Electronic Supplementary Information

Versatile PEG-derivatized Phosphine Oxide Ligands for Water-Dispersible Metal Oxide Nanocrystals

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Synthesis of PO-PEGs

3 mmol of dry Poly(ethylene glycol) methyl ether was dissolved in 7 ml of anhydrous tetrahydrofuran. 1 mmol of phosphoryl chloride was added to the mPEG solution and incubated at RT for 1 day. After removing the THF by evacuation, the resulting gel was incubated at 100 ºC under vacuum for 12 h.

Synthesis of amine-functionalized PO-PEGs

2 mmol of dry Poly(ethylene glycol) methyl ether was dissolved in 5 ml of anhydrous tetrahydrofuran. 1 mmol of phosphoryl chloride was added to the mPEG solution and incubated in RT for 1 day. After removing the THF by evacuation, the resulting gel was incubated at 100 ºC under vacuum for 12 h. At RT, 5 ml of THF and 3 ml of 1,2-ethylenediamine was added. After 12 h, THF and the remaining 1,2-ethylenediamine was evacuated.

Stabilization of Fe₃O₄ nanocrystals with PO-PEGs

Fe₃O₄ nanocrystals were prepared using the reported methods.¹ 10 mg of nanocrystals were dispersed in 2 ml of THF. 20 mg of PO-PEGs was added to the solution and dissolved. After removing the THF by evacuation, the nanocrystals were incubated at 150 ºC under vacuum for 1 h. 5 ml of water was added and the dispersed nanocrystals were filtered through 220 nm syringe filter. The unbound PO-PEGs were eliminated by ultracentrifugation column chromatography, or dialysis.

Conjugation of FITC with amine-functionalized Fe₃O₄ nanocrystals

Fe₃O₄ nanocrystals were stabilized with amino-functionalized PO-PEGs, and dispersed in carbonate buffer (pH: 9.4) to make a 2 mg/ml solution. 1 ml nanocrystals were mixed with 10 µl of a FITC solution (10 mg in 1 ml of DMSO) and incubated at RT for 1 h. The FITC conjugated nanocrystals were purified by column separation or dialysis.
Cell culture

A human breast cancer cell line, SKBR-3 was maintained in DMEM/F12 medium without folate at 37 °C in a humidified atmosphere containing 5 % of CO2. The cell culture media were supplemented with 100 units/ml penicillin, and 100 μg/ml streptomycin. The cells were regularly passaged and reseeded 24 h before the cellular uptake experiments.

In vitro cytotoxicity against SKBR-3 cells

The SKBR-3 cells were seeded in a 96-well plate at a density of $1 \times 10^4$ cells per well, and grown in DMEM/F12 medium (without folate) supplemented with 10% (v/v) fetal bovine serum for 24 h at 37 °C. The PO-PEGs stabilized Fe$_3$O$_4$ nanocrystals (5 nm) were added to the medium and the cells were incubated for 5 days at 37 °C. The concentrations of Fe$_3$O$_4$ nanocrystals used were 200, 100, 50, 25, and 12.5 μg of Fe/ml, respectively. The cell viability was determined using the MTT cell viability assay.²

In vitro optical and MR imaging of cancer cells

SKBR-3 Cells were plated in 100 mm dishes at a density of $1 \times 10^6$ cells per dish 24 h before cellular uptake. FITC conjugated PO-PEGs stabilized Fe$_3$O$_4$ nanocrystals (5 nm) were incubated in the SKBR-3 cell culture for 1 day at 37 °C in serum free medium. The Fe concentration was 0, 50 and 100 μM respectively. Then the cells were washed five times with cold PBS and imaged by using a fluorescent microscope. The cells were detached and centrifuged, and the resulting cell pellets were mixed with 0.5 ml of 0.8 % agarose solution. The mixture was transferred to an Eppendorf-tube for the MR measurement. The relaxation time of the labeled cells mixed with agarose were measured using a 3.0 T whole body MRI system (Philips, Achieva ver. 1.2, Philips Medical Systems, Best, The Netherlands) equipped with a 80 mT/m gradient amplitude and a 200 ms/m slew rate.
Table S1. Relaxation time of Fe₃O₄ labeled SKBR-3.

<table>
<thead>
<tr>
<th>Fe concentration (µM) in growing media</th>
<th>T₁ (ms)</th>
<th>T₂ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1490</td>
<td>192</td>
</tr>
<tr>
<td>50</td>
<td>1460</td>
<td>65.5</td>
</tr>
<tr>
<td>100</td>
<td>1440</td>
<td>58.5</td>
</tr>
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Fig. S1 MALDI-TOF mass spectra of a) reacted mPEG (Mn : 2000) and b) resulting PO-PEGs.
Fig. S2 $^{31}$P NMR spectra of PO-PEGs by various molecular weight of mPEG (a) Mn: 550 (b) Mn: 750 (c) Mn: 2000 (left: $^{31}$P NMR in CDCl$_3$ and right: $^{31}$P NMR in D$_2$O).
Fig. S3 Hydrodynamic diameter distribution of the PO-PEG stabilized iron oxide nanocrystals with core diameter of 8 nm. The data was obtained using light scattering method.
**Fig. S4** Characterization of as-synthesized organic dispersible and PO-PEG stabilized Fe₃O₄ nanocrystals. (a) XRD spectra of as-synthesized organic dispersible (black) and PO-PEG stabilized Fe₃O₄ nanocrystals (red) (Blue lines: JCPDS 19-0629, magnetite). (b) TEM images of as-synthesized organic dispersible (left) and PO-PEG stabilized Fe₃O₄ nanocrystals (right). (c) High resolution TEM images of as-synthesized organic dispersible (left) and PO-PEG stabilized Fe₃O₄ nanocrystals (right). (d) Size distribution of as-synthesized organic dispersible (left) and PO-PEG stabilized Fe₃O₄ nanocrystals (right).
Fig. S5 Photograph of magnetically attracted Fe₃O₄ nanocrystals dispersed in water with high iron concentration of 17 mg/ml.

Fig. S6 In vitro cytotoxicity of the PO-PEG stabilized Fe₃O₄ nanocrystals against SKBR-3 cells.

References for the Supporting Information