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Polyaspartic acid coated manganese oxide nanoparticles for efficient liver MRI†

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Abstract

We report in this communication a simple, facile surface modification strategy to transfer hydrophobic manganese oxide nanoparticles (MONPs) into water by using polyaspartic acid (PASP). We systematically investigated the effect of the size of PASP-MONPs on MRI of normal liver and found that the particles with a core size of 10 nm exhibited greater enhancement than those with larger core sizes.

With the rapid development of nanotechnology, inorganic nanoparticles (NPs) have shown great promise for diagnostic and therapeutic applications.^{1,2} Monodispersed NPs with uniform and controlled shape and size are typically synthesized by high temperature thermal decomposition methods.³ The as-synthesized hydrophobic nanoparticles always need to be functionalized with biocompatible molecules to allow water solubility. For manganese oxide nanoparticles (MONPs) which have been developed as T_1 contrast agents for magnetic resonance imaging (MRI), several polymer materials have been chosen for surface coating, the most common one being PEGylated phospholipid (PL-PEG).^{4–6} However, the r_1 relaxivity of PL-PEG coated MONPs is rather low (for example, the r_1 value of 20 nm MONPs reported by the Hyeon group⁶ was as low as $0.13 \text{ mM}^{-1} \text{ s}^{-1}$ on a 3.0 TMRI). Previously, our group reported that HSA coated MONPs showed a high r_1 of $1.97 \text{ mM}^{-1} \text{ s}^{-1}$.⁷ However, HSA coated MONPs are not highly water-soluble and tend to precipitate over time during storage. It is envisioned that surface coating may have a profound effect on the r_1 relaxivity of the MONPs and several different coating strategies have thus been tested to improve the r_1 relaxivity with moderate success.

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Polyaspartic acid (PASP) is a biocompatible and biodegradable polymer which has been used to coat quantum dots (QDs) and iron oxide nanoparticles.^{8–10} Compared to the hydrophobic inner layer of PL-PEG, it seems that the PASP layer is more hydrophilic and compact and thus more appropriate for MONPs coating and exerts efficient water exchange.

Here in this study, we systematically synthesized MONPs with three different core sizes (approximately 10 nm/25 nm/35 nm) *via* a thermal decomposition method¹¹ and coated with PASP *via* a simple, facile way to successfully transfer the hydrophobic MONPs into water. We examined size-dependent T_1 effect of PASP-MONPs and compared them with those coated with PL-PEG. PASP-MONPs were then applied for mouse liver imaging on a 7.0 T small animal MR scanner. To synthesize PASP-MONPs, 2 mg oleic acid stabilized MONPs (see ESI[†] for particle synthesis) in hexane were dried under argon and redispersed in 1 ml CHCl_3 , and were 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, nanoparticles in the supernatant were collected and purified by centrifugation, then redispersed in water. Representative TEM images of three different sized MONPs dispersed in hexane are shown in Fig. 1a–c. Fig. 1d shows that PASP was successfully coated onto the surface of MONPs to render water solubility. TEM images (Fig. S1[†]) found little morphological change to the MONP cores after coating with PASP. The dynamic light scattering (DLS) analysis showed that the hydrodynamic sizes of the PASP-MONPs in water with core sizes of 10, 25, and 35 nm were 43.82 ± 1.82 , 68.06 ± 0.95 , and 91.28 ± 3.73 nm, respectively (Fig. 2a and Table 1). Zeta potential results showed that PASP-MONPs were negatively charged (Fig. 2b) due to the multiple carboxyl groups on the particle surface, which was not affected by the core size of the MONPs. To test the stability of the particles in biological conditions, the PASP-MONPs at a lower concentration were observed during a 48 h incubation period in 10% serum (10% serum in PBS). Although the hydrodynamic size increased because of the serum adsorption, no obvious core size change was found (Fig. S1[†]).

To evaluate the T_1 reducing effect, the PASP-MONPs at different Mn concentrations were dispersed in 1% agarose and were investigated on a 7.0 T small animal MR scanner. Phantom images (Fig. 3a) demonstrated that T_1 signal intensity increased significantly with increasing Mn concentration. The T_1 enhancement is dependent on efficient interactions between water and surface manganese ions.¹² For NP-based T_1 contrast agents, a high surface to volume ratio (P) is essential because only the surface cations are active in affecting the longitudinal relaxation times of water protons.¹³ Smaller NP core size means a large P value and therefore, a higher r_1 relaxivity is expected (Fig. 3b). To further evaluate the advantages of PASP coating, MONPs with PL-PEG coating were subjected to phantom studies, and the results are shown in Fig. 3c and Table 1. Taking 10 nm MONPs for example, the r_1 relaxivity of PL-PEG-MONP-10 was $0.37 \text{ mM}^{-1} \text{ s}^{-1}$, while that of PASP-MONP-10 was $1.29 \text{ mM}^{-1} \text{ s}^{-1}$. This increase is attributed to the more hydrophilic nature of PASP coating. Unlike the case of PL-PEG, there is no hydrophobic zone between particle and the surroundings when using PASP as the coating. This led to a more effective water–manganese interaction and therefore, a higher r_1 .

Since PASP-MONPs have a relatively high T_1 shortening effect, we further assessed the *in vivo* performances of these particles in normal, healthy Balb/c mice on a 7.0 T small animal MRI scanner. The PASP-MONPs were intravenously (i.v.) injected at a dose of 5 mg kg^{-1} . T_1 -weighted MR images of the liver were taken at 0, 10 min, 4 h and 24 h post-injection (p.i.) to assess the signal enhancement. It was found that PASP-MONPs-10 induced the highest hyperintensities among all the three formulae at 4 h p.i. (Fig. S2[†]). To further

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quantify the contrast enhancement, we assessed the signal-to-noise ratio (SNR) of the liver at different time points. The regions of interest (ROIs) (ESI, Fig. S3[†]) were selected based on a previously reported protocol.¹⁴ Liver SNR was defined as $SNR_{liver} = SI_{liver}/SD_{noise}$ (where SI stands for signal intensity and SD stands for standard deviation). The average relative liver signal intensities of mice (SNR_{post}/SNR_{pre}) were plotted at different time points (Fig. 4a). Relative contrast enhancement, defined as signal increase $\Delta SNR = (SNR_{post} - SNR_{pre})/SNR_{pre}$, was also plotted at different time points (Fig. 4b). The signal intensity of the liver with PASP-MONPs was slightly increased at 10 min time point, reached the maximum at 4 h, and then decreased over time. Among the three sized nanoparticles, PASP-MONPs-10 showed the highest signal intensity increase at 4 h time point, with a ΔSNR value of 0.72 ± 0.06 , which is presumably due to the highest P and r_1 relaxivity of the PASP-MONPs-10.

Conclusions

In conclusion, hydrophobic MONPs were successfully transferred into water by coating with poly(aspartic acid) (PASP). These PASP-MONPs exhibit higher r_1 relaxivities over PL-PEG coated ones due to amore hydrophilic and compact coating, and associated with that, more efficient water–manganese interactions. The r_1 relaxivity decreases with the increase of MONP size. *In vivo* liver MRI with small animal models found that the smallest particles among all tested (PASP-MONPs-10) induced the most prominent hyperintensities. All these results indicated that PASP-MONPs-10 is an effective T_1 contrast agent for liver MRI. It is possible to conjugate the formula with targeting molecules to achieve site-specific targeting. The related research is underway.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

1. Nie S, Xing Y, Kim GJ, Simons JW. *Annu. Rev. Biomed. Eng.* 2007; 9:257–288. [PubMed: 17439359]
2. Hao R, Xing R, Xu Z, Hou Y, Gao S, Sun S. *Adv. Mater.* 2010; 22:2729–2742. [PubMed: 20473985]
3. Jun YW, Lee JH, Cheon J. *Angew. Chem., Int. Ed.* 2008; 47:5122–5135.
4. Park TG, Bae KH, Lee K, Kim C. *Biomaterials.* 2011; 32:176–184. [PubMed: 20934746]
5. Tremel W, Schladt TD, Shukoor MI, Schneider K, Tahir MN, Natalio F, Ament I, Becker J, Jochum FD, Weber S, Kohler O, Theato P, Schreiber LM, Sonnichsen C, Schroder HC, Muller WEG. *Angew. Chem., Int. Ed.* 2010; 49:3976–3980.
6. Lee JH, Na HB, An KJ, Park YI, Park M, Lee IS, Nam DH, Kim ST, Kim SH, Kim SW, Lim KH, Kim KS, Kim SO, Hyeon T. *Angew. Chem., Int. Ed.* 2007; 46:5397–5401.
7. Huang J, Xie J, Chen K, Bu L, Lee S, Cheng Z, Li X, Chen X. *Chem. Commun.* 2010; 46:6684–6686.
8. Jana NR, Erathodiyil N, Jiang J, Ying JY. *Langmuir.* 2010; 26:6503–6507. [PubMed: 20112990]
9. Zheng Y, Yang W, Wang C, Hu J, Fu S, Dong L, Wu L, Shen X. *Eur. J. Pharm. Biopharm.* 2007; 67:621–631. [PubMed: 17533123]

10. Held GA, Euliss LE, Grancharov SG, O'Brien S, Deming TJ, Stucky GD, Murray CB. *Nano Lett.* 2003; 3:1489–1493.
11. Park J, An K, Hwang Y, Park JG, Noh HJ, Kim JY, Park JH, Hwang NM, Hyeon T. *Nat. Mater.* 2004; 3:891–895. [PubMed: 15568032]
12. Baek MJ, Park JY, Xu W, Kattel K, Kim HG, Lee EJ, Patel AK, Lee JJ, Chang Y, Kim TJ, Bae JE, Chae KS, Lee GH. *ACS Appl. Mater. Interfaces.* 2010; 2:2949–2955. [PubMed: 20929249]
13. Park JY, Baek MJ, Choi ES, Woo S, Kim JH, Kim TJ, Jung JC, Chae KS, Chang Y, Lee GH. *ACS Nano.* 2009; 3:3663–3669. [PubMed: 19835389]
14. Huang J, Bu L, Xie J, Chen K, Cheng Z, Li X, Chen X. *ACS Nano.* 2010; 4:7151–7160. [PubMed: 21043459]

Fig. 1.
(a–c) TEM images of as-synthesized MnO nanoparticles ((a) 10 nm; (b) 25 nm; (c) 35 nm).
(d) PASP-MONPs were successfully transferred from hexane to water phase.

Fig. 2.
(a) Hydrodynamic diameter and (b) zeta potential comparison of different sized PASP-MONPs.

Fig. 3.

(a) T_1 -weighted MR images of PASP-MONPs in 1% agarose gel with various concentrations at 7 T; (b) $1/T_1$ versus Mn concentration with PASP-MONPs; (c) $1/T_1$ versus Mn concentration with PL-PEG-MONPs.

Fig. 4. (a) Quantification of relative SNR_{liver} collected before and after administration; (b) relative contrast enhancement (ΔSNR) of different sized PASP-MONPs after being injected into healthy Balb/c mice.

Table 1

The average hydrodynamic particle size, zeta potential, and r_1 relaxivity of PASP-MONPs

	PASP-MONPs			PEG-MONPs
	HD size/nm	Zeta/mV	$r_1/\text{mM}^{-1} \text{s}^{-1}$	$r_1/\text{mM}^{-1} \text{s}^{-1}$
MONPs-10	43.82 ± 1.82	-22.9 ± 0.27	1.29	0.37
MONPs-25	68.06 ± 0.95	-21.8 ± 0.92	1.06	0.22
MONPs-35	91.28 ± 3.73	-22.2 ± 0.71	0.88	0.12