Iron phosphide nanoparticles as a pH-responsive $T_1$ contrast agent for magnetic resonance tumor imaging†

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In this work, the potential of FeP nanoparticles as a pH-responsive $T_1$ contrast agent was investigated. The FeP nanoparticles have good biocompatibility and can significantly amplify $T_1$ magnetic resonance signals in response to the acidic microenvironment of solid tumors, holding great promise in serving as an acid-activatable $T_1$ contrast agent for tumor imaging.

Results and discussion

We first synthesized the FeP nanoparticles by a thermal decomposition method using Fe(acac)₃ as the iron precursor and triethylphosphine (TOP) as the phosphide precursor. To render the as-synthesized FeP nanoparticles water-soluble and biocompatible, we then modified these nanoparticles with poly(ethylene glycol) (PEG). Transmission electron microscopy (TEM) image shows that the FeP nanoparticles have a small size with the average particle size of 9.60 ± 1.73 nm (Fig. 1a). High-resolution TEM (HRTEM) image clearly reveals the lattice spacing of FeP nanoparticles, indicating the crystalline nature of the nanoparticles (Fig. 1a inset). The measured lattice spacing is about 0.27 nm, corresponding to the (011) plane of...
FeP. TEM-associated energy-dispersive X-ray spectroscopy (EDS) shows typical peaks of Fe and P (Fig. S1†). Moreover, X-ray diffraction (XRD) pattern confirms that the crystal phase of the as-synthesized nanoparticles is FeP (JCPDS no. 01-078-1443). These results suggest that FeP nanoparticles have been successfully synthesized. Fourier transform infrared (FTIR) spectrum presents the typical asymmetric and symmetric stretching bands (2918 cm\(^{-1}\) and 2850 cm\(^{-1}\)) and –C–O–C group vibrations (1000–1500 cm\(^{-1}\)), confirming the successful modification of PEG (Fig. S2†). \(^{26}\) Dynamic light scattering (DLS) measurements were used to investigate the hydrodynamic diameter of FeP nanoparticles (Fig. S3†). The hydrodynamic diameters of FeP nanoparticles in various solutions including water, phosphate buffered saline (PBS), and fetal bovine serum (FBS) are in the range of 20–25 nm. Furthermore, these hydrodynamic diameters have no obvious change over at least 7 days, indicating the good stability of FeP nanoparticles.

To investigate the pH-responsive \(T_1\) MRI performance of FeP nanoparticles, we dispersed the nanoparticles in buffers with different pH values and conducted the measurements. We first collected the \(T_1\)-weighted phantom images (Fig. 2a). Significant brighten signals can be detected when FeP nanoparticles are dispersed in acidic buffers (pH 5.0 and pH 6.0), suggesting that FeP nanoparticles generate \(T_1\) contrast enhancement at acidic conditions. In contrast, no obvious brighten signals are measured at pH 7.4, demonstrating that FeP nanoparticles have little contrast enhancement effect under neutral conditions. We then measured the longitudinal relaxivity \(r_1\) values of FeP nanoparticles (Fig. 2b). FeP nanoparticles have a relatively low \(r_1\) value (\(\sim 0.2 \text{ mM}^{-1}\ \text{s}^{-1}\)) at pH 7.4, and the value show little change over time, suggesting FeP nanoparticles have little \(T_1\) shortening effect under neutral conditions. In contrast, a gradual enhancement in \(r_1\) values can be observed when FeP nanoparticles are in acidic buffers. For example, the \(r_1\) value of FeP nanoparticles increases to 4.6 ± 0.2 mM\(^{-1}\) s\(^{-1}\) for pH 5.0 at 24 h. This value is close to that of commercial Gd-based MRI contrast agents such as Gd-DTPA and Gd-DOTA (4–5 mM\(^{-1}\) s\(^{-1}\) at 0.5 T). \(^{19,22,27}\) These results confirm that FeP nanoparticles can effectively shorten the \(T_1\) relaxation time of the surrounding water protons at acidic environments. To investigate this pH-responsive behavior of FeP nanoparticles, we further measured the release of Fe ions from FeP nanoparticles under different pH conditions by ICP-MS (Fig. S4†). FeP nanoparticles show very little release of Fe ions at pH 7.4 buffer. However, a significant increase in the release of Fe ions can be detected when FeP nanoparticles are in acidic environments. Paramagnetic Fe ions have the ability to shorten the \(T_1\) relaxation time of the water protons because of their high magnetic moment and long electron spin relaxation time. The pH-dependent release property makes FeP nanoparticles to be potential contrast agents for acid-triggered MRI. We further investigated the pH-responsive imaging ability of FeP nanoparticles in cells. MCF-7 cells were incubated with FeP nanoparticles and then were harvested at different time points for imaging. \(T_1\)-weighted images show that the \(T_1\) signals of MCF-7 cells gradually enhance with the increase of incubation time (Fig. 2c). Cells can uptake nanomaterials via endocytosis and the nanomaterials are trapped in endosomes and lysosomes. \(^{28}\) The acidic environment of endosomes/lysosomes trigger FeP nanoparticles to release Fe ions, thus resulting in the \(T_1\) signal enhancement inside the cells.

We then investigated the \textit{in vivo} acid-responsive MRI performance of FeP nanoparticles using MCF-7 tumor bearing mice as models. The biodistribution analysis confirms that FeP nanoparticles can effectively accumulate in tumor via enhanced permeability and retention (EPR) effect (Fig. S5†). \(T_1\)-weighted images of the mice were collected before and after the injection of FeP nanoparticles at different time points. Gradual brightening signals can be observed in tumor areas after the injection of FeP nanoparticles (Fig. 3a). To further quantify the contrast enhancement, we calculated the signal-to-noise ratio (SNR) in tumor region, and defined the contrast enhancement as the change of SNR, where \(\Delta\text{SNR} = (\text{SNR}_{\text{post}} - \text{SNR}_{\text{pre}})/\text{SNR}_{\text{pre}}\). The measured \(\Delta\text{SNR}\) values are 56.0 ± 23.8\%, 82.7 ± 13.6\%, 26.3 ± 8.6\% at 2 h, 8 h, 24 h after the injection, respectively (Fig. 3b). This time-dependent \(T_1\) signal change confirms that FeP nanoparticles can respond to acidic microenvironment of tumor, leading to the shortening effect of \(T_1\) relaxation in tumor area.

Biocompatibility is the key factor for a nanoparticle for biomedical applications. To investigate the biocompatibility of FeP nanoparticles, we first assessed the cytotoxicity of by tetrazolium-based colorimetric assay (MTT assay). FeP nanoparticles show no significant cytotoxicity on both MCF-7 and LO2 cells after being incubated with these cells for 24 h,
suggesting the little cytotoxicity of FeP nanoparticles (Fig. S6†).

We then evaluated the in vivo toxicity of FeP nanoparticles in mice. The mice were injected with FeP nanoparticles, and after 14 days, haematoxylin and eosin (H&E) stained histological images of major organs were collected to study the systemic toxicity of FeP nanoparticles. All major organs including heart, liver, spleen, lung, and kidney, maintain their typical tissue structures and exhibit no appreciable organ damage or inflammatory lesion, indicating the long-term safety of FeP nanoparticles (Fig. 4a). Moreover, blood biochemistry and hematology analyses of the mice were also performed (Fig. 4b). Various serum biochemistry parameters including aspartate transaminase (ALT), alanine aminotransferase (AST), blood urea nitrogen (BUN), and creatinine (CRE) maintain at similar levels as the controls and all fall within the normal reference intervals, suggesting that the injection of FeP nanoparticles does not affect the liver and kidney functions of mice. The hematology indices including white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and platelet count (PLT) also show no significant physiological difference comparing to the control group and maintain at normal levels, further confirming the long-term biosafety of FeP nanoparticles.

Conclusions

In summary, we have synthesized successfully FeP nanoparticles via a simple method. The as-prepared FeP nanoparticles exhibit pH-dependent MRI performance that the $T_1$ contrast signals could be significantly amplified in acidic environments. The in vivo imaging studies show that FeP nanoparticles can respond to the acidic microenvironment to generate significant $T_1$ contrast enhancement in tumor region. Moreover, the MTT assay indicates that FeP nanoparticles show very little cytotoxicity. The histological and hematological analyses confirm the in vivo long-term biosafety of FeP nanoparticles. We believe that this acid-responsive $T_1$ MRI contrast agent should have great potential in precise diagnosis of tumor.

Conflicts of interest

There are no conflicts of interest to declare.

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