**Design and Synthesis of Multifunctional Iron Oxide Nanoparticles for Cancer Imaging**

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**Statement of Purpose:** Synthetic super-paramagnetic iron oxide (SPIO) nanoparticles are widely used in many biomedical applications such as magnetic resonance imaging (MRI), targeted drug delivery, cell sorting, and hyperthermia. For MRI application, they are emerging as versatile ultra-sensitive nanoprobes for early diagnosis of cancer at the molecular and cellular levels.[1] For these applications, a facile and simple method to functionalize the surface of SPIO nanoparticles to achieve cancer specificity remains a considerable challenge. Herein we report a novel polyethylene glycol (PEG)-cysteine system to modify the surface of SPIO nanoparticles (**Fig. 1a**). Cysteine residue forms a stable binding to the surface of SPIO particles and PEG chains with different lengths minimize the biofouling and aggregation of SPIO nanoparticles, making those particles water-soluble. In addition, different functional groups, such as cancer targeting ligands or fluorescence probes can be easily conjugated on the other termini of PEG chain, which can lead to cancer targeting and fluorescent imaging functionalities.

**Methods:** Oleic acid stabilized SPIO nanoparticles with different sizes were synthesized by thermal decomposition of ferric precursor in organic solvent.[2] The morphology and size of the particles were characterized with transmission electron microscopy (TEM) and the magnetic properties of those particles were measured by alternating gradient magnetometer (AGM) and relaxometry. Cancer targeting peptides were conjugated on the end of PEG-Cysteine through standard solid-phase peptide synthesis method. These new peptides were directly coupling on the surface of SPIO by ligand-exchange process. Prussian blue staining and MR imaging of cancer cells were further applied to verify the targeting specificity of this novel system.

**Results:** In this study, we developed one step strategy to utilize well-defined magnetic nanocrystals by replacing the hydrophobic surfactants with cysteine terminated peptide. The replacement was achieved through carboxylate chelate binding between cysteine and iron atom, and the coating layer is further stabilized through the formation of disulfide linkage.[3] This surface modification was confirmed by FTIR spectroscopy, which showed a strong C-O-C stretching peak at 1100 $cm^{-1}$ from PEG units. Compared to other multi-step methods, our one-step method is simpler and easier to immobilize small targeting peptides on the SPIO surface. Lung cancer peptide (LCP, RGDLATLRQL), isolated from panning peptide library on the lung adenocarcinoma H2009 cell line, was conjugated on the PEG-Cysteine. For comparison, a scrambled peptide (SP, DALRLQGTLR) was also attached on the PEG terminal. Surface coated 6nm SPIO particles showed a hydrodynamic size of

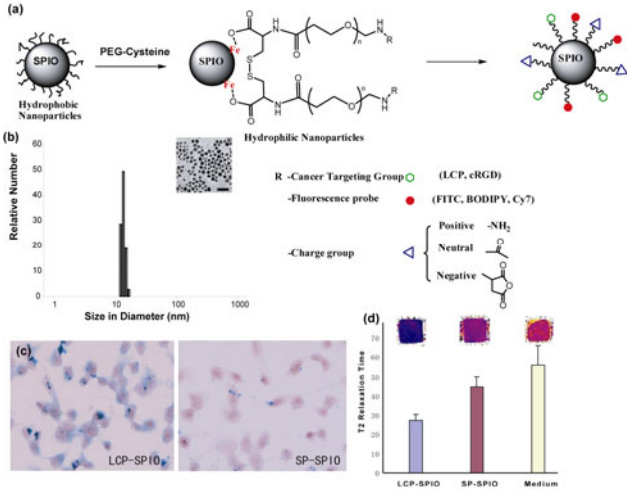


**Figure 1.** (a) Schematic of surface modification of SPIO nanoparticles by PEG-Cysteine. (b) Dynamic light scattering (DLS) histogram of LCP-PEG-Cysteine modified 6nm SPIOs in water; the inserted picture is TEM image of 6nm SPIOs (scale bar=20nm). (c) Prussian blue staining of H2009 cells treated with LCP-SPIO (left) and SP-SPIO (right) for 2 hours. (d) $T_2$ relaxation times and $T_2$-weighted images of H2009 cell lines treated with LCP-SPIO and SP-SPIO particles for 2 hours.

~15nm in diameter in water (**Fig. 1b**). LCP-SPIO or SP-SPIO has a high $T_2$ relaxivity of ~140 $s^{-1}mM^{-1}$.

*In vitro* specificity of LCP-SPIO to H2009 cell lines was determined by Prussian blue staining after 2 hrs incubation (**Fig. 1c**). Significantly more LCP-SPIO was taken up in H2009 cancer cells than SP-SPIO. H2009 cancer cell line was also used for MR imaging (4.7 T Varian scanner) and relaxivity study. As compared with cells incubated with SP-SPIO, the signal intensity (SI) of cells incubated with LCP-SPIO was remarkably decreased. More specifically, $T_2$ relaxation time for the cells incubated with LCP-SPIO for 2 hours was 16.7±2.5 ms, much lower compared to 35.6±3.1 ms with SP-SPIO (**Fig. 1d**).

**Conclusions:** Multifunctional SPIO nanoparticles were developed by introducing the PEG-Cysteine to the surface of monodispersed $Fe_3O_4$ particles. Cancer targeting ligand LCP was conjugated on the distal end of PEG. Specificity of the probes to the cancer cell line was demonstrated by MR imaging and Prussian blue staining. The facile surface chemistry and the multifunctional design of the proposed SPIO system provide a powerful nanoplatform for cancer molecular imaging applications.**References:**

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3. Nishio, K. Gokon, N. etc. *Chem. Lett*. **2006**, 35, 974-975.