.1.1. and the %ID/g was calculated according to the previous report.[1]

Analysis of serum ferritin (SF)

Serum ferritin level was measured by quantitative colorimetric ELISA assay (Nanjing Jiancheng Bioengineering Inst., Nanjing, PR China). The assay was performed in accordance to the given procedure. The absorbance was read at 450 nm using Microplate Reader.

Estimation of iron from Prussian Blue stained tissue sections

On the 21 st day, mice were sacrificed and organs (heart, liver, spleen, lung, kidney and pancreas) were collected. They were washed with ice cold saline to remove blood and preserved in 10% neutral buffered formalin. For analysis of iron content, Prussian blue stained histological tissue sections were also utilized. Optical micrographs of the tissue sections were acquired at

40 × magnification. About 8 images from different areas of the tissue sections were used to calculate the blue stained spots, and the total area of blue spots of each micrograph was determined by Image-Pro plus software.

Statistical analysis. Statistical analysis was performed with SPSS 18.0, and one-way analysis of variance was used to determine statistical differences among each group. Paired comparisons were considered significant if $p < 0.05$. All data were expressed as mean ± SD unless otherwise specified.

Table S1. The data of hydrodynamic sizes of MNPs in aqueous solution.

MNP	Diameter (nm)
MNP	4.9 ± 1.1
PEG_{1000}-MNP	6.5 ± 0.5
PEG_{2000}-MNP	6.9 ± 1.3
PEG_{5000}-MNP	7.5 ± 1.1
PEG_{1000}-MNP -Fe	10.8 ± 2.3
PEG_{2000}-MNP -Fe	7.8 ± 1.6
PEG_{5000}-MNP -Fe	8.2 ± 1.2

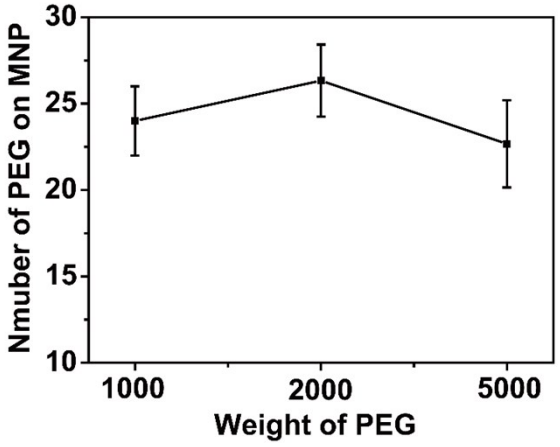

Figure S1. The relationship between the weight of the PEG and the number of PEG chain on every PEG-MNP.

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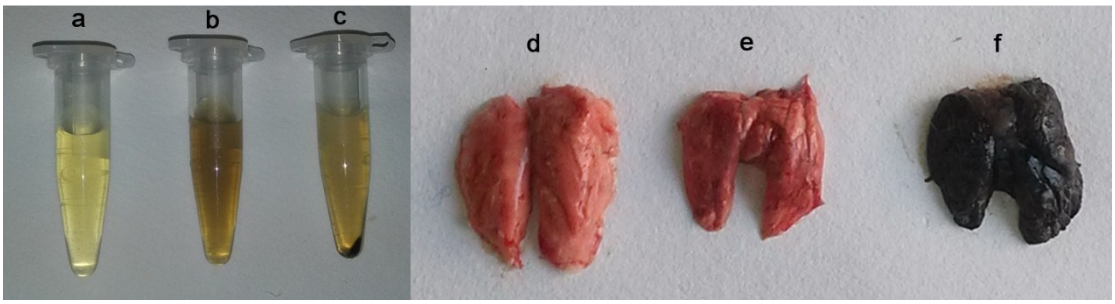

Figure S2. Comparative study of PEG-functionalized MNPs and naked MNPs *in vitro* (Iron-containing plasmas by adding a. PBS; b. PEG_{2000}-MNP; c. naked MNPs) and *in vivo* (the lungs of the mice injected with d. Saline; e. PEG_{2000}-MNP; f. naked MNPs.)

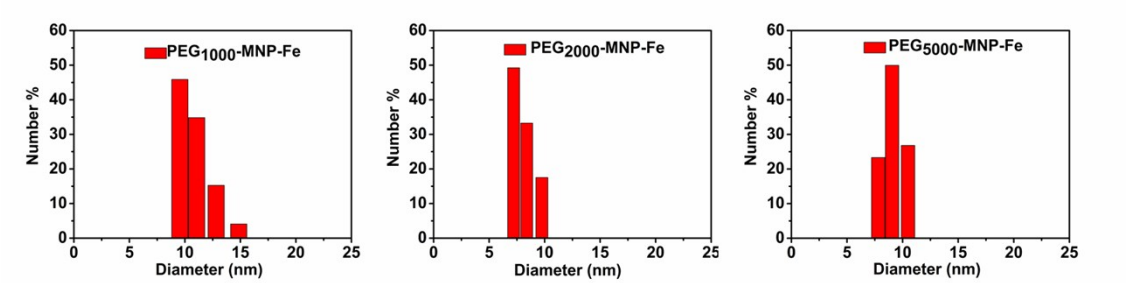

Figure S3. DLS of PEG_{1000}-MNP-Fe, PEG_{2000}-MNP-Fe and PEG_{5000}-MNP-Fe.

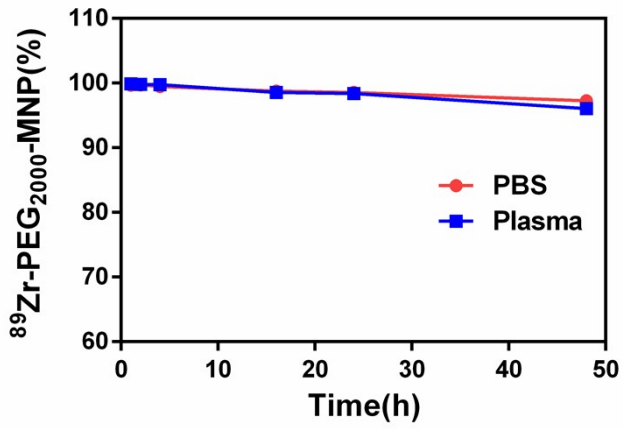

Figure S4. The stability of ^{89}Zr-labeled PEG_{2000}-MNP in plasma and PBS.

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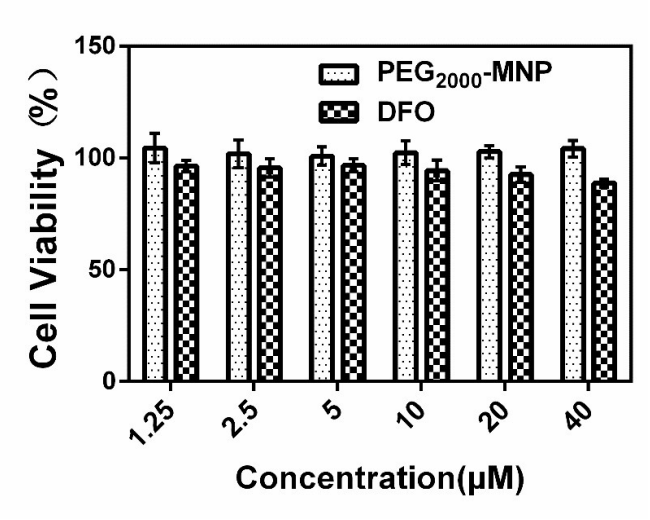

Figure S5. MTT assay using NIH-3T3 cells with PEG_{2000}-MNP and DFO at concentrations of 1.25, 2.5, 5, 10, 20 and 40 μM for 24 h incubation.

REFERENCES :

[1] M. Yang, Q. Fan, R. Zhang, K. Cheng, J. Yan, D. Pan, X. Ma, A. Lu, Z. Cheng, *Biomaterials* **2015**, *69*, 30.