A novel degradable polymeric-carrier for the encapsulation and selective release and imaging of magnetic nanoparticles

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Supplementary Information

Materials and methods

Materials

Ferric trichloride hexahydrate (FeCl₃·6H₂O), p-hydroxybenzaldehyde, 1-bromododecane, para-toluene sulfonic acid (PTSA), glycerol were all purchased from Shanghai Chemical Reagent Co. Ltd. as analytical regents and used without further purification. Azobisisobutyronitrile (AIBN) (Chemically pure, Shanghai Chemical Reagent Co. Ltd.) was recrystallised from ethanol anhydrous. Methacryloyl chloride was produced by Haimen Best Fine Chemical Industry Co. Ltd. and used after distillation. Hydroxyethylacrylate (HEA) was distilled under vacuum and stored at -15 °C under an inert gas atmosphere. Other reagents were commercially available and used as received.

Synthesis of superparamagnetic iron oxide nanoparticles (SPIONPs)
SPIONPs were synthesized according to the literature with some modifications.\textsuperscript{1} Briefly, 2.7 g (10 mmol) of FeCl\textsubscript{3}·6H\textsubscript{2}O and 9.1 g (30 mmol) sodium oleate were dissolved in a mixture solvent composed of 20 mL ethanol, 15 mL water and 35 mL hexane. After heating at 70 °C for 4 h, the organic layer was washed and evaporated to obtain iron-oleate complex. Then, 9.0 g (10 mmol) of the iron-oleate complex and 1.4 g of oleic acid (5 mmol) were dissolved in 50 g of trioctylamine at room temperature. The mixture was heated at 320 °C for 30 min and then cooled to room temperature. The black iron oxide magnetic nanoparticles were washed several times with ethanol and dried at 60 °C for 24 h.

**Synthesis of 4-n-dodecyloxybenzaldehyde (DBD)**

1-bromododecane (29.9 g, 120 mmol) was added dropwise to the mixture of p-hydroxybenzaldehyde (12.2 g, 100 mmol) and anhydrous potassium carbonate (20.7 g, 150 mmol) in 150 mL of acetone. After heated under reflux with stirring for 14 h, the mixture was filtered off and acetone was removed on a rotary evaporator. The crude product was purified by column chromatography (ethyl acetate-petroleum ether, 1:10). Anal. Calcd. for C\textsubscript{19}H\textsubscript{30}O\textsubscript{2}: C, 78.57 %, H, 10.41 %, O, 11.02 %. Found: C, 78.48 %, H, 10.53 %, O, 10.99 %. \textsuperscript{1}H NMR (400 MHz, CDCl\textsubscript{3}), \(\delta\) (ppm): 9.88 (s, 1H, CHO), 7.83 (d, \(J = 8.72\) Hz, 2H, C\textsubscript{6}H\textsubscript{4}), 6.99 (d, \(J = 8.69\) Hz, 2H, C\textsubscript{6}H\textsubscript{4}), 4.04 (t, \(J = 6.56\) Hz, 2H, CH\textsubscript{2}O), 1.86-1.76 (m, 2H, CH\textsubscript{2}CH\textsubscript{2}O), 1.46 (m, 2H, CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}O), 1.26 (m, 16H, CH\textsubscript{2}), 0.88 (t, \(J = 6.79\) Hz, 3H, CH\textsubscript{3}). \textsuperscript{13}C NMR (75 MHz, CDCl\textsubscript{3}) \(\delta\) (ppm): 191.12 (CHO), 164.50 (C), 132.24 (CH), 129.91 (C), 114.96 (CH), 68.65 (CH\textsubscript{2}O), 32.16 (CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{3}), 29.29-29.88 (CH\textsubscript{2}), 26.19 (CH\textsubscript{2}CH\textsubscript{2}CH\textsubscript{2}O), 22.94
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(CH₂CH₃), 14.39 (CH₃).

**Synthesis of 4-n-dodecyloxybenzalacetal (DBA)**

Acetal was synthesized according to the literature with some modifications. In a typical procedure, DBD (8.7 g, 30 mmol) reacted with glycerol (2.76g, 30 mmol) in 50 mL toluene using PTSA (0.5 g) as catalyst. The solution was refluxed with vigorous stirring for 14 h and the water formed by dehydrogenation reaction was removed by the oil-water separator. Then, the mixture was evaporated and washed with potassium carbonate solution (1 %, 80 mL) to remove the acid catalyst and any remaining glycerol. After that, the precipitate was filtered off and purified by column chromatography (ethyl acetate-petroleum ether, 1:2). Anal. Calcd. for C₂₂H₃₆O₄: C, 72.49 %, H, 9.95 %, O, 17.56 %. Found: C, 72.36 %, H, 10.04 %, O, 17.60 %. ^1H NMR (400 MHz, CDCl₃), δ (ppm): 7.41 (d, J = 8.51 Hz, 2H, C₆H₄), 6.90 (d, J = 8.67 Hz, 2H, C₆H₄), 5.51 (s, 1H, C₆H₄CH), 3.57-4.38 (m, 8H, C₆H₄OCH₂, CHCH₂O, CHOH, OH), 1.86-1.76 (m, 2H, CH₂CH₂O), 1.50-1.39 (m, 2H, CH₂CH₂CH₂O), 1.26 (m, 16H, CH₂), 0.88 (t, J = 6.78 Hz, 3H, CH₃). ^13C NMR (75 MHz, CDCl₃), δ (ppm): 191.15 (C), 132.25 (C), 127.36 (CH), 114.48 (CH), 101.93 (C), 72.48 (CHO), 68.28 (CH₂O), 64.21 (CHOH), 32.17 (CH₂CH₂CH₃), 29.29-29.88 (CH₃), 26.22 (CH₂CH₂CH₂O), 22.95 (CH₂CH₃), 14.39 (CH₃).

**Synthesis of 4-n-dodecyloxybenzalacetal monomer (DBAM)**

Methacryloyl chloride (2.3 g, 22 mmol) was added slowly to the anhydrous tetrahydrofuran (20 mL) solution containing DBA (4.0 g, 11 mmol) and triethylamine (4.5g, 44 mmol) and cooled to 0 °C in a water-ice bath. After constantly stirred for
another 12 h at room temperature, the mixture was filtered off to remove the byproduct. The obtained filtrate was concentrated and purified by column chromatography (ethyl acetate-petroleum ether, 1:8). Anal. Calcd. for C_{26}H_{40}O_{5}: C, 72.19 %, H, 9.32 %, O, 18.49 %. Found: C, 72.30 %, H, 9.28 %, O, 18.42 %. \(^1H\) NMR (400 MHz, CDCl\(_3\)), \(\delta\) (ppm): 7.41 (d, \(J = 8.39\) Hz, 2H, C\(_6\)H\(_4\)), 6.89 (d, \(J = 8.41\) Hz, 2H, C\(_6\)H\(_4\)), 6.30 (s, 1H, CCH\(_2\)), 5.65 (s, 1H, CCH\(_2\)), 5.52 (s, 1H, C\(_6\)H\(_4\)CH), 4.75 (s, 1H, CHO), 4.31 (d, \(J = 12.88\) Hz, 2H, CHCH\(_2\)O), 4.18 (d, \(J = 12.92\) Hz, 2H, CHCH\(_2\)O), 3.95 (t, \(J = 6.59\) Hz, 2H, C\(_6\)H\(_4\)OCH\(_2\)), 2.01 (s, 3H, CH\(_3\)), 1.81-1.72 (m, 2H, CH\(_2\)CH\(_2\)), 1.49-1.38 (m, 2H, CH\(_2\)CH\(_2\)CH\(_3\)), 1.26 (m, 16H, CH\(_2\)), 0.88 (t, \(J = 6.68\) Hz, 3H, CH\(_2\)CH\(_3\)). \(^{13}C\) NMR (75 MHz, CDCl\(_3\)), \(\delta\) (ppm): 167.45 (COO), 159.95 (C), 136.28 (C\(_6\)H\(_4\)), 132.25 (C), 127.57 (CH), 126.50 (CCH\(_2\)), 114.52 (CH), 74.21 (CH\(_2\)CO), 69.25 (CH\(_2\)CO), 68.28 (CHCH\(_2\)O), 32.16 (CH\(_2\)CH\(_2\)CH\(_3\)), 29.28-29.88 (CH\(_2\)), 26.24 (CH\(_2\)CH\(_2\)CH\(_3\)), 22.94 (CH\(_2\)CH\(_3\)), 18.52 (CCH\(_3\)), 14.39 (CH\(_3\)).

**Synthesis of amphiphilic copolymer poly (DBAM-co-HEA) (PDH)**

An amphiphilic copolymer was synthesized by free radical copolymerization of DBAM and HEA in cyclohexanone at 70 °C with AIBN as initiator. The ratio of the hydrophilic and hydrophobic units in the copolymer was controlled by using different feed ratios of the monomers. A typical procedure for synthesizing PDH with the feed ratio of 1: 3 was described as below. AIBN (5 mg), DBAM (300 mg, 0.7 mmol) and HEA (244 mg, 2.1 mmol) were dissolved in 2.0 mL of cyclohexanone and the tube was sealed and cycled between vacuum and nitrogen for three times. After 5 h reaction in oil bath, the mixture was poured into a large amount of dry hexane. The
precipitation was filtrated and dried under vacuum and then stored in a desiccator for further uses. 1H NMR (400 MHz, Acetone-\textit{d}6), $\delta$ (ppm): 7.44 (C$_6$H$_4$), 6.94 (C$_6$H$_4$), 4.21-3.76 (COOCH$_2$CH$_2$, CHCH$_2$O and C$_6$H$_4$OCH$_2$), 0.89-1.77 (OC$_{12}$H$_{25}$).

**Synthesis of PDH-coated SPIONPs**

Oleic acid-stabilized monodisperse SPIONPs (10 mg) and PDH (5 mg) was dissolved in tetrahydrofuran (1 mL). Then, 5 mL of distilled water was added to the above solution with vigorous shaking and the resulting colloid was stirred vigorously for 24 h to evaporate tetrahydrofuran. After that, the colloid was separated by centrifugation and the obtained PDH-coated SPIONPs were washed three times with distilled water to remove the unbound copolymer.

**Drug loading and release**

To evaluate the drug loading and release properties, a dye Nile Red was used as a hydrophobic model drug. Nile Red was loaded into PDH-coated SPIONPs by adding Nile Red acetone solution (40 $\mu$L, 1 mmol/L) to 0.8 mL of the above PDH-SPIONPs solution in tetrahydrofuran, followed by slowly addition of 10 mL phosphate buffer (20 mmol/L, pH=7.4). And the solution was shaken overnight to evaporate tetrahydrofuran and acetone completely. The drug release test was performed by suspending the PDH-coated SPIONPs in phosphate buffered saline (PBS) buffer (pH = 7.4) with shaking in a 37 °C water bath to maintain a constant temperature. To determine the release amount at any given pH values, the obtained colloid was divided into four equivalent parts and adjusted to different pH value by acetate buffer. The drug concentration was analyzed by measuring the fluorescence intensity.
Cell culture and preparation

Human hepatoma 7402 cell lines and L02 cell lines (purchased from Shanghai Cell Institute Country Cell Bank, China) were cultured as monolayer in RPMI-1640 medium supplemented with 10 % heat-inactivated fetal bovine serum at 37 °C in a humidified incubator (5 % CO₂ in air, v/v).

In vitro cytotoxicity

Sulforhodamine B (SRB) is used as an assay for assessing the effects of drug carriers in various concentrations. In brief, hepatoma 7402 cells was placed in 96-well plates (1.3 × 10⁴ cells per well) and four duplicate wells were set up in each sample. The culture medium was replaced with the medium containing different concentrations of PDH-coated SPIONPs (0, 0.391, 0.781, 1.563, 3.125, 6.25, 12.5, 25, 50 and 100 µg mL⁻¹) and cultured at 37 °C in a humidified incubator (5 % CO₂ in air, v/v). After cultured for 72 h, the medium was poured away and 10 % (w/v) trichloroacetic acid in Hank’s balanced salt solution (100 µL) was added and stored at 4 °C for 1 h. Then, the stationary liquid was discarded, the cells were washed with deionized water for five times before air drying and stained with 0.4 % (w/v) SRB solution (100 µL per well) for 30 min at room temperature. Following the remove of SRB, the cells were washed with 0.1 % acetic acid solution for five times. Bound SRB dye was solubilized with 10 mmol L⁻¹ Tris-base solution (150 µL, pH = 10.5). The test optical density (OD) value was calculated by the absorbance at 531 nm of each individual well measured with a spectrophotometer.

Cellular uptake of Nile Red
L02 cells and hepatoma 7402 cells were seeded in 96-well plates (1.3 × 10^4 cells per well) and incubated overnight at 37 °C in a humidified incubator. The dispersion was prepared in RPMI-1640 medium as described above and the concentration of the PDH-coated SPIONPs was 10 µg mL^-1. Cells were cultured with the above solution for 30 min and observed using fluorescence microscopy after washed three times with PBS. The fluorescence images were acquired using an Olympus IX-51 inverted microscope equipped with 100 W mercury-xenon arc lamp excitation light source and high speed CCD camera.

**MRI experiments**

MRI experiments were carried out with a 0.5 Tesla (T) superconducting unit (GE Vectra, International General Electric, Slough, UK). PDH-coated SPIONPs were loaded into the eppendorf tubes for imaging. Multisection T2-weighted fast spin-echo sequences were performed in at least two orthogonal planes to obtain MR phantom images. MRI signal intensity was calculated using the in-built software.

**Characterization methods**

TEM images were obtained using a TecnaiG220 electron microscope at an acceleration voltage of 200 kV. Number-average molecular weight (M_n), weight-average molecular weight (M_w) and polydispersity index (PDI) were measured by gel permeation chromatography (GPC) utilizing waters 1515 pump. Monomer conversion was determined by gravimetry. ^1H NMR and ^13C NMR spectra were measured by UNITY INOVA 400 MHz spectrometer and NMRststem-300 MHz (Varian) spectrometer, respectively. Room temperature emission and excitation
spectra were carried out using Edinburgh-920 fluorescence spectra photometer.

References


Table S1 Properties of PDH with different feed ratios

<table>
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<tr>
<th>Feed ratio (x:y)</th>
<th>Conversion (%)</th>
<th>$M_n$</th>
<th>$M_n/M_w$</th>
<th>Polymer composition (x:y)</th>
<th>Assemble with SPIONPs in PBS</th>
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<tbody>
<tr>
<td>3:1</td>
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<td>29400</td>
<td>1.9</td>
<td>1:6.1</td>
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<tr>
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<td>53600</td>
<td>2.6</td>
<td>1:7.9</td>
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* Colloid PDI determined by DLS.
Scheme S1 Synthetic route of DBAM
Figure S1 ¹H NMR spectra of PDH before (a) and after (b) degradation
Figure S2 Hydrolysis degree of PDH as a function of time at different pH at 37 °C.