A novel degradable polymeric carrier for selective release and imaging of magnetic nanoparticles†

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A water-soluble, pH-responsive copolymer was synthesized successfully and used as a polymeric carrier to deliver hydrophobic paramagnetic nanoparticles into cells. In an acidic environment, the nanoparticles aggregate as the copolymer degrades, resulting in the enhancement of an *in vitro* MRI signal.

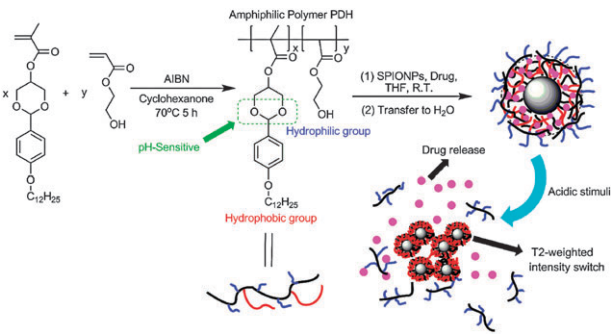
A large variety of functional polymers have been developed as carriers for biomedical applications owing to their chemically tailorable structure and storage capability.[1] In recent years, significant progress has been made in the design and synthesis of various amphiphilic copolymers as molecular carriers for drug delivery and release.[2] The amphiphilic polymers which form micelles *via* self-assembly can encapsulate the guest molecules. These are then triggered for release by external stimuli such as enzymatic, photoelectric, temperature and pH changes.[3] Most nano-carriers containing pH-sensitive covalent bonds are exploited for intelligent drug delivery because the pH value in endo- or lysosomes is around 5.0–6.5.[4]

Over the last decade, superparamagnetic iron oxide nanoparticles (SPIONPs) with favorable biocompatibility and chemical stability have been widely employed in biomedical applications especially as magnetic resonance imaging (MRI) contrast agents.[5] However, these mono-dispersed magnetic nanoparticles stabilized by oleic acid or by a hexadecylamine surfactant are usually poorly soluble in the body,[6] which has limited their application in biomedical applications. Consequently, several strategies such as ligand modification have been adopted for enhancing their water solubility.[7] These improvements, although valuable, are still affected by the complexity of the synthesis process and the limited functionality and stability of the synthesized polymer-coated magnetic nanoparticles in a physiological medium containing plasma protein and salts.[8] Therefore, a new strategy is required for future developments.

In this paper, we present a new type of water-soluble, pH-degradable polymer as a carrier of hydrophobic SPIONPs for MRI applications (Scheme 1). A novel long-chain 4-n-dodecyloxybenzalacetal monomer (DBAM) was synthesized and then polymerized with a water-soluble monomer, hydroxyethyl acrylate (HEA). Five copolymers were synthesized with different molar ratios and the content of the hydrophilic and hydrophobic groups could be determined from ^{1}H NMR spectra. Based on an evaluation of the polydispersity index, molecular weight and assembling properties of the copolymers (Table S1), the copolymer (PDH) polymerized with a monomer feed ratio of [DBAM] : [HEA] = 1 : 6 was chosen for further investigation. The as-synthesized amphiphilic copolymer nano-carrier has excellent biocompatibility and degradability properties. Oleic acid-capped SPIONPs with a uniform diameter of 18 nm were synthesized following the reported methodology (ESI†). The hydrophobic copolymer side-chain can insert into the oleic acid with the hydrophilic part on the surface to form water-soluble nanocomposites. Furthermore, this composite can also easily load hydrophobic drugs *via* hydrophobic interactions. Degradation of the polymer shell under weakly acidic conditions leads to the release of the SPIONPs and drugs from the polymeric carrier, resulting in the MRI signal switching and drug release, respectively. Our approach can achieve these outstanding dual functions, tumor diagnosis and therapy.

Fig. 1a shows a photograph of the SPIONPs before and after treatment with PDH. SPIONPs capped with oleic acid were initially dispersed in hexane. After PDH coating, the nanoparticle/polymer composite can transfer into water and form a homogenous aqueous solution. This water-soluble nanocomposite was stable in a phosphate-buffered solution for several weeks and is suitable for further use in biomedical applications.

The sulforhodamine B (SRB) assay is used to assess the cytotoxicity of PDH-coated SPIONPs at various concentrations (Fig. 1b).[9] No significant cytotoxicity is observed even at high concentration, which indicates the potential biocompatibility



Scheme 1 Schematic depiction of PDH-coated SPIONP synthesis and T2-weighted intensity switch caused by aggregation of SPIONPs and controlled release by degradation under weakly acidic conditions.

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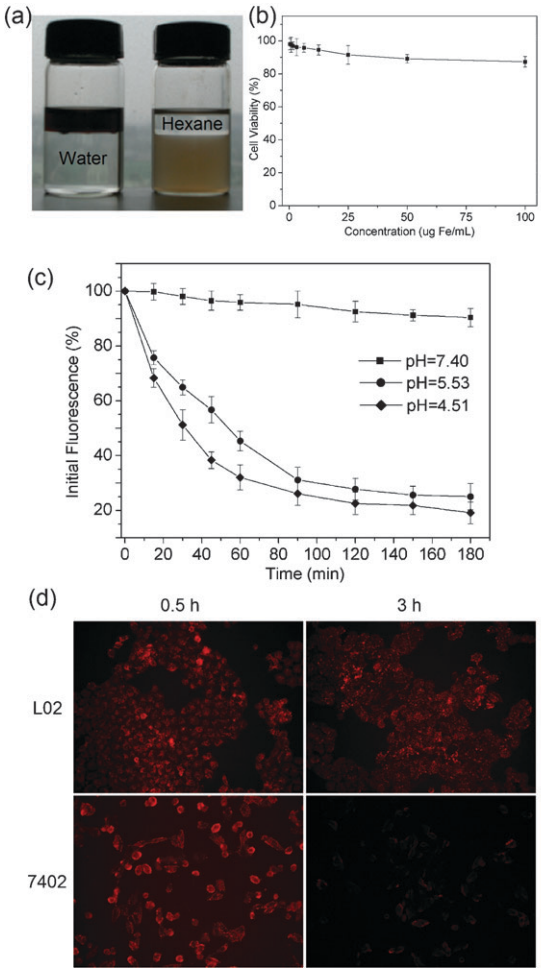

Fig. 1 Photograph for SPIONPs dispersion behavior before and after encapsulation by polymer nano-carrier (a), *in vitro* cell viability of PDH-coated SPIONPs (b), Nile Red fluorescence in PDH micelles as a function of time under different pH conditions and at 37 °C (c) and fluorescence microscopy images of L02 and 7402 cells treated with Nile Red-loaded PDH-coated SPIONPs for 30 min and 3 h (d).

of PDH-coated SPIONPs in medical applications. A typical ^{1}H NMR spectrum of the copolymer PDH in acetone-d_6 is shown in Fig. S1a. Degradation is observed by comparing spectra of the copolymer before and after adding a trace amount of acetic acid-d_4. The specific chemical shift appeared at δ = 9.87, which corresponds to the aldehyde group, while the resonance attributed to the protons of the water disappeared. This clearly indicates the obvious hydrolytic process of the copolymer chain under weakly acidic conditions (Fig. S1b).

The *in vitro* model drug release rate of PDH-coated SPIONPs was investigated at 37 °C and at different pH values as shown in Fig. 1c (for the *in vitro* degradation process, see Fig. S2). The model drug, hydrophobic fluorescent dye Nile Red, was encapsulated into the PDH-coated SPIONPs. The relative fluorescence intensity of the Nile Red released in the hydrophobic environment is higher than that in the hydrophilic environment. After 3 h treatment in solutions of different pH values (7.40, 5.53 and 4.51), the fluorescence intensity of the Nile Red decreased almost 75 and 80% at pH 5.53 and 4.51 (acidic environment), respectively, while little change was observed at pH 7.40. This result shows that the PDH-coated SPIONPs can also encapsulate hydrophobic molecules for selective release in a slightly acidic environment. *In vitro* experiments were used to further demonstrate the controlled releasing properties of our nanocomposites. Nile Red was used as a guest molecule and indicator, and L02 and 7402 were used as typical normal and tumor cell lines, respectively. After incubation for 30 min, the PDH-coated SPIONPs loaded with model drug were efficiently internalized in the cells by non-specific endocytosis (Fig. 1d). Compared with the incubation in the L02 cells, a different fluorescence intensity was observed in the 7402 cells presumably because of the different endocytic activity of the two cells. After incubation for 3 h, the fluorescence intensity decreased owing to the release of the model drug in the acidic endosomal/lysosomal compartments. With its pH-dependent degradation property, we believe that the dye-loaded PDH nano-carrier degraded in the low pH environment of the 7402 tumor cells and released the model drug continuously over the 3 h incubation period.

A photograph depicting the hydrolytic process for the PDH-coated SPIONPs colloid is presented in Fig. 2a. In a neutral environment, the nanoparticles were well dispersed in aqueous solution. After acidification for 10 min under acidic conditions (pH = 4.5), the colloidal sollution became cloudy, implying an increased aggregation of the hydrophobic SPIONPs.

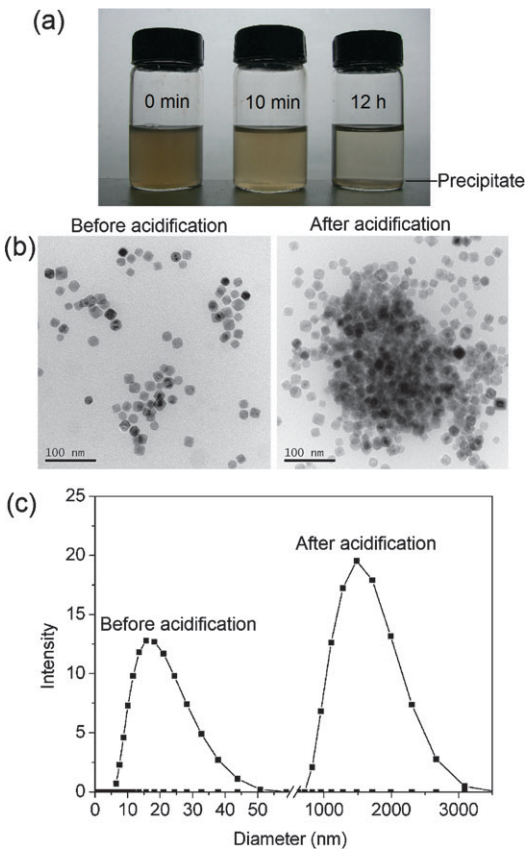

Fig. 2 Photograph of dispersion behavior of PDH-coated SPIONPs in PBS solution (pH = 7.40) and degraded under weakly acidic conditions (pH = 4.5) for 10 min and 12 h (a), TEM images (b) and DLS diagram (c) of PDH-coated SPIONPs before and after acidification for 10 min.

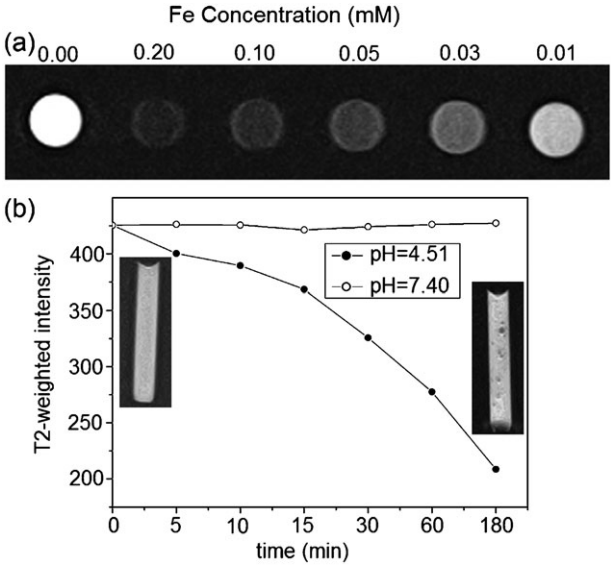

Fig. 3 T2-weighted MR images of the aqueous dispersion of PDH-coated SPIONPs at various Fe concentrations (a), and time-course of mean T2-weighted intensity of PDH-coated SPIONPs in solutions of different pH (b).

With continued hydrolyzation in an acidic environment, the nanoparticle aggregates settled at the bottom of the vial. The process is probably attributed to the agglomeration of the hydrophobic SPIONPs in a hydrophilic environment. Transmission electron microscope (TEM) images provide evidence of these assumptions. As shown in Fig. 2b, the colloid dispersed in aqueous solution gradually aggregates after 10 min under weakly acidic conditions. The result was further verified by dynamic light-scattering (DLS) measurements (Fig. 2c), which determine the diameters of the PDH-coated SPIONPs (about 20 nm) and SPIONPs in the aggregated state (1000–2000 nm).

In view of their low cytotoxicity and high colloidal stability, PDH-coated SPIONPs appear to be a suitable candidate as a magnetic resonance imaging agent. The T2-weighted images of PDH-coated SPIONPs suspended in water at various Fe concentrations (Fig. 3a) show a great contrasting effect. In addition, the MR signal increased with decreasing content of PDH-coated SPIONPs, confirming the capability of the SPIONP core in enhancing the transverse proton relaxation process. To further evaluate the influence of the PDH-coated SPIONP degradation process, magnetic resonance phantom imaging was used. The T2-weighted images of the PDH-coated SPIONPs before and after acidification are shown in Fig. 3b and the T2-weighted intensity was calculated using built-in software. As expected, the intensity of the colloid showed a significant T2-weighted intensity drop-off (from 425.48 to 389.57) in an acidic environment (pH = 4.51) for just 10 min in comparison to the unacidified control, which exhibited high sensitivity and rapid detectability by MRI. Furthermore, after acidification for 3 h, about 50% T2 intensity decrease was observed. The losses in T2-weighted intensity are probably attributable to the partial aggregation of hydrophobic SPIONPs, because the highly stable SPIONP clusters can efficiently change the spin–spin relaxation time of the adjacent water protons.[10]

In summary, we have successfully prepared a novel pH-degradable copolymer which can act as a carrier for the encapsulation of hydrophobic superparamagnetic nanoparticles. The core–shell nanoparticles show favorable biocompatibility and chemical stability. The hydrophobic drugs can also be encapsulated successfully through hydrophobic interaction. Furthermore, the obtained PDH-coated SPIONPs can selectively image and release guest drugs in a tumor by their degradation and aggregation under slightly acidic conditions. It is believed that additional potential applications may be possible owing to the material's aforementioned outstanding capability.

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