Supporting Information

Cationic poly-l-lysine-encapsulated melanin nanoparticles as efficient photoacoustic agents targeting to glycosaminoglycans for early diagnosis of articular cartilage degeneration in osteoarthritis

Liang Chen,‡[a] Ji Yu,‡[b] Xiaoming Hu,[a] Cao Cui,[c] Hong Liu,[a] Yufu Tang,[b] Baiwen Qi,[a] Yahui Niu, [a]Xiang Hu,*[a] Aixi Yu,*[a] Quli Fan,*[b]

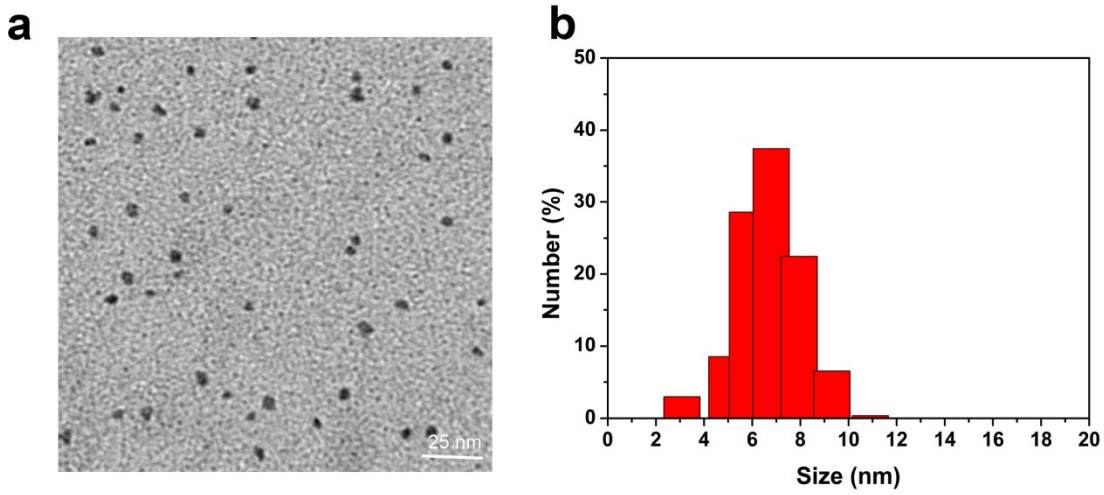

Fig.S1. (a) Representative TEM images and (b) DLS of MNPs in PBS (PH = 7.4) solution.

Table S1. The data of hydrodynamic size and zeta potentials of MNPs and PLL-MNPs in aqueous solution.

	Diameter (nm)	Zeta potential (mV)
MNP	5.8±2.1	-22.2±12.1
PLL-MNP	42.5±1.6	+32.5±9.3

Stability of PLL-MNPs

We generally use the change of the hydrodynamic size distribution of PLL-MNPs in PBS with time to evaluate its stability. The PLL-MNPs (1 mg) were dispersed to the solution with 50% PBS (20 mL). Its hydrodynamic size was collected at 0 h, 6 h, 12 h, and 24 h by DLS. After 24 h dispersed, it was showed that the hydrodynamic sizes of PL-MNPs and the polydispersities were little changed. Thus, we concluded that the PL-MNPs are likely to be stable in PBS (Fig. S3).

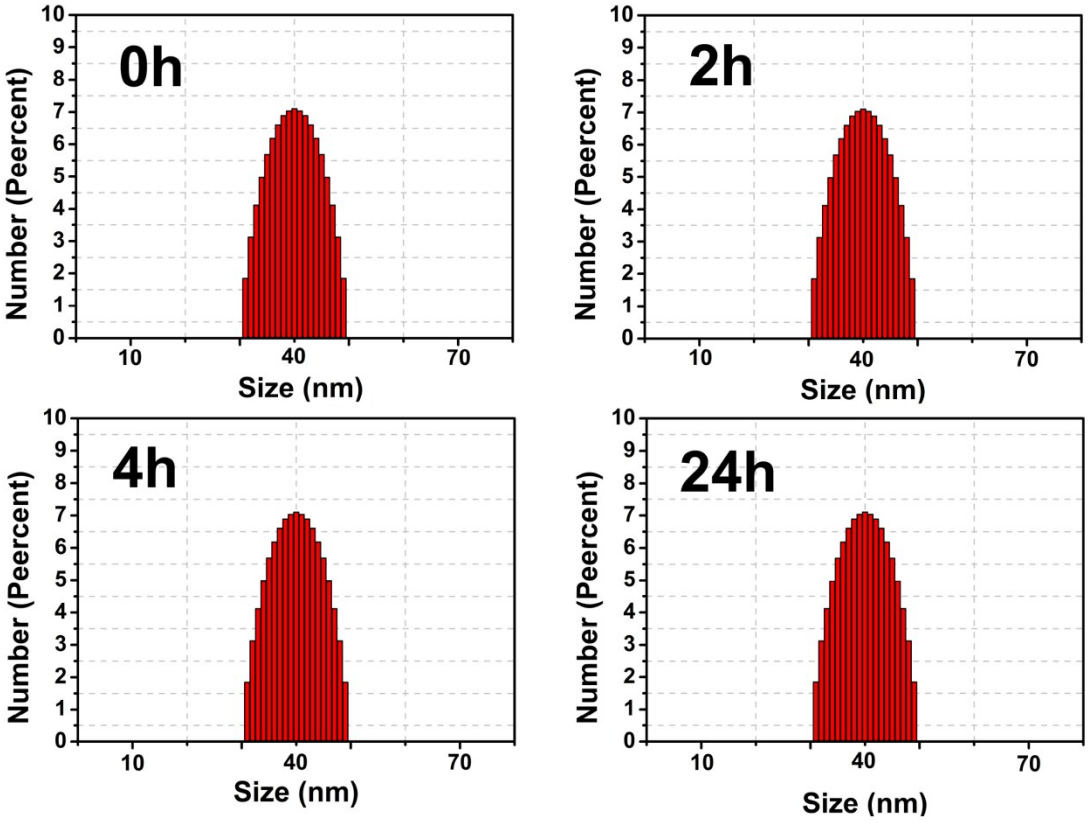

Fig. S2. Stability of the PLL-MNPs hydrodynamic size during different incubation time in PBS.

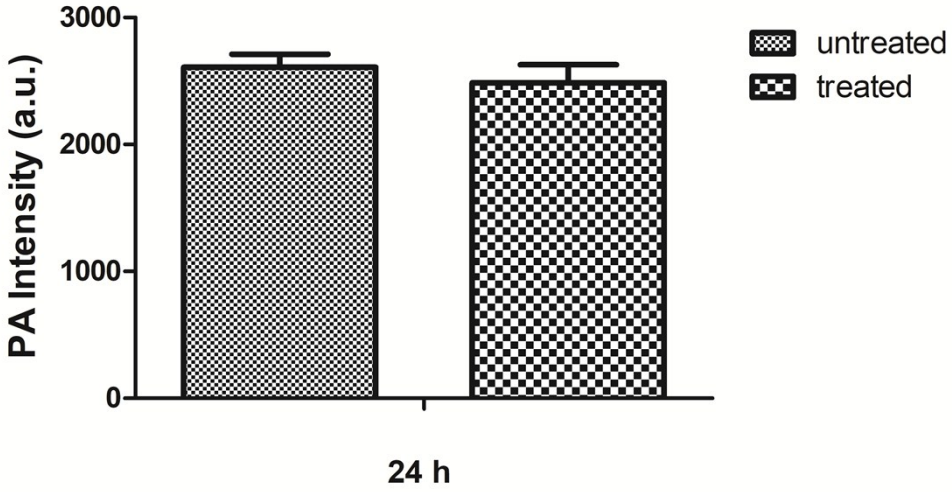

Fig. S3. The corresponding PA intensity of the collagenase treated and untreated cartilage 24 h after incubated with PLL-MNPs. There were no obvious differences of

PA intensity between the two groups ($p > 0.05$).

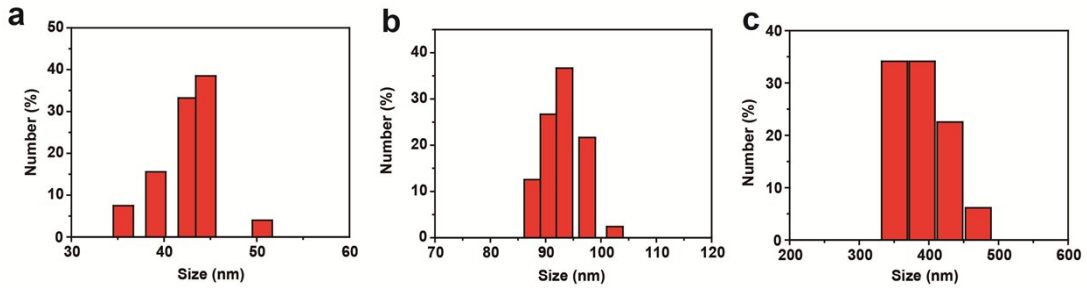

Fig. S4. (a) DLS of PLL-MNPs in PBS (without proteoglycans) solution. (b) DLS of proteoglycans in PBS solution. (c) DLS of PLL-MNPs in PBS (with proteoglycans) solution.

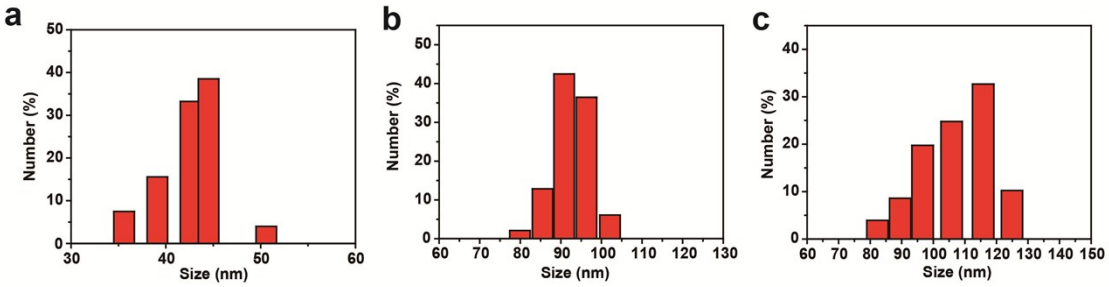

Fig. S5. (a) DLS of PLL-MNPs in PBS solution. (b) DLS of collagen type II in PBS solution. (c) DLS of PLL-MNPs in PBS (with collagen type II) solution.

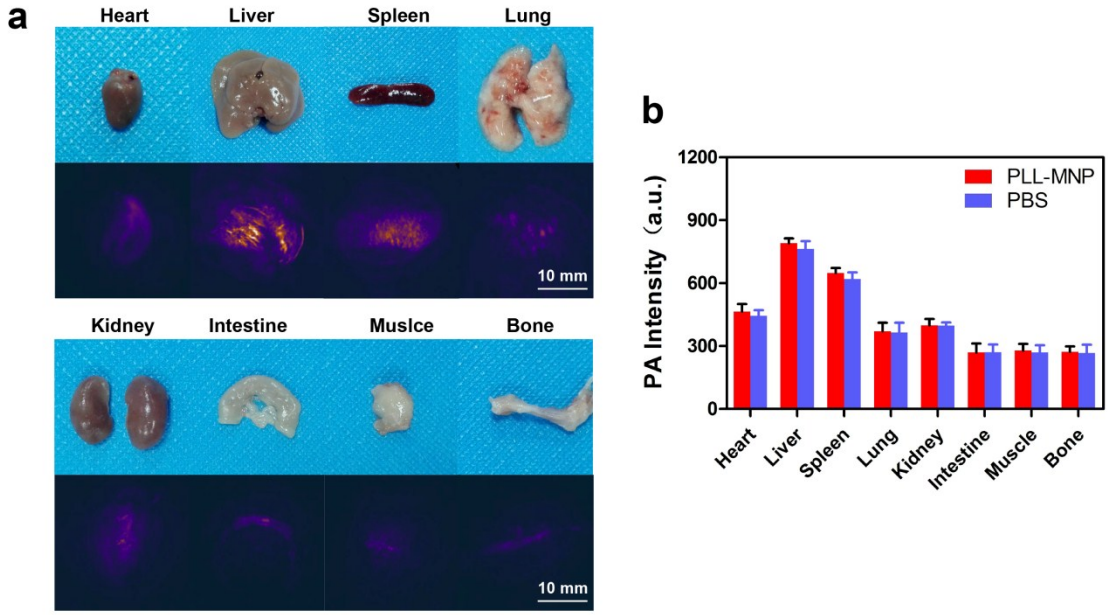

Fig. S6. *Ex vivo* PAI of major organs 24 h after intra-articular injection of PLL-MNPs

or PBS. (a) Photograph and PA image of major organs resected 24 h after PLL-MNPs injection. (Heart, liver, spleen, lung, kidney, intestine, muscle and bone). (b) PA quantification of major organs. The PA data were acquired at 680 nm. Error bars were based on Standard Deviation (SD) (n = 3)

Biochemical assessment of GAG content in vitro

Cartilage samples were prepared for biochemical analys is of GAGs content (total GAG content per mg cartilage wet weight) using the1, 9-dimethylmethylene blue (DMMB) colorimetric assay (Sigma St Louis, MO, USA).[1] Cartilage including deep zone, was separated from the subchondral bone using a razorblade. The wet samples were weighed and lyophilized for 24 h, and the dry weights of lyophilized tissues were obtained. The resulting samples were digested in papain (Sigma) solution (0.5 mg mL^{-1} in 0.05 mM sodium phosphate, 2 mM dithiothreitol (DTT), 1 mM ethylene diaminete traacetic acid (EDTA), pH 8.0) at 65 ^{o}C for 24 h, and they were then diluted by PBS (10 mM, pH = 7.4) from 10 to 100 times for the following assays.[2] Chondrointin-4-sulfate (Sigma) was used for calibration in this study. It was dissolved in PBS at concentrations from 10 to 100 mg mL^{-1}, in order to derive a calibration curve. Each chondroitin-4-sulfate calibration solution (10 mL) was added with100 mL of DMMB solution (40 mM NaCl; 40 mM glycine; 46 mM DMMB, pH 3.0) in a 96-wellplate. The absorbance at 520 nm was monitored using a plate reader (TECAN, Morrisville, NC). The total GAG weight per milligram wet weight of the cartilage for each sample was calculated.

REFERENCES

1. R. W. Farndale, C. A. Sayers and A. J. Barrett, *Connective tissue research*, 1982, 9, 247-248.
2. P. N. Bansal, N. S. Joshi, V. Entezari, B. C. Malone, R. C. Stewart, B. D. Snyder and M. W. Grinstaff, *Journal of orthopaedic research : official publication of the Orthopaedic Research Society*, 2011, 29, 704-709.