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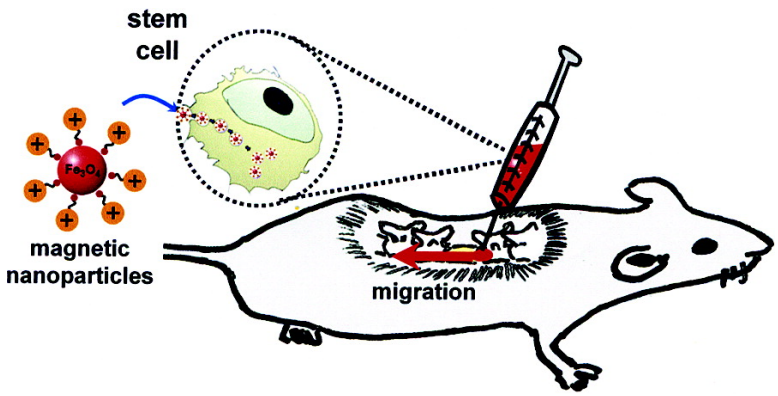

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Surface Modulation of Magnetic Nanocrystals in the Development of Highly Efficient Magnetic Resonance Probes for Intracellular Labeling

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Recently, much attention has been given to the development of novel biomedical applications of inorganic nanocrystals[1−3] which possess unique optical,[4] magnetic,[5] and electronic properties.[6] Among this group, magnetic nanocrystals serve as excellent magnetic resonance imaging (MRI) probes which can be used in the noninvasive in vivo monitoring of molecular and cellular events.[7−10] Especially, in vivo cellular MR tracking with magnetic nanocrystal probes has the potential of being a powerful technique for determining the history and the fate of cells[11] and for evaluating cell-based therapies.[12] However, their practical utilization has still been limited, and to have successful in vivo cellular MR tracking it is necessary to develop an efficient magnetic labeling protocol which can enhance the MR signal of the targeting cells.

Several iron oxide-based magnetic labeling systems have been developed for monitoring stem cell migration[13−16] and tracking lymphocytes.[17,18] Included in this group are derivatized HIV−Tat peptide MION,[19,20] Feridex−poly-L-lysine (PLL) conjugates,[15] and nanocrystals encapsulated in dendrimers.[10] However, probes based on these materials typically have difficulties for the successful MR cellular imaging due to either relatively low cell transport efficiencies or the use of extra-high-molecular weight transport facilitating agents which can often cause unwanted side effects such as nanocrystal aggregation and cytotoxicity at a high dose level.[21]

In this study, we present a surface-modulated and highly biocompatible magnetic iron oxide (Fe_3O_4) nanocrystal probe that can be used for efficient intracellular labeling and their MRI applications. Specifically, we describe the preparation of these probes and the results from studies exploring their (1) transport into various cell types and MR contrast effect, (2) cytotoxicity, and (3) application in in vivo monitoring of neural stem cell migration in rat spinal cord.

High-quality iron oxide (Fe_3O_4) nanocrystals were prepared by thermal decomposition of $Fe(CO)_5$ in hot dioctyl ether following a modification of the known procedure.[22] X-ray diffraction (XRD) analysis shows that the nanocrystals are highly crystalline Fe_3O_4 magnetite (Supporting Information). TEM analysis reveals that the nanocrystals are spherical (~12 nm) and have a high monodispersity (σ < 5%) (Figure 1a). These nanocrystals are coated with hydrophobic ligands (i.e., lauric acid) and are only soluble in organic solvents (Figure 1a, inset). However, in their use as intracellular magnetic probes, it is necessary to appropriately modify the nanocrystal surface with hydrophilic ligands to promote solubility in aqueous media. For this purpose, (3-carboxypropyl)trimethylammonium chloride was ligated to the nanocrystal surface (Scheme 1a). The carboxylate end of the ligand binds to the surface iron, and the appended ammonium cation makes the nanocrystal hydrophilic. In a similar manner, 2-carboxyethyl phosphonate was ligated to the nanocrystal surface (Scheme 1b), producing a material having anionic phosphonate groups to promote water solubility.

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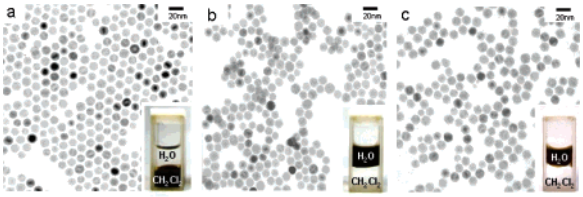
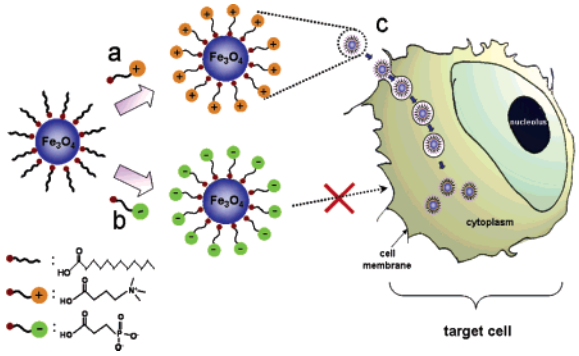


Figure 1. TEM images and solubility tests (inset) of as-synthesized iron oxide (a), cationic WSIO (b), and anoinic WSIO nanocrystals (c).

Scheme 1. Schematics of the Ligand Exchange Procedure and Utilization in Cellular Labeling[a]



[a] Surface exchange of as-synthesized Fe_3O_4 nanocrystals with either cationic (a) or anionic ligand (b) and their utilization for cell labeling (c).

The ligated nanocrystals are now fairly stable and well dispersed in water (Figure 1, b and c inset). TEM analyses of the water-soluble iron oxide (abbreviated as WSIO) coated with (3-carboxypropyl)trimethylammonium chloride (cationic WSIO, Figure 1b) and with 2-carboxyethyl phosphonate (anionic WSIO, Figure 1c) show that each retains its individual size without aggregation.

Studies were conducted to evaluate the transport capabilities of the WSIO nanocrystals into fetal rat neural stem cells. The transport efficiencies of the WSIO nanocrystals were compared to that of the previously well-studied and popular Feridex−PLL system.[15] Cells in culture media containing cationic WSIO, anionic WSIO, and Feridex−PLL (iron concentration: 25 μg/mL) were independently incubated for 30 h. The extent of intracellular labeling by the three different types of iron oxide nanocrystals was determined on harvested cells by using Prussian blue staining. As the staining results show (Figure 2a), transport of cationic WSIO nanocrystals into the neural stem cells takes place efficiently. In contrast, no Prussian blue staining is observed for cells treated with anionic WSIO (Figure 2b) and only a small amount of staining is observed for Feridex−PLL treated cells (Figure 2c). These cell transport tendencies can also be inferred from macroscopic color changes of cells after the nanocrystal treatments. A deep dark-brown color of cationic WSIO-treated cells (Figure 2a, inset) is observed, which is indicative of the binding of WSIO nanocrystals to the cells. In contrast, no noticeable color change is observed for anionic WSIO-

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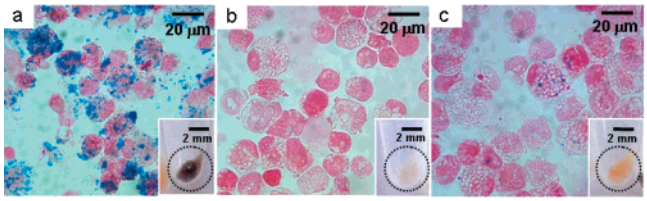

Figure 2. Macroscopic cell images (inset, circled) and Prussian blue-stained cell images of cationic WSIO (a), anoinic (b), and Feridex−PLL (c) treated neural stem cells.

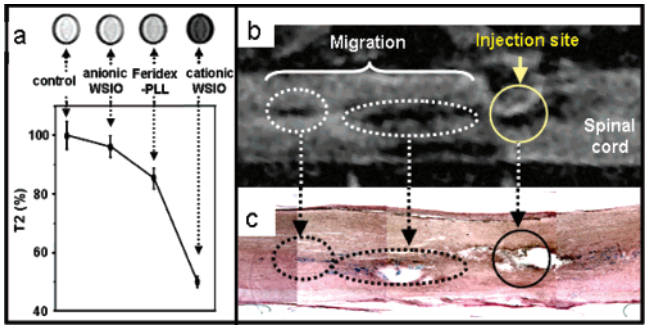

Figure 3. In vitro MR images (a) of anoinic WSIO-, Feridex-PLL-, cationic WSIO-treated neural stem cells. In vivo MR tracking (b) and histological examination by using Prussian blue (c) of cationic WSIO nanocrystal-labeled neural stem cells in a SD rat spinal cord.

treated cells (Figure 2b, inset), and pale brown color is seen for Feridex−PLL-treated cells (Figure 2c, inset). These results indicate that cationic WSIO nanocrystals are efficiently transported into the neural stem cells.

Such excellent cell transport capability of cationic WSIO nanocrystals results in the significant MR signal enhancement of the labeled cells. In the T_2-weighted MR images, a marked darkening of the MR image arising from cationic WSIO-treated cells is observed as compared to those from cells incubated with anionic WSIO and Feridex−PLL (Figure 3a). Consistently, a significant T_2 value drop of ~50% is obtained for cationic WSIO treated cells, while only ~3 and ~14% drop in T_2 values are observed for anionic WSIO and Feridex−PLL treated cells, respectively (Figure 3a).

To prove the versatility of the cationic WSIO nanocrystals as cellular MR probes, we examined intracellular labeling of a variety of other cell types, including tumor (MDA-MB-231), fibroblast (NIH3T6.7), and ovary (CHO) cell lines. Prussian blue staining data show that all cell lines are efficiently labeled by cationic WSIO nanocrystals (Supporting Information).

The excellent cell transport capabilities of the cationic WSIO nanocrystals prompted us to investigate an in vivo application. Before doing this, it was first necessary to assess if these nanocrystals have any deleterious biological properties. Consequently, we determined if they have cytotoxic effects on neural stem cells by using the Trypan blue dye exclusion assay. The cationic nanocrystals are noncytotoxic at iron concentrations at least as large as 100 μg/mL, dose levels that are above those typically used for intracellular labeling (Supporting Information).

With this information in hand, we explored a preliminary application of the cationic WSIO nanocrystals to in vivo MR tracking of neural stem cell migration. Fetal rat neural stem cells (5×10^5), labeled with cationic WSIO, were transplanted into the thoracic spinal cord of a Sprague−Dawley rat. After three weeks, the longitudinal migration of cationic WSIO-labeled neural stem cells (Figure 3b, dotted circle) was clearly observable in MR images as an elongated dark region along the spinal cord starting at the implant site (Figure 3b, solid circle). Histological examination by using Prussian blue staining which detects the location of iron shows stained regions that reasonably closely match those characterized by using MR imaging (Figure 3c).

In summary, a novel, noncytotoxic, and highly efficient magnetic nanocrystal probe for intracellular labeling and in vivo MR tracking has been developed. By simple modulation of the nanocrystal surface charge properties, we have been able to prepare magnetic nanocrystals that efficiently label a variety of cell types. Since cell membranes are known to be weakly negatively charged,[23] it is expected that only cationic WSIO nanocrystals easily anchor to cell membranes through electrostatic interactions and are internalized into cells by way of a charge-mediated endocytosis process.[24] Finally, the excellent intracellular labeling capability of the newly developed cationic WSIO has led to a system for a preliminary, but successful, MRI monitoring of the migration of neural stem cells in vivo in rat spinal cord. The combination of the cell-labeling strategy developed here and the development of highly sensitive nanocrystal MR probes will enable long-term MR monitoring of cell-based medical treatments and cancer metastasis.

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Supporting Information Available: XRD of as-synthesized iron oxide nanocrystals, infrared (IR) spectra, cytotoxicity test, details of the ligand exchange procedure, T_2 relaxivity, Prussian blue staining results for other cell types, MR imaging procedures, cellular distribution of WSIO nanocrystals, and complete ref 10. This material is available free of charge via the Internet at http://pubs.acs.org.

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