MULTIFUNCTIONAL CATIONIC SUPERPARAMAGNETIC NANOPARTICLES FOR GENE DELIVERY AND IMAGING

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Introduction

There has been significant progress in the development of magnetic nanoparticles for cancer diagnosis and therapy. For example, superparamagnetic iron-oxide nanoparticles (SPIONs) are applied as magnetic resonance (MR) imaging contrast agents for tumor detection. In addition, SPIONs are also used for located hyperthermia therapy. Recently, a new tendency has emerged which focuses on integrated magnetic nanoparticles which are designed for combined MR imaging and therapy. Gene therapy holds great promise for the treatment of a broad of human diseases including cancer. In this study, we thus aim to design multifunctional SPIONs for MR imaging and gene therapy.

Experimental

Materials. SPIONs were prepared according to the previous description [1]. Cationic SPIONs were synthesized by the coupling of SPIONs with 600Da-polyethylenimine via an EDC/NHS activation reaction.

Characterization. Particle size and surface charge of SPIONs were measured at 25 °C using a Nano ZS90 instrument (Malvern, UK) with the dynamic light scattering (DLS) technology.

MR imaging. SPIONs were dispersed in pH 7.4 PBS buffer at iron concentrations. *T2*-weighted MR phantom images were obtained using a 1.5-T Bruker magnet.

In vitro gene transfection and cell viability assays. Transfection experiments were performed towards MCF-7 cells using a pCMV-GFP reporter plasmid. The cells were incubated with the complexes of pEI-SPION (1 μg DNA per well) in the wells of a 24-well plate for 1 h in DMEM medium. Afterwards, the medium was replaced with 0.5 ml of fresh DMEM full medium and the cells were allowed to incubate further for a total of 48 h. All transfection experiments were carried out in triplicate. A linear pEI (ExGen 500), prepared at an N/P ratio of 6/1 was used as a positive control. GFP expression was determined by measuring the fluorescence intensity using fluorescence plate reader at an excitation wavelength of 488 nm and emission at 509 nm. The total cellular protein content in cell lysate from each well was estimated using Bradford reagent by taking BSA as a standard. The fluorescence intensity of GFP was normalized against total protein content in cell extracts. The data are presented as arbitrary units (A.U.)/mg of cell protein. Metabolic activities of MCF-7 cells were evaluated by Alamar blue assay

Results and Discussion

Water-soluble SPIONs were prepared via a one-pot hydrolysis reaction of anhydrous $FeCl_3$ at a high temperature (200 ºC) in the presence of the coating agent, poly(acrylic acid) (PAA). The content of the carboxylic acid groups in PAA-coated SPIONs was determined to be ~7 mmol/g by acid-base titration assay. The SPIONs could be used for MR imaging. As shown in Figure 1A, a significant negative contrast enhancement (signal darkening) was observed at an iron concentration of 0.5 or 1 mg/mL. Moreover,

T2 relaxation time (*T2*-RT) decreased from about 941 to 29 ms with increasing iron concentration from 1/16 to 1 mg/mL. Next, the SPIONs were chemically modified with polyethylenimine oligomer (pEI, 600 Da) via an EDC/NHS activation reaction, to give a group of cationic pEI-SPIONs (Figure 1B). Different feed mole ratios of the amine (NH_2) in the pEI to the carboxylic acid (COOH) in the SPIONs were applied. DLS experiments showed that by increasing the feed mole ratio from 2/1 to 8/1, the particle sizes of the pEI-SPIONs decreased from 53.9 to 26.5 nm, but their surface charges clearly shifted from a negative charge (-26.4 mV) to positive charges (Table 1). The data thus suggest that the pEI was successfully immobilized on the surface of the SPIONs.

Table 1. The characteristics of pEI-SPIONs

No.	acronym	Feed ratio [a]	Size (nm)	Zeta-P. (mV)
1	SPION	0	59.5±0.6	-26.4±0.7
2	pEI2-SPION	2/1	53.9±4.5	+21.4±0.7
3	pEI4-SPION	4/1	32.1±0.6	+27.1±0.5
4	pEI8-SPION	8/1	26.5±0.5	+29.3±2.1

[a] mol $_{NH2}$/ mol $_{COOH}$

Gene transfection of pEI-SPIONs/DNA complexes was evaluated *in vitro* by using MCF-7 cells and GFP plasmid as reporter gene and their metabolic activities were evaluated by Alamar blue assay. The complexes of the pEI8-SPIONs led to the highest transfection efficiency (Figure 2a). Moreover, the complexes, prepared at a pEI8-SPIONs/DNA mass ratio of 8/1, induced higher level of gene expression compared to that of the linear pEI (ExGen 500) as a positive control. Importantly, these pEI-SPIONs revealed very low cytotoxicity with cell viability more than 80% (Figure 2b). The results indicated that PEI8-SPIONs have high potential for gene therapy.

Conclusions

We have demonstrated that cationic superparamagnetic iron-oxide nanoparticles could be successfully designed and applied for combined magnetic resonance imaging and gene delivery.

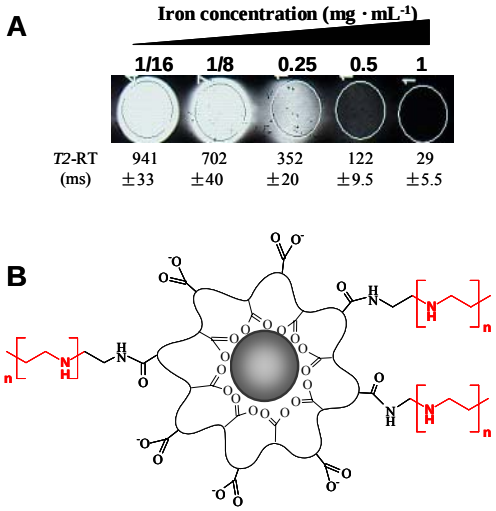

Figure 1. (A) *T2*-weighted MR phantom images and *T2*-RT of the SPION in PBS buffer pH 7.4 at different iron concentrations, (B) schematic illustration of pEI-SPIONs.

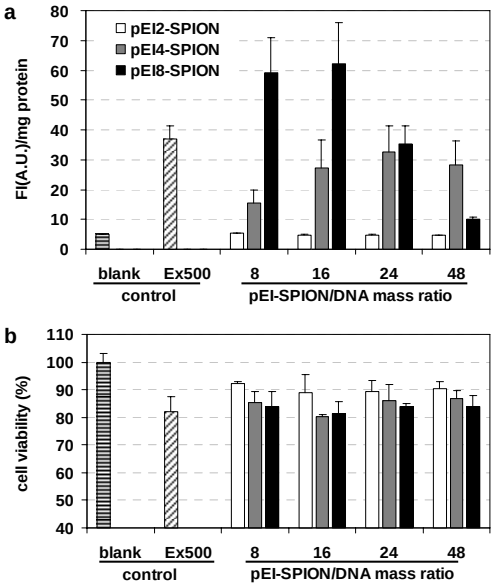

Figure 2. Transfection efficiencies (a) and cell viabilities (b) of pEI-SPIONs/DNA complexes at different mass ratios towards MCF-7 cells.

References

1. Ge, J. P. et al. One-step synthesis of highly water-soluble magnetite colloidal nanocrystals, *Chem. Eur. J.*, **13** (2007), 7153.