

Electronic Supplementary Material for

Polyaspartic Acid Coated Manganese Oxide Nanoparticles for Efficient Liver MRI

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1. Experimental Section

1.1 Preparation of MnO nanoparticles (MONPs)

The 25 nm MONPs were synthesized by following a previously published protocol.¹⁻² 1.24 g Mn-oleate was added into 10 mL of 1-octadecene (Aldrich Chemical Co., 90 %) to yield a transparent red solution. The mixture solution was heated to 160 °C and stay at this temperature at Ar₂ protection for 1 h to remove water and oxygen and then heated to 300 °C with vigorous stirring. The reaction system was maintained at 300 °C for 1 h and then cooled down to room temperature. 20 mL of hexane was added into the product, followed by the addition of 80 mL acetone to precipitate the nanoparticles. The precipitate was collected by centrifugation and washed for two more times to remove excess surfactant and solvent. As the reaction time increased from 1 hr to 2 h, the particle size of the MnO nanoparticles increased from 25 nm to 35 nm. When 1-hexadecene was used as a solvent instead of 1-octadecene and the aging was performed at 280 °C, and remained this temperature for 10 min, 10 nm particles were obtained.

Mn-oleate complex was prepared by following the previously protocol, 1.98 g manganese chloride tetrahydrate (MnCl₂•4H₂O, 10 mmol, Sigma-Aldrich, 98 %) and 6.09 g of sodium oleate (20 mmol, TCI, 95 %) were added to a mixture composed of 10 mL of ethanol, 40 mL of distilled water and 50 mL of n-hexane. The resulting mixture was heated to 70 °C and stirred overnight. The final product is washed several times by using water, then heated to 50 °C to remove hexane.

1.2 Preparation of PASP-MONPs

2 mg oleic acid stabilized MONPs in hexane were dried under argon and redispersed in 1 mL CHCl₃, and was added into a PASP solution (1 mg in a mixture of 1 mL water and 1 mL methanol). The mixture was stirred and heated at 45 °C for 1 h and then cooled to room temperature. After stirring for another 3 h, the nanoparticles in the Supernatant were collected, added more Ethanol and precipitated by centrifugation at 8,000 g for 10 min. Then the nanoparticles were washed another two times by

adding Methanol centrifuged at 14,000 g for 30 min. The excess PASP was removed by using centrifugal filters(Amicon Ultra-0.5ml 100 K) at 8,000 g for 10 min(three times). The water-soluble PASP-MONPs were characterized by Fourier transform infrared (Perkin-Elmer spectrum GX spectrophotometer), DLS(Zetasizer Nano series (Zen3600) from Malvern with zetasizer software 6.0 as the interface), TEM(FEI Tecnai12).

1.3 Phantom studies with MONPs

T_1 relaxivities of PASP- and phospholipid-PEG- MONPs were assessed on a 7.0 T small animal MRI scanner (GE Healthcare). The particles were dispersed in 1 % agarose gel in 0.2 mL tubes with elevated Mn concentrations(measured by ICP-AES). The MR images were acquired with the following spin echo sequence: TE = 4.5 ms, TR = 250 ms, thickness = 1 mm, FOV = 3cm × 3cm, Echo 1/1.

1.4 Balb/c mice in vivo MRI study

Normal, healthy Balb/c mice weighing 20-30 g were anesthetized by breathing 2 % isoflurane in oxygenenriched air with a facemask, The PASP-MONPs were administered into the tail vein of each mice with a dose of 5 mg Mn (measured by ICP-AES) per kg of mice body weight. The T_1 -weighted MR images of the liver were obtained with a TSE technique using a 7.0 T small animal MRI scanner (GE Healthcare) equipped with a homemade surface coil. T_1 weighted fast spin-echo images were acquired before, and 10 min, 4 h, 24 h after administration of the MONPs in 3 animals, with the following parameters: TE = 4.5 ms, TR = 250 ms, thickness = 1 mm, FOV = 3cm × 3cm, Echo 1/1. The signal intensities in defined ROIs were analyzed by Image J (National Institutes of Health).

2. Supplementary results

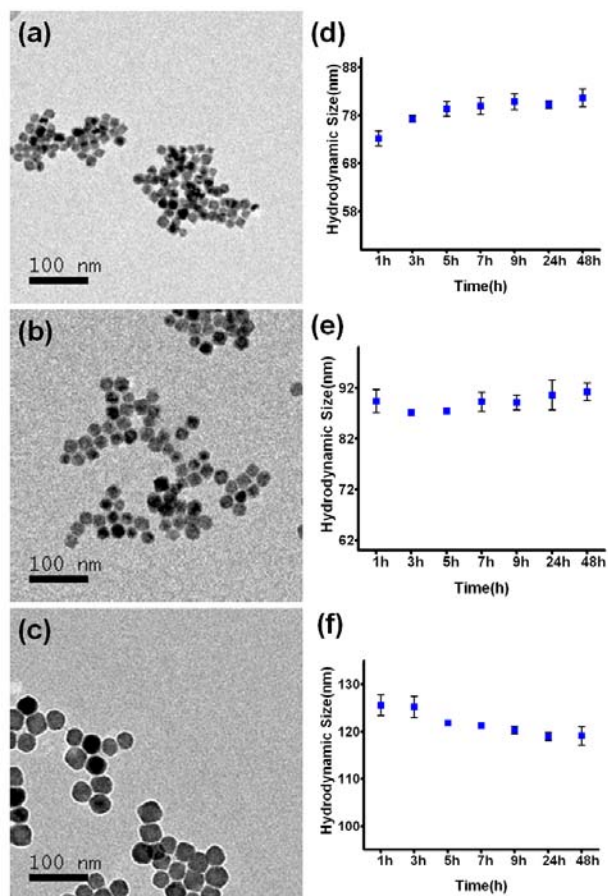


Fig. S1 TEM images of PASP-MONPs with three kinds of core sizes: (a) 10 nm; (b) 25 nm; (c) 35 nm (Due to particle drying on TEM grid, PASP-MONPs are closely located). Size change of PASP-MONPs when incubated in PBS(with 10 % serum) at 37 °C for 48 h, monitored by DLS with three kinds of core size: (d) 10 nm; (e) 25 nm; (f) 35 nm.

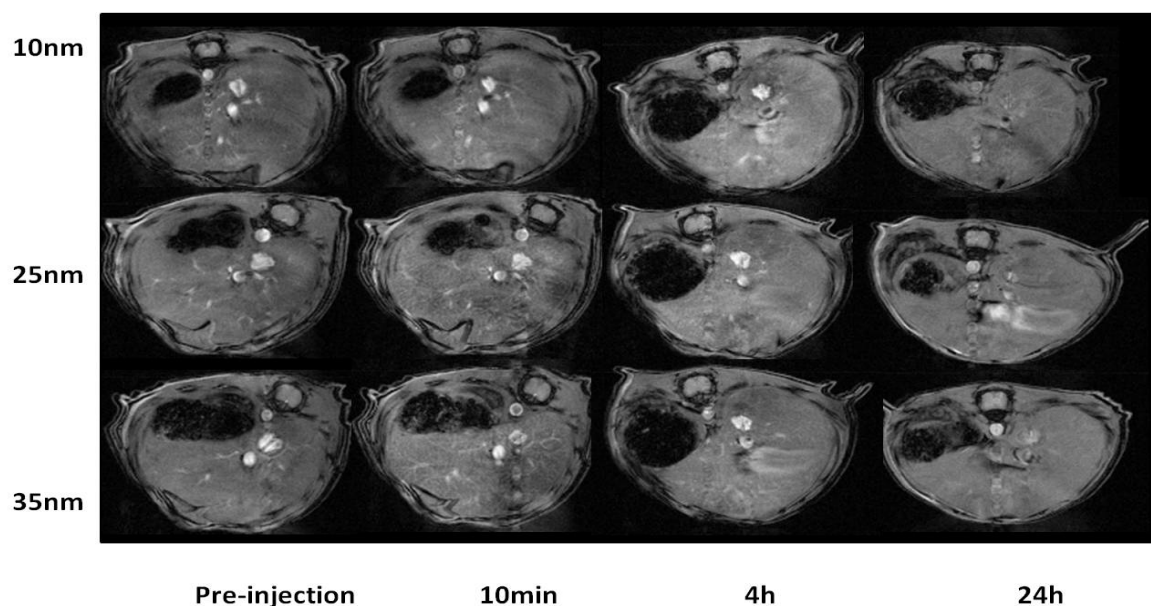


Fig. S2 *In vivo* mice liver MR images at different time points after intravenous administration of PASP-MONPs at a dose of 5 mg/kg.

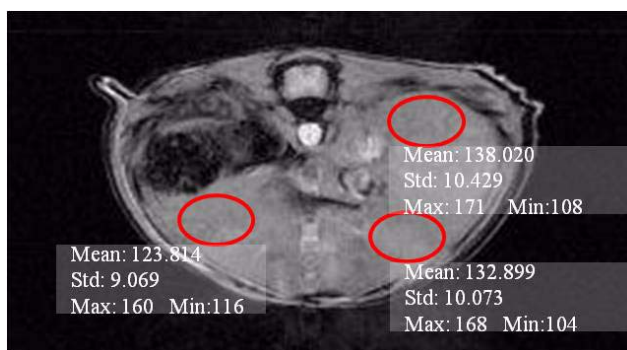


Fig. S3 Examples of regions of interest (ROIs) selected on an in vivo MR image at mice liver site.

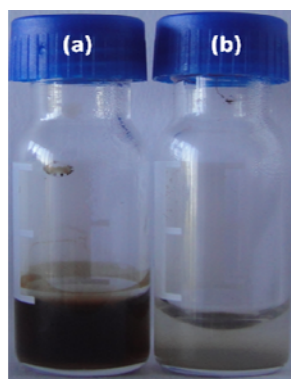


Fig. S4 After being placed for 4 months, compared to (a)PASP-MONPs, (b)PL-PEG-MONPs showed a lot of aggregation.

1. J. H. Lee, H. B. Na, K. J. An, Y. I. Park, M. Park, I. S. Lee, D. H. Nam, S. T. Kim, S. H. Kim, S. W. Kim, K. H. Lim, K. S. Kim, S. O. Kim and T. Hyeon, *Angew Chem Int Edit*, 2007, **46**, 5397-5401.
2. J. Park, K. An, Y. Hwang, J. G. Park, H. J. Noh, J. Y. Kim, J. H. Park, N. M. Hwang and T. Hyeon, *Nat Mater*, 2004, **3**, 891-895.