Enhanced cellular uptake of aminosilane coated superparamagnetic iron oxide nanoparticles in mammalian cell lines

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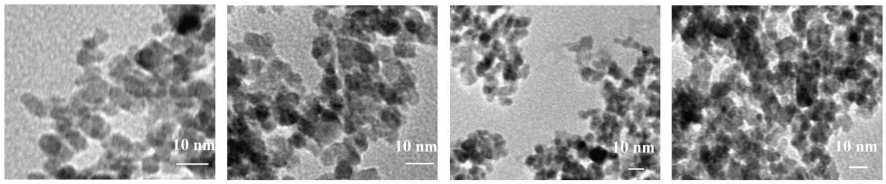
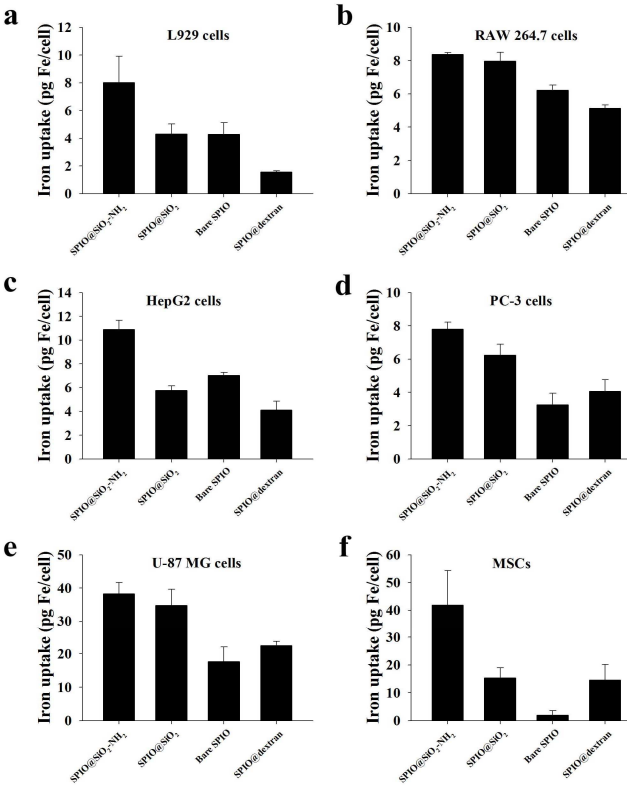
Introduction: To date, dextran coated SPIO nanoparticles of ferumoxides (Feridex) and ferucarbotran (Resovist) are clinically approved for liver MRI, and carboxydextran coated SPIO nanoparticles of Ferumoxtran (Sinerem) have undergone clinical trials for MRI evaluation of lymph node metastasis. SPIO nanoparticles has further important potentials for in vivo stem cell tracking, magnetic separation, hyperthermia therapy, and anticancer drug delivery. For many these applications, optimized cellular uptake of SPIO nanoparticles by target cells is a critical step. One strategy to modulate the cellular uptake efficiency or specificity of SPIO nanoparticles is to modify their surface coating. In addition to dextran and carboxydextran, a number of surface coatings for SPIO nanoparticles, including polyethylene glycol (PEG), polyvinyl alcohol (PVA), dendrimers, starch and silica, have been reported. Among these coating materials, silica is regarded as a biocompatible material. Silica coating has the advantages of preventing the aggregation of particles in liquid and improvement of their chemical stability. Moreover, the silica for nanoparticles coating can be terminated by a functional group which can be covalently attached to a specific ligand by various coupling agents. Organosilanes such as tetraethyl orthosilicate (TEOS), aminopropyltriethoxysilane (APTES) or (3-amino-propyl) trimethoxysilane (APTMS) are commonly used to produce functionalized thin films on the silica coatings of nanoparticles to bring a variety of applications. Among them, APTES is one of the most frequently used to produce the aminosilane (SiO_2-NH_2) coating to help protein and cell adhesion. SiO_2-NH_2 coated SPIO (SPIO@SiO_2-NH_2) nanoparticles prepared by alkaline hydrolysis of APTES have been reported by us and other groups. In our previous study, we applied the SPIO@SiO_2-NH_2 nanoparticles for rabbit mesenchymal stem cells (MSCs) labeling (1). It was shown SPIO@SiO_2-NH_2 nanoparticles had higher labeling efficiency than SiO_2 coated SPIO (SPIO@SiO_2) nanoparticles (1). However, whether SPIO@SiO_2-NH_2 nanoparticles offer an universal higher internalization efficiency than other surface coatings in different mammalian cell lines remains unknown. In this study we attempted to evaluate and compare the intracellular uptake of SPIO@SiO_2-NH_2 with other three nanoparticles, namely SPIO@SiO_2, bare SPIO, and dextran coated SPIO (SPIO@dextran) nanoparticles, in a variety of common mammalian cell lines.

Material and Methods:



Mono-dispersed SPIO@SiO_2-NH_2, SPIO@SiO_2, bare SPIO and SPIO@dextran nanoparticles were synthesized (Fig. 1). These four nanoparticles possessed a similar SPIO core size of 7 nm, and together with their coating, the overall sizes were 7-15 nm. The MRI relaxivity $r2$ was 106.8±15.1, 155.8±14.6, 83.2±16.0 and 101.4±18.2 $mM^{-1}s^{-1}$ respectively. The mouse macrophage cells (RAW 264.7), mouse fibroblast cells (L929), human hepatoma cells (HepG2), human prostate cancer cells (PC-3), and human glioblastoma cells (U-87 MG) were obtained from American Type Culture Collection (ATCC, Manassas, VA, USA). The mesenchymal stem cells (MSCs) were primarily derived from mouse bone marrow. For SPIO labeling, 5,000-10,000 of each type of cells were seeded into each well of the 24-well plates. After 12 h incubation, the culture medium was replaced with the serum-free DMEM containing different types of SPIO nanoparticles with 4.5 μg/ml iron concentration. The cells were further incubated for 24 h. Colorimetric method was used to study the iron concentration for SPIO nanoparticles or cell samples labeled by SPIO nanoparticles (2).

Fig. 1. TEM images of synthesized SPIO@SiO_2-NH_2, SPIO@SiO_2, bare SPIO and SPIO@dextran nanoparticles. Dark dots represent the core of a single SPIO nanoparticle measuring approximately 7 nm in diameter. The coating is observed as a thin and white layer around each single iron oxide core.



Results: The intracellular iron content for the four types of SPIO nanoparticles in six cell lines is demonstrated in Fig. 2. Comparing the iron uptake of four types of SPIO nanoparticles in the six different cell lines, each cell group treated with SPIO@SiO_2-NH_2 nanoparticles showed the highest iron content. For SPIO@SiO_2, bare SPIO and SPIO@dextran nanoparticles, there were variations of labeling efficiency among different cell lines, with SPIO@SiO_2 tended to rank as the second, and bare SPIO and SPIO@dextran tended to have lower labeling efficiency. For the same SPIO nanoparticles, the intracellular iron contents in different cell lines were quite variable. For SPIO@SiO_2-NH_2 nanoparticles, the highest accumulation was observed in U-87 MG cells and MSCs.

Fig. 2. Intracellular iron content study in L929, RAW 264.7, HepG2, PC-3, U-87 MG and primary cultured mouse mesenchymal stem cells (MSCs) after 24 h incubation of SPIO nanoparticles with iron concentration at 4.5 μg/ml.

Discussion: In the current study, all these six mammalian cell lines showed the highest cellular uptake for SPIO@SiO_2-NH_2 nanoparticles. That the intracellular iron contents in U-87 MG and mouse MSCs were nearly four-fold of any other type of cells may be partly due to both U-87 MG and MSCs have bigger cell sizes than the rest cell lines. We have previously reported that surface amine modification enhances labelling efficiency for rabbit MSCs of SPIO@SiO_2-NH_2 nanoparticles by 4-fold compared to SPIO@SiO_2 nanoparticles (1). That finding agreed well with the current result of mouse MSCs (Fig. 2f). Under pH value less than 8, the protonation of amino groups on aminosilane-modified magnetic nanoparticles occur, resulting surface positive charges. Due to the hydroxyl group present on the surface of SPIO@SiO_2 and bare nanoparticles, these two nanoparticles are negative charged. It is also known that plasma membranes possess large negatively charged domains, which should repel anionic nanoparticles, but cationic surfaces have been shown to facilitate cellular internalisation. This may partially explain SPIO@SiO_2-NH_2 nanoparticles have higher cellular-labelling efficiency. This study shows U-87 MG the glioblastoma cell line had 4-fold higher SPIO@SiO_2-NH_2 nanoparticles accumulation when compared with other cell lines. It is possible that human glioblastoma may be particularly suited for SPIO@SiO_2-NH_2 mediated cellular imaging or targeted therapy.

[This study is partially supported by HK ITF (ITS/066/09) and by HK RGC grant SEG_CUHK02] **References**: 1) Wang HH, Wang YX, Leung KC, et al. Chemistry 2009; 15:12417. 2) Gupta AK, Gupta M. Biomaterials 2005; 26:1565